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Abstract Book

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1

THE ZMAPP, ZMAB, AND MB-003 COCKTAIL ANTIBODIES INHIBIT EBOLA VIRUS BY BINDING TO NON-IDENTICAL EPITOPES ON THREE DOMAINS OF THE GLYCOPROTEIN

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Cocktails of monoclonal antibodies (MAbs) that target the Ebola virus (EBOV) surface glycoprotein (GP) have been used under emergency compassionate treatment protocols in fifteen patients. However, the detailed epitope binding sites for the MAbs in ZMapp, ZMAb, as well as the related MB-003 cocktail, have not been fully elucidated. In this study we resolved the amino acid epitopes of all six MAbs in these cocktails, as well as the commonly used reference MAb KZ52. Comprehensive alanine scanning (shotgun mutagenesis) was used to create 641 EBOV GP variants that were individually expressed in human cells to enable identification of the most energetically important GP residues required for the binding of each MAb. Identification of epitope residues for these MAbs helps explain their breadth of reactivity against different EBOV species, predict viral evasion against these MAbs, and design new cocktails of MAbs that may offer improved complementarity.

2

HETEROGENEITIES IN THE CASE FATALITY RATE IN THE EBOLA OUTBREAK IN WEST AFRICA

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The ongoing Ebola outbreak in West Africa is the largest on record with over 24,000 case and 10,000 deaths officially recorded by April 2015, the true burden likely considerably higher - a humanitarian catastrophe for which we were not adequately prepared. The case fatality ratio (CFR, proportion of cases that are fatal) is a key indicator of disease severity useful for gauging the appropriate public health response or for evaluating treatment benefits if estimated accurately.We analysed the VHF databases of the three most heavily affected countries Guinea, Liberia and Sierra Leone. The overall CFR in was 68.4% (95% CI: 67.4% - 69.5%) in confirmed and probable cases, with age the most important modifier of survival probabilities: the CFR decreased throughout childhood with a minimum around 15 years of age, then increasing monotonically with age.We classified Treatment Centers (TCs) into 6 types. The CFR varied significantly between TC types: it was higher among cases who were not hospitalized or whose hospitalization status was unknown than that of hospitalized patients ($p<0.001$). Furthermore, the CFR varied between districts and between TCs more than would be expected by chance. We developed a statistical analysis to detect outliers in CFR between districts ($n=41$) and TCs ($n=32$) with 10 or more cases, adjusting for known factors influencing survival and identified twelve districts - eight (two in Liberia and three each in Guinea and Sierra Leone) with significantly higher and four (two in Sierra Leone and one each in Guinea and Liberia) with significantly lower CFR than the average, while there were two TCs with significantly higher and two TCs with significantly lower CFR than average. From the current dataset we cannot determine whether the observed variation in CFR seen by district or TC reflects real (perhaps care-related) differences in survival or was caused by differences in reporting practices or case ascertainment. We will update these results with the latest dataset available in October 2015.

3

PRECLINICAL DEVELOPMENT OF AN EBOLA VIRUS VACCINE BASED ON RECOMBINANT SUBUNITS EXPRESSED IN INSECT CELLS

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Soluble recombinant Ebola virus glycoproteins (GP) and matrix proteins (VP24 and VP40) were generated in the Drosophila S2 cell expression system and purified by immunoaffinity chromatography. The immunogenicity of recombinant subunits and admixtures formulated with or without clinically relevant adjuvants was evaluated in mice, guinea pigs and macaques. Strong antigen-specific IgG titers as well as virus neutralizing titers were observed after administering two or three doses of adjuvanted formulations. In mice and non-human primates subunit proteins were also shown to elicit cell mediated immune responses. Analysis of secreted cytokines in batch-cultured, antigen-stimulated splenocytes or PBMC's demonstrated antigen-induced Th1 and Th2 type responses.Recombinant vaccine candidates were tested in mice for protection against challenge with mouse-adapted EBOV. All vaccine formulations containing EBOV GP generated protective responses and serum transfer from such animals into naïve mice demonstrated that humoral immunity alone can be fully protective. Furthermore, the transfer of immune splenocytes into naïve mice showed that recombinant GP and VP24 subunits elicit functional T cell responses that lead to protection against live virus challenge.Immunogenicity and efficacy studies in guinea pigs were focused on optimized antigen dosing, antigenic balance and adjuvantation. Multiple formulations consistently produced strong antibody responses and demonstrated 100% protective efficacy in the EBOV guinea pig model.Results from studies in two species of non-human primates suggest that vaccination with GP+VP40+VP24 and an emulsion-based adjuvant consistently produces high anti-EBOV IgG and virus neutralizing titers. This prevents viremia subsequent to live virus challenge and protects animals from terminal EBOV disease. These studies suggest that we have defined a viable Ebola virus vaccine candidate based on non-replicating viral subunits.

4

PROTEIN-LEVEL SPECIFICITY OF ANTIBODY RESPONSES TO EBOLA AND MARBURG VIRUSES BY HUMAN SURVIVORS OF INFECTION

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The lack of approved Ebola vaccines or therapeutics has contributed to the high mortality rate of the current outbreak. Clinical evidence suggests that serum from recovered individuals may be effective in the treatment of active cases. The epidemic caused by ***Zaire ebolavirus*** has infected >20,000 people, yet previous outbreaks involving other species of filoviruses also resulted in many fatalities. It follows that variability in human responses to infection can influence the therapeutic effects of antibody products. We examined antibody specificity with sera collected from survivors of outbreaks that were caused by ***Marburg marburgvirus***(MARV), ***Bundibugyo ebolavirus*** (BDBV), or ***Sudan ebolavirus*** (SUDV). To measure antibody responses, we used a protein microarray that displayed NP,VP40, and GP antigens from isolates of the six species of filoviruses that are primate pathogens. Analysis of the microarray data by hierarchical clustering revealed clear separation of positive signals from negative controls. The control samples clustered according to geographical region, with non-infected US subjects exhibiting

the lowest background levels of antibodies. Statistically significant antibody responses to autologous antigens were observed for all three outbreak cohorts. Consistent with amino acid sequence similarities, NP was most cross-reactive and exhibited the highest level of antibody responses, while antibody responses to GP were the most specific. Antibodies to GP, NP and VP40 were also long-lived, as observed for Gulu SUDV survivors 14 years after infection. While most sera were not monospecific, antibodies from MARV survivors presented the lowest level of cross-reactivity with heterologous antigens. Our results suggest that there is a considerable amount of variability in human responses to Ebola and Marburg viruses. The observed anti-GP antibodies, which are important for blocking virus entry into cells, were primarily directed towards the infecting species, whereas antibodies to VP40 and NP may also serve as biomarkers of infection.

5

COMPARISON OF RIFT VALLEY FEVER VIRUS PREVALENCE AMONG COMMUNITY MEMBERS AND SLAUGHTERHOUSE WORKERS IN WESTERN KENYA

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Rift Valley fever virus (RVFV) is a virus of the Bunyaviridae family that infects both domesticated animals, primarily livestock, and humans. The spread of RVFV and the occurrence of zoonotic infections are both considered to be related to the handling and trading of infected livestock. RVFV is also transmitted by mosquitoes, yet determining the contribution of mosquito-borne transmission towards RVFV infection in humans is difficult, mainly due to limited surveillance. The goal of this study was to measure the seroprevalence of RVFV in a rural community that may have exposure to livestock through animal herding and husbandry (n=2071), and compare this to the seroprevalence of RVFV in persons employed in slaughterhouses in the study area in western Kenya (n=720). Samples were screened in-house for anti-RVFV IgG by indirect ELISA. Of the community samples, 16 samples (0.77%, CI95 0.44 to 1.25) were positive for RVFV IgG, whereas 19 of 720 samples (2.64%, CI95 1.6 to 4.1) from slaughterhouse workers were positive for RVFV IgG (p=0.0003). Anti-RVFV IgG concentrations in positive samples were higher overall in the slaughterhouse worker samples than those of the community samples (geometric mean concentrations of 5.583 AU/ml (p=0.0292) versus 3.522 AU/ml (p=0.0003), respectively). Variation in anti-RVFV antibody concentrations between the two groups may reflect inherent differences in the nature of the exposure to RVFV and inoculum size. Risk factors for the community samples include occupation as a farmer (OR 4.8, CI95 1.1 to 21.2, p=0.040) and age of >36 years (OR 8.2, CI95 2.0 to 34.2, p=0.004). Risk factors associated with the slaughterhouse samples include age of >36 years (OR 2.8, CI95 0.8 to 10.2, p=0.12), position as a slaughterman compared to other occupations in the slaughterhouse (OR 3.3, CI95 1.0 to 11.2, p=0.06), and working with only cattle (OR 3.9, CI95 0.8 to 18.0, p=0.08) instead of in a mixed-ruminant slaughterhouse. These results confirm the presence of RVFV in western Kenya, of which direct contact with livestock may increase risk for RVFV transmission, and suggest that mode of transmission influences host immune response to RVFV.

6

CO-CIRCULATION OF HUMAN MONKEYPOX VIRUS AND VARICELLA-ZOSTER VIRUS (CHICKENPOX) IN THE SANKURU DISTRICT, DEMOCRATIC REPUBLIC OF CONGO

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Monkeypox virus (MPXV) is considered the most important virus in the orthopoxvirus genus since the eradication of smallpox (variola). MPXV causes similar clinical features to chickenpox, caused by varicella zoster virus (VZV). There have been reports of the co-circulation of MPXV and VZV in Central Africa. However, a sustained outbreak of both viruses has never been confirmed. Here, we use data from a 2005-2007 active surveillance program for human MPX in Kasai Oriental province, Democratic Republic of Congo (DRC) to show that co-circulation is occurring in 9 health zones of the Sankuru District. Demographic and contact tracing information was collected as well as samples of vesicles, crusts, or fluid from active lesions. . Samples were tested for both MPXV and VZV by PCR and test results were aggregated into the following categories: -MPXV/-VZV, +MPXV/-VZV, -MPXV/+VZV, and +MPXV/+VZV. Proportions were compared across the 9 health zones to determine which zones had the highest incidence of co-circulation. Cases with GPS coordinates were plotted geographically by time period to assess the spatiotemporal spread of the outbreaks. Additionally, an adjacency matrix of contacts was used to create a graph of the contact network. All analyses were completed using R and Excel. Of the 1115 cases investigated during the active surveillance period that were tested for MPX V and VZV, 53% tested positive for MPXV only, while 24% were only VZV positive. Approximately 8% of cases were negative for both viruses and 14% had evidence of MPX/VZV co-infection. At least one +MPXV/+VZV case was detected in each of the health zones, with Lodja and neighboring Djalo Ndjeka containing the most significant amount of both +MPXV/+VZV and +MPXV/-VZV.Although MPXV and VZV have been confirmed to co-infect humans, their co-circulation within the same outbreaks has never been confirmed, and its epidemiological significance is little understood. Here, we show the chains of transmission where MPXV and VZV both thrived, as well as potential geographical factors involved in co-circulation success.

7

OUTBREAK INVESTIGATION OF KAYSANUR FOREST DISEASE (KFD) IN WAYANAD DISTRICT, KERALA, INDIA 2015

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Kyasanur Forest disease (KFD), a tickborne viral hemorrhagic fever, is endemic to Karnataka State but has been reported spreading to bordering states of Tamil Nadu and Kerala. Infected ticks transmit infection to monkeys, the amplifying host, which disseminate infection (hotspots). Vaccination of high risk populations is the primary strategy for controlling KFD along with use of personal protection measures. On February 6, 2015, Kerala reported its first KFD outbreak in an area adjoining Chikenji forest range of Wayanad district. The outbreak was investigated to assess its extent and identify risk factors. Residents of Sulthan Bathery taluk (sub-district) who during 25 December 2014 - 13 March 2015 presented with sudden onset of fever, headache, and myalgia were defined as

cases. For each case we selected two healthy controls matched for age, sex and place of residence and they were interviewed regarding recent exposures using a structured questionnaire. Entomological and monkey death investigations were also conducted. We identified 113 cases (attack rate 6.9 cases/10,000 persons) with a case fatality rate of 5.3%. Most cases (96%) were over 14 years of age and 62% were females. None had received vaccination prior to this outbreak. Of 81 cases laboratory tested, 43 (53%) confirmed for KFD virus (KFDV) by reverse transcription polymerase chain reaction (RT-PCR). Among the enrolled 59 cases and 118 controls, a recent visit to forest (OR=4.8, 95%CI=2.1-10.6), grazing animals in forest (OR=3.1, 95%CI=2.2-6.9), exposure to monkey death (OR=4.1, 95%CI=2.2-7.2) and collection of heaps of leaves around house (OR=1.9, 95%CI=1.09-2.8) were significantly associated with the disease. The tick vector (Hemophysalis spinigera) for KFD was found in abundance in the affected forest area. Out of 18 monkey deaths (Macaca radiate species) reported, 5 tested positive for KFDV by RT-PCR. The evidence suggests transmission of KFDV within the district as both the virus and the vector have been found. We recommended vector control, use of personal protective measures and an effective vaccination policy for prevention of KFD outbreaks in future.

8

FAILURE OF DIHYDROARTEMISININ-PIPERAQUINE IN NORTHERN CAMBODIA FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM*

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Since artemisinin drug resistance was first observed in 2006, a series of artemisinin-based combination therapies (ACTs) have been selected for the treatment of ***Plasmodium falciparum*** malaria in Cambodia; partner drugs with favourable pharmacological properties are selected in order to minimise the impact of artemisinin resistance. Dihydroartemisinin-piperaquine (DHA-PQP), introduced as first-line treatment for ***P. falciparum*** in Cambodia in 2009, is the most recent of five artemisinin-based combination therapies (ACTs) to be recommended by World Health Organization (WHO). Although DHA-PQP initially demonstrated a high efficacy, there has been recent evidence of parasitological and clinical failure. This study presents the data collected in several 2014-15 therapeutic efficacy studies using DHA-PQP for the treatment of ***P. falciparum*** in Cambodia. This study utilised the standardized WHO protocol for assessment of antimalarial treatment efficacy. It was conducted in the northern Cambodian provinces of Siem Reap, Stung Treng and Mondulkiri between August 2014 and February 2015, during the malaria transmission season. A total of 120 patients infected with ***P. falciparum*** were included. Efficacy data and risk of PCR-adjusted recrudescence up to Day-42 (D42) were evaluated using intention-to-treat and Kaplan-Meier analysis. High DHA-PQP treatment failure rates were observed at D42: 62.5% in Siem Reap, 40% in Stung Treng and 10% in Mondulkiri. In the context of regional artemisinin resistance, these results are suggestive of ***P. falciparum*** resistance to Piperaquine. Piperaquine is a bisquinoline and is structurally similar to chloroquine, for which there is known resistance in Cambodia. This is the first time that the northern provinces of Cambodia have been found to have higher treatment failures and a higher proportion of D3-positive participants than the western provinces. It is concerning that western and northern Cambodia have a high volume of population movement, which is likely to contribute significantly to the spread of spread of multidrug resistance; this population movement is a considerable hindrance to the elimination of malaria. These findings demonstrate that it is becoming increasingly difficult to find a highly effective antimalarial treatment for ***P. falciparum*** in both western and northern Cambodia. There is an urgent need for new antimalarial regimens for these populations.

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SAFETY AND EFFICACY OF PYRONARIDINE-ARTESUNATE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* IN WESTERN CAMBODIA

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The first signs of artemisinin resistance were reported from Pailin Province in western Cambodia. There are now increasing reports of parasitological and clinical failure following most widely used ACTs indicating resistance to both the artemisinin compound and the partner drugs. There is an urgent need for new malaria treatment options, including new ACT regimens, especially in areas of known multi-drug resistance. The combination of pyronaridine-artesunate could be an alternative option for the treatment of uncomplicated ***Plamodium falciparum*** malaria in western Cambodia. In an open-labelled, controlled clinical trial conducted in two sites in western Cambodia (Pailin and Pursat provinces), patients with ***P. falciparum*** mono-infection were treated with pyronaridine-artesunate and were followed up for 42 days. The primary endpoint was PCR-adjusted adequate clinical and parasitological response (ACPR) by day 42, and secondary endpoints included: PCR-adjusted ACPR on day 28, parasite positivity rate on day 3, liver function test and other safety outcomes, and prevalence of molecular markers of drug resistance. One hundred and twenty-three patients were enrolled and completed the full course of treatment and 42 days of follow-up. Fourteen (14/116=12.1%) patients had RT-PCR confirmed recrudescence, all of which carried the C580Y K13-propeller mutant allele associated to artemisinin resistance. Recrudescent infections at the Pailin site (n=8), all shared the same ***msp1***, ***msp2*** and ***glurp*** genotypes as well as 10-SNPs bar coding, but all occurred within a single village. Transient elevations of liver enzymes occurred in 15 (12%) patients but values returned to normal by day 28 in the majority patients. The WHO criterion for introducing a new first-line malaria treatment (i.e. an adequate clinical and parasitological response in more than 95% of patients at follow-up on day 28) was not met western in Cambodia. However, pyronaridine-artesunate was observed to be a safe drug and well tolerated with no reports of severe adverse effects including no indications of significant liver toxicity.

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TRANSMISSION-BLOCKING EFFICACY OF SINGLE DOSE PRIMAQUINE ADDED TO ACT IN CAMBODIANS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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In low transmission areas, single dose primaquine is recommended by the WHO to be given alongside artemisinin-based combination therapy to ***Plasmodium falciparum*** patients to prevent transmission. While multiple studies have shown that this intervention reduces post-treatment gametocytemia, no clinical studies have directly measured its effect on infectiousness to mosquitoes in those already treated with ACT. We conducted an open-label clinical trial in 101 Cambodian patients with normal to moderately deficient G6PD-activity deficiency randomized to either 45mg single dose primaquine or no primaquine on Day 3 of dihydroartemisinin-piperaquine (DP) therapy. Human-to-mosquito infectivity was assessed using membrane feeding assays pre-treatment and at days 4, 7, and 14 on 300 ***Anopheles dirus*** mosquitoes. We examined 50 of the roughly 220 engorged mosquitoes per feed for the presence of midgut oocysts, saving the rest for PCR and genetic analyses. Only 6/101 (5.9%) patients were infectious to mosquitoes prior to DP treatment. On day 4 post-treatment, 3/6 remained infectious (1 of 3 had received primaquine 20hrs earlier) and 3 more patients became infectious (none of whom had received primaquine). By week 1 post-treatment, 4/6 patients remained infectious, none of whom had been treated with primaquine. This transmission-blocking effect was statistically significant (0/48 patients vs. 4/49 patients transmitted in the PQ vs no PQ group at week 1, p=0.043). There were no clinically significant adverse hematologic events. Details of mosquito infection, gametocyte, and PCR endpoints will be discussed. This study supports the transmission-blocking utility of a 45mg dose of primaquine in subjects without severe G6PD deficiency. We are currently studying the lower dose of primaquine (0.25mg/kg) recently recommended by the WHO for all patients without G6PD screening.

11

IMMUNITY TO MALARIA AND EMERGING ARTEMISININ RESISTANCE

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Resistance to artemisinin, the first-line treatment against falciparum malaria, was first reported in Western Cambodia in 2009. Recently, the Tracking Resistance to Artemisinin Collaboration (TRAC) a unique multicentre artemisinin therapeutic efficacy study, including 15 sites of varying malaria transmission across Asia and Africa, confirmed that resistant falciparum malaria (defined by a slow clearing phenotype and the presence of the kelch13 molecular marker) is firmly established in Western Cambodia, Thailand, Eastern Myanmar, and Southern Vietnam, and is emerging in Northern Cambodia and Southern Laos. Naturally acquired immunity to malaria has the ability to clear parasites and is an important host factor to understand in the context of understanding the emergence of resistant parasites. To date, there have been no large multinational studies of malarial immunity or any investigations into how variations in population levels of immunity may impact on the geographical spread of artemisinin resistance. We aimed to quantify variations in ***Plasmodium falciparum*** antibody responses and their impact on parasite clearance time after artemisinin treatment across multiple populations experiencing varying levels of malaria transmission. We determined antibody levels and function to a panel of ***P. falciparum*** antigens representing various stages of the life-cycle to enable us to identify immune biomarkers that predict parasite clearance time in 16 sites and 9 countries participating in TRAC. We show that i) host-immunity to ***P. falciparum*** varies across populations and is lowest in areas where the prevalence of kelch13 mutations and the slow clearing phenotype is the highest; ii) ***P. falciparum*** antibodies are associated with faster ***P. falciparum*** clearance times, even in areas of relatively low immunity; iii) ***P. falciparum*** antibodies have the biggest impact on ***P. falciparum*** clearance in the presence of slower-clearing parasites carrying kelch13 mutations. We conclude that immunity is an important confounder in the assessment of emerging artemisinin resistance and may also contribute to the emergence of artemisinin resistance in the region.

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METABOLIC PROPERTIES OF *PLASMODIUM FALCIPARUM* SUB-POPULATIONS ASSOCIATED TO ARTEMISININ RESISTANCE IN CAMBODIA

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The undergoing WHO Malaria elimination program is threatened by emergence and the potential spread of the ***Plasmodium falciparum*** artemisinin resistant parasite. Artemisinin resistant parasites have emerged in the western part of Cambodia, where chloroquine and pyrimethamine drug resistance emerged in the past. Recent reports have shown (1) presence of several ***P. falciparum*** sub-populations in Cambodia and (2) evidence that mutations in the propeller domain of the K13 gene are major determinants of artemisinin resistance in Cambodian parasite population. To characterize the Cambodian parasite sub-population metabolic properties and identify genetic evidence associated to the acquisition and the transmission of artemisinin resistance, a reliable SNP variant calling pipeline based on analysis of signal parameters in comparison with 3D7 reference genome was applied to around 170 NGS Cambodian genome sequences recovered from ENA database. In addition, a barcode approach based on LUMINEX technology was used to screen for parasite population structure in Cambodia. Genome wide analysis revealed presence of major hot spots of variation and specific haplotypes among the Cambodian sub-populations. The annotation for sub-population specific gene set based on proteins and domain associated GO terms, pathways and networks (co-expression, metabolism, Y2H, ⋯) was determined. Distribution of K13 mutant alleles provide genetic evidence that acquisition and transmission of artemisinin resistance is related to parasite population structure in Cambodia. Presence of admixture parasite sub-population was found as a major risk for artemisinin resistance transmission. Based on the barcode analysis, a new sub-population located in south Cambodia, was associated with the most common C580Y K13 allele. Parasite sub-populations differed in metabolic capacities and specific genes in some sub-populations were associated to various housekeeping

functions including cytoskeleton and ubiquitination, likely involved in K13 protein interaction. Our findings question the origin and the persistence of the ***P. falciparum*** sub-populations in Cambodia.

13

COMPARISON OF TWO REGIMENS OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN PREGNANT WOMEN IN THE DEMOCRATIC REPUBLIC OF CONGO

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Malaria in pregnancy is a major cause of maternal and newborn morbidity and mortality. Artemether-lumefantrine (AL) is an effective 3-day therapy, however its pharmacokinetic properties are altered in pregnancy resulting in reduced plasma concentrations. The aim of this study was to compare an extended regimen of AL to the standard one in a group of pregnant women (PG) and a control of non-pregnant women (NP) with uncomplicated falciparum malaria. Ninety-six patients were randomly allocated to 5-day (extended) or 3-day (standard) regimen. AL was administered DOT and with milk. Drug plasma concentrations were characterized for each patient. Therapeutic efficacy was assessed using the standard 42-days WHO protocol. Tolerability and safety (including pregnancy outcomes) were also assessed. The maximum concentrations (Cmax) were significantly higher in the PG-5d arm than the PG-3d arm: 75 ng/mL (range 29 to 118) vs. 52 ng/mL (27 to 130), p=0.008. Similarly the median day 7 lumefantrine plasma level was higher in the PG-5d arm, 1545 ng/mL (537 to 3650) vs. 597 ng/mL (216 to 928). The day-42 efficacy, PCR unadjusted, was similar in the PG-5d and PG-3d arm, 90% vs. 91%. The 4 pregnant women with a recurrent episode of malaria had a day-7 level of lumefantrine between 320 and 750 ng/mL. One patient only (PG-3d) had a value below 280 ng/mL, the critical cut-off predicting treatment failure. Results were comparable in the control group of non-pregnant women. There were no hematological or biochemical abnormalities but more patients in the 5-d regimen had GI symptoms. Active placental malaria at delivery was observed in 6 cases in the 3d arm and 3 in the 5d arm (p=0.02). Four neonatal deaths (unrelated to study treatment) were reported: 3 stillbirths in the 3d arm and 1 neonatal sepsis in the 5d arm. Thirty-nine babies (87%) were followed-up for 1 year and displayed a normal physical and neurological development. Further analyses are currently being performed. The extended regimen improved the exposure to lumefantrine in pregnancy with a good safety profile.

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COMPARING ARTEMETHER-LUMEFANTRINE AND CHLOROQUINE--WITH AND WITHOUT PRIMAQUINE--FOR THE TREATMENT OF UNCOMPLICATED VIVAX MALARIA IN ETHIOPIA: A RANDOMIZED CLINICAL TRIAL

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Chloroquine (CQ) is currently the first line treatment for ***Plasmodium vivax*** monoinfections in Ethiopia and artemether-lumefantrine (AL) for those with mixed infections or ***P. falciparum***. Treatment of hypnozoite stages with primaquine (PQ) is not widely practiced due to G6PD deficiency-related safety concerns. To determine the safety and efficacy of primaquine radical cure and confirm the ***P. vivax*** schizontocidal efficacy of AL and CQ, we conducted a 4-armed randomized open label controlled trial comparing AL and CQ--with and without PQ (0.25mg/kg once daily x 14 days). The study was conducted in two sites in Oromia State: Bishoftu and Bulbula. Patients older than 1 year of age presenting with uncomplicated vivax malaria were randomly assigned to one of four treatments: AL alone, AL+PQ, CQ alone and CQ+PQ. Initial efficacy outcomes were measured for day 28 and 42, but follow up was continued for 12 months irrespective of recurrence. Patients with recurrent episodes after 42 days were treated with the same treatment as on enrolment. Between October 2012 and December 2014 a total of 399 patients were enrolled. Preliminary analyses revealed the uncorrected efficacy rate at day 42 was 81.2% (95% CI: 71.8-87.7) following CQ, 70% (95% CI: 59.5-78.4) after AL, 98.8% (95% CI: 91.9-99.8) after CQ+PQ, and 93.3% (95% CI: 85.3-96.8) after AL+PQ. The risk of recurrence at 12 months was 60% (96% CI: 43.6-74.4) in the CQ arm, 66.6% (95% CI: 50.3-97.8) in the AL arm, 20.5% (95% CI: 10.8-35.5) in the CQ+PQ arm and 37.8% (95% CI: 15.4-43.0) in the AL+PQ arm. The full analysis of the completed study including additional laboratory testing results will be presented. The high risk of recurrence at day 42 likely reflects emerging resistance in the CQ arm and lower post treatment prophylaxis following AL. The addition of the standard 14-day dose PQ was well tolerated and reduced recurrences, but the risk of recurrence at 12 months was significantly higher when combined with AL compared to CQ.

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THE CONTRIBUTION OF HUMORAL IMMUNITY TO CLINICAL OUTCOMES IN AN AREA OF MULTIDRUG-RESISTANT *PLASMODIUM FALCIPARUM* IN CAMBODIA

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Mutations in the kelch 13 propeller gene of ***Plasmodium falciparum*** have been implicated as molecular markers of artemisinin resistance associated with prolonged parasite clearance times. We recently reported that Cambodian patients with the k13 C580Y mutation were 5.4 times more likely to fail dihydroartemisinin-piperaquine (DP) therapy for uncomplicated ***P. falciparum*** infection than those with non-C580Y mutations. However, 47% of those with C580Y mutation did not recrudesce, suggesting other factors involved in cure, particularly pre-existing immunity. We compared clinical outcomes among 93 evaluable patients with antibody levels by median fluorescent intensity (MFI) and seropositivity rates to 4 ***P. falciparum*** antigens (MSP1, AMA1, CelTOS, CSP) using a multiplexed bead array (Luminex®). Approximately 90% of patients were seropositive for MSP1, AMA1 and CelTOS while 56% were seropositive for CSP. Volunteers who were seropositive for AMA were more likely to have complete cure (ACPR) at 42 days compared to those with malaria recurrence (53% vs 8% p=0.003). Seropositivity for AMA was also associated with ACPR versus recrudescence in those with the C580Y k13 mutation (p=0.04) but not the R539T mutation (p=0.1). PfMSP1 titers at admission in those who went on to ACPR were higher versus those who had recrudescence (unpaired t-test p=0.025). Neither MFI nor seropositivity for any antigen was predictive of parasite clearance half-life or day 3 positivity. Humoral immunity, particularly the presence of AMA1 antibodies, appeared to contribute to the cure of malaria infection in populations residing in areas of multi-drug resistance, suggesting efficacious vaccine could play an important adjunctive role in malaria elimination in the region.

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DISSECTING THE FUNCTIONAL DIFFERENCES AMONG SPECIFIC HUMAN IGG SUBCLASSES AGAINST *PLASMODIUM FALCIPARUM* MEROZOITES

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Individuals living in malaria endemic areas develop natural immunity that protects them from symptomatic malarial disease, and antibody responses against blood stage antigens play a major role. Some antibodies target the invasive form of the parasite, the merozoite, and are able to inhibit erythrocyte invasion. Antibodies targeting ***Plasmodium falciparum*** erythrocyte binding antigen 175 (EBA-175) have been shown to inhibit binding to its erythrocyte receptor, glycophorin A. Interestingly, naturally acquired antibodies to EBA-175 and other merozoite antigens are typically skewed towards either IgG1 or IgG3, and the IgG3 responses are generally more strongly associated with protection. It remains unclear how the important differences in the functional effector responses between IgG subclasses impact malaria immunity. To assess this, we used a well-characterised invasion inhibitory monoclonal antibody (mAb) to EBA-175 (R217). We determined that Papua New Guinean children, following natural infection, acquire antibodies that target the same EBA-175 epitope as the mAb R217. We then expressed this R217 mAb as recombinant chimeric human IgG1, IgG2, IgG3 and IgG4 and examined potential functional differences in a series of ***in vitro*** functional assays. These chimeric antibodies contained the same Fv region as R217 but had different human IgG backbones. These chimeric human mAbs showed similar epitope-specificity, affinity, glycosylation patterns and the ability to inhibit parasite growth and EBA175-glycophorin A binding interactions. Subclass specific differences were observed for other functional effector responses including complement deposition and opsonic phagocytosis. These findings suggest that human IgG subclasses do mediate differences in functional immunity and have important implications for assessing outcomes from vaccine studies.

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FCRL5 EXPRESSION IS ASSOCIATED WITH *PLASMODIUM FALCIPARUM* EXPOSURE AND DEFINES A FUNCTIONALLY DISTINCT SUBSET OF ATYPICAL MEMORY B CELLS

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Atypical memory B cells (MBCs) are associated with ***Plasmodium falciparum*** (Pf) exposure, and have been hypothesized to be functionally exhausted due to long-term Pf exposure. Recent reports by our group and others have found that a majority of atypical MBCs express FCRL5, not FCRL4 as previously thought, however, the functional relevance of FCRL5 expression has not been assessed. We tested the hypothesis that poor antibody recall was associated with FCRL5 expression, and that FCRL5 expression relates to Pf exposure. When we stimulated FCRL5+ and FCRL5- atypical and classical MBCs ***in vitro*** with the poly-clonal B cell mitogen CpG, we found that FCRL5+ MBCs, either classical or atypical, had decreased antibody recall responses compared to their FCRL5- counterparts (mean of 1.7 and 33.7 antibody secreting cells per 1000 atypical MBCs, FCRL5+ and FCRL5- respectively, p=0.03). We also assessed the frequency of FCRL5+ atypical MBCs from individuals living in an area of intense Pf exposure compare to age matched individuals with more moderate exposure, and found that frequencies of FCRL5+ atypical MBCs were increased in individuals with higher Pf exposure. Since FCRL5 expression was functionally important and associated with Pf exposure, we tested whether Pf infected red blood cells could directly induce expression of FCRL5 on B cells. Preliminary findings indicate that infected red blood cells can induce FCRL5 expression on class-switched B cells from non-Pf exposed individuals in a dose dependent fashion (MFI of 500 to 1400 with iRBC), and this FCRL5 induction was augmented with the addition of CpG (MFi of 2500 with CpG and 6000 with CpG + iRBC). These findings suggest that induction of FCRL5 on B cells may be, at least in part, non-specific or innate-like since these B cells were unlikely to have memory responses, but we are also currently testing the hypothesis that FCRL5 expression may be antigen-specific in people highly exposed to Pf. In summary, our findings suggest that FCRL5 expression is strongly associated with B cell exhaustion and exposure to Pf, and may be driven, at least in part, through non-specific or innate-like mechanism(s) during infection.

18

CHARACTERIZATION OF IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* GAMETOCYTES

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Drugs and vaccines targeting ***Plasmodium falciparum*** transmission stages have recently gained prominence as necessary tools for malaria elimination and eradication. Current transmission-blocking vaccine strategies target the stages in the mosquito while the developing sexual stages in the human host, or gametocytes, have so far been neglected. However, we would argue that targeting these stages has tremendous potential to decrease the global burden of malaria. As in asexual sequestration, the recently described gametocyte sequestration process in human bone marrow is likely mediated by interactions between specific host receptors and adhesins on the surface of infected red blood cells (iRBCs). Antibodies recognizing early-stage gametocytes could confer protection by 1) inhibiting binding necessary for entry and/or development in the bone marrow, 2) increasing killing by effector cells, and/or 3) inducing phagocytosis by macrophages and neutrophils. Hypothesizing that early-stage gametocytes entering into or developing inside the bone marrow are targets of host antibody responses, we performed the first systematic characterization of immune responses targeting gametocytes. After establishing a flow cytometry assay to screen Malawian patient sera for antibody recognition of gametocyte-iRBCs, we were able to identify samples that are significantly positive for the gametocyte-iRBC surface. Some of these positive sera also recognize asexual-iRBCs while others uniquely recognize gametocyte-iRBCs, particularly early gametocyte-iRBCs. Immunofluorescence microscopy confirms that early gametocytes are recognized more than later gametocytes. We are currently further defining the stage specificity of the response, using qRT-PCR to correlate gametocyte densities with immune responses, and using proteomics to identify the corresponding target antigens. Understanding the human immune response elicited by ***Plasmodium*** gametocytes and the target antigens involved is essential for understanding host-pathogen interactions and designing transmission-blocking vaccine strategies.

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COMPLEMENT AND ANTIBODY-MEDIATED ENHANCEMENT OF RED BLOOD CELL INVASION BY *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum malaria results in close to one million deaths worldwide each year due to repeated cycles of red blood cell (RBC) invasion and destruction. Attempts to develop a vaccine to block RBC invasion have failed. Although antibodies induced against merozoite vaccine antigens show inhibition of invasion ***in vitro*** in the presence of heat-inactivated (HI) serum (to remove complement), there is poor efficacy ***in vivo***. The reasons for this discrepancy are unknown. We hypothesized that complement activation and opsonization of merozoites actually enhances complement receptor 1 (CR1)-mediated invasion of RBCs. We tested this hypothesis by studying the effect of mouse monoclonal anti-merozoite surface protein 1 (MSP-1) antibody 5.2 (mAb5.2) and antibodies from recipients of a merozoite vaccine (MSP-1_{42}) for their ability to enhance or inhibit invasion in the presence or absence of complement. Invasion of RBCs was enhanced by fresh serum relative to 3 min, 5 min, or 30 min HI serum. Furthermore, addition of mAb5.2 to fresh serum increased the enhancement effect. Addition of C2 and Factor B to 3 min HI serum, but not to 30 min, rescued the enhancement of invasion in the presence of mAb5.2. Likewise, Compstatin, a C3 specific inhibitor, and soluble CR1 w(sCR1) ere able to negate the enhancing affects of mAb5.2 in fresh serum but not that of fresh serum alone. Purified antibodies from MSP-1 vaccinees showed inhibitory activity in C3/C4-inactivated serum, but inhibition was drastically reduced in C3/C4 reconstituted serum. Our results demonstrate that anti-merozoite antibodies in the presence of complement can enhance RBC invasion via CR1 constituting a novel mechanism of immune evasion by ***P. falciparum***. These findings are of great importance to the efforts to develop a merozoite blocking vaccine since understanding the mechanisms of parasite immune evasion will aid in the development of more effective vaccines. Further studies will be needed to understand the relevance of our findings to ***in vivo*** models.

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PLASMODIUM FALCIPARUM INDUCES DIFFERENTIAL CHANGES ON DENDRITIC CELLS IN SYMPTOMATIC AND ASYMPTOMATIC PATIENTS FROM THE AMAZON REGION

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Dendritic cells (DCs) play an important role in the induction and regulation of immune responses via antigen-presentation, co-stimulation and production of cytokines and chemokines. Circulating circulating DCs are essential for adequate immunity as they continually replenish the pool of tissue-residing DCs. In malaria, the functionality of DCs remains elusive because of immunomodulatory properties of the ***Plasmodium falciparum*** parasite. Changes in peripheral populations of DCs during acute ***P. falciparum*** malaria were characterized in other setting transmission, but no data are available for ***P. falciparum*** infections in the Peruvian Amazon. We characterized peripheral populations of DCs in uncomplicated malaria patients infected with ***P. falciparum*** and in healthy controls living in the same area of endemicity and exposed to relatively low levels of malaria transmission. Cryopreserved PBMCs from ***P. falciparum*** infected patients (symptomatic and asymptomatic) and endemic controls were stained with an antibody mixture containing lineage-specific mAbs to CD3, CD14, CD16, CD19, CD20, and CD56 conjugated with FITC (lin-FITC), antibodies to CD11c conjugated with APC and CD123 conjugated with PE, and antibodies to HLA-DR conjugated with PerCP. 50000 events were analyzed in a C6 Accury flow cytometer (BD). HLA-DR+CD123+lin–cells were defined as plasmocytoid dendritic cells (PDC) and HLADR+CD11c+lin– as myeloid dendritic cells (MDC). The results showed that the absolute number of PDC and MDC increased in both symptomatic (3,48 x10e7 cells/L) and asymptomatic (2,96 x10e7 cells/L) ***P. falciparum*** patients compared to control individuals (1,83 x10e7 cells/L). Furthermore, it was found a greater absolute number of PDC but not MDC in symptomatic patients versus asymptomatic patients and controls. Here, we also observe the relationship between increased levels of plasma IL-10 and numbers of DC in patients with clinical Pf infections. In conclusion, ***Plasmodium falciparum*** is associated with a clear increase in the absolute number of plasmacytoid DCs (CD123+) phenotype in symptomatic patients.

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MONOCYTE SUBSET PHENOTYPES AND FUNCTIONS IN KENYAN CHILDREN WITH UNCOMPLICATED MALARIA

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Monocytes/macrophages play an important role in innate and adaptive immunity to malaria. Human blood monocytes are classified into 3 subsets according to levels of expression of CD14 and CD16, surface molecules

functionally significant to tissue migration, and inflammatory cytokine production. Cryopreserved PBMC were obtained from 23 children at presentation with acute uncomplicated malaria and at 6 weeks following recovery, 17 healthy child controls, and 14 healthy adult controls in western Kenya. We determined the proportions of monocyte subsets and levels of TLR2, TLR4, CD36, PD-L1, CD86, and BAFF expression. Children with acute malaria had an increased proportion of intermediate "inflammatory" monocytes (CD14hiCD16+) compared to 6 week recovery samples and healthy children and adults (p values <0.03). Compared to 6 week recovery samples and healthy child controls, acute malaria was associated with increased TLR2,TLR4, BAFF, and PD-L1 expression on intermediate monocytes and decreased CD36 and CD86 expression on classical and nonclassical monocytes (p values <0.05). An increased PD-L1/CD86 ratio was seen on all 3 monocyte subsets during acute malaria compared to 6 week recovery and healthy child controls (p values <0.001). Functional assays of these subsets, including phagocytosis and cytokine production, are in progress, and results will be correlated with phenotypic data. These data indicate that monocyte surface expression phenotypes are altered with acute malaria, and these changes are subset-specific.

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I GET HEIGHT WITH A LITTLE HELP FROM MY FRIENDS: HERD PROTECTION FROM SANITATION IN RURAL ECUADOR

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Infectious disease interventions, such as vaccines and bednets, have the potential to provide herd protection to non-recipients. Similarly, improved sanitation in one household may provide community-wide benefits if it reduces contamination in the shared environment. Sanitation at the household-level is an important predictor of child growth, but less is known about the effect of sanitation coverage in the community. From 2008 to 2013, we took repeated anthropometric measurements on 1,314 children under five years of age in 24 rural Ecuadorian villages. Using mixed effects regression, we estimate the household and neighborhood effects of sanitation on child growth.Sanitation coverage at the neighborhood level was strongly associated with child height, as those with 100% coverage in their neighborhood had a 5 fold reduction in the odds of being stunted (OR 0.21, 95%CL 0.05-0.84) compared to those with 0% coverage. Children from households with improved sanitation had a slightly lower odds of being stunted (OR 0.70, 95%CL 0.43-1.15). This protective effect of neighborhood sanitation is manifested primarily among girls during the second year of life, the time at which growth faltering is most likely to occur. Discussion-Our study highlights that a household's sanitation practices can provide herd protection to overall community. Studies which fail to account for the positive externalities that sanitation provides will underestimate the overall protective effect. Future studies could seek to identify a threshold of sanitation coverage, similar to a herd immunity threshold, above which increases in coverage have no marginal benefit.

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SCALE-UP OF MOBILE-TO-WEB COMMUNITY-LED SANITATION ACROSS 13,000 RURAL VILLAGES IN ZAMBIA: CHALLENGES AND OPPORTUNITIES

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The Ministry of Local Government and Housing in Lusaka has adopted community-led total sanitation (CLTS) as its intervention of choice for driving progress toward an open defecation free (ODF) Zambia. CLTS is a behavior-change intervention that generates community engagement and impetus to achieve ODF. As with all public health interventions, accurate targeting and timely monitoring are critical for sustainable, efficient uptake, however the traditional, paper-based systems used to monitor CLTS and other community-based interventions are slow, unwieldy and expensive. We instituted a mobile-to-web monitoring system in 29 districts to target intervention efforts and track community progress toward ODF. Community-level sanitation action groups collect household-level hygiene and sanitation information on paper forms, aggregate to the community-level and submit via mobile phone into the DHIS2 decision support platform. Automated data validations, dashboards and reports are then fed back to community, district, traditional leadership and central level stakeholders. Monthly reporting rates in the system exceed 85% thanks to the dedication of community volunteers and traditional leaders, further reinforced by informed, timely feedback provided by districts using DHIS2. National progress toward ODF is substantial. Over 1,500,000 new users of improved sanitation have been produced in less than 2 years. Further one full district is already certified as ODF, the first documented instance of its kind in southern Africa and more likely to be certified later in 2015. Cost savings have been substantial. Districts implementing CLTS through the mobile-to-web platform save, on average, 34% in total implementation costs compared to districts implementing CLTS without mobile-to-web. Zambia is now scaling mobile-to-web CLTS to 46 districts and the system is being augmented to include indicators measuring village-level access to safe, clean drinking water. We will discuss challenges, solutions and opportunities in developing and sustaining real-time monthly monitoring across thousands of villages in resource-deprived settings.

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PROGRESS TOWARD COMMUNITY ACHIEVEMENT OF OPEN-DEFECATION FREE BEFORE AND AFTER INVOLVEMENT OF TRADITIONAL LEADERS IN ZAMBIA

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Community-led total sanitation (CLTS) is a behavior change intervention intended to cease the practice of open defecation, a leading risk factor for the transmission of diarrheal disease and soil-transmitted helminths. CLTS engages district and sub-district agents who 'trigger' communities by clearly illustrating fecal transmission between open-defecation piles and common food sources, rousing the collective disgust and pride of residents; households are subsequently encouraged to build latrines in effort to become open defecation free (ODF). The Zambia Ministry of

Local Government and Housing implements CLTS through community champions who use mobile phones to transmit monthly data on latrine coverage and adequate sanitation for each of roughly 13,000 villages in rural districts. These data post to an instance of DHIS2, a web-based decision management system, which then pushes feedback to various stakeholders. We devised one such automatic feedback loop - a mechanism for routing intervention impact data - for traditional leaders who are outfitted with tablet computers which display DHIS2 data for the relevant chiefdom. The mobile-to-web CLTS system has been rolled out in 14,020 villages across 29 districts in Zambia, with plans to scale nationwide. A total of 47 out of 71 chiefdoms have been oriented into the system. Before chiefdom orientation only 12.8% of triggered villages achieved ODF; after chiefdom orientation mean progress was doubled with 25.6% of remaining villages achieving ODF. Further nine Chiefdoms are verified as ODF. The engagement of traditional leaders is an important consideration for interventions requiring community engagement, though there are certain limitations. Some of the chiefs in Zambia are less engaged in the push toward ODF and some chiefdoms are in dispute limiting their effectiveness in engaging the community. In Zambia feedback of data empowers traditional leaders and is likely to have impact in other sectors beyond sanitation. In areas without traditional leaders feedback to community-level stakeholders may elicit a similar response in the community.

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SOIL-TRANSMITTED HELMINTH CONTAMINATION OF SOIL IN RURAL KENYAN HOUSEHOLDS

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Globally, about 1.5 billion people are infected with at least one species of soil-transmitted helminth (STH). We developed a method to test for STH in soil and conducted a pilot study to determine the prevalence of STH in soil among rural households in Kenya. We adapted the US EPA method for detecting and enumerating ***Ascaris*** in biosolids via microscopy to identify STH eggs in soil. Soil was seeded with a known number of ***Ascaris suum*** eggs and then processed to determine a recovery efficiency of 73%. Viability of the eggs was not determined, although an incubation step could easily be added to allow determination of viability. We conducted a pilot study from June to September 2014 in Kakamega, Kenya to characterize the prevalence of soil-transmitted helminth contamination of household soils using a version of the method described above. Field staff collected soil samples from the household entrance and the latrine entrance (if present) from each household. We found that 27% of households (N=67) had at least one type of STH egg in the soil. ***Ascaris*** was the most common STH detected at the household-level (19%), followed by ***Trichuris*** (9%), and hookworm (1%). Prevalence of any STH egg in soil was slightly higher at the household entrance (19%, N=67) compared to the latrine entrance (11%, N=62), but the difference was not statistically significant (p=0.31). Among positive samples, the median STH concentration was 0.7 eggs/g dry soil. Detection of soil-transmitted helminth eggs in soil at almost one-third of households suggests that child exposure to soil may be a substantial health risk. Contamination of the soil at both the house and latrine entrances could indicate that there are multiple sites of helminth transmission and exposure within the home. The results from this study will inform soil sampling methods in a large randomized controlled trial to assess the impact of improved sanitation on levels of STH in household soil.

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WASH FOR WORMS: A CLUSTER RANDOMIZED CONTROLLED TRIAL OF THE IMPACT OF A COMMUNITY-BASED WASH PROGRAM ON SOIL-TRANSMITTED HELMINTH INFECTIONS IN TIMOR-LESTE - MID-POINT RESULTS AT SIX MONTHS FOLLOW-UP

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Soil-Transmitted Helminths (STH) are most prevalent in communities lacking adequate clean water, sanitation and hygiene (WASH). Deworming programmes with anthelminthic drugs are highly effective in reducing morbidity but rapid reinfection occurs if there is no reduction in environmental contamination with infective stages, impeding the sustainability of STH control programmes based on deworming alone. "WASH for Worms" is a cluster randomised controlled trial (RCT) assessing the impact of a community-based WASH intervention, implemented by WaterAid Australia, on infection with intestinal parasites following mass albendazole (ALB) chemotherapy in villages in Timor-Leste. In this trial, initiated in 2012, twelve intervention villages receive the WASH programme and ALB treatments every six months. Twelve control villages receive only the six-monthly ALB. All villages are followed-up for two years after the first ALB distribution. Infection prevalence and intensity is measured by a modified qPCR. An overview of the study design and implementation progress will be presented. Additionally, the prevalence and intensity of STH infections at baseline and after the first (6-monthly) follow-up will be discussed and compared across trial arms. At baseline the prevalence of STH in the 24 villages was high, with more than 70% of the 2225 participants who provided stools infected with at least one STH, mostly comprising Necator americanus (62.3%) followed by Ascaris lumbricoides (30.4%). At the first follow-up the overall prevalence of STH infection decreased to 46.3%, with 34.6% of the 1630 participants who provided stools infected with N. americanus and 21.0% infected with A. lumbricoides. In the intervention arm, N. americanus decreased from 62.8% to 32.2% whereas A. lumbricoides decreased from 31.6% to 22.0%. In the control group, N. americanus decreased from 61.8% to 36.9% whereas A. lumbricoides decreased from 29.2% to 20.1%.This trial is the first reported RCT evaluating the impact of integrated WASH and deworming programmes on infection with STHs; and will provide essential evidence for scaling up integrated programmes for STH control.

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FECAL MARKERS OF ENVIRONMENTAL ENTEROPATHY ARE ASSOCIATED WITH ANIMAL EXPOSURE AND CAREGIVER HYGIENE IN RURAL BANGLADESH

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Recent estimates by the World Health Organization (WHO) report that a quarter of children under five years of age are stunted globally. There is a growing body of literature indicating an association between stunting and environmental enteropathy (EE), a disorder defined by abnormal intestinal morphology, reduced intestinal barrier function, and increased inflammation. To determine if household unsanitary environmental conditions were significantly associated with environmental enteropathy and stunting in children, we conducted a cross-sectional study of 216 children(<30 months) in rural Bangladesh. Stool was analyzed

for fecal markers of environmental enteropathy: alpha-1-antitrypsin, myeloperoxidase, and neopterin combined to form an environmental enteropathy disease activity(EE) score, and calprotectin. We observed a significant association between having an animal corral in a child's sleeping room and elevated EE scores (1.0 point difference, 95% confidence interval (CI): 0.13,1.88) and stunting(height for age z-score <-2) (Odds Ratio(OR): 2.53, 95% CI: 1.08,5.43), after adjusting for potential confounders. In addition, children of caregivers with visibly dirty hands had significantly elevated fecal calprotectin(µg/g)(384.1, 95% CI: 152.37, 615.83). These findings suggest that close contact with animals and caregiver hygiene are potential risk factors for environmental enteropathy in young children. These findings are consistent with the hypothesis that unsanitary environmental conditions can potentially lead to environmental enteropathy in susceptible pediatric populations.

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HOUSEHOLD SANITATION AND HYGIENE INDICATORS OF ENTERIC PATHOGEN TRANSMISSION AND CHILDHOOD DIARRHEAL EXPOSURE RISK IN MIRZAPUR, BANGLADESH

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The effectiveness of water quality, sanitation and hygiene (WASH) interventions in reducing diarrheal disease can be strengthened through the identification of enteric pathogen transmission pathways. Our aim was to determine associations between significant diarrheal pathogens among rural Bangladeshi children and potential pathogen sources and household risk factors that may make up such transmission pathways. Stools collected from children aged < 59 months with moderate-to-severe diarrhea (MSD) and matched healthy controls enrolled in the Bangladeshi component of the Global Enteric Multicenter Study (GEMS) were screened for enteric pathogens. Multinomial logistic regression was used to determine associations of ***Shigella flexneri, Cryptosporidium*** spp**,** enterotoxigenic ***Escherichia coli*** (ETEC), rotavirus and ***Aeromonas*** outcomes with WASH measures. Children from households with improved sanitation facilities and disposed children's feces had lower ***S. flexneri*** and ***Cryptosporidium*** diarrhea risk. ***Cryptosporidium*** diarrhea risk was higher when cow dung was used as fuel and mothers did not wash hands before eating. Children from households with toilets and that disposed children's feces had lower ETEC infection risk when no handwashing was practiced after cleaning a child, following defecation and before cooking, respectively. Rotavirus diarrhea was lower among children from households with deep tube wells when no hand washing was practiced after handling of animals. Finally, children from households with improved sanitation facilities and whose mothers washed hands before nursing had lower ***Aeromonas*** diarrhea. We have identified household sources and factors that are critical points in pathogen transmission pathways and children's exposure to selected enteric pathogens and shown how distinct hygiene behaviors may modify these pathways. These findings have important implications for the development of more cost-effective intervention that reduce pathogen exposure risk and overall diarrheal burden through targeted interventions that focus on critical points in pathogen-specific transmission pathways.

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HIGH RATES OF CHAGAS DISEASE CO-INFECTION IN HIV-INFECTED BOLIVIANS

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Chagas disease, caused by protozoa ***Trypanosoma cruzi***, affects 8-12 million Latin Americans. Reactivation Chagas disease with microscopy-positive parasitemia can manifest with severe neurological and cardiac symptoms in patients with advanced HIV, but there are few systematic studies of the syndromes of coinfection. We recruited hospitalized HIV-infected patients from the emergency or inpatient departments of Hospital San Juan de Dios in Santa Cruz, Bolivia to better characterize the epidemiology and clinical spectrum of HIV/***T. cruzi*** coinfection. All subjects underwent interview, physical examination, and laboratory testing. Parasitemia was quantified using RT-PCR. ***T. cruzi*** infection was defined as a positive result by 2 serological tests or parasites detected by microscopy or PCR. CD4 counts and HIV viral loads were performed by the Bolivian government agency CENETROP. 157 HIV-infected subjects (54 women, 103 men) were recruited, and 37 of 150 (25%) were infected with ***T. cruzi***. 28 subjects (80% of ***T. cruzi***-infected subjects) had PCR-detectable parasitemia ranging from 0.71-556,726 parasites/ml. Sex was not related to ***T. cruzi*** infection, but coinfected subjects were almost 6 years older (p=0.01). ***T. cruzi*** infected subjects had higher CD4 counts (median 307 vs. 134, p=0.03); among coinfected subjects, CD4 counts were lower in subjects with PCR-detectable parasitemia (median 161 vs. 563, p=0.09) and HIV viral loads were higher (median 597 vs. 65,357.5 p=0.01). Prevalence of ***T. cruzi*** infection in HIV-positive people in our study was high, reflective of the high burden of disease in Bolivia. Parasites were detectable by RT-PCR in most coinfected subjects and were more prevalent in those with advanced HIV. A more refined definition of reactivation Chagas disease is needed that incorporates the increased sensitivity of PCR technology and characteristic clinical manifestations in order to define the epidemiology of HIV/***T. cruzi*** coinfection and guide studies of treatment and prophylaxis of these patients.

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A PROSPECTIVE COHORT STUDY TO ASSESS THE EFFECT OF COTRIMOXAZOLE PROPHYLAXIS IN HIV-EXPOSED CHILDREN ON MALARIA IN SOUTHERN MALAWI

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A number of studies on the use of malaria chemoprophylaxis have reported an increase in episodes of malaria and other morbidities in the year following termination of prophylaxis. Cotrimoxazole prophylaxis (CPT) is given to HIV exposed but not infected children until HIV infection can be excluded and the child is no longer exposed through breastfeeding. CPT may provide effective prophylaxis against malaria, and may modulate

the development of malaria-specific immunity. It is unclear if cessation of chemoprophylaxis in these circumstances is associated with a rebound in malaria disease. We have studied the protective efficacy of CPT and determined if there was an increased risk of malaria after cessation. We recruited a birth cohort at 6 weeks of age of HIV exposed (HIV-exp) children who were prescribed CPT for 12 months at the PMCT clinic in Zomba, Malawi. Simultaneously age and residency-matched non-HIV exposed children who were not on CPT were recruited from the community. Both cohorts were systematically followed until their second birthday. HIV-exp children who stopped breastfeeding (usually at 12 months) were tested for HIV and those who tested negative had CPT stopped. All children were encouraged to come to the research clinic at anytime for all morbidities. Data on malaria-related and all-cause OPD visits, admissions and deaths was collected over the 2 years of follow-up. We recruited 500 HIV exposed and 500 HIV non-exposed children. Recruitment and follow-up is now complete and analysis is on going. Results will be presented at the conference and will enhance an understanding of the implications of this HIV prevention strategy on malaria and hence directly inform the policies on HIV and malaria control in areas where both diseases are endemic.

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RISK FACTORS, SEXUAL BEHAVIOR AND HIV PREVALENCE AMONG MEN WHO HAVE SEX WITH MEN (MSM) IN LOME, TOGO

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Men who have sex with other men (MSM) have been consistently identified to be at higher risk of HIV infection and transmission. In Togo, there is limited epidemiological information about HIV prevalence, risk factors and behaviors that render them at higher risk of infection. Three hundred and fifty four MSM ≥18 years of age were recruited using respondent driven sampling (RDS) for a cross-sectional survey in Lome, Togo. Participants completed a structured questionnaire. Participants were tested for HIV and syphilis. Statistical analysis included RDS-weighted proportions, bootstrapped confidence intervals and logistic regression models. Mean age was 22 years; 71.5% were between 18 and 24 years. Ever having anal sex without a condom was reported by 155 (43.8%) MSM. In the final RDS-weighted multiple logistic regression model, higher risk of HIV infection was observed among MSM who were 25 years or older (RDS aOR=7.4, 95% CI=2.3-23.6) and among those reporting community/social stigma and discrimination (RDS aOR=2.1,95%CI=1.2-3.9). MSM who reported having sex with another man for the first time when they were 18 years of age or older had lower risk of HIV infection (RDS aOR=0.03, 95% CI=0.0-0.03). RDS weighted HIV prevalence was 9.2% (5.4%-13.2%) and syphilis RDS weighted prevalence was 1.3% (0.0-2.9%). Results indicate that HIV prevalence in MSM is approximately three times that of the general population, making it a priority group for HIV prevention, care and treatment services in Togo. Individual level, social, community and public policy factors might all be affecting the HIV epidemic within this key population and should be seen as targets for interventions. Multilevel approach is required to address risk of HIV infection. Community and social interventions to address stigma and policy change should be key to fight the epidemic in Lome.

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INTRA-VAGINAL PRACTICES AND STRETCHING OF THE *LABIA MINORA* MAY CONTRIBUTE TO AN INCREASED RISK OF STI/HIV INFECTION IN MOZAMBIQUE

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The magnitude of risk for HIV and sexually transmitted infection (STI) acquisition among women in sub Saharan Africa (SSA) is unparalleled. In 2011, HIV prevalence among pregnant women in Mozambique was estimated to be 16%. Intra-vaginal drying/ tightening and stretching of the ***labia minora*** may contribute to an increased risk of STI/HIV infection. We sought to describe the intra-vaginal and labia stretching practices of women in Zambézia Province, Mozambique. A 2014 population-based survey gathered information from 3892 female heads of household from 255 enumeration areas across 14 districts. Kriging analyses using Geographic Information Systems (GIS) were used to map spatial patterns of traditional vaginal practice prevalence in three heavily sampled districts, and statistical analyses were conducted to estimate associations of covariates with vaginal practices. To determine if specific vaginal practices were associated with HIV infection, we modeled the probability of HIV diagnosis at last pregnancy among 810 women who reported HIV test receipt at ANC using multivariable logistic regression with robust covariance estimates. Among all women surveyed, 56% were planning to use intra-vaginal substances for drying/ tightening in the next year. Almost 100% of women who had heard of labia stretching reported they either had or planned to undergo labia stretching in the coming year. From inspection of GIS maps, both practices varied remarkably intra- and inter-district. There was a bivariate association between women who planned to use intra-vaginal substances and HIV infection (p=0.049), but this relationship was not detected in our multivariable model (p=0.21). Given the association between intra-vaginal agent use and STIs/ bacterial vaginosis and the potential link between vaginal infections and HIV acquisition, understanding local practices may be an essential first step to addressing high rates of HIV in young women.

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A CLINICAL SNAPSHOT OF HOSPITALIZED, NEWLY DIAGNOSED, HIV-POSITIVE MALAWIAN CHILDREN REVEALS OPPORTUNITIES FOR IMPROVED HIV HEALTHCARE DELIVERY

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In Malawi, all HIV-positive children under 5 years old qualify for antiretroviral treatment (ART), but CD4+ T cell quantification (CD4 count), when available, or WHO clinical staging of HIV severity is used to determine ART eligibility for children ≥5 years old. Few studies have examined the clinical or immunological status of older children with newly diagnosed HIV infection. We studied children ≥ 18 months of age admitted to Queen Elizabeth Central Hospital in Blantyre, Malawi, from May 2013-June 2014. Initial HIV testing followed the WHO-recommended serial testing algorithm using HIV rapid diagnostic tests (RDT) Determine and Uni-Gold. Guardians of 222 children with positive HIV RDT consented to CD4 count and HIV-1 RNA PCR (HIV VL) while in hospital. We examined age, discharge diagnosis, total and percent CD4 count, HIV VL, and clinical HIV stage using 2013 WHO guidelines. Median age of the children was 48 months (range 18-192 months) and median absolute CD4+ T-cell count was 541 cells/mm3 (range 1-3888 cells/mm3). Of the 182 subjects with HIV VL results, 16 had low (<1000 copies/ml) or undetectable HIV

VL, prompting confirmatory HIV Western Blot and Enzyme Immunoassay. Ten out of 16 patients had positive confirmatory HIV serological testing, consistent with elite-controller status, while 6 had negative confirmatory test results, indicating that 3.3% of children incorrectly reported to have positive HIV RDTs were not HIV seropositive. The most common reasons for hospitalization were sepsis (n=43, 19%), pneumonia (n=42, 19%), malaria (n=38, 17%), malnutrition (n=36, 16%), and meningitis (n=14, 6%). There was poor correlation between clinical and immunologic staging. Of patients aged ≥5 years (n=106), 33% (n=35) qualified for ART by CD4 count but would have been ineligible by WHO clinical stage alone. These findings highlight the importance of regular quality assessment and need for confirmatory testing prior to initiating ART, and support the current WHO algorithm of routine staging of all children, initiation of ART in children who are WHO stage III or IV, and obtaining CD4 count in children who are stage I or II.

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MENTAL HEALTH OF HIV+ FEMALE CAREGIVERS IN RURAL UGANDA. IMPLICATIONS FOR RESEARCH AND PROGRAMMING

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In Sub-Saharan Africa the intersection of mental health and HIV/AIDS frequently constitutes a double burden for HIV+ caregivers. The physiological and psychological effects of HIV disease, the effect of antiretroviral therapies, social stressors (including stigma and discrimination), and financial strains associated all inform the mental health status of women living this the disease. Findings from three studies in rural eastern Uganda assessing mental health of HIV+ caregivers of HIV infected and affected children showcase potential research and clinical implications for global health. In our first two studies, results suggest that caregiver's depression can significantly bias parent-reported ratings. First, caregivers (N=150) with greater depressive symptoms (as measured by the Hopkins Symptom Checklist-HSCL) reported their children as having more behavioral problems related to executive functioning (as measured by the Behavior Rating Inventory of Executive Function-BRIEF) (b=11.0, t(150)=3.97, p<0.01), with the association being stronger in HIV-infected as opposed to HIV-exposed children. Caregivers (N=118) from a second study with higher depressive symptoms in the HSCL reported more Internalizing (b=.25, t(118)=2.29; p=.02) and Total Problems (b=.22, t(118)=2.23; p=.03) in the Child Behavior Check List (CBCL) evaluating their children. Finally, a third study including 288 HIV-positive women; most of who were the biological mothers (98%) of HIV affected children, we found that lower family support was significantly associated with higher depressive symptoms (b= -.10, t (286)= -2.61, p= .01), while higher socio-economic status predicted less depression (b= -.16 t (34)= -.47, p=.01). Findings highlight the relevance of maternal mental health and on identifying and addressing the mental health concerns of HIV infected mothers, which can allow for research design and adjustment, for planning community-level programs aimed at identifying and treating depression, and enhanced child development through maternal well-being.

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TRACKING DEVELOPMENT ASSISTANCE FOR THE PREVENTION AND TREATMENT OF HIV/AIDS, 1990-2014

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Millennium Development Goal (MDG) six focuses on halting the spread of HIV/AIDS. Since this goal was established, more resources have been devoted to combat HIV/AIDS in low- and middle-income countries than any other cause of illness. This presentation will cover findings related to tracking the development assistance for health (DAH) disbursed for the prevention and treatment of HIV/AIDS. We extract data from the Institute for Health Metrics and Evaluation's Financing Global Health 2014 report. This report systematically tracks DAH from 1990 to 2014, splits disbursed DAH into 15 health focus areas, and draws data from all the major international development agencies engaged in combating HIV/AIDS. The sources of the funds, primary channels of delivery, and country recipients of DAH are reported. Since 2000, $100.4 billion of DAH has been provided for HIV/AIDS. In 2014 alone, $10.9 billion was disbursed. Between 2000 and 2010, DAH for HIV/AIDS grew at an annualized rate of 22.5%. Since 2010, the annualized rate of growth has only been 0.7%. The United States government was the largest source of DAH for HIV/AIDS during this period, proving $56.5 billion, or 56.3% of the total, since 2000. 81.0% of the DAH for HIV/AIDS from the US was channeled through US government agencies, while 9.2% was channeled through the Global Fund to Fight AIDS, Tuberculosis, and Malaria. The Bill & Melinda Gates Foundation was the largest private organization providing DAH targeting HIV/AIDS, furnishing $3.3 billion since 2000. Of DAH for HIV/AIDS that could be tracked to a single recipient country, 38.6% was disbursed to sub-Saharan Africa. Across all recipient countries, an average of $3,600 of HIV/AIDS DAH per HIV/AIDS disability-adjusted life-year (DALY) was disbursed between 2000 and 2014. With the post-MDG era in sight, these estimates and trends hold lessons for future global health ambitions and the unfinished agenda of the MDGs.

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THE ABILITY OF CHIKUNGUNYA TO REEMERGE IN A PREVIOUSLY EXPOSED POPULATION

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Chikungunya is a mosquito-transmitted alphavirus, causing potentially severe disease manifestations that can last for many months or even years. There have been a number of large-scale outbreaks in recent years. However, it is unclear if the virus will become established in these populations. This knowledge gap is unsurprising as the locations of chikungunya outbreaks are often unpredictable and local immunity levels at the start of an epidemic are usually unknown. In addition, many surveillance systems have not historically recorded cases. To help address this gap, we used data from two studies conducted in the same community in Cebu, Philippines 39 years apart (in 1973 and 2012). In both studies, individuals of all ages (N=150 in the 1973 study, N=854 in the 2012 study) were tested for evidence of historic exposure using neutralization tests. The proportion of seropositive individuals varied

greatly by age in both studies. In particular, no one under the age of 14 in the 2012 study had been exposed (out of 316 tested) compared to 28% overall, suggesting an absence of chikungunya since before 2000. We used the age of individuals and serostatus to estimate the proportion of the population that was infected in each year between 1950 and 2012. We used historical census records to estimate the proportion of the population that remained susceptible to infection at any time point. Overall our models identified four short-lived outbreaks since 1950 (in 1968, 1986, 1993 and 1999), with an average of 24% of the susceptible population infected following each introduction (range: 16% to 37%). These dates are consistent with historical case reports from the area. We estimated that at least half of the population remained susceptible in any year with an average of 56% unexposed, suggesting there existed a significant pool of people capable of becoming infected at any time point. These findings are consistent with occasional introductions resulting in brief but rapid dispersal of the virus, followed by long absences. Local environment, in particular climactic factors and regional human movement patterns may drive these observations.

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CLINICOPATHOLOGIC CHARACTERISTICS AND IMMUNOLOCALIZATION OF VIRAL ANTIGENS IN CHIKUNGUNYA-ASSOCIATED FATAL CASES- PUERTO RICO, 2014

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Chikungunya virus (CHIKV)-related deaths are uncommon and usually occur in neonates exposed intrapartum, older adults, or people with underlying medical conditions. We describe the epidemiology and histopathologic findings for people that died and had evidence of acute CHIKV infection during an outbreak in Puerto Rico in 2014. We identified patients who died following an acute febrile illness and had CHIKV RNA detected by RT-PCR in a pre-mortem serum specimen, or in serum or tissue specimens collected at autopsies. Immunohistochemical staining for CHIKV antigen was performed on post-mortem tissue specimens. Data from medical records, autopsy findings, family interviews, and diagnostic test results were compiled. We identified 26 people who died in Puerto Rico during May-December 2014 and had laboratory evidence of acute CHIKV infection. Median age was 61 years (range: 6 days-85 years) and 16 (62%) were male. All had ≥1 underlying medical condition, most frequently hypertension (54%), diabetes (46%), and obesity (35%). Seven (27%) people died at home without seeking medical care. Median day of death post-illness onset was 6 (range: 1-28) for the 18 (69%) cases where these data were available. Of 21 cases with post-mortem tissue available for evaluation, CHIKV antigen was detected in 10 (48%). Common histopathologic findings included intraalveolar hemorrhage and edema. Viral antigen was detected in multiple organs, predominantly in mesenchymal tissues and cells of the mononuclear phagocytic system. CHIKV RNA was detected in the serum or tissue of 26 people who died during a chikungunya outbreak in Puerto Rico. All had comorbid conditions and most were older adults. Half of the patients evaluated had viral antigen detected in post-mortem tissue. Evaluation of autopsy tissue from patients infected with CHIKV provides evidence on the pathologic consequences of the disease that cannot be gained by diagnostic laboratory testing alone. This underscores the importance of enhanced surveillance, autopsies, and tissue-based diagnostic testing in understanding mortality associated with an emerging infectious disease.

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EXPOSURE OF EPITOPE RESIDUES ON THE OUTER FACE OF THE CHIKUNGUNYA VIRUS ENVELOPE TRIMER DETERMINES ANTIBODY NEUTRALIZING EFFICACY

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Chikungunya virus (CHIKV) is a reemerging alphavirus that causes a debilitating arthritic disease and infects millions of people and for which no specific treatment is available. Like many alphaviruses, the structural targets on CHIKV that elicit a protective humoral immune response in humans are poorly defined. Here we used phage display against virus-like particles (VLPs) to isolate seven human monoclonal antibodies (MAbs) against the CHIKV envelope glycoproteins E2 and E1. One MAb, IM-CKV063, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), demonstrated high-affinity binding (320 pM), and was capable of therapeutic and prophylactic protection in multiple animal models up to 24 h post-exposure. Epitope mapping using a comprehensive shotgun mutagenesis library of 910 E2/E1 mutants with alanine mutations demonstrated that IM-CKV063 binds to an intersubunit conformational epitope on domain A, a functionally important region of E2. MAbs against the highly conserved fusion loop have not previously been reported but were also isolated in our studies. The fusion loop MAbs were broadly cross-reactive against diverse alphaviruses but were non-neutralizing. Fusion loop MAb reactivity was affected by temperature and reactivity conditions, suggesting that the fusion loop is hidden in infectious virions. Visualization of the binding sites of 15 different MAbs on the structure of E2/E1 revealed that all epitopes are located at the membrane-distal region of the E2/E1 spike. Interestingly, epitopes on the exposed topmost and outer surfaces of the E2/E1 trimer structure were neutralizing, whereas epitopes facing the interior of the trimer were not, providing a rationale for vaccine design and therapeutic MAb development using the intact CHIKV E2/E1 trimer.

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ARTHRITIS PATHOGENESIS IN TWO MOUSE MODELS OF CHIKUNGUNYA VIRUS INFECTION

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Chikungunya virus (CHIKV) is an alphavirus spread by ***Aedes aegypti*** and ***Ae. albopictus*** mosquitoes. Currently, an outbreak affecting more than 1 million people is occurring in the Caribbean and the Americas, representing a significant emerging threat to the United States. Generally, infection results in high fever and arthralgia, with resolution of symptoms in a few weeks. However, in 20-60% of cases, people develop a painful chronic relapsing arthritis lasting months to years. Currently there are no commercially available vaccines for CHIKV and treatment is mainly supportive. While persistent arthritis can be a significant and debilitating component of the disease, little is known about the pathogenesis. We hypothesize that CHIKV-induced arthritis is an erosive and inflammatory arthritis exhibiting articular degeneration and osteoclastic bone resorption. To investigate this, two mouse models exhibiting a wide spectrum of disease severity were employed: IRF $3/7^{-/-}$ mice and wild-type C57Bl/6 mice. Mice were intradermally or subcutaneously inoculated with 10^5 pfu of virus [Southeast Asian strain SVO 476-96] in the hind footpad or adjacent to the stifle; control mice were inoculated with a sterile PBS solution.

IRF 3/7$^{-/-}$ mice were sacrificed at various timepoints post inoculation and analyzed by micro-computed tomography to assess for changes in bone volume, density and morphometry and by histopathology to determine the pathogenesis of arthritis. Additionally, various serological parameters will be evaluated to assess for correlation with disease severity and potential utility as novel prognostic markers. Virus inoculated mice developed significant swelling associated with the inoculation site by 2 days post inoculation (dpi) and at 6 dpi had a mild pleocellular tenosynovitis and moderate to marked cellulitis and myonecrosis of adjacent tissues. Control mice had no significant gross or histological lesions. The C57Bl/6 mouse experiment is currently ongoing. Detailed characterization of the pathogenesis of CHIKV-induced arthritis could provide novel targets for disease prevention or alternative treatment modalities.

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CENTRAL NERVOUS SYSTEM ENTRY OF NEUROINVASIVE ALPHAVIRUSES IN MICE FOLLOWING PERIPHERAL ROUTE OF INFECTION

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Alphaviruses most often associated with neuroinvasive disease include EEEV, VEEV, and WEEV. Alphavirus entry into the CNS of infected vertebrates after peripheral challenge is associated with hematogenous spread of virus although the precise entry sites are not well described. We determined the site of CNS entry following footpad inoculation of CD-1 mice using a combination of ***in vivo/ex vivo*** bioluminescence imaging, CLARITY, and traditional histological examination methods. We found a consistent pattern of virus entry among imaged brains that implicated circumventricular organs as the initial site for neuroinvasion. Confirmatory histological analyses of the imaged tissues, led to the finding that CNS entry by EEEV, VEEV, and WEEV likely occurs in areas of the CNS where the blood-brain barrier is naturally absent. These areas include the hypothalamus, the subfornical organ, the pineal gland, and the area postrema. Importantly, these results reveal a previously unrecognized method of alphavirus entry into the CNS.

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SRC FAMILY KINASE INHIBITORS BLOCK ALPHAVIRUS STRUCTURAL AND NONSTRUCTURAL PROTEIN TRANSLATION *IN VITRO*

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Alphaviruses are arthropod-transmitted positive-sense single-stranded RNA viruses that derive evolutionarily from the New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses] and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinctive virus-dependent pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. However, all viruses intimately rely on host cellular machinery for crucial components of their replication cycles. Herein we screened inhibitors of host kinases using a CHIKV replication assay. We have identified a number of kinases as playing essential roles in CHIKV replication. Specifically, we found that inhibition of host Src family kinases blocks Alphavirus replication. Src family kinase inhibition blocked late events of virus replication reducing the release of infectious particles. While viral RNA amplification was unaffected by kinase inhibitor treatment, we saw a reduction in viral protein expression and reduction in titer and genomic copy number in the supernatant. Targeting host factors involved in Alphavirus replication represents an innovative, perhaps paradigm-shifting strategy for antiviral therapeutic development.

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A ROLE FOR THE INTERFERON-STIMULATED EXONUCLEASE, ISG20, AND ITS REGULATED GENES IN THE RESTRICTION OF CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIKV) is an arthritogenic alphavirus that has spread globally, causing a pandemic of acute febrile disease and viral arthritis across Asia, Europe and the Americas. CHIKV and related alphaviruses are controlled to varying degrees by the activity of effector proteins induced by type I interferon (IFN). We previously used a systems biology approach to identify interferon-stimulated genes with potential roles in controlling alphavirus infection. This analysis identified ISG20, a 3'-5' interferon-induced exonuclease with specificity for RNA substrates, as having a potent antiviral effect against CHIKV and other alphaviruses. In the current studies, ISG20 restricted CHIKV replication immediately after entry through a disruption of early viral gene translation. This restriction appeared to be independent of direct exonuclease activity on the viral RNA. RNA deep sequencing analysis of cells overexpressing ISG20 revealed cell-wide changes in gene transcription. Notably, ISG20 overexpression induced a number of other known antiviral genes, including IFIT1, independent of IFN production. We further examined the role of IFIT1 as a potential mediator of ISG20 restriction of CHIKV due to its related function in translation inhibition. Using overexpression of mouse IFIT1 in murine fibroblasts, we demonstrated a similarly potent restriction of CHIKV as seen with ISG20. Our findings demonstrate a role for IFIT1 in the restriction of CHIKV replication and provide a possible mechanism by which ISG20 may indirectly inhibit translation of incoming CHIKV genomes. Continuing work is aimed at determining the importance of these two antiviral proteins using knockout mice in an established mouse model for CHIKV pathogenesis coupled with ***in vivo*** imaging (IVIS) for longitudinal monitoring of disease progression in individual mice.

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MINIMAL TISSUE FACTOR EXPRESSION REDUCES BLOOD BRAIN BARRIER PERMEABILITY AND SUSCEPTIBILITY TO NEUROLOGICAL SYMPTOMS IN EXPERIMENTAL CEREBRAL MALARIA

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Sequestration of ***Plasmodium falciparum***-infected erythrocytes in the brain results in a severe neurological syndrome, cerebral malaria (CM). Although the extent to which coagulation is responsible for severe disease is incompletely understood, thrombosis and endothelial disruption likely play a significant role in CM pathogenesis and may provide useful diagnostic and therapeutic targets. To assess the role of Tissue Factor (TF) in CM-induced blood brain barrier (BBB) disruption and neurological

symptoms, mice with a null mutation in TF that are transgenic for human TF expressed at 1% of the normal level (low TF; LTF), mTF heterozygous littermates (LTF+/-) and TF-intact C57BL/6J (B6) mice were infected with ***P. berghei*** ANKA, a CM-inducing malaria strain, and were serially sacrificed between days 4 (ED4) and 6 (ED6) post-infection. TF procoagulant activity was assessed in homogenized brain samples using a one-step clotting assay. To assess the extent of BBB permeability, mice were injected with Evans blue dye and intensity of staining in the brain was quantified. Brain pathology was assessed in H&E-stained histological sections. Pro-inflammatory cytokines were measured in plasma and tissue by ELISA. The strains exhibited varying susceptibility to CM; 47% (B6), 100% (LTF+/-), or 30% (LTF) of mice succumbed to CM on ED5, and 75% (B6) or 0% (LTF) succumbed on ED6. TF activity was increased in brains of CM-positive (CM+) B6 (100-fold, ED5; 10-fold, ED6), LTF +/- (1000-fold, ED5) and LTF (100-fold, ED5) mice versus those with uncomplicated malaria. Though extensive hemorrhage was seen in histological sections from CM+ B6 and LTF+/- mice, minimal to no hemorrhage was seen in brains of CM+ LTF mice. Extensive Evan's blue staining was seen in brains of CM+ B6 and LTF+/- mice; however, LTF mice that exhibited neurological symptoms showed minimal, focal Evan's blue staining. Increased TF activity in brains of CM+ mice and reduced BBB permeability of LTF mice together suggest TF is playing a significant role in the pathogenesis of CM. Ongoing studies are assessing the mechanisms by which this occurs, with emphasis on thrombin-dependent PAR1 signaling.

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TCR USAGE IN PATHOGENIC CD4+ AND CD8+ T CELLS DURING EXPERIMENTAL CEREBRAL MALARIA

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T cells are major mediators of the pathogenesis of experimental cerebral malaria (ECM) in mice. While $CD4^+$ T cells exert their pathogenic effect during the induction phase of disease, brain sequestered $CD8^+$ T cells target endothelial cells of the blood brain barrier for destruction during the effector phase of disease contributing to the symptoms of ECM. Although it is understood that T cells play a critical role in the pathogenesis of ECM, the biology and diversity of the T cells that are expanded during ECM are poorly understood. Specifically, it is unclear whether a dominant clone of pathogenic T cells is expanded during ECM or whether a rare population is sufficient to induce ECM. To determine T cell receptor (TCR) usage by pathogenic T cells in ECM, we isolated highly pure populations of $CD4^+$ and $CD8^+$ T cells during the induction phase (day 3 post-infection) from spleen and during the effector phase (day 6 post-infection) from brain and spleen of ***P. berghei*** ANKA-infected C57BL/6 mice by fluorescence activated cell sorting (FACS). We then sequenced the complementarity-determining region 3 (CDR3) of the TCRβ gene by template-switch anchored RT-PCR on RNA isolated from sorted cells. Analysis is currently being performed using the IMGT/V-QUEST alignment tool for TCR nucleotide sequences. The T cell populations under investigation are malaria specific ($CD49b^+CD11a^+$) $CD4^+$ and $CD8^+$ T cells. TCR sequences analysis and their association with ECM will be presented. A better understanding of the antigen repertoire recognized by these pathogenic T cells may facilitate the design of an anti-disease vaccine that could prevent the activation and migration of pathogenic T cells during a severe malaria episode.

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IMMUNOPATHOLOGY ASSESSMENT OF CENTRAL NERVOUS SYSTEM IN *PLASMODIUM COATNEYI*-INFECTED RHESUS MACAQUE AS A CEREBRAL MALARIA MODEL

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Cerebral malaria (CM) is one of the major manifestations of severe falciparum malaria causing not only significant numbers of deaths but also neuro-disability in surviving patients. Relevant animal models represent an important way of investigating the pathogenesis of falciparum malaria and potential adjuvant neuroprotective treatments. ***Plasmodium coatneyi***-infected rhesus macaques (Macaca mulatta) have been proposed as a suitable non-human primate model for human CM; however, the pathogenesis of disease in the model has not been well characterized. Here we investigated the immunohistochemical changes of the central nervous system (CNS) in ***P. coatneyi***-infected rhesus macaques. Animals were splenectomized and infected with ***P. coatneyi*** which progressed to high levels of parasitemia (32-39%). All infected monkeys developed severe lethargy, markedly decreased mentation without coma, anemia, mild to moderate thrombocytopenia, and signs of hepatic and renal dysfunction. After euthanasia and necropsy, sections of the cerebrum, cerebellum, brain stem, and spinal cord were immunostained with glial fibrillary acidic protein (GFAP) as a marker of astroglial activation, and β-Amyloid precursor protein (βAPP) to detect axonal injury, both of which have been demonstrated in brain from human cases of CM. Staining patterns of fibrinogen were also examined to detect blood-brain barrier leakage. Generalized astrogial activation was not seen, whereas significant axonal injury was detected most predominantly in the brainstem and spinal cord. In addition, we evaluated cytokine expression levels in cerebrospinal fluid (CSF) from malaria-infected animals in comparison to controls. These demonstrated increases in inflammatory biomarkers including IL-8, IL-15, MCP-1, and TGF-ο, which may be involved in the immunopathology of CM. A better understanding of CM pathogenesis and pathophysiological processes will facilitate research into therapeutics in the treatment of severe falciparum malaria.

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PROTEOMIC ANALYSIS OF *PLASMODIUM FALCIPARUM* 3-DAY LIVER STAGE PARASITES

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Malaria caused by the ***Plasmodium falciparum*** parasite is one of the most deadly diseases in the world causing over 600,000 deaths. Developing a vaccine that targets the pre-erythrocytic stage of ***Plasmodium falciparum*** (Pf) would be facilitated by identification of antigens that are the targets of protective immune responses. The goal of this study was to describe the proteome of Pf liver-stage parasites cultured in cell-free environment for three days and compared it to previously identified sporozoite and other parasite's stages proteomes. The first proteomics analysis of a 3-day axenic cultured sporozoites identified 1,517 Pf proteins with 175,481 peptide spectrum matches and 65% of the proteins were identified with multiple peptides. These 3 days liver-stage parasites were grown in an axenic culture with a final count of 9E7 of which 72% transformed into liver-stage mimicking parasites based on morphology and expression of new liver stage proteins. The sample was electrophoresed followed by in-gel digestion with trypsin. Peptide samples were analyzed by LC-MS/MS with exclusion runs using the LTQ Orbitrap

Velos. 7% of the proteins identified were unique. They previously have not been identified in Pf sporozoites, or asexual or sexual stages erythrocytic stages, and include proteins which are members of the VAR gene family. We will present data describing the molecular function of the proteins uniquely expressed during the parasite's liver-stage development.

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UIS2, A UNIQUE PHOSPHATASE REQUIRED FOR THE DEVELOPMENT OF *PLASMODIUM* LIVER STAGES

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Plasmodium salivary sporozoites, the infectious form of the malaria parasite, are dormant while inside salivary glands of *Anopheles* mosquitoes. During dormancy, protein translation is inhibited by the kinase UIS1 that phosphorylates serine 59 in eIF2α. De-phosphorylation of eIF2α-P is required for the transformation of sporozoites into liver stages. In mammalian cells the de-phosphorylation of eIF2α-P is mediated by protein phosphatase 1 (PP1). Instead, we report here that in malaria sporozoites the eIF2α-P phosphatase is UIS2. Both ***uis1*** and ***uis2*** are highly transcribed in salivary gland sporozoites, but the translation of ***uis2*** is inhibited by the Pumilio protein Puf2. The translational repression of ***uis2*** is alleviated when sporozoites developed into liver stages. UIS2 belongs to the PP2C/PPM phosphatase family. While most eukaryotic phosphatases attach transiently to their substrates, UIS2 binds tightly to phosphorylated eIF2α, but does not recognize unphosphorylated eIF2α, raising the possibility that high throughput searches may identify chemicals that disrupt the interaction and prevent malaria infection.

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CHARACTERIZATION OF THE EXPANDED ACYL CO-A SYNTHETASE GENE FAMILY

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Plasmodium falciparum has the ability to quickly adapt its genome to selective pressures encountered in the human host by acquiring single nucleotide polymorphisms, recombination or gene duplications. The acyl Co-A synthetase (ACS) gene family is one example of such duplication and recent positive selection. In ***P. falciparum*** and ***P. reichenowi*** four conserved orthologs of ACS are predicted to perform classical ACS function while nine paralogs have expanded and diverged from the ***Pf***ACS9 ortholog. ACSs activate fatty acids (FA) scavenged from the host, which can then be used for protein modification, phospholipid biosynthesis, FA elongation, and beta-oxidation. To characterize the function of individual family members, we tagged single genes and observed different subcellular localization and protein abundance throughout the asexual lifecycle. Using different protein extraction methods, we also observed distinct membrane associations for individual family members. Taken together, these results suggest different roles for individual ACSs and potential neo-functionalization. The CRISPR/CAS system allowed us to generate knock out parasites lines for ***Pf***ACS5, ***Pf***ACS8, ***Pf***ACS9 and ***Pf***ACS12. None of the knock out lines showed a major growth defect in complete media when compared to the parental line. We are currently testing different growth conditions to further characterize the biological function of the individual ACSs. We hypothesize that the expansion and recent positive selection of the ***Pf***ACS gene family are the consequence of metabolic pressures driving parasite evolution, and understanding FA metabolism will give us insight into key metabolic pathways that might serve as potential targets for novel antimalarials.

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PLASMODIUM SPP. PREFERENCE FOR IMMATURE RED BLOOD CELLS: THE MISSING RECEPTOR?

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Plasmodium vivax is the predominant form of malaria outside of Africa and is an important public health and economic development problem in many countries. The blood stages of ***P. vivax***, which cause the disease, have a strong preference to infect immature red blood cells, or reticulocytes. In contrast, the other major human malaria parasite, ***P. falciparum***, can infect mature and immature red blood cells. Understanding unique mechanisms regulating ***P. vivax***-specific host cell preferences is fundamental for basic research of this parasite, limiting our ability to maintain long term continuous cultures of ***P. vivax***, and consequently hinders development of more effective drugs and vaccines to prevent vivax malaria. In this study we have investigated surface receptors present subpopulation of reticulocytes (CD71neg/CD71low/CD71med/CD71high) preferentially infected by clinical isolates of ***P. vivax***. We discovered ***P. vivax*** had better invasion rates into CD71med/CD71high reticulocytes, which occurred also with laboratory lines of ***P. falciparum***, suggesting that there may be a common receptor on immature reticulocytes for all ***Plasmodium*** species. Further characterization of these subpopulations revealed that cell surface markers CD49d-alfa4 integrin, CD44, as well as mitochondria were more abundant on the CD71med/CD71high reticulocytes. Nevertheless we could not find positivity for CD49d, CD44 as well as CD36 for any red blood cells hosting the four ***P. vivax*** ring stage's frozen isolates from Thailand that we tested. Binding assays with PvSal1 DBP into different population of reticulocytes confirmed a better binding to CD71med/CD71high reticulocytes consequently to the higher expression of DARC in reticulocytes compared to mature erythrocytes. Consistent with the preferred invasion patterns, ***in vitro*** binding assays with ***P. vivax*** recombinant RBP1 showed a sharp increase in binding into CD71med/CD71high. These results reveal new insights into the molecular basis for the preference of ***P. vivax*** to invade very immature reticulocytes.

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EFFECT OF DEWORMING ON NUTRITIONAL INDICATORS, COGNITIVE ABILITIES AND SCHOOL PERFORMANCE AMONG SCHOOLCHILDREN IN RURAL CHINA: A CLUSTER-RANDOMIZED CONTROLLED TRIAL

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Chronic infection with soil-transmitted helminths (STH) among school-aged children has been correlated with nutritional deficiencies, cognitive impairment, and lower rates of school attendance, but the effectiveness of deworming in improving these outcomes is unclear. This aim of this cluster-randomized controlled trial was to examine the impact of a deworming intervention on STH infection prevalence, infection intensity, nutritional indicators, cognitive abilities, and school performance among 2,028 school-aged children in rural Guizhou Province, China, where STH prevalence is over 40 percent. The intervention involved biannual administration of a 400 mg dose albendazole accompanied by cartoon

pamphlets about STH infection, treatment, and prevention. Specific outcomes measured at baseline and follow-up were STH infection prevalence, infection intensity (fecal egg count), anemia prevalence (Hb < 115 g/L), stunting prevalence (HAZ < -2), underweight prevalence (WAZ < -2), processing speed index (PSI), working memory index (WMI), school attendance rates, and normalized scores on the Trends in International Mathematics and Science Study (TIMSS). Follow-up evaluation after 12 months found that in this population with light-intensity infection, deworming significantly reduced both infection prevalence and infection intensity in the intervention group (n=1000) relative to the control group (n=1028). However, our results found no evidence that the deworming intervention improved outcomes of nutritional indicators, cognitive abilities, or school performance. Main implications of this trial for future studies are two-fold: (1) researchers should quantify and report infection intensity (fecal egg counts) for accurate epidemiological characterization of the sample population; and (2) evidence from future randomized-controlled trials is needed to assess the effect of deworming on key outcomes in populations with moderate and high-intensity infections.

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PERFORMANCE AND COST-EFFECTIVENESS OF STOOL-BASED MULTIPLEX REAL-TIME PCR FOR THE DETECTION OF HUMAN SOIL-TRANSMITTED HELMINTHS ACROSS DIFFERENT STUDY SETTINGS

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The sensitivity of currently used copromicroscopic tests for the diagnosis of soil-transmitted helminths (STH) can vary markedly among studies and is especially low for the detection of individuals with light infections. With the current effort to control NTDs, including STHs, infection intensities will decrease substantially and infections may be missed. The sensitivity of copromicroscopic diagnosis across settings may also be variable due to difficulties in standardising diagnostic procedures. To overcome these limitations, new sensitive molecular diagnostic tools have been developed to allow the simultaneous species specific detection of DNA of ***Ancylostoma duodenale, Necator Americanus, Ascaris lumbricoides*** and ***Strongyloides stercoralis*** (ANAS) in human stool. To evaluate whether STH diagnosis based on the ANAS real-time PCR allows a more accurate and standardised assessment of infections, we compared its qualitative and quantitative performance to standard copromicroscopic methods across five different study settings in Ecuador, Indonesia, Kenya, Malawi and Mozambique. Sensitivities of diagnostic tests were estimated based on a Bayesian latent class analysis approach. The cost-effectiveness of real-time PCR and copromicroscopic methods was compared across the settings. Our study showed that the sensitivity of the ANAS real-time PCR for the detection of hookworms, A. lumbricoides and S. stercoralis was high across all study settings (72.5- 99.6%) while sensitivity of copromicroscopic methods varied dramatically (8.2- 95.3%). DNA loads and egg counts were correlated; however, the agreement in the identification of moderate and heavy infections was fair to poor. In conclusion, real-time PCR based STH diagnosis is a sensitive tool for comparable assessment of hookworm, ***A. lumbricoides***, and ***S. stercoralis*** infections across different settings and its performance is less influenced by deviation from sample processing protocols than copromicroscopic methods. More research is needed to better understand the relationship between DNA load and observed egg counts for the assessment of infection intensities.

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USE OF QUANTITATIVE REAL-TIME PCR TO MEASURE ACQUISITION OF ENTERIC PARASITIC INFECTIONS DURING THE FIRST 5 YEARS OF LIFE: OBSERVATIONS FROM A BIRTH COHORT IN RURAL ECUADOR

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Over 2 billion humans are estimated to be infected with gastrointestinal parasites worldwide. These fecal-oral transmitted parasites are indicator infections of poverty and are associated with limited access to basic services. Standard microscopy of stool samples for detection of these parasites has a low diagnostic sensitivity compared to quantitative real-time PCR (qPCR). There are few data on the epidemiology of gastrointestinal infections from the rural tropics in pre-school children. We analyzed stool samples collected longitudinally during the first 5 years of life in a birth cohort, the ECUAVIDA cohort, set in a rural District of Quinindé, Esmeraldas Province, in tropical Ecuador using a random sample of 400 of 2,404 newborns recruited into the cohort. Stool samples collected at 1, 2, 3, and 5 years of age, were analyzed using a high throughput multi-parallel qPCR for the presence of intestinal helminths (***Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Trichuris trichiura***) and protozoa (***Cryptosporidium spp., Entamoeba histolytica*** and ***Giardia lamblia***). ***Giardia*** and ***Ascaris*** were the most prevalent parasites in children at all ages: ***Giardia*** (31.5%, 45.6%, 52.1%, and 43.3%); ***Ascaris*** (6.8%, 12.9%, 16.4%, and 14.4%) at 1, 2, 3 and 5 years, respectively. The prevalence of most enteric parasites increased through 3 years of age after which the prevalence leveled off or fell, except for ***Cryptosporidium*** was most frequently detected during the first 2 years of life (5.3% at 1 and 5.9% at 2 years). Infections with ***E. histolytica*** and ***A. duodenale*** were of low prevalence (<4%) during the first 5 years of life in the cohort. Furthermore, the burden of infection increased comparing the 1 and 5 year olds, for ***Ascaris*** (0.72 fg/µl to 2.3 fg/µl, $p < 0.05$) and ***Giardia*** (0.84 fg/µl to 6.7 fg/µl, $p < 0.05$), respectively. Signifying increasing rates of parasitic infections and intensity of burden. Future analyses will determine the impact of these enteric parasites on growth and the development of the immune response during the first 5 years of life.

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WHAT ARE THE BENEFITS OF COMMUNITY WIDE TREATMENT FOR THE CONTROL AND ELIMINATION OF HOOKWORM?

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The WHO treatment guidelines for soil-transmitted helminth (STH) infections focus on targeting children. However, unlike the other STH infections, the majority of hookworm infections are harboured by adults. This untreated burden may have important implications in controlling both hookworm's morbidity and transmission. This is particularly significant given recent increased interest in investigating STH elimination strategies. We used a deterministic model of the dynamics of STH transmission to evaluate the impact of child-targeted verses community-wide treatment against hookworm in terms of preventing heavy infections (a proxy for morbidity) and the timeframe for breaking transmission. Furthermore, we investigated how community-wide treatment may influence the long term programmatic costs of preventive chemotherapy for hookworm. We found that a large proportion of the overall prevalence of heavy infections is unaffected by the current targeted strategy. Furthermore,

biannual targeted treatment offered little additional benefit. Annual community-wide treatment was markedly more effective in controlling heavy infections, and was the only scenario in which breaking transmission was possible - reducing the required programme duration. Due to these reductions in programme duration it is possible for community-wide treatment to generate long term cost savings compared to using the current targeted strategy - even if it notably increases the distribution costs of the programme. In conclusion, community-wide treatment is notably more effective for controlling hookworm morbidity and transmission, and could even be cost saving in many settings. This shows that it is not optimum to treat the different STH infections in the same way, and highlights the need for further consideration of community-wide treatment for hookworm control.

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MULTI-PARALLEL QPCR PROVIDES INCREASED SENSITIVITY AND DIAGNOSTIC BREADTH ALLOWING FOR IMPROVED EVALUATION OF THE IMPACT OF DEWORMING PROGRAMS FOR SOIL-TRANSMITTED HELMINTHS (STH)

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Although chronic morbidity resulting from STH infections can be reduced by anthelmintic treatment, inconsistent diagnostic tools make it difficult to measure the impact of deworming programs. In order to quantify the variability in different STH diagnostic measures, we intensively screened 1671 people in four villages for helminth infections using the Kato-Katz (KK) method. We retrospectively screened these samples using multi-parallel qPCR. We treated everyone with albendazole, and collected ***Ascaris lumbricoides*** expelled post-treatment. Three months later, we re-screened and re-treated 1225 people and collected expelled worms. Between baseline and follow-up the prevalence by KK fell from 8 to 5% for ***A. lumbricoides***, and from 5 to 2% for hookworm. Expelled ***A. lumbricoides*** worms were highly aggregated, and there was a strong correlation (Spearman r=.64; p<0.0001) between the number of adult worms expelled and the intensity of infection pre-treatment. When compared to qPCR results, KK missed 20% of ***A. lumbricoides*** infections and 80% of ***N. americanus*** infections. While the limit of sensitivity for qPCR is such that a single ***A. lumbricoides*** egg is consistently detected, false negative results were common using KK at infection intensities below 2000 eggs per gram. Among a subsample of 246 individuals, 22% were found to be infected with ***Giardia lamblia***, and 16% with ***Entamoeba histolytica,*** based on qPCR. No infections with ***Trichuris trichiura, Ancylostoma duodenale, Strongyloides stercoralis*** or ***Cryptosporidium parvum*** were detected. Our data suggest that KK may be an inadequate tool for measuring worm burdens, especially in areas where hookworm and/or Strongyloides are common or where intensity of infection is low. As it becomes important for deworming programs to distinguish between populations where STH infection is controlled and those where further treatment pressure is required, multi-parallel qPCR (or similar high throughput molecular diagnostics) may offer new diagnostic tools for STH control programs.

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INDIVIDUAL-BASED MODELLING OF HOOKWORM INFECTION: PREDICTED FEASIBILITY OF ACHIEVING CONTROL AND ELIMINATION BY 2020

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Globally, 440 million people are infected with hookworms, the majority living in developing countries. High parasite loads contribute to development of anaemia, particularly in children and women of childbearing age (WCBA). In recognition of the hookworm disease burden, the WHO has set the target to implement annual or semi-annual preventive chemotherapy (PCT) for pre-school and school-aged children and WCBA in endemic areas with an overall coverage of at least 75% by 2020. The associated parasitological goal is to achieve <1% prevalence of heavy hookworm infection in these PCT target populations (and thus prevent most morbidity). As part of the NTD Modelling Consortium, we evaluated the feasibility of achieving control (prevalence of heavy infection <1%) or even elimination of hookworm infection, given currently recommended intervention strategies. To this end, we developed an individual-based model for transmission and control of soil-transmitted helminths that synthesizes all relevant available information on hookworm biology, and captures heterogeneities in transmission and PCT participation. Model predictions were compared to longitudinal parasitological data spanning five years, collected pre-control and during PCT. In general, model predictions suggest that elimination of hookworm infection is not possible by means of PCT, unless a broader age group is targeted (e.g. including all adults). Controlling levels of heavy infection (<1%) is however very well possible through annual or semi-annual PCT (depending on pre-control endemicity) applied to current target populations at 90% coverage. Sensitivity analysis showed that individual systematic non-participation is a key determinant for achieving control, and in particular, elimination. In conclusion, we present the first individual-based model for hookworm infection and a first-time comparison of mathematical model predictions to longitudinal data. Model predictions suggest that hookworm infection can be controlled with PCT, but probably cannot be eliminated with the current strategy.

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EVALUATION OF SEROPREVALENCE OF PARASITIC DISEASES IN U.S-BOUND REFUGEES FROM BURMA (MYANMAR)

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The U.S. Refugee Resettlement Program resettles ~60-80,000 refugees to the United States annually. The Centers for Disease Control and Prevention provides guidance for pre-departure and post-arrival management of health conditions, including voluntary testing and presumptive treatment for parasites. There has been limited evaluation of the current program management strategies for parasitic diseases in refugees. A large cohort of refugees of Burmese origin has recently been resettling annually from Thailand. Serological testing for several parasitic diseases, including cysticercosis, lymphatic filariasis (LF), and strongyloidiasis, was undertaken from samples collected in camps in Thailand. Blood specimens were collected at the initial medical screen (approximately 3-6 months prior to departure for the U.S), before departure, and, when possible, after arrival in the U.S. All patients were offered presumptive parasitic treatment with albendazole and ivermectin at the initial medical screen and at departure. To date, 964 (48%) of the 2003 individuals enrolled have been tested for antibody responses against antigens from ***Strongyloides*** (NIE), cysticercosis (T24H), and LF (Bm14, Bm33 and Wb123) using the Luminex multiplex platform. At baseline, specimens from 5% of participants

reacted against T24H and none were positive against all three LF antigens. Seropositivity against NIE was 15%. After treatment, NIE-specific antibody levels decreased in 65% of reactive individuals with 14.5% of individuals converting to seronegative. Changes in titers as well as decreases in median fluorescent intensities (MFI) of the responses were observed in non-reverters. Especially noticeable were changes in individuals with high magnitude responses; 82% (18/22) showed decreases in the magnitude of the response (average of 88% decrease in MFI) and titers post treatment. The data suggest that presumptive treatment impacts strongyloides infection rates among U.S.-bound refugees from Thailand. Measuring the magnitude of the NIE antigen response may prove a useful tool in monitoring treatment efficacy.

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DEVELOPMENT OF A PROOF-OF-CONCEPT *DE NOVO* BACTERIOPHAGE THERAPEUTIC AGAINST MULTIDRUG RESISTANT *ACINETOBACTER BAUMANNII* WOUND INFECTIONS

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Multidrug Resistant (MDR) bacterial infections have become a significant threat to civilian and military populations alike. Recently bacteriophage have remerged as a promising alternative therapeutic. Our objective was to develop a phage cocktail that could serve as an antibacterial therapy for MDR ***Acinetobacter baumannii*** wound infections. We isolated and purified 31 lytic phage capable of killing clinical isolates of MDR ***A. baumannii*** from DC metro area wastewater. From this modest library, we have assembled a proof-of-concept 5-member phage cocktail that is highly effective in treating mice harboring a full thickness wound infected with the model clinical isolate MDR ***A. baumannii*** 5075. Mice harboring a wound infected with 5075 were treated with our phage cocktail intraperitoneally and topically, and compared to untreated controls. In these experiments our cocktail: I) significantly reduced the ***A. baumannii*** bioburden in the wound-bed, II) prevented the invasion of ***A. baumannii*** into tissue surrounding the wound and thus promoted tissue preservation/vitality in adjacent areas, III) prevented the infection-associated increase in wound size seen in untreated controls, IV) decreased the morbidity of the wounded/infected mice as measured by a significant reduction in infection-associated weight-loss, and V) promoted rapid wound healing at a rate similar to that of uninfected controls. Based on the success of this initial cocktail our goal is to develop a broad-spectrum phage cocktail that kills at least 90% of ***A. baumannii*** clinical isolates. To do so, we have developed a 70-member MDR ***A. baumannii*** diversity set composed of divergent clinically relevant strains for improved phage isolation. We have also begun phage isolation from sites around the world, including: NAMRU6-Peru, NAMRU3-Egypt, NAMRU2-Cambodia, and FT Benning, GA. Thus, we are building a large and diverse phage library from which new cocktails can be compounded for the treatment of MDR ***A. baumannii*** wound infections.

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A RANDOMIZED, CONTROLLED TREATMENT TRIAL FOR THE VERRUCOUS STAGE OF *BARTONELLA BACILLIFORMIS* INFECTION IN PERU

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There are no clinical trials that study the effect of alternative therapies for the verrucous stage of ***Bartonella bacilliformis*** infection. Our main objective was to compare the standard treatment with rifampin versus azithromycin in terms of time to resolution of the verrucous stage and time of resolution of the bacteremia. We conducted a randomized, controlled treatment trial. Participants were recruited in Caraz, Peru (northern Andean mountains) were randomly allocated in two groups of treatment (rifampin and azithromycin). At each arm, they received the treatment during two weeks, and they were followed up at 7, 14, 30, and 60 days. At each visit, we assessed the number of lesions, size of lesions, and number of body areas involved as well as the time to resolution of the verrucous rash of ***B. bacilliformis*** infection and the duration of the bacteremia associated with the verrucous rash. 125 participants were recruited (62 in the rifampin groups and 63 in the azithromycin group). We found that azithromycin reduced the time of resolution of baseline bacteremia (8.26 ± 1.79 days for rifampin vs. 7.60 ± 1.77 days for azithromycin, $p < 0.001$) and the time of reduction of verrucous lesions (16.15 ± 16.52 days for rifampin vs. 13.50 ± 11.25 days for azithromycin, $p = 0.786$). In addition, azithromycin reduced the average size of the lesions over time compared to rifampin. The results of the study showed that azithromycin had similar or better effects than those of rifampin for reducing the time of resolution of the verrucous rash and the time of resolution of initial bacteremia. Also, it had a similar effect in reducing the number of lesions, the average size of lesions and the number of body regions involved, becoming a valid alternative for the treatment of the verrucous stage of ***B. bacilliformis*** infection.

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REGULATION OF B12 BIOSYNTHESIS BY PATHOGENIC *LEPTOSPIRA*

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To better understand Leptospira virulence and pathogenic mechanisms, we sequenced and annotated the genomes of two strains of a novel species ***Leptospira licerasiae*** prevalent in the Peruvian Amazon and then compared the gene content of these genomes with those of L. interrogans, ***L. borgpetersenii*** and ***L. biflexa***. These comparative genome studies revealed a 17-gene B12 biosynthesis gene cluster (cob I/III) restricted to infectious species and a non-coding RNA regulatory element in the 5′ un-translated region of the cluster. Because these genes and associated regulatory elements were present in infectious Leptospira, we hypothesized that, in contrast to current dogma, infectious Leptospira should grow in the absence of B12. Using bioinformatics approaches to determine the distribution of the previously identified cob I/III cluster amongst >300 recently sequenced and annotated high quality draft Leptospira genomes, we confirm that both the cluster and associated regulatory element are widely and uniquely distributed amongst infectious strains. Second, we tested whether infectious Leptospira synthesize B12 de novo. We created a chemically defined minimal medium and demonstrate that whereas ***L. interrogans*** and ***L. licerasiae*** grow indefinitely in the absence of B12, growth of the non-pathogen, L. biflexa, was inhibited in minimal medium without B12 supplementation. Finally, we confirmed by qRT-PCR that expression of cob I/III is modulated by exogenous B12. Because B12 is sequestered ***in vivo*** in mammals, this capacity of

infectious Leptospira to respond to diminishing levels of exogenous B12 and in response synthesize this essential nutrient could have important implications during Leptospira pathogenesis.

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A MINIMAL EPITOPE COMBINATION VACCINE CAN PROTECT AGAINST ALL STRAINS OF *STREPTOCOCCUS PYOGENES* IRRESPECTIVE OF EMM TYPE AND VIRULENCE PHENOTYPE

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Infections caused by ***Streptococcus pyogenes*** (group A ***Streptococcus***, GAS) and their sequelae are responsible for over 500,000 lives lost prematurely each year. A synthetic peptide, J8, from the conserved region of the M-protein combined with diphtheria toxoid (DT) has shown efficacy against disease that follows intraperitoneal inoculation of bacteria. By developing a murine model for infection that mimics human skin infection we show that the vaccine can also protect against pyoderma and bacteremia caused by multiple GAS strains. However, the vaccine was significantly less effective against hyper-virulent CovR/S mutant GAS strains and this correlated with up-regulation of SpyCEP and the strains' abilities to degrade CXC chemokines (IL8, MIP2, KC) thus preventing neutrophil chemotaxis. Chemokine proteolysis is mediated by bacterial SpyCEP. By combining J8-DT with an inactive form of recSpyCEP, we developed a vaccine that can block chemokine degradation thus permitting opsonic antibodies to kill the bacteria. Mice receiving the combination vaccine were strongly protected as evidenced by between a 100-fold to 1,000-fold reduction in bacterial burden following challenge. We then used a peptide array to identify the minimal epitope within SpyCEP. A twenty amino acid peptide ('S2') was one of several epitopes identified using serum from recSpyCEP-immunized mice; however, this epitope was the sole target for anti-SpyCEP antibodies that could protect IL8 from streptococcal SpyCEP-mediated proteolysis. It was of great interest that this epitope was also recognized by human serum from healthy individuals naturally exposed to GAS. Serum from mice immunized with S2-DT could completely protect IL8 from GAS-mediated proteolysis and human serum from healthy donors showed a partial ability to protect IL8. We then combined S2-DT with J8-DT to develop a minimal epitope vaccine and showed that the combination vaccine could protect mice against pyoderma and bacteremia due to all strains of GAS that we have tested irrespective of their emm type and independent of their CovR/S phenotype. This vaccine is now being prepared for a Phase I human trial.

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INVESTIGATING THE ROLES OF T CELL-MEDIATED IMMUNITY DURING SCRUB TYPHUS WITH A NEWLY DEVELOPED MOUSE MODEL

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Scrub typhus, a long neglected but important tropical disease, is caused by a Gram-negative obligately intracellular coccobacillus, ***Orientia tsutsugamushi***. Scrub typhus is a serious global public health problem that causes illness in one million people each year, the majority in the Asia-Pacific region. Without appropriate diagnosis and treatment, the disease can cause severe multiorgan failure with a mortality rate of 7-15%. However, the mechanisms behind the interactions between ***O. tsutsugamushi*** and host immunity are largely neglected and unknown. Using the newly developed intravenous (i.v.) mouse model, we discovered that host immunity was skewed towards T_h1 responses from 12 days post infection (dpi) until 3 months post infection. Our flow cytometry data determined that more $CD8^+$ T cells than $CD4^+$ T cells appeared in the spleen of infected mice after 12 dpi. We also found that T_{reg} cells and the proportion of T cell producing IL-10 levels in T cells were significantly increased from 6 dpi, which was in parallel with the body weight loss and increased bacterial loads. Our latest studies with $CD8^{-/-}$ mice and their wild type (WT) C57BL/6 control counterparts determined the important role of $CD8^+$ T cells. After being administered one LD_{50} of ***O. tsutsugamushi***, all $CD8^{-/-}$ mice expired by 11 dpi while half of the WT mice survived. Bacterial loads in the lung, kidney, liver and blood of $CD8^{-/-}$ mice were significantly higher than those in WT mice. IFN-γ mRNA levels in the lungs of $CD8^{-/-}$ mice were significantly higher than in WT mice. There was no statistically significant difference in the mRNA levels of IL-10 in the lung, liver, spleen, and kidney between $CD8^{-/-}$ and WT mice. We also found a greater pro-inflammatory immune response in the tissues of WT mice than in $CD8^{-/-}$ mice. More studies are necessary to better understand the role of host immunity during ***Orientia*** infection. This will benefit the control of scrub typhus as well as the development of a vaccine and improved therapy.

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ACUTE GASTROINTESTINAL INFECTION AND OTHER HEALTH PROBLEMS ABOARD THE USNS COMFORT DURING CONTINUING PROMISE 2011: A HUMANITARIAN ASSISTANCE/DISASTER RESPONSE MISSION

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Disease and non-battle injuries (DNBI) can significantly impact operational missions and compromise readiness. No study to date has examined DNBI rates aboard hospital ships on humanitarian aid/disaster response (HADR) missions. Given their nature, these HADR training and operational missions utilize significantly different personnel and result in unique exposures compared to operational deployments. From April to September 2011, US military, partner nation military, non-governmental organization personnel, and merchant marines participated in Continuing Promise 2011, a HADR training mission aboard hospital ship USNS COMFORT (T-AH 20). We conducted health surveillance for the purpose of assessing DNBI trends and improving force health protection during the deployment. The data collected were from two sources: (1) weekly DNBI aggregate data from the medical treatment facility sick-call clinic; and (2) an enhanced weekly, self-reported, surveillance questionnaire (eDNBI). Additionally, a case series study of acute gastrointestinal illness (AGI) was conducted via culture-independent microbiology on stool specimens. Clinic-based DNBI were obtained from an average weekly force of around 900 personnel. In addition, 3,156 self-report surveys (~15% of personnel each week) were collected. The self-report survey respondents were a representative sampling of the entire ship's crew. The leading syndrome-specific cause of average weekly visits to the ship's clinic was AGI, followed by dermatological conditions and acute respiratory illness (ARI) (2.1, 1.9, 1.5 per 100 person-weeks, respectively). eDNBI rates were similarly represented in the top three, although in reverse order, with 11.2 ARI, 8.7 dermatological, and 7.4 AGI average cases per 100 person-weeks. AGI were responsible for a majority of duty days lost (201/325, 61.8%). Among 51 AGI cases, one or more pathogens were identified in 71% of cases, with ETEC and norovirus as leading causes. These data highlight important disease and injury burden on HADR missions and may be useful for future planning.

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RISK FACTORS, GUT FUNCTION BIOMARKERS AND GROWTH DEFICIT ASSOCIATED WITH ENVIRONMENTAL ENTEROPATHY AND MALNUTRITION: THE CASE-CONTROL MAL-ED STUDY IN FORTALEZA, CEARÁ, BRAZIL

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In Fortaleza, located in the poorest region of Brazil, there is a large socioeconomic and cultural disparity that maybe influences the prevalence of malnutrition and its serious consequences for growth and cognitive development in children. The aim of this study was to evaluate the variables related to the child and the mother, as well as environmental and socio-economic factors associated with environmental enteropathy (EE) and malnutrition (MN) in children from Fortaleza, Ceará, Brazil. The study design was a case-control prospective study. The protocol was approved by the local Internal Review Board (IRB) (Universidade Federal do Ceará), a national IRB (CONEPE) and IRB at University of Virgínia. The study period was from August/2010 to September/2013; Inclusion criteria for the case: z score weight-for-age (WAZ) -1; and all children aged 6-24 months. As for 30Nov2013 we screened 484 and enrolled 402 children in the study protocol. The results showed preliminary analysis on 244 children (126 controls and 118 cases) for risk association with EE and MN. The age mean ± standard deviations was 11.83 (5.2) for controls and 15.00 (5.5) for cases ($p<0.001$). Sex distribution was similar on both groups. The mean ± standard deviations for z-score on WAZ was 0.03 (0.97) for controls compared to -2.70 (0.78) on cases ($p<0.0001$). The multiple linear logistic regression and Wald test analysis showed three variables that hold significant results with <5%, which were increased in control for birth weight, head circumference and number of pregnancies compared to cases. Calprotectin (CAP) was increased on both (cases and control) and it was significantly higher on malnourished compared to nourished children. Higher myeloperoxidase (MPO) and alpha1-antitrypsin (A1AT) were associated with impaired "catch-up" growth. These data showed protective risk factors associated with nourished compared to malnourished children. Elevated CAP, MPO and A1AT are associated with gut inflammation and impaired growth in these children.

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THE QUECHUA PEOPLE OF SOUTHERN PERU: A DESCRIPTIVE ANALYSIS OF CLINIC ATTENDEES

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This project builds upon a 2014 study, "The Epidemiology of Disease among the Quechua People of Southern Peru: A Pilot Study". In this present research, we will analyze data (N = 839) related to common primary care issues among the Quechua People of southern Peru, e.g., diabetes, hypertension, anthropometric measures, etc. Up to this time, previous studies have focused their attention primarily on biogenetic issues such as hemoglobin variants. As such, day-to-day primary care issues have eluded the attention of researchers. Data concerning primary care complaints among the Quechua, an underserved, rural---and largely unstudied--population with regard to common medical issues were collected during a two week primary care clinic based in the remote town of Pampichiri in the Andahuaylas region of southern Peru. The findings in this study, one of the first of its kind, highlight primary care health issues among Quechua clinic attendees.

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KNOWLEDGE AND PRACTICES OF VILLAGE HEALTH TEAM MEMBERS IN EARLY DETECTION AND CARE FOR CHILDREN WITH SEVERE ACUTE MALNUTRITION AT COMMUNITY LEVEL: A CASE STUDY OF A RURAL COMMUNITY IN UGANDA

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Background Malnutrition remains a serious problem for young children in many developing countries. In Uganda, highest malnutrition rates are in the South West where 40% of children have chronic malnutrition; 5% acute malnutrition.The first level of government provided healthcare are community based Village Health Team (VHTS) with very basic health knowledge. The study determined how VHT members identified and managed children with early malnutrition in SW Uganda. Methods Cross sectional survey of VHTs in randomly selected parishes of Bushwere and Ryamiyonga in SW Uganda who attended two sessions in August and September 2013.A pretested malnutrition knowledge and management questionnaire was used.The project received ethical approval from MUST-REC and was funded by MicroResearch Results A total of 59 VHTS from the 2 parishes were interviewed. Their mean age was 37 ± 9y (range 22-60y); 75% were female. 95% had initial 5-day training for their VHT role;90% had served in their position for > 5 years.50% of VHT had mid upperarm circumference (MUAC)training for nutrition assessment, none had received a MUAC tape.83% could correctly classify the local food crops in their respective food groups. 88% identified body swelling, changes in skin or hair colour and having protuberant abdomen signs of malnutrition in children.However, only 8% of VHTs selected measuring MUAC as a method to identify malnutrition.Knowledge on feeding options when mother is HIV+ showed that 97% were only aware of outdated options; e.g no breastfeeding or for a short time only.. 89% reported offering feeding advice; 42% reported advising referral to health unit to parents when severe malnutrition was noted in their children. Conclusion VHTs had adequate knowledge of feeding of children and could correctly classify foods into three major food groups.They were able to describe late signs of malnutrition but unable to detect early signs possibly due to lack of MUAC tapes. VHTs would benefit from refresher courses on (a) recommended nutrition for infant and young children of HIV+ mothers,(b) training on MUAC use and measurement and should be given MUAC tapes and normal values for age.

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EFFECT OF COOKING METHODS ON THE CONCENTRATION OF OXYTETRACYCLINE RESIDUES IN CHICKEN

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Antibiotics are used in poultry industry to obviate disease, enhance growth and increase production. However, the use of these drugs often results in the accumulation of violative levels of residues in tissues. Consumption of such poultry meat would potentially adversely affect human health through the development of resistant pathogenic microorganisms and hypersensitivity reactions in sensitized individuals. Although meat is always heat treated before consumption, which should ordinarily render

the residues innocuous, some drugs are heat stable and therefore would persist at residue violative levels. Since tetracyclines are the most frequently used antibiotics in poultry production in Nigeria, this study was therefore embarked upon to find out the effect of cooking methods (boiling, microwaving and roasting) on the concentration of oxytetracyclines (OTC) in poultry meat and organs. Muscle and liver tissues were harvested from birds that were treated with OTC either by intramuscular injection or orally in drinking water and analysed for residues using the three plate test (TPT) and the enzyme-linked immunosorbent assay (ELISA). TPT at two different pH levels reduced the inhibition zones of raw muscles between 34-49%, 67-69.6% and 53-56% for microwaving, boiling and roasting respectively but the difference in the means was not statistically significant ($P > 0.05$). TPT however, significantly ($P < 0.05$) reduced the inhibition zones of raw liver between 79- 80.9%, 57-60.29% and 88-89.71% for microwaving, boiling and roasting respectively, at both pH levels. ELISA determined a slight increase in mean OTC concentration in microwaved (1.2%) and roasted (0.3%) muscle tissues with a slight decrease by boiling (3.5%) but the differences were not significant. Boiling and roasting however significantly ($P<0.05$) reduced OTC concentration in liver tissues by 2.83% and 3.17% respectively. The reduction of OTC concentrations by heating will give a ray of hope for those in developing countries who are exposed to violative levels of OTC residues due to non-enforcement of laws against antimicrobial use in livestock and poultry production.

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EFFECTIVENESS OF LONG LASTING INSECTICIDE NETS ON UNCOMPLICATED CLINICAL MALARIA: A CASE-CONTROL STUDY FOR OPERATIONAL EVALUATION

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In a context of large scale implementation of malaria vector control interventions such as LLINs, it is an urgent need to monitor their effectiveness. Case-control study could be an alternative tool for this evaluation since this study design avoids many of the ethical issues inherent to longitudinal and experimental studies. The present study aimed to use the case-control approach to evaluate the post-deployment effectiveness of LLINs. A case-control study took place in two health districts in Benin; Ouidah-Kpomassè-Tori (OKT) in South and Djougou-Copargo-Ouaké (DCO) in North. Children aged 0-60 months recruited in populations were included. Cases were children with a high axillary temperature (≥37.5°C) or a reported history of fever during the last 48 hours with a positive RDT. Controls were children with neither fever nor signs evoking malaria with negative RDT. The necessary sample size was at least 396 cases/1,188 controls per area. The main exposure was "Sleeping all night under LLINs two weeks preceding the survey". The conditional logistic regression model taking into account clustering random effect was used for analysis. The protective effectiveness (PE) of LLINs was calculated as (PE=1-OR). The declared use of "LLINs all night since two weeks" ranged was 17.0% and 27.5%, respectively, in cases and controls in OKT area and 44.9% and 56.5% in cases and controls, respectively, in DCO area. The use of LLINs conferred 40.5% [95CI: 22.2%-54.5%] and 55.5% [95CI: 28.2%-72.4%] of PE in the OKT and the DCO area, respectively. Differences in PE were observed according to the education level of the mother. The case-control study appeared to be a relevant and suitable epidemiological tool for operational evaluation of LLINs effectiveness in the real condition after their deployment. In the context of a mass distribution of LLIN, the use of LLINs conferred protection up to 40% against the occurrence of uncomplicated malaria cases in children.

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ASSOCIATION OF CAUSAL BELIEFS ABOUT SHOE WEARING TO PREVENT PODOCONIOSIS: A BASELINE STUDY

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Podoconiosis is a neglected tropical disease caused by long term barefoot exposure to volcanic clay soil. Susceptibility to podoconiosis is hereditary but disease can be prevented if individuals at high risk consistently wear shoes. Our previous qualitative research identified various domains of beliefs about the causes of podoconiosis held by members of the community. This study aimed to quantitatively evaluate the prevalence of these beliefs and to assess their association with observed shoe-wearing behavior. A baseline study was conducted in 2013, in six communities endemic for podoconiosis in Wolaita zone, southern Ethiopia. A total of 1800 respondents (600 affected and 1200 unaffected parents of an index child aged between 3 and 6 years) took part in the study. The care-giver who spent the "most time with the [index child] and knew the child's daily habits the best" was asked to complete the baseline survey with the index child in mind. Two versions of the enumerator-administered survey were created with measures assessed in parallel for the affected and unaffected household respondents. Associations among measures were assessed using linear regression models. Respondents from affected families were significantly more likely to report that the index child wore shoes than respondents from unaffected families ($p<0.001$). Accuracy of understanding about podoconiosis was significantly lower among respondents from unaffected than affected households ($p<0.001$).Beliefs about heredity among affected respondents were negatively associated with reported shoe wearing of the index child (OR=0.67, 95% CI 0.55-0.83). Associations of causal beliefs with shoe wearing were moderated by risk perceptions for both groups. Interventions aimed at preventing podoconiosis and improving shoe wearing should consider family-oriented education on hereditary susceptibility targeting both affected and unaffected families in resource limited settings.

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GLOBAL HEALTH WITHIN BORDERS: OUTLOOK OF HEALTH SCREENING AMONG IRAQI AND SUDANESE REFUGEE COMMUNITY AND THE IMPACT OF LANGUAGE BARRIER THE PATIENT SELF-ADVOCACY

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The CDC estimates that between 50 to 70 thousand refugees are resettled in US each year. Caring for this highly diverse population represents a unique opportunity and challenge to practice international health and community outreach within US borders. We report health screening finding among refugees at an urban clinic. We aim to review the immunization status of adult immigrants, identify missed opportunities for vaccination and analyze the impact of fluency in English have on compliance with recommended schedule A retrospective chart analysis was conducted in 101 patients seen at a refugee health reference center in Philadelphia from 2012 to 2014. Variables measured included demographic, medical and anthropometric data, vaccination statuses and fluency in English. For the purpose of this study, full vaccination encompassed influenza, MMR, Tdap and varicella. English fluency was

determined by provider documentation. Our demographically diverse population was originated from 16 different countries, largely young (median age 33.2), comprising mostly Iraqi (50%) and Sudanese (14.8 %) refugees. They had high burden of transmissible diseases: LTBI (19.8%), presumed parasitic infections (7.9), syphilis (4%). In regards to vaccination, 5.9% were susceptible to varicella and 1.9% had incomplete MMR vaccine. Among non English speakers, 58% had received full vaccination versus 71% of English speakers. Much like Americans, refugees exhibited high rates of or chronic diseases and mental illness. One in every three patients was smoker and obesity rates were 20.1% Mental health screening was positive in 10.9%. Length of stay > 1 year was not correlated to increase in BMI, contrary to previous literature reports. No differences in BMI, smoking rates, vaccinations rates were found among ethnic groups. In conclusion, ability to speak English represents a significant barrier to patient self-advocacy, despite availability of interpretation service and patient navigation resources. Targeted interventions of health English literacy are likely to improve vaccination compliance and health indicators among refugees

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USING RAPID ETHICAL APPRAISAL (REA) TO DESIGN A CONTEXTUALIZED CONSENT PROCESS FOR BIOMEDICAL STUDIES THE ETHIOPIA

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Conducting biomedical studies in developing countries can be difficult partly due to poor knowledge about research process and research ethics. The situation is complicated when the disease of interest is thought to be familial and a reason for stigmatisation. We used a Rapid Ethical Appraisal tool to assess local factors that were barriers to getting genuine informed consent prior to conducting genetic study of podoconiosis (non-filarial elephantiasis) in two provinces in Ethiopia. The tool involves In-depth Interviews and Focus Group Discussions with patients, healthy community members, field workers, Institutional Review Board members, elders, religious leaders, and administrators who work closely with patients. We compared our findings with two earlier studies that employed this method in another part of Ethiopia and north western Cameroon. Most of the study participants did not differentiate research from routine clinical diagnosis. The decision to participate in a study was influenced by the presence of trusted community members during the consent process and the type of biological sample sought. Many believed podoconiosis to be hereditary; some also considered it to be a communicable disease that can be transmitted by wearing patients' shoes and washing with the same basin the patients have used. Participants better understood genetic susceptibility concepts when analogies drawn from their farming experience were used. Understanding the concerns of local people in areas where research is to be conducted will help to design contextualized consent processes appropriate for all parties and will ultimately result in getting genuine consent.

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COMMUNITY-BASED LARVAL SOURCE MANAGEMENT WITH LARVIVOROUS FISH TO CONTAIN VECTORS OF JAPANESE ENCEPHALITIS THE DISTRICT GORAKHPUR, UTTAR PRADESH, INDIA

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Community-based larval source management with larvivorous fish to contain vectors of Japanese Encephalitis in district Gorakhpur, Uttar Pradesh, India Susanta K Ghosh1, Vijay P Ojha2, Satyanarayan Tiwari1, Milind Gore2 and Neena Valecha2 1National Institute of Malaria Research, ICMR complex, Devanahalli, Bangalore 562110, India 2National Institute of Malaria Research, Sector 8, DWARKA, New Delhi 110077, India 3National Institute of Virology, Field Unit, BRD Medical College, Gorakhpur-273013, Uttar Pradesh, India Japanese Encephalitis (JE) is a mosquito-borne viral disease mostly prevalent in rice growing areas in the Asia-Pacific region. In India JE is endemic in 24 states and union territories. About 75% of Indian JE cases are reported Uttar Pradesh, and district Gorakhpur also contributed nearly 75% cases from Uttar Pradesh. We undertook a study to contain the JE vectors in three endemic blocks in district Gorakhpur using larvivorous fish involving the local administration and community. Baseline larval survey data in July-August 2013 revealed JE vectors Culex tritaneorynchus, Cx. vishnui, Cx. pseudo vishnui breed in ponds, wells, rice fields and borrow pits. During summer months only ponds and wells support the vector breeding. Based on our malaria vector control experience in Karnataka, we targeted ponds with mosquito fish Gambusia affinis and wells with Guppy fish (Poecilia reticulata). Geographical reconnaissance (GR) of all water sources were mapped at village level. Workshops were organized involving the staff of local health and Block Development Officers, Panchayat (Local self-government) members. Focused group meetings in each village were also organized. Monthly monitoring of vectors in the post fish-intervention data revealed a reduction of 78% on breeding indices. Here, rice fields have very limited role as they get dry up in the summer months, and no breeding of vector was observed in rice field post fish intervention period. Our study showed that larvivorous fish has a potential role to contain vectors of JE. Study is undergoing to expand this programme in other areas of the district and other JE endemic areas.

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STIGMA TOWARDS A NEGLECTED TROPICAL DISEASE: FELT AND ENACTED STIGMA SCORES AMONG PODOCONIOSIS PATIENTS IN NORTHERN ETHIOPIA

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Podoconiosis, or non-filarial elephantiasis, is a neglected tropical disease (NTD) characterised by swelling of the lower legs. When left untreated, this disfiguring condition has a significant social impact. This study aimed to describe the stigma experience among podoconiosis patients in Dembecha, Northern Ethiopia and assess potential associations between stigma and sociodemographic determinants. The study was conducted in May 2012 in Northern Ethiopia. A questionnaire-based cross-sectional study design was used and stigma was assessed using a validated podoconiosis stigma scale including 'felt' and 'enacted' stigma domains. Enacted stigma includes the experience of discrimination such as abuse, loss of employment or prejudicial attitudes, while felt stigma is the perceived fear of enacted stigma. A multivariable linear regression model was used to explore determinants that may be associated with stigma. A total of 346 clinically confirmed podoconiosis patients participated in

the study. The total mean score ofall stigma scale items was 30.7 (Range = 0 to 96). There was a higher mean score of scale items in domains of feltstigma (21.7; Range = 0 to 45) as compared to enacted stigma (9.0; Range = 0 to 51). The total mean score of all stigma scale items appeared to increase with disease stage. A final adjusted linear regression model found an association between stigma and factors including monthly income, duration lived in the current residence, and disease stage, after controlling for confounders. In conclusion, podoconiosis is a stigmatized disease with a clear social impact. This paper documented the burden of podoconiosis-related stigma and identified associated factors. Programs aimed at preventing and treating podoconiosis should incorporate interventions to mitigate both felt and enacted stigma.

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PODOCONIOSIS PREVALENCE AND PREVENTION EDUCATION IN RWANDA AFRICA

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Non-filarial elephantiasis (also known as Podoconiosis), is a noninfectious neglected tropical disease caused by prolonged exposure of bare feet to irritant volcanic soils. The focus of our research has been to determine the number of individuals in the Northern Province with this condition in an attempt to treat those with the condition and to prevent any new cases from occurring through a focused prevention education program. The Imidido Project team began registering those with Podoconiosis in March of 2013 and to date we have 183 registered in Musanze with a population of 368,267. The Imidido Project expanded services to Kinoni in the Burera District with 72 registrants and a population of 339,200. Our preliminary results indicate that less than 1% of the population in the Northern Province is suffering from Podoconiosis. High risk individuals have been a focus for our prevention education team and our preliminary data indicates that of the 183 persons with Podoconiosis only 2 family members of the 183 registrants have signs and symptoms of Podoconiosis since the prevention education team began its work visiting families in their homes. Podoconiosis prevalence is less than 1% of the population in the Northern Province; however, the prevention education team focuses on building awareness in schools, churches, communities and healthcare centers throughout the Northern Province with hopes of eradicating this neglected tropical disease in Rwanda Africa.

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PREVENTING MATERNAL MORTALITY THE THE DEMOCRATIC REPUBLIC OF THE CONGO: EFFECT OF THE ORGANIZATIONAL MODEL OF HEALTH CARE

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The Democratic Republic of the Congo (DRC) has the third highest number of maternal deaths (MD) and one of the highest risks of MD (730/100,000live births) in the world. Reaching Millennium Development Goal 5 (≤138/100,000 live births) in 2015 is out of the question; appropriate care is often not accessible. This study aimed to determine the impact of differentorganizational models of mother-child health (MCH) services on maternal mortality (MM).Using data on the MM ratio, cause of MM, and MCH services use from the Multiple Indicator Cluster Survey 4 (MICS4), we modeled the reduction in MM as a function of the variation in the level of use of services for the period from 2010-2015. We evaluated scenarios based on the model of service provision: family-based or informal care, community-based care, and clinical care. For each model, the rate of MCH services use was progressively varied between 60 and 90%. This rate was not varied for services for which the use was already above the set threshold. In 2010, 16,390 MD occurred in DRC. If universal (>90%) coverage of all types of services, on the continuum, were assured to women over one year, more than 48.5% of these deaths could be avoided. Cost-effective MCH services are well-known. Universal coverage across the mother-child continuum constitutes the strategy most likely to significantly reduce MM in DRC. To achieve this level of performance, the entire health care system must be strengthened.

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HOUSEHOLD CHARACTERISTICS ASSOCIATED WITH MALARIA-INTESTINAL HELMINTHS CO-INFECTIONS AMONG RURAL DWELLERS THE SOUTHWEST, NIGERIA

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Malaria and intestinal helminth infections occur concurrently in tropical regions due to similarity of favorable breeding conditions, the effects on morbidity outcomes cannot be over-emphasized. The household environment is a major determinant and influences the risk of infectious diseases. This study explored household related risk factors associated with the occurrence of both infections particularly among rural dwellers. A two stage sampling technique was employed to recruit 647 household representatives and/or head of households from 35 communities. At the household of the enrollees, data was collected using a pre-tested questionnaire and an observation checklist. About 47% of the respondents were males and mean age was 46.0 ± 1.3 years, 51.1% had no formal education. Farming (51.0%) and trading (31.8%) were the major occupations of the people. The Well was mentioned as the predominant source of water for domestic use (84.0%) and drinking (81.0%). About 85.8% defecated in the bush. Only 7.7% used bednet as malaria preventive material, 7.5% slept under the net the night before and only 9.7 had window/door screens. Very few (2.5%) had had an Indoor residual spray in the last 1 year preceding the study. Several (61.2%) managed malaria at home first before seeking any other provider. Of all respondents, 20% said the health facility was far from their house. A number of (33.7%) had passed out worms in the last one year, 49.3% dewormed once in the last one year while 35.3% said they never deworm. About 54.6% had overgrown vegetations, 49.3% had uncovered containers with water, 11.6% had stagnant water around the household environs. A number (26.5%) of the respondents recalled that they had fever two weeks before the study. Household characterististics and risky practices associated with malaria and intestinal helminths abound in most of the households and environs visited in the study.

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EVALUATION OF A UNIVERSITY-NGO PARTNERSHIP TO ADVANCE EQUITY THROUGH NURSING EDUCATION

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Nurses provide 90% of health care worldwide today, yet global health nursing is a field still in its infancy. There is growing recognition of the need to elevate the leadership role of nurses, especially in resource-limited settings. In Haiti, few public nursing schools, limited opportunities for continuing education and high levels of nurse migration weaken an already overburdened health system. In 2014, the University of California, San Francisco (University of California San Francisco) developed the University of California San Francisco Global Health Nursing Fellowship to advance nursing competency and leadership in Haiti, while also training US-based nurses in global health care delivery in resource-limited settings (RLS). Partnering with the non-profit Partners In Health, the fellowship aims to support PIH's Nursing Center for Excellence trainings and mentorship for the local nursing workforce. The 10-month fellowship is offered to US-based advanced practice nurses. Fellows

spend 50% of their time in Haiti creating educational opportunities and modeling team-based care, and the rest of their time at University of California San Francisco mentored as associate clinical faculty. This study was undertaken to address two interrelated, unmet needs in nursing education: clinical, didactic and leadership support for nurses in low-resource settings; and post-graduate global health training for US-based nurses. The collaboration has completed its inaugural year and offers an evaluation here. Successes include: 1) piloting a structured format for bed-side teaching; 2) modeling the role of a dedicated in-patient nurse educator; and 3) increasing fellows' understanding of local burden of disease, health inequities and barriers to care delivery. Challenges include: 1) engaging overburdened nursing staff and leadership; 2) aligning the learning needs of the fellows with the contextual realities of a hospital in a RLS; 3) reinforcing collaborative relationships with physicians to improve interprofessional rounding. These early results demonstrate a potential model for international university-NGO partnerships that can advance health equity, benefiting both US-based learners as well as the local nurse workforce.

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ASSESSING THE COST-EFFECTIVENESS OF DIFFERENT VACCINATION STRATEGIES FOR CHILDREN IN THE DEMOCRATIC REPUBLIC OF CONGO

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While measles mortality has been reduced more than 78%, the disease remains one of the major causes of childhood vaccine preventable diseases globally. Measles immunization requires a two-dose schedule and only countries with strong, stable immunization programs have been able to rely on routine services to deliver the second measles dose. In the Democratic Republic of Congo (DRC), the second dose of measles vaccine is administered via supplementary immunization activities (SIAs), due to inadequately low routine immunization coverage. We used a decision analysis with a Markov model based on published and unpublished data to compare the cost-effectiveness of two different strategies for the second dose of Measles Containing Vaccine (MCV) to one dose of MCV through routine immunization services over a 15-year time period for a hypothetical birth cohort of 3 million children. Compared to strategy 1, strategy 2 (MCV2 by SIA) would prevent a total of 279,110 measles cases and 6,795 deaths and save U.S. $2.26 million. Compared to strategy 1, strategy 3 (MCV2 by RI) would prevent a total of 207,996 measles cases and 5,074 measles-related deaths and save U.S. $0.71 million. Strategy 2 was both cost-saving and dominated the other two strategies, yielding the fewest deaths and the lowest total program costs over the 15-year time period for the hypothetical cohort. Vaccination recommendations should be tailored to each country, offering a framework where countries can adapt to local epidemiological and economical circumstances in the context of other health priorities. Our results reflect the synergistic effect of two doses of MCV and demonstrate that the most cost-effective approach to measles vaccination in DRC is to continue the administration of the second dose by mass campaign.

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COMPARING ANTHROPOMETRIC MEASURES OF INDIGENOUS SCHOOL-AGED CHILDREN THE GUERRERO, MEXICO TO AVAILABLE INTERNATIONAL, NATIONAL AND LOCAL GROWTH REFERENCES

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Malnutrition, both acute and chronic, continues to be a major health burden for children in resource poor countries. Nutritional surveillance studies in the form of anthropometric measures are useful screening tools to identify children at risk. Established international measures of wasting (< - 2SD weight-for-height, BMI-for age Z-score), being underweight (< - 2SD weight-for-age Z score), and stunting (< - 2SD height-for-age Z score) are now more commonly used in the field. The Comisión Nactional para el Desarrollo de los Pueblos Indígenas reports the indigenous population of Mexico lives in the poorest and most disadvantaged states. This cross-sectional study conducted in February 2013 examined the severity of nutritional deficiencies in the form of grown parameters for indigenous children in two communities in the state of Guerrero, Mexico. Anthropometric measures were collected for children in the communities of Buena Vista (n= 72) and Nuevo Zaragoza (n= 51). The data was entered into the World Health Organization (WHO) AnthroPlus program, and z-scores were generated for height-for-age (HAZ), weight-for-age (WAZ), and BMI-for-age (BAZ). Statistical analysis was performed using simple, independent t-test comparing the collected variables to published mean z-scores from the World Health Organization, Mexico, and the state of Guerrero. The sampled indigenous population had significantly lower z-scores for each t-test performed (All p-values < 0.001). This small study suggests nutritional disparities exist in this mostly indigenous region of Mexico. Specifically, children demonstrated evidence of stunting and are at risk for wasting and being underweight as indicated by their z-scores. The authors believe this information may be useful to public health, policy-makers, governmental and non-governmental organizations seeking to improve health parameters in this region through innovative partnerships addressing this disparity. Understanding the environmental and social influences on health and nutrition in these communities with future studies will allow for site appropriate interventions and stronger advocacy for children at risk.

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DEVELOPMENT OF A COMMUNITY HEALTH WORKER PROGRAM FOR RURAL HAITI

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Access to health care poses an essential challenge for Haiti. To bridge such gaps in health care access, rural communities in a number of countries have instituted a system of community health workers (CHWs). These CHWs are individuals selected from within the community who are trained to serve in both direct patient care and health education. In April 2015, a pilot study will be conducted to investigate (1) the current state of access to primary and emergency health services in the rural community of Mussotte, Haiti, (2) the attitudes of community members toward existing health services and health providers, and (3) the extent of interest in a community health worker program. A cluster sampling method will be used to select approximately 50 subjects for participation in a household survey. Data analysis will include a regression analysis for factors influencing knowledge, attitudes, and perceptions about need for community health workers. Our findings will not only further our understanding of the current health care needs in Mussotte but also allow

for interventions that will more effectively address the existing structural and cultural barriers to care. Ultimately, we hope that this research will create a platform for improvement in access to quality medical services in Mussotte, as well as other rural communities in Haiti which face similar barriers to care.

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ADVOCACY AND TROPICAL DISEASES: ETHICAL RAMIFICATIONS OF THE WEST AFRICAN EBOLA VIRUS DISEASE PUBLIC HEALTH RESPONSE

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As important as it is to devote significant resources to the west African Ebola virus disease (EVD) epidemic, against the backdrop of insufficient resources for public health, the scope and scale of the response has had and will continue to have consequences for other public health problems, including diseases such as malaria, responsible for greater morbidity and mortality. A highly visible public health problem like EVD, from which the U.S. public perceives themselves at risk, provides a rare opportunity for public health officials to advocate for resources for immediate and long-term public health investments, including in parts of the world that are often overlooked. However, there is no substitute for direct advocacy for less publicly compelling public health problems like neglected tropical diseases. This presentation critically examines policy and public health implications of defining public health crises in ways that garner substantial public and political attention. Public health officials often find themselves advocating for resources for the current emergency against a backdrop of political and budgetary constraints, including emphasis on national interest and short-term funding cycles. As a result, public health officials are often required to make difficult choices that might immediately set back other public health priorities, such as vaccine-preventable diseases. Short-term advocacy in the context of a public health emergency can have positive long-term implications, including increased funding and capacity of the public health workforce. Yet these longer term dividends are not automatic, do not always extend to the most intractable public health problems, and often do not obviate the short-term trade-offs that might result from prioritizing crisis response. We must acknowledge these tensions and explicitly engage with the ethical dimensions of tropical diseases and other persistent public health problems, including their social and economic determinants. It is sustained investment in health infrastructure that will better equip systems to manage and overcome public health crises.

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DECREASING INEQUITY OF INSECTICIDE-TREATED NET (ITN) OWNERSHIP IN SUB-SAHARAN AFRICA COUNTRIES FROM 2003-2014

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The increase in funding for malaria control in the past decade resulted in an increase in ownership and use of insecticide treated nets (ITNs) in many countries in sub-Saharan Africa (SSA). However, with the shift in programmatic focus from high risk target groups to universal coverage there is a need to ensure equal access to ITNs for all sub-populations regardless of socioeconomic status. This study assessed change in disparity in ITN ownership among different socioeconomic groups using wealth quintiles in 19 malaria-endemic countries in SSA that had at least two national household surveys with ITN data between 2003 and 2014. One survey must have been published between years 2003-2008 (baseline) and the other survey published between years 2009-2014 (endline). The authors used Lorenz Concentration Curve and Index (C-Index) to assess equity in household ITN ownership between wealth quintiles. C-Index values range between -1 to 1. A value of 0 suggests no difference in outcomes among different socioeconomic groups. Across all countries ITN ownership significantly increased between baseline and endline with the greatest improvement in Tanzania and Rwanda where ownership increased by 68%. Equity in ITN ownership increased in 17 out of 19 countries particularly South and East African countries. In a pooled multi-country analysis a significant reduction in wealth inequality was seen in areas of medium/high malaria transmission (PfPR2-10≥5%) between baseline (C-Index 0.10, 95% CI: 0.09;0.18) and endline surveys (C-Index -0.01, 95% CI: -0.00;-0.02). These findings show the tremendous achievement of ITN ownership across sub-Saharan Africa in increasing ITN coverage and reducing inequity in the past 10 years.

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MATHEMATICAL MODELLING APPROACHES TO ESTIMATING THE OPTIMUM STRATEGY FOR THE ERADICATION OF YAWS

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Yaws is a re-emerging endemic treponemal infection. The WHO aims to eradicate yaws by 2020 with a strategy that is based on mass treatment of the entire population with a single dose of oral azithromycin treatment. Previous mass treatment campaigns based on penicillin in the 1950's and 1960's failed due to inadequate coverage, in particular of latent cases, and failures of surveillance post the initial round of treatment. Subsequent successes in Ecuador and India have suggested that, with prolonged intervention and high coverage, disease elimination can be achieved. However, the number of rounds of treatment or the coverage that will be required to achieve eradication are currently unknown. This information will be vital if the aim of yaws eradication is to be achieved. Cost and time constraints limit the ability of traditional randomised studies to fully evaluate all possible control strategies but mathematical modelling approaches may allow a detailed exploration of the number of rounds and coverage that will be required to achieve the goal of yaws eradication. We developed a stochastic model of yaws transmission in an endemic setting. Baseline parameters were estimated using robust epidemiological data derived from study sites in Papua New Guinea and the Solomon Islands. We applied different control strategies varying both the coverage and number of rounds of treatment. We then used the model to assess the predicted impact of different control strategies including the probability of achieving eradication for each control strategy. Using this data we are able to suggest critical thresholds that must be achieved to interrupt local transmission of yaws and achieve the goal of yaws eradication. This work contributes significantly to our understanding of the predicted impact of community mass treatment with azithromycin and provides important data to inform the design and roll-out of yaws control programmes worldwide.

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THE "EMD SERONO GLOBAL HEALTH" APPROACH

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Launched in 2014 to address key unmet medical needs for children from developing countries suffering from tropical diseases, EMD's Global Health unit is a R&D platform focusing primarily on malaria and schistosomiasis. Under its "One Merck for Children" concept, the goals are to develop innovative, affordable, implementable and integrated health solutions including new pediatric medicines, tailored diagnostics & associated

delivery & eHealth technologies through leveraging from EMD's cross competencies and in partnership with leading Global Health institutions and organizations in both developed and developing countries. To address the need of new antimalarial to continue fighting against emergence of resistance, EMD Serono Global Health aims at building a small sustainable portfolio of molecules on selected key existing gaps in the current fight against malaria: long lasting, liver & gametocyte acting compounds. Also, in collaboration with EMD Millipore, a new malaria diagnostic assay is being developed to measure levels of parasitemia as well as identification of the infectious type in very small amount of blood to address pediatric sample limitations. This assay will be compatible with an existing point of care compact flow cytometry platform (MUSE) that has already demonstrated its capacity to measure with very high sensitivity and specificity counts and % of CD4T cells during its clinical trials in African countries. For schistosomiasis, there is a pressing need to treat preschool children and the current Pediatric Praziquantel Consortium is actively developing a suitable formulation of the L-enantiomer of PZQ. Beyond closing treatment gaps by developing a new pediatric PZQ formulation, the PZQ usage to tackle other helminthic diseases is also considered by building a small drug discovery portfolio to complement PZQ as a single drug. It also aims at identifying options to co-develop diagnostic tools, addressing the impact on co-infections, on female/male genital schistosomiasis and contributing to strengthen the Merck Praziquantel Donation Program by enhancing the R&D competence at EMD in the area of human schistosomiasis.

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USE OF M-HEALTH AT COMMUNITY LEVEL TO PROMOTE GOVERNANCE AND EQUITY WITHIN THE HEALTH SYSTEM: DESIGN, IMPLEMENTATION AND CHALLENGES THE RURAL HEALTH DISTRICT, BURKINA FASO

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The use of mobile phone has been described as offering a remarkable potential to deliver primary health care, provide antenatal are services and remind patients about follow-up appointments. In Burkina Faso, high maternal mortality rates and persistent numbers of people living with HIV are priorities to address by government. A strong primary health care approach is required to ensure that people are able to access adequate, affordable and equitable health services and guidance within their community. Here we described the potential of an innovative mobile phone platform that helps to overcome barriers of access to health service information by community members in remote areas. A community-based mobile phone project was implemented to enhance better access to health information, better health care delivery for mother, newborn and people living with HIV. An interactive voice system was developed and incorporated major local languages to overcome literacy barrier. Overall 423 pregnant women, 319 newborn mothers and 116 HIV/AIDS patients were followed-up by the mobile phone system in 2014 by 62 community health workers. An average 177 patient's reminder for appointment was completed. There was an 8% increase of antenatal care uptake and 3.5% for newborn BCG vaccine coverage. Better compliance of HIV patients to antiretroviral services was also noted. However, running mobile devices in remote areas is challenging. About 29% of cell phone and 24% of solar recharging system were changed. Users also faced a regularly network breakdown for the major phone company. Community data integration within national health system and system interoperability were a big challenge to address. Use of mobile phone at community level is undoubtedly a powerful tool to increase their equitable access to health care information and participation local health care governance. However the issue of cell phone robustness, ease of use and availability of sustainable source of energy need to be more explored.

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SCALING UP MALARIA RAPID DIAGNOSTIC TESTS IN PRIVATE SECTOR SUPPLY CHAINS IN UGANDA: ADAPTING MULTI-CRITERIA DECISION ANALYSIS AS A METHODOLOGY TO UNDERSTAND DECISIONMAKING AND PREFERENCES

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Diagnosis of malaria is important in order to ensure early and effective treatment, to facilitate public health surveillance, and to prevent drug resistance. Rapid diagnostic tests (RDTs) are an important tool in resource-constrained settings, as they do not rely on costly lab equipment and specially trained personnel. In Uganda's private sector clinics and drug shops, which is where the majority of patients first seek care, diagnosis of malaria is often presumptive and patients receive neither RDT nor microscopy. Several studies have focused on the patient perspective (e.g. willingness to pay) but much less understood about the supplier perspective (e.g. willingness to stock). This study aimed to understand stocking strategies for agents across the malaria RDT supply chain in Uganda using multi-criteria decision analysis. This methodology was adapted to be relevant and understandable for agents in Uganda so that we could analyze business decisions incorporating aspects such as selling price, purchase cost, sales volume, complexity of regulations, waste management, and training available. Data surveys and semi-structured interviews were collected from 28 private sector retailers (i.e., shopkeepers, pharmacists, clinic managers), two first line buyers, three distributors, and two manufacturers. Analysis resulted in value functions for all agents and quantified the tradeoffs among decision criteria. Our results offer critical insights for understanding how to engage the private sector in scaling up usage of malaria RDTs. The study also demonstrates how to adapt the multi-criteria decision analysis methodology for studying supply chains in resource-constrained contexts.

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FIELD EVALUATION OF HOME-BASED AND CLINIC-BASED MULTIPLEX DEVICES FOR TRANSMISSION OF RESULTS VIA A SECURE BIO-SURVEILLANCE NETWORK IN IQUITOS, PERU

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The Defense Threat Reduction Agency (DTRA) is partnered with the U.S. Naval Medical Research Unit No. 6 to evaluate the speed and efficiency of result reporting from novel multiplex diagnostic devices to a novel bio-surveillance ecosystem (BSVE). Handheld devices ("buddy care") will be observed for ease-of-use in homes of local residents in Iquitos, Peru. Participants' comprehension of basic instructions followed by self-administration of one lateral flow test will be observed and documented. Clinic-based devices ("role 1") will be used by healthcare professionals on febrile patients in one of six clinics throughout the city. Both of the buddy care devices (ChemBio DPP Febrile Illness Test and the InBios Active-Dengue-Melioidosis Detect Rapid Test) will be distributed throughout an existing cohort in the city of Iquitos. First, movement teams will recruit up to five households of non-febrile participants in each block to use buddy care tests under supervision. Second, a maximum of thirty households per block will receive one of the buddy care devices and will then be asked to either perform the test alone or while receiving assistance by

trained medical personnel in cases of febrile symptoms. The role 1 devices will be distributed in six Iquitos medical clinics where NAMRU-6 trained phlebotomists will enroll febrile participants for the study. All results from both buddy care and role 1 devices will be uploaded into the BSVE and compared with results from gold-standard, laboratory-based testing. All protocols and informed consent documents have been approved, and city blocks within the cohort area where the buddy care devices will be distributed have been selected. The role 0 portion of this study is set to begin by mid-April, which includes observations for ease-of-use by non-febrile participants, as well as distribution of buddy care devices in the homes. To achieve success, we need greater than 90% accuracy in transmission of results to the BSVE, as well as 75% and 85% sensitivity for the buddy care and role 1 devices compared with the gold standard tests, respectively. Preliminary tests uploading results into the BSVE have been promising.

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DISPARITIES THE QUALITY OF ANTENATAL CLINIC CARE THE KENYA: ANALYSIS OF KENYA DEMOGRAPHIC HEALTH SURVEY 2008 - 2009

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ANC provides the opportunity for early detection and treatment of pregnancy anomalies, and to deliver preventive health services. However, detailed information about the quality and content of ANC in practice in Kenya is scanty. We reviewed data from the 2008/9 KDHS, a nationally representative survey and analysed data from women aged 15-49 years. Descriptive data summaries were presented as proportions while association was measured as prevalence odds ratios. In this study 50.9% of women sought ANC services either in health centres or dispensaries. Maternal age, regional residence, urban residence, wealth index, education and the media influenced ANC initiation and ANC 4+ visits. There were coverage gaps existing on iron-folate supplementation (66.1%), tetanus toxoid (66.5%), presumptive/preventive treatment for malaria with SP (38.7%) and education on pregnancy complication (44.3%). Nearly 24 % of women missed the screening for complication during pregnancy. Quality of ANC service provided is associated with type of health facility. Facilities common in rural communities and informal urban settlements had lower quality service. Even though ANC visit is high, quality of service varies greatly. Women attending clinics at dispensaries and government health centres are likely getting the lowest quality service, presenting disparities in a rural-urban context. It is important that more resources, including equipment and skilled health workers be availed in these facilities to reverse this trend.

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FACTS AND RUMORS: SOCIAL MEDIA REACTION TO INFORMATION AND MISINFORMATION ON EBOLA

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We analyzed the misinformation circulating on Twitter and Sina Weibo (the leading Chinese microblog platform) at the outset of the 2014 Ebola epidemic. We retrieved Twitter and Weibo data created within 24 hours of the WHO announcement of Public Health Emergency of International Concern (Batch 1) and seven days later (Batch 2). We obtained a 1% random sample of the Twitter universe, of which tweets containing the keyword Ebola were analyzed. We retrieved all Weibo posts with Chinese keywords for Ebola for analysis. Trending and fading analysis was performed for keywords, hashtags and web links. We identified misinformation by manual coding and categorization of randomly selected sub-datasets. Ebola-related misinformation constituted a minority of Twitter and Weibo contents. The predominant content was information released by public health agencies and the major news agencies. Two misinformed speculated "treatment" predominated in Twitter posts. Saltwater was speculated to be protective against Ebola in the first batch of tweets, but faded a week later. "Nano-silver" was on the top 10 trending Twitter list. Chinese microblogs focused on the Chinese government sending medical assistance to Africa. In conclusion, in the 2014 Ebola epidemic, Twitter and Weibo are platforms that circulate outbreak news and scientific health information.

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A QUALITATIVE EVALUATION OF STAKEHOLDER PERSPECTIVES ON THE MILLENNIUM VILLAGE PROJECT SUCCESSES AND CHALLENGES IN SAURI, KENYA

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The Millennium Villages Project (MVP) is a 10-year, multi-sectorial, rural development program that strives to achieve the Millennium Development Goals at an annual cost of US$110 per capita through implementation of evidence-based interventions across sectors, including agriculture, health, and education. Focusing on early implementation of the first MVP site in Sauri, Kenya, perspectives held by major stakeholders (N=27) in the planning and implementation of project activities were examined using semi-structured interviews. Key stakeholders represented implementing agency and partners from village sector committees (VSCs), local and regional government agencies, international health agencies, non-governmental organizations, and academic and policy institutions. Interviews were recorded, transcribed, and analyzed using NVivo10, a qualitative software. Interviews were coded inductively and independently by three researchers to ensure high inter-rater reliability. Data suggest differing views among stakeholders around program successes. Additionally, although MVP sought to impact changes in three areas -namely, agriculture, health, and education- during its early phase, most emphasized positive effects in health and agriculture, with fewer mentioning achievements on education. Distinctions in expressed challenges were found with most indicating planning difficulties regarding development and mobilization of VSCs and others identifying implementation barriers associated with program adoption and adaption. Conflict was apparent in stakeholder comments around resolving village-level power struggles and reconciling conflicts between MVP activities and government policies. Findings also suggest enhancement in community cohesion and capacity. This qualitative evaluation of stakeholder perspectives on MVP successes and challenges contributes to the current debate at national and international levels in setting and implementing rural development policies.

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DISTRIBUTION OF *PHLEBOTOMUS ORIENTALIS* IN MERTI KENYA

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Visceral leishmaniasis (VL) is a neglected tropical disease in East Africa whose principle vector of the disease casing agent is the sand fly ***Phlebotomus martini*** and ***P. orientalis***, however not much is known on the distribution and biogeography. The sandfly, ***P. orientalis*** is a principal vector of leishmaniasis in Sudan and Ethiopia, where it has been associated with black cotton soil and Acacia seyal and Balanites aegyptica trees. The range of ***P. orientalis*** has not historically involved Kenya, but

recent surveillance data has revealed that these sandflies are present in high numbers sufficient for it to sustain transmission. The objective of the current study was to explore the distribution of ***P. orientalis*** in Merti and Isiolo, Kenya by sampling in multiple habitats. Sampling was conducted for five nights using CDC light traps baited with dry ice placed along a river bed, in a village next to houses, a cattle camp, and next to a goat shed in open fields. The village, cow and goat sheds, were more than two kilometers from the river in this rural area of Kenya. All sampled sand flies were separated into Phlebotomus and Sergentomyia genera. Identification was completed on all Phlebotomus and 10% of the Sergentomyia sandflies collected. A total of 344, ***P. orientalis*** were sampled over the five nights of trapping. Human dwellings had 27% (94), representing four ***P. orientalis*** per trap per night, the river bed had 29% (101) representing three ***P. orientalis*** per trap night. Cow and goat sheds had 25% (86) and 19% (63) representing 14 and 32 ***P. orientalis*** sandflies per trap night, respectively. The majority of the sand flies caught were females. A few of the males sampled had unrotated genitalia; implying that all habitats sampled were breeding habitats. This is the first recorded sampling of ***P. orientalis*** in animal and human dwellings in Kenya. This study illustrates that ***P. orientalis*** is more widely distributed than previously thought. Further studies that investigate the possible blood meal sources and natural infection rates are needed because of the evidence of this leishmanisis vector breeding near human dwellings.

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THE IMPACT OF FOUR YEARS OF SEMI-ANNUAL IVERMECTIN TREATMENT ON TRANSMISSION OF *ONCHOCERCA VOLVULUS* AND THE FEASIBILITY OF ONCHOCERCIASIS ELIMINATION THE SOME GHANAIAN COMMUNITIES

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Since 2009 onchocerciasis endemic villages in Ghana have shifted from annual to semi-annual ivermectin treatment due to a change in the control strategy by the African Programme for Onchocerciasis Control (APOC) from morbidity control to elimination of infection. A standard annual dose of ivermectin kills close to 99 % of skin microfilariae and temporarily halts microfilariae production by female adult worms. We used entomological techniques to assess the impact of semi-annual ivermectin treatments on ***Onchocerca volvulus*** transmission and to explore the feasibility of onchocerciasis elimination in Ghana. Adult female ***Simulium damnosum*** s.l. were collected from 17 onchocerciasis endemic communities, which have been receiving annual rounds of ivermectin treatment, using human land catches and analysed for parity rates and ***O. volvulus*** infection. ***O. volvulus*** transmission indices were estimated from manual fly dissection data. A total of 53,675 female blackflies were analysed after four years of vector collection, 19,156 (35.7 %) of which were parous and 776 flies were infected with ***O. volvulus*** larvae. 265 (0.5 %) flies haboured 439 L3s in the head representing an overall infectious rate of 0.82 %. Monthly biting rates (MBR) varied from a minimum of 0 bites in the dry season to a maximum of 10579 bites in the wet season while the monthly transmission potential (MTP) ranged from 0 to 320.3 infective bites. The vector infectivity rates also varied from 0 to 219.5 L3s per 1000 parous flies. After four years of semi-annual treatment seasonal biting rates, ranging from 15.3 to 15,694 bites, remained high and transmission of ***O. volvulus*** had reduced drastically in all communities except New Longoro, Tainso, Agborlekame I and Wiae. Infection appears to have been interrupted in 4 of the 17 communities with seasonal transmission potentials (STP) below postulated thresholds of under 20 L3s per 1000 parous flies after three years of semi-annual ivermectin treatment. The implications for onchocerciasis elimination in Ghana will be discussed.

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HIGH-THROUGHPUT MULTIPLEX FRAGMENT ANALYSIS FOR IDENTIFICATION OF *ANOPHELES GAMBIAE* SL IN MALARIA ELIMINATION ERA

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Identification of mosquito vector species is important in control of vector-borne disease as it provides better understanding of local vector species involved in disease transmission. Large scale frequent sampling is common in entomological surveillance in malaria elimination programs and requires robust genotyping method. Although various molecular assays have been developed for vector identification in regional control programs, they are not suitable for large sample size vector surveillance. Therefore, a robust, rapid, high-throughput and portable genotyping method is needed where species of as large as a thousand mosquito specimens can be identified from a single PCR plate within a short period to inform timely control measures. Here, we describe the optimization and validation of a novel method, multiplex fragment analysis (MFA) to enable routine and rapid identification of large scale surveillance specimens. PCR amplification of mosquito specimens was performed using modified forward oligonucleotide primers of two widely-used protocols followed by fragment separation with capillary electrophoresis and detection by laser using DNA analyzer. Up to a thousand mosquito specimens were pooled together in a single PCR plate and identified by differences in their sizes and colors. Validation was done with previously genotyped genomic DNA of 1,056 specimens. Agreement in species identification between the novel MFA and current methods was compared using Kappa statistics and turn around times for specimen processing were calculated. The novel MFA showed ~100% agreement with current methods in identifying An. arabiensis, An. gambiae ss, An. colluzzii and An. melas. The estimated turn around time for processing 1,056 specimens using the novel method was 20 working hours (~3 working days) but 54 working hours (~7 working days) with classical methods. The novel MFA is rapid, reproducible and highly applicable for large scale entomological surveys compared to the existing methods. It is also less-laborious and has better genotyping resolution than the current gel agarose electrophoresis-based methods.

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SYSTEMATICS OF THE *AMBLYOMMA MACULATUM* GROUP OF SPECIES *(ACARI: IXODIDAE)*

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The "***Amblyomma maculatum*** group" currently includes 5 species: ***A. maculatum*** Koch, 1844; ***A. neumanni*** Ribaga, 1902; ***A. parvitarsum*** Neumann, 1901; ***A. tigrinum*** Koch, 1844 and A. triste Koch, 1844. ***Amblyomma maculatum***, ***A. triste***, and ***A. tigrinum*** exhibit a striking morphological similarity, more obvious between the first two species. This fact has led to misleading identifications more than once in the past. As the distribution areas of the three species sometimes overlap, identifications can become even harder. The three species are vectors of pathogens of public health importance, such as ***Rickettsia parkeri***. Therefore, a correct identification is the first step towards establishing control and prevention strategies. The main objective of our work was to reassess the taxonomic status of ***A. maculatum, A. triste*** and ***A. tigrinum*** by using 6 different molecular markers. Tick specimens were obtained from different North, Central and South American countries. The molecular markers employed were the fast evolving 12SrDNA, 16SrDNA, D-loop, COI, COII (mitochondrial) and ITS2 (nuclear) genes. The phylogenetic analyses were consistent in identifying three different genetic clades. One of them, corresponding to ***A. tigrinum***, exhibits values of genetic divergence from the other species high enough to consider it a separate species, whereas preliminary phylogenetic results could be consistent with ***A. maculatum*** and ***A. triste*** being conspecific.

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HOUSE INFESTATION DYNAMICS OF *TRIATOMA DIMIDIATA* IN SAN LORENZO, ECUADOR

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Triatoma dimidiata is the main vector of Chagas disease in Ecuador. It is an invasive species widely distributed in house parameters along the tropical pacific coast. Despite the Ecuadorian Ministry of Health's goal to eliminate the vector by 2017, its vectorial capacity and infestation patterns remain to be elucidated. This study fills the gap by providing new knowledge in the extent of distributional patterns and infestation rates of ***T. dimidiata*** in a remote, high-risk community. Triatomines were collected during November 2013 and April 2014 in the rural community of 205 houses in San Lorenzo, Ecuador. Houses were noted as fully searched, partially searched, or closed and mapped using Picasa GPS to show insect distribution. Intra- and peri-domiciliary searches were performed in a two-man team for ***T. dimidiata.*** The data were used to calculate entomological indices including infestation index, density, crowding, and colonization index. Positive houses and those in the perimeter were sprayed with deltamethrin at 25 mg a.i./m^2. A total of 435 ***T. dimidiata*** (324 of which were nymphs) were collected in November in 28 houses, 27 of which had peri-domiciliary infestation. 388 (301 nymphs) specimens were found in April in 24 peri-domiciliary houses. Vectors were most frequently discovered in bird nests and construction materials stored outside of homes. Colonization indices of 89.9% and 100% respectively show vector reproduction was occurring in almost all homes. An infestation index of 15.6% and 21.4% respectively shows nearly a quarter of the community housed ***T. dimidiata***. Re-infestation was observed in 8 houses, possibly due to insufficient insecticide capacity. New infestation occurred in 5 houses, notably in those located next to closed homes. Our data suggest that ***T. dimidiata*** house infestation in San Lorenzo is not randomly distributed, primarily peri-domiciliary, well established and reproducing. The quantity of specimens collected emphasizes its importance as a Chagas vector and suggests that increased community surveillance and effective insecticides are needed to attain ***T. dimidiata*** elimination in Ecuador.

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SURVEILLANCE REPORTS AND RECOLONIZATION OF *TRIATOMA INFESTANS* FOLLOWING AN URBAN VECTOR CONTROL CAMPAIGN IN AREQUIPA, PERU

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In the city of Arequipa, Peru, a Chagas disease vector control campaign has been ongoing since 2003. After the attack phase of insecticide residual spraying, treated areas enter a surveillance phase, which mainly consists of resident reports of vector return to health posts. We previously analyzed homeowner reports received between 2009 and 2012 and developed multivariate models to identify risk factors, collected during the attack phase, Multivariate models provided the the surveillance phase of the campaign confirm that nonparticipation in the initial treatment phase is a major risk factor (odds ratio [OR] 21.5, 95% CI 3.35-138). Infestation during surveillance also increased over time (OR 1.55, 95% CI 1.15-2.09 per year). In addition, we observed a negative interaction between non- participation and time (OR 0.73, 95% CI 0.53-0.99), suggesting that recolonization by vectors progressively dilutes risk associated with nonparticipation. Here we use these models to identify high-risk households for survey, and test the sensitivity and specificity of the models to detect infestation in a new dataset, consisting of vector reports between 2012 and 2015. We then field-test the model by conducting additional active search for vectors in the city.

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A SURVEY OF TICK SPECIES *(ACARI: IXODIDAE)* AND SCREENING OF TICK-BORNE RICKETTSIAL PATHOGENS IN BELIZE, CENTRAL AMERICA

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Tick-borne rickettsial infections are emerging as an important public health concern in travel medicine. These pathogens are transmitted to humans and animals by the bite of ixodid ticks. There is a lack of data on rickettsial pathogens associated with ixodid tick species in Belize, Central America. This study was conducted to investigate the presence of ***Rickettsia*** species in ticks from three of the six districts of the country. Ticks were collected from domestic animals and by tick-drag sampling in 23 different villages in northern and western Belize in November 2014 and February 2015. A total of 1,966 ticks were collected and morphologically identified to species. They were then pooled, according to species and life stage, for DNA extraction and screening for ***Rickettsia*** by real-time PCR (qPCR) using genus-specific primers targeting the ***17kDa*** gene. Positive samples were tested by PCR using primers specific for spotted fever group (SFG) and typhus group (TG) ***Rickettsia*** followed by sequencing for confirmation. The majority of ticks collected were ***Amblyomma mixtum*** (previously known as ***Amblyomma cajennense***), ***A. maculatum***, ***A. ovale***, ***Dermacentor nitens*** and ***Rhipicephalus sanguineus***. Additionally, a small number of ***R. microplus***, ***Ixodes affinis*** and ***I. boliviensis*** were captured. Thus far, a pool of ***A. mixtum*** has been confirmed with spotted fever group ***Rickettsia*** infection. Data from this study will be used for mapping tick distribution as well as modeling the risk of rickettsial infections associated with ixodid tick species in Belize.

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THE ECOLOGY OF *WOLBACHIA* IN NATURAL MOSQUITO HOSTS

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Wolbachia are a group of endosymbiotic bacteria infecting 25-76% of arthropods. The newly developed strategy of combating mosquito-borne diseases by releasing Wolbachia-transinfected mosquitoes into disease-afflicted regions is based on the ability of Wolbachia to suppress many human pathogens in insects, including dengue fever virus and human malaria parasites, and to spread through insect populations by the mechanism of cytoplasmic incompatibility. While aspects of Wolbachia ecology have been studied in recently trans-infected mosquitoes and in several other arthropod hosts, little is known about the ecology of Wolbachia in natural mosquito hosts. In mosquito species such as container-breeder ***Aedes notoscriptus*** and salt marsh inhabitant ***Culex sitiens***, Wolbachia infection frequencies in the field range from 25-85% and 50-100%, respectively. Ecological factors such as the role of ovarian microbiota in maternal transmission and the effect of environmental conditions on larval Wolbachia titre may be responsible for the patchy distribution of Wolbachia infections in these species. The results of

experiments exploring these ecological phenomena in ***Ae. notoscriptus*** and ***Cx. sitiens*** will be presented. Findings may be relevant to the success of releasing Wolbachia transinfected mosquitoes for disease control.

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PROTEOMIC AND GENOMIC ANALYSIS OF *SARCOPTES SCABIEI*

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Scabies is a pruritic skin disease caused by the burrowing of the mite ***Sarcoptes scabiei.*** Symptoms mimic other skin diseases and thus it is difficult to diagnose. No reliable blood or molecular diagnostic test is available. The aim of this project was to identify scabies mite proteins, including those that may be useful in the development of a diagnostic test, using a combined proteomic and genomic approach. Scabies mite extract was separated by 2-dimensional electrophoresis and 844 Coomassie Blue stained protein spots were excised, subjected to trypsin digestion and analyzed by MALDI-TOF/TOF mass spectrometry (MS). In parallel, a draft genome of ***Sarcoptes scabiei*** var. ***canis*** was generated from paired end sequences using DeBruijn graph-based assembly methods. Assembled contigs covered 56.2 megabases with a contig N50 of 11.1 kb. The assembly was used to predict the ***S. scabiei*** proteome. Maker was used for structural annotations of 10-12,000 protein-coding genes. Roughly 70% of the predicted proteins could be assigned to an orthologous group, and were given natural language identifiers based on their homology to other proteins. The assembled genome and predicted proteome were then used to help deduce the origins of peptides identified by mass spectrometry. Deduced sequences that aligned to tryptic fragment sequences determined by MS were then searched by BLASTp vs. the NBCInr database (with taxonomy restricted to Acari) leading to the identification of > 150 proteins. Only 14 proteins hit to previously-identified scabies proteins with 12 yielding significant hits to dust mite homologs. Most other sequences (~100) aligned to proteins in other mites and ticks while the remainder possessed conserved protein domains. These data will now allow us to determine the identity of the proteins to which scabies patients produce antibodies, including those that may be good candidates for inclusion in a diagnostic test.

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HEAT TREATMENT TO CONTROL TRIATOMINE VECTORS OF CHAGAS DISEASE

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Currently, treatment to control triatomine vectors of ***Trypanosoma cruzi***, the causative parasite of Chagas disease, is mostly based on the use of insecticides through indoor residual spraying campaigns. These spraying campaigns are costly and expose a large number of people to chemicals. In the US and other countries, pests from the Hemiptera order, such as bedbugs, are controlled with heat treatment. Heat treatment has the advantages of preventing the development of insecticide resistance and being environmentally friendly. We first tested, under laboratory conditions, the effect of different temperatures on the survival and reproduction indexes of ***Triatoma infestans***, the most important vector of ***T. cruzi*** in the southern cone of South America. We found that ***T. infestans*** shows susceptibility to moderately high heat temperatures beginning at 48°C. We designed and implemented a transportable greenhouse chamber with low-cost and common materials. The chamber was big enough to contain household items such as mattresses, clothing, brick piles, etc. We placed ***T. infestans*** of different stages and eggs on top and within the household items. We field tested the chamber and found that on days with sufficient sun, it reaches temperatures that kill 100% of ***T. infestans*** and completely reduce the viability of triatomine eggs. The use of this chamber could complement the work of insecticide spraying campaigns in instances in which the use of insecticide would be cumbersome and much insecticide would be wasted (e.g. large piles of rocks, bricks, or other construction materials), reluctance of dwellers to accept insecticide spraying because of the presence of animals, or in the case of infestation of kissing bugs within mattresses or other items that would otherwise have to be destroyed or damaged to remove the insects.

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IDENTIFICATION, DIVERSITY AND DISTRIBUTION OF POTENTIAL SAND FLY VECTORS IN ENDEMIC AREAS OF LEISHMANIASIS AT THE PERU, BRAZIL, AND BOLIVIA TRI-BORDER REGION

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The Peru-Brazil-Bolivia tri-border region is a highly endemic area for leishmaniasis in the Amazon, yet information about the diversity and distribution of sand fly vectors is limited. Recent expansion of the New World visceral leishmaniasis vector, ***Lutzomyia longipalpis***, into non-endemic regions in Brazil and Bolivia could pose a serious risk to populations in Peru where neither the disease nor the vector are found. The goal of this study was to characterize the sand fly fauna and identify potential leishmaniasis vectors in two communities near the Peru-Brazil-Bolivia tri-border. Sand flies were collected in Flor de Acre and Villa Primavera (Tahuamanu, Madre de Dios, Peru) from February-September 2014, using CDC light traps, CDC UV traps, and Shannon traps. A total of 6,185 sand flies were identified to the genera ***Lutzomyia*** (49 species) and ***Brumptomyia*** (2 species). The most abundant species were ***Lu. yucumensis*** (32%), ***Lu. whitmani*** (19%), ***Lu. davisi*** (8%), and ***Lu. carrerai*** (4%); all reported as cutaneous leishmaniasis vectors in the Amazon. The subgenus ***Trichophoromyia*** was also abundant (17%), among which ***Lu. auraensis***, potential cutaneous leishmaniasis vector, was identified. ***Lutzomyia longipalpis*** was not recorded. Sand fly species number (36) was comparable between sites but species composition differed. Sand fly abundance was higher in Flor de Acre (5,561) than in Villa Primavera (624); the Shannon-Weaver diversity index (H) was lower in Flor de Acre (H=0.73) than in Villa Primavera (H=0.93). Potential sand fly vector abundance was higher in Flor de Acre (97%) than in Villa Primavera (75%), which could be linked to leishmaniasis transmission. We provide information about the diversity and abundance of putative cutaneous leishmaniasis vectors in the Peruvian side of the Peru-Brazil-Bolivia tri-border where ***Lu. longipalpis*** is still absent. Recent results in the Brazilian side confirmed the abundance of the subgenus ***Trichophoromyia*** among which ***Lu. aurensis*** was recorded; however, ***Lu. longipalpis*** was also absent. Future studies will determine ***Leishmania*** infection rates of these sand flies to predict disease transmission potential.

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INVESTIGATING THE ROLE OF TSETSE PGRP-LA IN THE FLY'S RESISTANCE TO TRYPANOSOMES

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Tsetse flies (***Glossina*** spp.) are the sole vectors of protozoan African trypanosomes, which cause Human and Animal African Trypanosomiases (HAT and AAT, respectively) in sub-Saharan Africa. While most tsetse flies are highly refractory to parasite infection, a small proportion of individuals are susceptible and thus responsible for disease transmission. Tsetse's

ability to immunologically detect trypanosomes following ingestion of an infectious blood meal is of paramount relevance to infection outcomes. The insect immune system relies on several Pattern Recognition Receptors (PRRs), among which the PeptidoGlycan Recognition Proteins (PGRPs) play a central role. PGRPs form a conserved family of proteins that function to sense Microbe Associated Molecular Patterns (MAMPs), trigger innate immune pathways, modulate immune responses, or present direct anti-microbial activity. The tsetse fly genome encodes six different PGRPs. Using real-time quantitative PCR we show that ***pgrp-la*** is significantly up-regulated in the gut associated proventriculus organ (cardia) of trypanosome-infected flies. RNAi-mediated knockdown of ***pgrp-la*** expression facilitates the establishment of parasite infections in tsetse. Thus, we suggest that the PGRP-LA may play a key role in parasite detection and the subsequent regulation of host immune responses. Unraveling the immune mechanisms that underlie tsetse detection of pathogenic trypanosomes may lead to the development novel disease control strategies based on enhancing the fly's ability to perceive and immunologically respond to the presence of parasites.

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EVIDENCE FOR POPULATION REPLACEMENT AND ECOLOGICAL ADAPTATION IN *ANOPHELES DARLINGI* FROM THE PERI-IQUITOS REGION OF PERU

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The Neotropical malaria vector, ***Anopheles darlingi***, was reintroduced into the Iquitos, Loreto, Peru area during the early 1990s, where it caused a major epidemic (158,115 reported cases in 1997) of ***Plasmodium vivax*** and ***P. falciparum***. We investigated the population genetic structure of ***An. darlingi*** sampled before and after the introduction of insecticide treated nets (ITNs) for evidence of population change, and tested current samples of ***An. darlingi*** for a signature of ecological adaptation to highway versus riverine habitat, linked to forest cover. Several analyses of microsatellite loci from seven settlements (2006) and nine settlements (2012-2014) in the Iquitos area detected distinctive populations with little overlap, although it is unclear whether this population replacement is associated with ITN distribution or climatic events. Interestingly, this current population of ***An. darlingi*** is most closely related to mosquitoes collected in northwestern Bolivia in 1991. Two highly admixed subpopulations, A and B, identified within the current population, were differentiated by habitat with B significantly overrepresented in highway, and both in near-equal proportions in riverine. There is strong evidence of population expansion in both subpopulations, and moderate genetic differentiation between them. Habitat and forest cover had a significant effect on human biting rate (HBR), such that risk of ***Plasmodium*** transmission, as measured by entomological inoculation rate (EIR), in peridomestic (within village) riverine settlements was three-fold higher than in peridomestic highway settlements. Subpopulations A and B may be in an early stage of differentiation triggered by anthropogenic alterations to local habitat.

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COMPARATIVE MICROBIOME OF *TRIATAMA INFESTANS*, VECTOR OF CHAGAS DISEASE

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The microbiota of the insect gut has been shown to be important for several vector-pathogen relationships; for example, the bacterium Wolbachia has profound effects on mosquito life span and fitness as a vector of dengue and other infections. Triatomine bugs are the vector of Chagas disease, a chronic parasitic infection caused by ***Trypanosoma cruzi*** that affects 8-12 million Latin Americans. We used deep sequencing to describe the hindgut microbiome of the most important vector of ***T. cruzi***, ***Triatoma infestans***. We compared the diversity and composition of the gut microbiome of 3 groups of triatomines: 1) lab-raised ***T. cruzi***-infected, 2) lab-raised ***T. cruzi***-uninfected, and 3) uninfected bugs caught in households in Arequipa, Peru. We conducted amplicon sequencing of the V4 region of bacterial 16S RNA using Ion Torrent. We analyzed 3-4 bugs per group, using 2-3 replicate PCRs per sampled bug. Diversity between individuals and groups was compared using MG-RAST, EstimateS, and QIITA. Rarefaction curves were approaching their asymptote suggesting most genera were likely detected in the majority of samples. The microbiomes of wild-caught bugs were more diverse than the microbiomes of either lab-raised group, though this was largely driven by samples from one fifth-stage nymph whose gut contained more than 300 species (twice that of the next most-diverse sample). ***Enterococcus*** and ***Arsenophonus*** were the predominant genera found in lab raised bugs, with the exception of one ***T. cruzi***-uninfected bug where ***Morganella*** predominated. In contrast, there was no one predominant genus identified in wild-caught bugs, and ***Enterococcus*** and Enterbacteriaciae were not substantial components. There were not large differences in α diversity between lab-raised ***T. cruzi***-infected and -uninfected bugs. Future studies should examine geographic differences in triatomine microbiome composition and diversity, microbiome changes with bug stage and infection status both in the lab and in the wild, and the effect of antibiotic-mediated disruption of the triatomine gut on susceptibility to infection by ***T. cruzi***.

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PHYLOGEOGRAPHY OF *TRIATOMA DIMIDIATA*, A MAJOR VECTOR OF CHAGAS DISEASE, IN BELIZE

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Triatoma dimidiata is the main triatomine vector of Chagas disease throughout Central America and southern Mexico. Throughout this broad distribution range, differences in vector behavior have been observed that could likely impact the vectorial capacity of local insect populations. Coupled with recent publications regarding the intraspecific genetic variability within ***T. dimidiata*** which have successfully distinguished five groupings within what is now designated ***T. dimidiata*** sensu lato, these observations support the need for additional research. Extensive investigation regarding the phylogeography of ***T. dimidiata*** s.l. has revealed broad patterns describing the divergence and genetic isolation of groupings with the species complex. Here, we characterize the genetic profiles of vectors collected from northern and central Belize, a region which has been strongly underrepresented in the relevant literature. The data presented here appear to lend support to previously reported trends in the divergent evolution and geographic radiation of the subgroupings

within ***T. dimidiata*** s.l. As the genetic profiles of these seemingly isolated populations are further defined, it is possible that concomitant behavioral attributes with implications for efficient vector control may be revealed.

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EVIDENCE OF GENE FLOW IN FEMALE OF *ANOPHELES GAMBIAE* S.S RESULTING OF MASS CROSSING OF *AN. COLUZZII* AND *AN. GAMBIAE* S.S GILES

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Hybridization between ***Anopheles coluzzii*** and ***An. gambiae*** Giles has been increasingly reported in sub Saharan African countries over the past decade. ***An. coluzzii*** (previously referred to as ***An. gambiae*** M molecular form) and ***An. gambiae*** s.s Giles (previously referred to as ***An. gambiae*** S molecular form) were considered to be reproductively isolated, yet hybrid specimens have been found in the field. This phenomenon was studied in laboratory by crossing ten virgin females and males of each form in separated small cages and allowed them to mate. The resulting progeny were analyzed using PCR methods to detect molecular forms. An average of 50 mosquitoes including males and females were analyzed per generation, giving approximately 1000 mosquitoes for the two crossing ways of the experiments and the five generations analyzed. The results showed 100% hybrid females at the first progeny (F1 generation) and 100% males carrying the parent female's phenotype. A decrease in the hybrid proportion was observed from the second generation which was due to the fact that the male were not hybrid (analysis of progenies is still going on). On the other hand, it has been noted a shift of the male forms, which were inversely changed following the female parent form in the two experiments at the first progeny stage. This study confirmed the ability of M and S molecular forms to hybridize. Further monitoring is required to understand the extent of hybridization in the field. A better understanding of the interaction between these two species is required, particularly in the context of differing resistance genotypes.

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LESSONS LEARNED, CHALLENGES AND PROSPECTS AFTER SIX YEARS OF EXPERIENCE IN THE IMPLEMENTATION OF INDOOR RESIDUAL SPRAYING IN BENIN, WEST AFRICA

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From 2008 to 2013, a prevention intervention against malaria based on Indoor Residual Spraying (IRS) supported by the President's Malaria Initiative (PMI) of the US Government was implemented in Benin. This intervention protected more than 350,000 people in the south and over 650,000 people in the north. From 2008 to 2012, Ficam M, a Bendiocarb-containing product was used for house spraying and in association with Pirimiphos methyl EC (Actellic EC) in 2013. Entomological Monitoring-Evaluation (M&E) is based on IRS impact on Human Biting Rate (HBR), Entomological Inoculation Rate (EIR) and blood meal inhibition in ***Anopheles gambiae***, the main malaria vector in the study area.The purpose of this project was to draw attention to the lessons learned during the M&E, new challenges and future prospects for the success of IRS in Benin and generally in Africa. The main strength of the intervention was a large-scale operation in which more than 80% of the structures were treated, thanks to the massive support of the population. In addition, a drastic reduction of the Entomological Inoculation Rate of ***An. gambiae*** in areas under IRS were observed in the first 4 months following the treatment of structures. However there were many challenges including the high cost of IRS implementation and the identification of suitable areas to implement IRS. This was because of the low short residual effect of the insecticides recommended for IRS and the difficulties to manage vector resistance to insecticides. These indicated challenges are accompanied by suggested solutions. For example, the presence of international NGOs supporting the implementation of IRS in Africa, particularly in Benin, should be limited in time to allow local organizations with the relevant skills in terms of IRS planning and implementation to take over. Such organizations must ensure a better partnership with the NMCPs. Concerning insecticide resistance management, we proposed various ways among which an alternation of IRS campaigns with LLINs distribution campaigns. IRS will then be implemented every 3 years. Between the three years, two years of extensive use of LLINs will be inserted.

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ARTIFICIAL COURTSHIP SONGS FOR CONTROL OF ADULT MOSQUITO POPULATIONS

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Traditional methods of controlling adult mosquito populations involve the use of insecticides in form of residual sprays or embedment of the drugs in bed nets. These methods apart from causing contamination of environments and posing possible direct effects on human health; they are now faced with the threat of being rendered ineffective as a result of the development of insecticide resistance. Dispensing of insecticides in the form of residual sprays or insecticide-treated bed nets implies individual efforts at each specific household which may be impossible especially for poor or unwilling individuals. Moreover, these traditional methods are applicable only inside houses while not effectively preventing malaria transmission taking place outdoors. There is therefore a need to device new ways which are safer, insecticide-resistance-proof, while at the same time offering communal protection to individuals staying both indoors and outdoors. Since courtship songs are a crucial event leading to mating and consequently reproduction in mosquitoes, targeting of mosquito populations during this event by interrupting the mating process may lead to collapse of mosquito populations within as a large area as a village. Based on the knowledge of particular mating-songs frequencies and their patterns, we have developed artificial courtship songs which imitate mosquito natural mating songs with the hope that when these songs are played from a stationed sound transmitter will disrupt the swarming events in mosquitoes at a given radius thus leading to unsuccessful mating and thus collapse of local mosquito populations with time. Initial semi-field experiments have been able to show collapse of a caged population of mosquitoes when treated with certain sound frequencies similar to those produced during mating events, while the control cage population without treatment continued to propagate. Further research is needed to develop high capacity sound delivery systems that may be applied across a wider range to cover the size of typical village to enable field intervention programs targeting to control adult mosquito populations.

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INSECTICIDE RESISTANCE MUTATIONS MODULATE *ANOPHELES GAMBIAE* HOST SEEKING BEHAVIOR

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Major means of malaria vector control are based on use of insecticides. Their efficiency is threatened by widespread resistance mechanisms. In addition to the physiological resistance mechanisms already well studied, the issue of the behavioral modulation as cause or consequence of the resistance is largely overlooked. Nevertheless there are evidences that insecticide-based control tools alter mosquito behavior before any contact, suggesting that the mosquitoes can detect the presence of the insecticide. In the present study, we tested this hypothesis by investigating the behavioral responses of different resistant genotypes (differing by presence of L1014F (***Kdr***) mutation and ***Ace-1***) of ***Anopheles gambiae*** to host odors and insecticide treated equipment. Behavior experiments involving ***kdr***-carrier mosquitoes, showed that heterozygous were more actives than two other genotypes. Moreover, homozygous resistant preferred host behind the permethrin treated net than host behind untreated net.

For ***Ace-1***-carrier mosquitoes, results showed that mutation and the duplication of the resistance gene impact negatively the spontaneous activity of mosquitoes and their perception of host odors. Nevertheless, duplication seems to decrease this negative effect. We did not evidencing any significant effect of insecticide on host choice for these mosquitoes. Our results confirm the interaction between insecticide resistance mutations and behavior. Moreover, ***Kdr*** resistant mosquitoes can perceive insecticide on net and adapt their behavior in response of it. Our original study highlighted the urgent need for further investigations of chemical ecology of malaria vector in a vector control pressure context.

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A LOW COST DEVICE FOR CONTROLLING OUTDOOR HOST SEEKING MOSQUITOES

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Residual transmission of malaria is maintained by mosquito vectors biting at dusk and dawn outside houses. Outdoor baited traps have been promising,yet,difficult to implement in poor resource areas because of the expensive source of Carbon dioxide (CO_2), delivery of synthetic attractants like CO_2 and power source. This study aims to design a passive outdoor host seeking device (OHD) and assessing the efficacy of OHD when incorporated with attractive synthetic blends, non-repellent bioactives (e.g. bendiocarb) and natural CO_2 to attract and kill malaria vectors outside houses. Experiments were conducted to assess efficacy of OHD using rectangular chamber (2.06x1.50x 1.47m) inside semi field system at Ifakara Health Institute in Tanzania. The device was either treated or untreated during experiments. The OHD device was hanged inside and outside rectangular chamber. The installation of the device outside the chamber, involved the use of a fan to suck out natural CO_2 from human volunteer sleeping inside the chamber. Group of 100 female ***Anopheles arabiensis*** were released outside the chamber and left to forage overnight. Next morning mosquitoes were recaptured and identified as either dead or alive. The live mosquitoes were held in the insectary to record 24 hours mortality rates. The proportion of dead mosquitoes was compared between treated and untreated. Each experiment, treated or untreated was replicated three times.When OHD was hanged in the center of the chamber with synthetic attractants, the treated OHD improved its killing effect than untreated device. The percentage of attracted and killed mosquitoes were 18% for the worn socks, 36% for mbita strips and 33% for Ifakara strips. When the source of natural CO_2 was added into OHD, the mortality rates were 65% for worn socks, 65% for Mbita strips and 51% for Ifakara strips treated device. Therefore,the source of natural CO_2 sucked by the fan improved the attractiveness of OHD 2-3 times than with no CO_2. Further studies are ongoing to test the OHD installed outside the chamber with no fan, hanged near bed net inside houses, hanged outside houses at the eave level and near cow sheds.

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PROFILING INSECTICIDE RESISTANCE AND OUTDOOR MALARIA TRANSMISSION IN ETHIOPIA: IMPLICATIONS FOR SUSTAINING CONTROL

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Abstract Indoor Residual Spraying (IRS) and long-lasting insecticidal nets (LLINs) are key components in malaria prevention and control strategy in Ethiopia. However, the development of resistance by vectors to insecticides recommended for IRS and/or LLINs could affect insecticide-based malaria vector control. We assessed the susceptibility levels of Anopheles arabiensis to insecticides used in malaria control, characterize basic mechanisms underlying resistance and their biting activity from southwestern Ethiopia. Susceptibility status of An. arabiensis was assessed using WHO bioassay tests against insecticides used in public health. Mosquito were screened for knockdown resistance (kdr) and insensitive acetylcholinesterase (ace-1R) mutations using AS-PCR and PCR-RFLP, respectively. Populations of An. arabiensis from the study site were highly resistant to DDT, permethrin, deltamethrin and malathion. However, the mosquito populations were susceptible to bendiocarb, propoxur and Pirimiphos methyl.The West African kdr allele was found with a frequency ranged from 95% to 100%. Ace-1R mutation was not detected. Baseline levels of metabolic resistance were assessed in population of An. arabiensis for esterases, mixed function oxidases (MFO), glutathione s-transferase (GST) and insensitive acetylcholinesterase (ACHE). The results of the biochemical assays showed that there were highly elevated activities of esterases and MFO in the mosquito population. However, elevated activities of glutathione s-transferase and insensitive acetylcholinesterase were not observed. Populations of An. arabiensis showed both endophagic and exophagic behavior with peak biting activity from 19:00h to 22:00h. The observed multiple-resistance coupled with outdoor and early biting behaviour in populations of An. arabiensis could profoundly affect malaria vector control programme in Ethiopia. This needs an urgent call for implementing integrated vector control intervention, rational resistance management strategy and looking for new alternative vector control tools.

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PLASMODIUM FALCIPARUM MULTIPLICITY OF INFECTION PRE- AND POST-VECTOR CONTROL CAMPAIGNS IN NCHELENGE DISTRICT, ZAMBIA

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Plasmodium falciparum malaria is holoendemic in Nchelenge District, Zambia where the primary vectors are ***Anopheles gambiae*** s.s. and Anopheles funestus s.s.. In Nchelenge District, indoor residual spray (IRS) and long-lasting insecticide net (LLIN) campaigns were conducted to reduce malaria transmission. In other settings, decreased malaria transmission as a result of LLIN/ITN use can lead to changes in the genetic diversity of ***P. falciparum***. It is therefore critical to monitor the effect of the recent vector control interventions in Nchelenge District, focusing on multiplicity of infection (MOI)_defined as the number of genetically distinct ***P. falciparum*** clones present in a given infection. A pre-IRS analysis of three parasite gene loci from Anopheles mosquitoes indicated that 93.9% of mosquitoes harbored polyclonal infections with an average complexity of infection of 6.4 unique clones. Preliminary data from human dried blood spot (DBS) samples suggests the MOI in humans is lower. This comparative analysis is limited, however, by the low number of genetic loci analyzed, as well as the fact that whole mosquito samples must be considered both diploid and haploid while human samples are only haploid. Following these preliminary results, we will use a SNP-based barcoding assay which characterizes 24 parasite loci to analyze DBS samples from study participants as well as salivary gland samples containing haploid parasite from mosquitoes. By comparing the MOI between vector and host as well as pre- and post-IRS, we can monitor the effect of vector control strategies on parasite genetic diversity. We hypothesize that decreased malaria transmission due to vector control strategies will lower the MOI in both vector and human samples with possible implications for acquired immunity, likelihood of disease severity, and rate of drug resistance development.

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EFFICACY AND PERSISTENCE OF PIRIMIPHOS-METHYL (ACTELLIC 300CS) FOR INDOOR RESIDUAL SPRAYING IN ZANZIBAR

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Indoor Residual Spraying (IRS) is a principal vector control intervention for malaria control in Zanzibar. In 2006, Zanzibar Malaria Control Programme introduced IRS with lambda-cyhalothrine (ICON 10WP/CS). Following detection of pyrethroid resistance in 2010, insecticide resistance mitigation plan was proposed and IRS with Bendiocarb started in 2011. As a resistance management strategy, Actellic 300CS replaced the Bendiocarb from 2014. The study investigated residual efficacy of Actellic 300CS sprayed on common surfaces of human dwellings in Zanzibar. Bioefficacy tests aimed to determine mortality of female Anopheles mosquitoes exposed to sprayed surfaces and identify onset of specific decline in toxic effect of Actellic 300CS deposits applied to different surfaces. Six houses with different wall surfaces (mud wall, oil and water painted walls, lime washed wall, un-plastered cement block wall and un-plastered stone blocks) were sampled from each district of Zanzibar. Actellic 300CS was sprayed on surfaces at a dose of one gram of active ingredient/m2. Ten susceptible females ***Anopheles gambiae*** s.s (age range 2-5 days old) were introduced through a sucking tube into a cone exposed on sprayed surfaces. Subsequent tests were undertaken on monthly basis using the World Health Organization (WHO) guideline. Insecticide resistance testing was also undertaken to investigate susceptibility of local malaria vectors against Actellic 300CS using WHO protocols. Twenty five unfed females ***Anopheles gambiae*** s.l (age range 2-5 days) were introduced into a tube containing Actellic impregnated paper (0.25%) for one hour and kept in holding tube with 10% sugar solution. Mortality was counted at the end of 24hrs holding period. Baseline tests conducted one day post-spraying revealed 100% mortality on all sprayed surfaces. Bioassay tests conducted over 214 days showed 100% 24 hours mortality on all sprayed surfaces. Results of resistance tests showed that malaria vectors in Zanzibar are 100% susceptible to Actellic 300CS. Based on the findings collected through bioassay testing, Actellic 300CS is highly effective and appropriate for IRS in Zanzibar.

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DOES HETEROGENEITY IN INSECTICIDE RESISTANCE CONTRIBUTE TO MALARIA HOTSPOTS IN THE GAMBIA?

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Malaria transmission hotspots consistently have higher than average transmission intensity and are predicted to become increasingly common as malaria continues to decline. Little is known about the role of insecticide resistance in maintaining hotspots. The status of insecticide resistance was investigated in vector populations from six local pairs of villages from across The Gambia, comprising of a high and low malaria sero-prevalence village within each pair. Larvae and blood fed ***Anopheles gambiae*** s.l. were collected from each village to generate adults for use in World Health Organization insecticide bioassay tests. Of 1047 mosquitoes asssayed, 23.5% were An. arabiensis, 31.2% ***An. gambiae***, 43.3% ***An. coluzzii***, 2.04% were hybrids of ***An. coluzzii*** × ***An. gambiae***. In 3 village pairs, species population and composition varied significantly between high and low transmission villages. Resistance to DDT and deltamethrin was heterogeneous within and among species, but most prevalent in ***An. gambiae*** s.s. from eastern Gambia. Resistance was strongly associated with the target site (kdr) mutation L1014F (DDT, OR=256.7, (95% CI 48.6 - 6374.3, p<0.001) and deltamethrin, OR= 9.14, (95% CI 4.2 - 21.4, p<0.001). A metabolic resistance mutation, Gste2-114T in ***An. gambiae*** s.s. also conferred significant resistance to both DDT (OR=3.4, 95% CI 1.4 - 9.2, p = 0.006) and deltamethrin, (OR= 3.4, 95% CI 1.2 - 10.3, p=0.024. Resistance to DDT was more likely to be found in villages with high malaria sero-prevalence, (Wilcoxon test, p=0.025) but this was not the case for deltamethrin, p= 0.238). Whilst causality of relationships requires further investigation, variation in vector species and insecticide resistance is associated with malaria sero-prevalence setting in The Gambia. Our results suggest that in areas with heterogeneous malaria transmission, the role of the vector should be investigated to guide malaria control interventions.

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BREEDING CONDITIONS INFLUENCE SUSCEPTIBILITY TO INSECTICIDES IN MOSQUITOES

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As insecticide resistance increasingly threatens malaria control programs, it is very important to understand the processes and factors that interact to produce observed phenotypes. The contribution of the environment and breeding conditions to the susceptibility of the mosquito has been largely ignored. In this study, we evaluate how temperature, population density (crowding) and nutrition during the larval stage interact to influence the susceptibility of the adult mosquito to public health insecticides. Larvae of ***Anopheles gambiae*** (KISUMU) and ***Anopheles stephensi*** were bred under different combinations of temperature, population density and nutrition using a factorial experimental design. Emerging adults were tested against the lethal concentration of permethrin that would kill 50% of the mosquito population under standard rearing conditions in the World Health Organization insecticide susceptibility tests. . As a secondary endpoint to mortality, mosquito body weight was measured and included in the data analysis. Additional experiments explored the relationship between immediate knock down and 24 hours mortality, as these endpoints are often used interchangeably. Mosquitoes bred under different conditions showed significant differences in body sizes and mortality. Dry weight was strongly related to mortality (OR = 0.0000992, p < 0.001) in both experiments but was not significantly associated with time-to-knockdown (coeff -6.70; P = 0.176). In conclusion, the breeding conditions of mosquito larvae have a significant impact on the dry weight as well as susceptibility status of the adult mosquito. It is therefore important to incorporate the size of the mosquito when studying insecticide susceptibility in mosquitoes

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AGRICULTURAL PRACTICES SUSCEPTIBLE TO TRIGGER THE DEVELOPMENT OF INSECTICIDE RESISTANCE IN MALARIA VECTORS

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Vector control is a main component of all malaria control strategies. Unfortunately the effectiveness of vector control is more and more affected by the increasing phenomenon of vectors resistance to insecticides. The different approaches proposed by the Global Plan for Insecticide Resistance Management (GPIRM) to overcome this situation of resistance assumes that the vector control itself is the main source of resistance; whereas the agricultural surfaces constituted sometimes by gigantic mosquito breeding sites polluted with pesticides could exert a

resistance selection pressure on mosquito larvae. The present study has been carried out in the rice perimeters of the locality of Tiassale located in the south of the Cote d'Ivoire to highlight farming practices that could trigger the development of resistance to insecticides. We have investigated the management of different pesticides used against crop'pests, for soil fertilization, or weed. The questionnaire covered among others, the procurement of products, the doses of application, the frequencies of treatment, and all the hygiene rules relating to the use or storage of products. We have also determined the residues of various pesticides in the mosquito breeding sites located within the farms. The results of this study are in the process of analysis and will be presented during the scientific exchanges.

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SAVE MOSQUITOES, SAVE MONEY: A RESAMPLING ANALYSIS TO DETERMINE HOW MANY MOSQUITOES ARE NEEDED TO TEST A LONG-LASTING INSECTICIDAL NET

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The reference method for testing the insecticide activity of Long-Lasting Insecticidal Nets (LLIN) distributed in the field needs a hundred live female mosquitoes per LLIN. To test 100 nets, one needs an entomological facility capable to produce 10,000 two days-old females plus the mosquitoes needed for maintenance of female breeding. If one could reduce the number of mosquitoes needed to test the effectiveness of LLIN, the human and animal resources, costs, and the duration of nets evaluation would be equally reduced, enhancing the ability of entomology labs to evaluate the effectiveness of LLIN. The WHOPES protocol proposes to tests the insecticide bio-efficacy of LLIN by cutting equal and predetermined positions' areas in each of the 5 sides of the net with 4 cones in which 5 mosquitoes are introduced. A LLIN is considered as valid if mortality after 24 hours is ≥80% or if Knock-Down rate (KD) after 60 minutes is ≥95%. We resampled a database of 200 LLIN collected from the population in Madagascar and tested appropriately, of which 41.1% were considered as valid. Each random resampling was performed 10,000 times. Receiver Operating Characteristic (ROC) curves for 1, 2, and 3 cones showed excellent performances of the mortality criterion while KD demonstrated a low reproducibility. Using 2 cones instead of 4, and considering mortality only, had 99.0% sensitivity and 98.2% specificity. The average error in the measured proportion of valid LLIN was 0.8%. The 95% confidence intervals (CI) of sensitivity and specificity narrowed while the sample size increased, and the 95% CI of the difference between 2-cones-testing and 4-cones-testing proportions of valid LLIN didn't exceed 5% when the sample was ≥40 LLIN. As a conclusion, testing the bio-efficacy of LLIN with twice less mosquitoes provides a fair evaluation of the proportion of LLIN valid when considering mosquitoes' mortality only, and a sufficient sample size (e.g. ≥40 LLIN). We propose to focus on mortality in the evaluation of the bio-efficacy of LLIN. This protocol will help entomology labs to double their capacity in testing the effectiveness of LLIN or divide its cost by two.

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FINE-SCALE PATTERNS OF PYRETHROID RESISTANCE IN *AEDES AEGYPI* FROM YUCATAN, MEXICO

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As observed with other medically important arthropods, the strong reliance on pyrethroid insecticides to control ***Aedes aegypti***, the principle vector of dengue and chikungunya viruses, has led to the evolution of insecticide resistance. The "knock-down resistance" (***kdr***) mechanism arises from point mutations on the voltage-gated sodium channel gene, and it confers resistance to pyrethroids in ***Ae. aegypti***. Understanding the dynamics of resistance at a fine scale within urban environments is key to both managing resistance and maintaining vector control efficacy. In this study, we analyzed the within-city distribution of ***kdr*** alleles in ***Ae. aegypti*** populations in time and space given heterogeneous selection pressures. During two consecutive years, 2013-2014, we collected 2,227 adult mosquitoes from inside 580 homes in four towns of Yucatan, Mexico. In each town, we sampled 5 blocks, with the exception of one town in which we sampled 24 blocks to better understand fine-scale dynamics. For each mosquito, we used PCR to detect the V1016I and F1534C ***kdr*** mutations. Additionally, we conducted CDC bottle bioassays to characterize phenotypic resistance to pyrethroids. Frequencies of the resistant alleles in 2013 ranged from 0.47 to 0.74 for 1016I and from 0.59 to 0.96 for 1534C. Intensive sampling of one small town, about 16 square kilometers, showed that ***kdr*** frequencies are highly heterogeneous between blocks, ranging from 0.18 to 0.64 for 1016I and from 0.36 to 0.73 for 1534C mutation. Spatial analyses showed a statistically significant difference from homogeneity in the allele frequencies, indicating an absence of spatial clustering (Weighted K function, $p<0.05$). High variability in the frequency of pyrethroid application suggests that heterogeneous, sporadic insecticide applications could be contributing to the observed differences in resistance patterns observed at a fine scale. Understanding the scale at which resistance arises and can be maintained in ***Ae. aegypti*** can aid in developing novel intervention strategies that exploit the fitness cost of the resistance alleles in sub-populations that are not heavily controlled with insecticide.

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STEROL CARRIER PROTEIN, SCP2, IS CRITICAL FOR *PLASMODIUM* TO ESTABLISH INFECTION IN *ANOPHELES STEPHENSI*

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Mosquitoes are the vectors of multiple diseases which account for over 700 million deaths annually global wide. Little is known about lipid metabolic interactions between mosquitoes and parasites. Lipids are essential components of cell membranes and have key roles in different signaling pathways. We found that malaria parasites (***Plasmodium berghei***) infection resulted in significant alterations in metabolic profiling in ***Anopheles stephensi***. Sterol carrier protein (AsteSCP2), a soluble protein that facilitates the uptake of lipids in mosquitoes, is responsible for promoting parasites invasion. Silencing SCP2 impaired the ability of ***Plasmodium*** to establish infection in mosquitoes. In addition, AseSCP2 helps to maintain homeostasis of microbiota. Knocking down SCP2 led to significantly reduction of total bacteria number in comparison to dsGFP controls. Thus, SCP2 plays a vital role in controlling both ***Plasmodium***

infection and microbiota proliferation. Further experiments need to be done to investigate mechanisms of influence of SCP2 on parasites infection and microbiota homeostasis.

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LAND USE, AN ENVIRONMENTAL RISK FACTOR FOR A VERY HIGH MALARIA TRANSMISSION

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The goal of the study is to investigate if local agricultural practices have an impact on malaria transmission in four villages located in the same geographical area within a radius of 15 kilometers in southern Benin. Among the villages, one (Itassoumba) is characterized by the presence of a large fish farming area on which several fish ponds are dug. The three others (Itakpako, Djohounkollé and Ko-Koumolou) are characterized by traditional food-producing agriculture. Human biting rate (HBR) was evaluated using human-landing catches, two nights per month from july 2011 to june 2012. Collected mosquitoes were identified morphologically. Species molecular identification was also performed using PCR. Female ***Anopheles*** mosquitoes were tested for the presence of ***Plasmodium falciparum*** antigen using ELISA technique in order to determine the sporozoitic index [S]. The entomological inoculation rate (EIR) was also calculated (EIR = HBR x [S]). ***An. coluzii*** (93.7%) was identified as the main malaria vector. The EIR ranged from 9.7 to 21.7 infected bites of ***An. gambiae*** per human per year in Djohounkollé, Itakpako and Ko-Koumolou against 1159.7 in Itassoumba ($p<0.0001$). The heterogeneous character of malaria epidemiology was confirmed. Land use through fish ponds creation contributed to the development of suitable and permanent breeding sites for ***Anopheles*** mosquitoes. That led to a drastically high malaria transmission in Itassoumba. We recommend that the human dwellings be located far from these fish farming activities so that the populations can avoid to be exposed to the high rate of infected bites. It is also important to target the exact areas where high transmission is persisting such as Itassoumba so that the control operations can be more prioritized and focused in these areas.

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EFFECTS OF NEUTRALIZING ANTIBODIES AND HUMAN COMPLEMENT PROTEINS ON DENV INFECTION LEVELS IN *AEDES AEGYPTI*

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Dengue virus (DENV) causes the most common vector-borne viral disease in humans living in the tropics. While secondary infection with DENV is frequently associated with severe disease, the majority of DENV infections are mild or asymptomatic. Protection against DENV infection may depend on neutralization capacity of antibodies (ab) in serum. Transmission of DENV occurs when a mosquito takes a blood meal from a DENV infected host. The blood meal of a mosquito consists of host cells, fluids and immune factors. Previous studies have shown that immune factors may remain active in the arthropod midgut and retain the ability to interact with pathogens and affect their viability several hours after ingestion. Antibodies transferred from the host may then also block pathogen infectivity in the vector. So far, no studies have evaluated the effect of neutralizing antibody titers and complement activity in human blood on DENV infectivity of ***Aedes aegypti***. Thus, we decided to evaluate these effects by experimentally infecting mosquitoes with both field-collected and laboratory strains of DENV2 in mixture with human serum samples. Serum contained varying titers of neutralizing antibodies against serotype-specific DENV (Exp), and we also included control sera (Ctl) with no previous history of DENV exposure. Serum was either inactivated (IA) or non-inactivated (NIA) serum at the time of the feeding. Using quantitative Real-Time-PCR, we found no significant difference in relative viral RNA quantity between mosquitos fed with Exp or Ctl serum 1h after blood meal, although mosquitoes receiving inactivated serum form both groups had higher relative viral RNA quantity than those that ingested blood with non-inactivated sera. However, at 3h post feeding, mosquitoes receiving Exp sera had significantly higher virus concentration than those fed with Ctl sera. Our findings indicate that heat inactivation of human serum increases DENV infectivity in mosquitoes and that the presence of anti-DENV antibodies in blood potentially play an important role in viral disease transmission to mosquitoes.

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DIFFERENTIAL EXPRESSION IN DENGUE-INFECTED *AEDES ALBOPICTUS* REVEALS GENES IMPORTANT FOR ANTIVIRAL RESPONSE

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The Asian tiger mosquito, ***Aedes albopictus*** is an important vector of dengue virus, which is responsible for recent epidemics in urban temperate and subtropical regions. Its ability to inhabit colder zones than the major epidemic vector, ***Ae. aegypti*** poses risks to expand the epidemic or endemic areas. We investigated transcriptomes of dengue-infected and -uninfected ***Ae. albopictus*** using Illumina sequencing technology. This study reveals how mosquito gene expression is modulated in early time points following dengue infection.

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CHANGES IN MALARIA VECTOR DYNAMICS POST-IRS IN NCHELENGE DISTRICT, ZAMBIA

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Nchelenge District of northern Zambia, lying along Lake Mweru and sharing a border with the Democratic Republic of the Congo, experiences high transmission of malaria despite almost a decade of malaria control interventions, including implementation of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). From October to November in 2014, an IRS campaign using the organophosphate pirimiphos-methyl as the residual insecticide was implemented in Nchelenge, targeted mainly to households lying along Lake Mweru. In association with the Southern Africa International Centers for Excellence in Malaria Research (ICEMR) project, Centers for Disease Control light-trap (CDC LT) collections have been ongoing in Nchelenge for several years at households throughout the study site, both in IRS-targeted and IRS-negative homes. These collections were compared to evaluate possible differences in mosquito abundance and foraging behaviors that may have resulted from vector control. In addition, pyrethroid spray catch (PSC) and barrier screen collections were conducted to assess resting behaviors of vector mosquitoes in Nchelenge in IRS and non-IRS zones. The data resulting from these studies will increase our understanding of malaria vector dynamics and transmission in highly endemic regions, with implications for future vector control.

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EVALUATION OF INTERVENTIONS AIMING AT INTERRUPTING MALARIA TRANSMISSION IN BAGAMOYO, TANZANIA

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The persistence malaria transmission despite of well-planned vector control programs, early diagnosis and treatment with artemisinin combination therapy (ACT) in many settings, threat the available control measure. In 2012, World Health Organization, estimated that there are about 207 million cases of malaria and 627, 000 deaths are related to malaria. 90% of these deaths occur in sub-Saharan Africa. In responding to these challenges, the Malaria Eradication Research Agenda (malERA) initiative was conceive as a rigorous scientific consultative process to identify knowledge gaps and new tools that will be needed to eliminate and eradicate malaria globally. The malERA pointed out the need to include transmission-blocking interventions to interrupt malaria transmission by targeting the infectious gametocytes carriers that are responsible to maintain malaria transmission. In response to the recommendation, Ifakara Health Institute (IHI) has established a level 3 insectary laboratory and Phase I Clinical Trial Facility to be used to evaluate different interventions such as vaccines and drugs aiming at interrupting malaria transmission both at individual and community levels. Some interventions have already been evaluated using these platforms. Recently, we have evaluated whether, ARCO and EURARTESIM have the potential to clear post treatment gametocytes reservoir. This study involved adult aged 18 and above with uncomplicated malaria. Participants were assigned to either of the interventions and admitted at the facility for three (3) days to monitor treatment then discharged home. On day seven post treatment, direct skin feeding using blood naïve lab-reared sterile mosquitoes was done. Analysis of midgut by PCR is going on at Nijmegen, Netherland and results will be available soon hoping to present them during the coming ASTMH meeting.

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SPATIO-TEMPORAL DISTRIBUTION AND ABUNDANCE OF IMMATURE STAGES OF *AEDES AEGYPTI* ON THE SOUTHERN COAST OF KENYA

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In endemic areas, ***Aedes aegypti***, the principal vector of dengue and chikungunya viruses breeds in a variety of container habitats both indoors and outdoors. Understanding of the vector ecology is essential for effective vector control. In Kenya, where dengue and chikungunya are prevalent, little is known about larval ecology of the vector. As part of a larger study, all indoor and outdoor water- holding containers that might harbor ***A. aegypti*** larvae and pupae were examined monthly from March to October 2014 in 20 selected houses from a Msambweni (rural site) and Ukunda (Urban site). Of 1928 containers inspected in Msambweni (1194) and Ukunda (734), 3.7% and 10.6% were positive for ***A. aegypti***, respectively. Seven out of the 16 container habitat types were commonly found harboring ***A. aegypti*** immature stages - animal watering containers, water drums, water tanks, buckets, Jerry-cans, tires and food tins. In the rural site, , the most persistent container habitat types were buckets and water tanks, while in the urban site, the animal watering containers, Jerry cans and tires were most persistent. Of 9,269 larvae and 919 pupae of mosquitoes collected, 83% and 78% of the collected larvae and pupae, respectively, were ***A. aegypti***. House, Container and Breeding indices were always higher in the urban site (46%, 10% and 2.1, respectively) in comparison to the rural site (33%, 4% and 0.7, respectively, $p<0.0001$), and outdoors compared to indoors ($p<0.0001$). In both sites, all indices were high in May, June and July, after rainy periods, and lowest in March and October, after dry periods, suggesting a lagged correlation with prior rainfall. In conclusion, key container habitats were identified in the two study sites and ongoing entomological surveys will help determine the most productive habitats. Targeting productive container habitats for dengue and chikungunya vectors will make vector control efforts affordable and feasible in the study area.

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BEHAVIOR OF *ANOPHELES DARLINGI* IN THREE COMMUNITIES IN THE PERI-IQUITOS REGION OF AMAZONIAN PERU

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Malaria transmission in the peri-Iquitos region of Amazonian Peru has been designated as seasonal and hypoendemic with recently described hyperendemic hotspots. Despite relatively recent distribution of LLINs, before the start of the study, malaria in Amazonian Peru persists and increased substantially in 2014 compared to previous years. ***Anopheles darlingi***, the main malaria vector, is known for its variable behavior depending on locality and environment. To evaluate vector biology metrics in relation to seasonality and malaria transmission, mosquito collections were carried out in three localities (Lupuna, Cahuide and Villa Buen Pastor) in the peri-Iquitos region, Loreto, Peru in 2011-2012. HLC, SHA and CDC trap types were compared for effectiveness in a Neotropical setting. Abundance, human biting rate, and EIRs were measured to provide an updated view of transmission patterns post-LLINs distribution. HLC collected significantly more anopheline mosquitoes than Shannon traps and CDC light traps. ***An. darlingi*** was the most prevalent species in all three villages (84% overall). Biting patterns varied depending on trap type, season and village. EIRs varied temporally and spatially and the highest (2.52) occurred during the 2012 malaria outbreak in Cahuide. Unexpectedly we found high Infection rate 1.47 (57 mosquitoes analyzed) and 1.75 (52 mosquitoes) outside the normal malaria transmission season, coincident with a second local outbreak in CAH. Our data underscore the importance of HLC as the most meaningful collection method for measuring vector biology indices in this amazon region. Our study clearly demonstrated microgeographic differences in Anopheles. darlingi peak biting times, biting patterns, infectivity and EIR. The trend of an increase in outdoor biting together with early evening infected mosquitoes may undermine the effectiveness of LLINs as a primary malaria intervention. Anopheles. darlingi was the most abundant species and the only one infected with ***Plasmodium***, confirming its importance as the major malaria vector in the area. HLC is still the most effective trap for ***An. darlingi*** in this region.

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SEMINAL INFLUENCES: THE ROLE OF MALE TRANSFERRED 20E IN *ANOPHELES GAMBIAE* REPRODUCTIVE FITNESS

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Reducing the burden of malaria induced mortality and morbidity via targeting of the mosquito vector requires an increased investment into understanding the reproductive ecology of Anopheles mosquitoes. With increasing levels of insecticide resistance threatening the efficacy of existing vector control strategies, the induction of sexual sterility in natural vector populations is an attractive alternative. However, a lack of knowledge regarding many of the basic elements of ***Anopheles*** mating hampers development of these strategies. Recently, our group has demonstrated that the suite of mating induced physiological and behavioral changes in ***An. gambiae*** females is largely mediated by the receipt of the steroid hormone 20-hydroxyecdysone (20E) as part of the male mating plug. We have also shown that females choose to mate with males that transfer higher levels of male 20E during mating. Here, we demonstrate through GC/MS analysis that males whose mating attempts are accepted and rejected exhibit different chemical contact cue profiles, a sensory modality that females can use for mate discrimination. Furthermore, behavioral assays reveal that a transgenic line of ***An. gambiae*** males deficient in 20E synthesis suffers a cost in terms of their mating competiveness relative to a control line, further underscoring the role of male synthesized 20E in mechanisms of pre-copulatory mate choice. Finally, we also analyze whether male 20E levels are heritable, and reveal that 20E transfer provides females with both direct and indirect benefits. Taken together, these results provide compelling evidence that 20E is a key factor determining reproductive fitness for both sexes across sequential episodes of sexual selection. This work provides critical insights into the mating ecology of a major disease vector while extending our understanding of mating system dynamics in both swarming and monandrous insect species.

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ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES INFECT DIVERSE VECTORS OF SOUTHEAST ASIA AND AFRICA

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Artemisinin-resistant ***Plasmodium falciparum*** parasites are rapidly spreading in Southeast Asia, yet very little is known about their transmission. This knowledge gap, and the possibility of their future spread to sub-Saharan Africa, endangers global efforts to control malaria. Studies on the population genetic structure of ***Plasmodium falciparum*** isolates from Cambodia revealed drug-resistant parasites that fell into highly structured groups, as distinct from each other as from African parasite isolates. The discovery of Kelch13-propeller polymorphism, a new marker for artemisinin resistance, helped to further resolve these parasite populations. To investigate the transmission dynamics of these parasites, we performed membrane feeding assays with Cambodian clinical isolates from several distinct parasite populations recently shown to be artemisinin resistant in patients and ***in vitro***, to infect native and non-native mosquito vectors. We found that that multiple artemisinin-resistant and artemisinin-sensitive isolates successfully infected two Southeast Asian vectors, ***Anopheles dirus*** and ***An. minimus***, as well as the major African vector, ***An. gambiae***, and also produced human-infective sporozoites. The ability of artemisinin-resistant parasites to infect highly diverse Anopheles species, combined with their higher gametocyte prevalence in Cambodian patients, may explain their rapid and extensive spread in Southeast Asia and further challenge regional efforts to contain and eliminate them.

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MOLECULAR CHARACTERIZATION OF METABOLIC FACTORS REQUIRED FOR SPERM FERTILITY AND STORAGE IN THE MAJOR MALARIA VECTOR *ANOPHELES* MOSQUITOES

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The application of vector control methods based on the use of insecticides has yielded resounding success in reducing the incidence of malaria and its impact on global health. The insurgence and spread of insecticide resistance in mosquito populations however is threatening these control methods, and new strategies are urgently needed. Among these, the use of sterile insect techniques (SIT) to control malaria vector populations and thus reduce disease transmission has gained renewed attention. To strengthen our knowledge of the use of SIT, an understanding of the molecular mechanisms essential for survival and functionality of sperm and reproductive success of ***Anopheles*** mosquitoes is absolutely required. To this end, we set out to characterize metabolic pathways essential for sperm production and function. Our data indicate that sperm function depends on key rate-limiting enzymes involved in lipid metabolism. Further molecular dissection of these pathways is underway to assess whether their impairment will affect fertilization of eggs after mating. This study may identify new targets to reduce natural mosquito populations and hence impact malaria transmission.

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF ANOPHELINE MOSQUITOES AND THEIR BEHAVIORAL PATTERNS IN UYO, SOUTH-SOUTH NIGERIA

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Adult mosquito vectors were collected from two areas in Uyo, Nigeria where no information exists on the major malaria vectors associated with human malaria. Samples collection was carried out between May and October 2013 using Knockdown and Human Landing Catches (HLC) techniques. A Molecular Method using Polymerase Chain Reaction (PCR) was used to further characterize and identify ***Anopheles gambiae*** sibling species. A total catch of 1,300 mosquitoes was recorded out of which 700 was used for morphological identification. A total of 90 (12.8%) of these were identified as female anopheles mosquitoes consisting of 21(23.3%) Anopheles nili and 69 (76.7%) ***An. gambiae*** complex. A PCR based test on the ***An. gambiae*** complex identified 66(96.0%) as ***An. gambiae*** sensu stricto. The study also revealed that the resting behaviour of ***An. gambiae*** complex species in this area is endophilic whereas the resting behaviour of An. nili is exophagic/exophilic. The peak biting activity of ***An. gambiae*** complex species occurred at 2300 hours (indoor) and 1900 hours (outdoor) in July whereas that of An. nili occurred at 2200 hours (indoor) and 1800 hours (outdoor) in June. The total number of ***An. gambiae*** collected was more than ***An. nili*** and Human Biting Rates (HBR) recorded for ***An. gambiae*** was higher than ***An. nili***. It is concluded from the study that there is a need for a comprehensive knowledge on the behaviour and heterogeneities that exist within and among malaria vector species in Uyo if the goal of malaria elimination is to be achieved. It is recommended from the study that more insecticide treated nets should be used in this area for effective control of malaria vectors in Uyo, South-South geopolitical zone and generally, in Nigeria.

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RESPONSE OF MOSQUITOES TO OVITRAPS SET IN DIFFERENT COLOURED CONTAINERS AT THEIR NATURAL BREEDING SITES AND THE BIO-INSECTICIDAL ACTIVITY OF *BACILLUS* SPP ON MOSQUITO LARVAE

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The behavioural choices of female adult mosquitoes to different colour substrate and the bio insecticidal activities of ***Bacillus*** species were studied in order to develop surveillance and monitoring systems for vector control. Five different colour containers; viz, black, blue, green, yellow and white were selected for the ovitraps and observed daily for mosquito eggs. A total of 1149 mosquitoes belonging to three genera, ***Aedes, Anopheles*** and ***Culex*** were collected from the five ovitraps in the study site. The highest occurring species was ***Culex*** species 963 (83.8%), followed by ***Aedes*** 170 (14.8%), and Anopheles 16 (1.4%). The colour preference for the mosquitoes was in this order: black 53.8 % (618), blue 23.2 % (266), green 10.4 % (119), yellow 8.5% (98) and white 4.2% (48). High numbers of ***Culex*** and ***Aedes*** species were found ovipositing in black 563 (58.5%), blue 238 (24.7%) and yellow 98 (10.2%) containers with relatively few numbers in green and white containers. The bio-insecticidal activity of three different ***Bacillus*** spp (***B. thuringiensis, B. subtilis***, and ***B. cereus***) at different treatment levels (0%, 10%, 20%, 30%, 40%, and 50% of breeding water) were introduced on three different mosquito genus; ***Aedes***, ***Anopheles***, and ***Culex*** and observed for mortality over 72 hours. ***B. cereus*** at concentrations of 30% and 40% was very effective on all the three mosquito species. ***B. subtilis*** showed total mortality (100%) on ***Aedes*** species at all concentrations after 72 hours. ***B. thuringiensis*** was more effective on the ***Culex*** and ***Aedes*** larvae as compared to the ***Anopheles***. ***B. cereus*** and ***B. subtilis*** must be considered as bio-insecticide for controlling ***Aedes***, ***Culex***, and ***Anopheles***. These findings are significant for mosquito vector control programmes and could be employed for future mosquito control campaigns in the Navrongo community, following further investigations.

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MOSQUITOES SPECIES DIVERSITY AND ABUNDANCE IN NEIGHBORHOODS WITH PREVIOUS ARBOVIRUS ACTIVITY IN IQUITOS, PERU, 2010-2013

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Arbovirus infections with alphavirus (Venezuelan Equine Enchephalitis (VEE), Mayaro), and orthobunyavirus (Guaroa, Oropuche, Group C) have been observed through clinic-based surveillance in the Amazonian City of Iquitos, Peru since the 1990s. Small outbreaks have occurred within urban neighborhoods in this isolated city of approximately 400,000 people. To identify the vectors transmitting these alphaviruses, we carried out mosquito collections in 4 neighborhoods with a history of VEE transmission. Two collection activities were done in the northern part of the city that are completely flooded by the Nanay River a few months of each year. The other two neighborhoods, located in the center and south of the city, are situated near rivers but do not flood. A total of 116 separate collections using two CDC light traps with dry ice (1800-0600) per neighborhood were carried out between 2010 and 2013. After species identification, mosquitoes were pooled and stored for virus testing. We estimated species diversity using the Shannon Index (H'). We collected 29,938 mosquitoes belonging to 49 species during that period. Species diversity (H') ranged from 0.86 to 2.05. The neighborhoods with the highest levels of seasonal flooding during a few months out of the year (houses located on stilts), had lower species diversity than the more urbanized neighborhoods. The most abundant species collected were ***Culex declarator/mollis*** (56.3%), ***Culex quinquefasciatus*** (18.5%), ***Aedeomyia squamipennis***(5.0%), ***Culex*** (***Melanoconion***) spp. (3.2%), ***Culex*** (***Melanoconion) ocossa*** (3.1%), ***Culex*** (***Aedinus) amazonensis*** (2.8%), ***Mansonia indubitans/titillans*** (1.9%). Although mosquito densities were lower than those observed in nearby rural communities, urban areas of Iquitos support a broad range of species that are known vectors of arboviruses. Of these the most notable were from the ***Culex*** (Melanoconion) group, previously incriminated as vectors of Venezuelan Equine Encephalitis virus.

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TRANSMISSION PATTERNS AND RISK CLUSTERING OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

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Understanding spatial patterns of dengue virus (DENV) transmission and underlying human behavioral and environmental factors are important to effective control. We assessed clustering and transmission factors for DENV among residents of Puerto Maldonado, Peru, a city in the southern Amazon Basin. We conducted a cross-sectional demographic and serosurvey and knowledge, attitudes, and practices (KAP) assessment in randomly selected households in 2012. Serum samples were screened by ELISA for DENV antibodies with confirmation by plaque reduction neutralization test to distinguish between primary and secondary infections. We used an ordinal model in SaTScan to assess spatial patterns adjusting for other covariates (available services and infrastructure, residence time, income) and created an ordinal multivariate model introducing variables measuring the distance of households to potential vector and infection sources (***i.e***. markets, cemeteries, hospitals, flooding areas, river shore). Data were collected from 270 households, over 60% of which were migrants to the city. Primary DENV infections were noted in approximately 40% of households and secondary infections in over 25. We identified five clusters of high DENV seroprevalence. The most likely cluster had a radius of 0.75 Km in which primary and secondary cases were noted in 15% and 30% of households, respectively. In the multivariate analysis, higher income (OR 1.6, 95% CI 1.1-2.3) and higher KAP scores (OR_{Q1} REF; OR_{Q2}: 1.5, 95% CI 0.7-3.0; OR_{Q3}: 2.2, 95% CI 1.1-4.4; OR_{Q4} 2.6, 95% CI 1.3-5.4) were positively associated with DENV infection, while odds of infection decreased with increasing distance (meters) from flooding areas (OR 0.999, 95% CI 0.998-0.999). No association was noted with migration time, distance to other features in the city or the presence of services and infrastructure. We found clustering of DENV infection in Puerto Maldonado, with increased risk surprisingly associated with higher income and KAP score. We speculate that higher income serves as proxy for increased exposure time to DENV in the city.

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CHARACTERIZATION OF CHIKUNGUNYA VIRUS INFECTIONS IN CHILDREN IN MANAGUA, NICARAGUA

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Chikungunya is a viral disease transmitted by ***Aedes aegypti*** and ***Ae. albopictus*** mosquitoes. In late 2013, chikungunya virus (CHIKV) was introduced in the Caribbean island of St. Martin. Since then, over 1,250,000 chikungunya cases have been reported by PAHO and most countries in the Americas report autochthonous transmission of CHIKV. In Nicaragua, the first imported case was described in July 2014 and the first autochthonous case in September. We analyzed the epidemiology and clinical presentation of chikungunya in two prospective pediatric cohort studies in Managua, Nicaragua: a community-based cohort study and a hospital-based study. Suspected chikungunya cases in both studies and cases with undifferentiated fever in the community cohort were screened by RT-PCR for CHIKV infection. From September 2014 to February 2015, a total of 96 and 83 chikungunya cases were identified in the community cohort and the hospital study, respectively. In the community cohort, cases were equally distributed by sex; however, more males presented to the hospital (67%, p=0.001). Most chikungunya cases were identified from November to January (community cohort: 83%, hospital: 92%). In the first six months of the epidemic, the incidence of symptomatic CHIKV infection in children aged 2-14 years in the community cohort was 4.6 cases per 1,000 person-months (95%CI: 3.8-5.7). Clinical presentation in the community cohort ranged from undifferentiated fever (13%) to children requiring hospitalization (16%). CHIKV-positive children were older than the rest of the children in the cohort study (9.9 vs. 8.1 years, p<0.001), and CHIKV-positive children presenting with typical chikungunya symptoms were older than those with undifferentiated fever (10.1 vs 8.1 years, p=0.03). A detailed analysis of acute symptoms in our chikungunya cases is underway. Additionally, patients with confirmed CHIKV infection will be followed longitudinally to characterize chronic symptoms associated with CHIKV infection. Finally, healthy annual serum samples collected from cohort participants in March 2014 and 2015 will be used to estimate the rate of subclinical CHIKV infections.

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CONTINUOUS OUTBREAK OF CHIKUNGUNYA VIRUS IN THE PHILIPPINES CAUSED BY 2 GENOTYPES, 2011-2014

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Chikungunya (CHIKV) is a mosquito-borne infection that caused large outbreaks in several tropical countries. Prior to 2011, the last reported outbreak of Chikungunya in the Philippines was in 1996 involving a small agricultural village. Limited information about the virus from the country is available. Here, we report the circulation of 2 Chikungunya genotypes causing its re-emergence in the Philippines. Serum samples collected from patients presenting with fever, rash, and joint pains from several provinces were tested for Chikungunya IgM. Samples collected <5 days after onset of symptoms and with negative IgM were tested for CHIKV RNA. The partial E1 gene was amplified using one-step RT-PCR and followed by direct Sanger sequencing. Phylogenetic analysis was performed using neighbor joining method using Kimura-2 parameter model (K2+G) on the partial E1 gene (733nt) by MEGA 6.05. Of the 6,549 serum samples collected from 2011 to 2014, 53% have detectable anti-Chikungunya IgM. CHIKV RNA was detected from 105 samples while 31 samples were sequenced for partial E1 gene. Most of the Philippines strains were grouped into Asian genotype and clustered into the same branch, which showed high similarity with the strains reported from Indonesia and Malaysia. Three samples from Davao have the East/Central/South African (ECSA) genotype. And have the alanine to valine substitution in the codon 226 (A226V) which increased the transmissibility of the virus. Chikungunya has caused outbreaks throughout the country. Initially detected in 2 provinces in southern Philippines in 2011, it increased to 30 provinces with reported cases in 2012 and to almost 90% of the provinces in 2013 and continued to invade new areas in 2014. In southern Philippines, strains of 2 genotypes, Asian and ECSA, circulated in the same time. During this outbreak, an ECSA genotype with the A226V mutation was reported in the country. The determination of the epidemic transmission route of CHIKV may be helpful to fully understand the epidemiology and molecular evolution of the virus into the country as well as its role in the ongoing Caribbean outbreak.

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EPIDEMIOLOGIC CHARACTERISTICS AND CLINICAL MANIFESTATIONS OF CHIKUNGUNYA IN A NAIVE POPULATION, PUERTO RICO 2015

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Chikungunya (CHIK) is a mosquito-borne disease caused by the chikungunya virus that was first detected in the Americas in October 2013, with the first laboratory confirmed Puerto Rico case detected in May 2014. Common clinical features are: fever, rashes and arthralgia/arthritis; rarely atypical or severe manifestations occur. Risk groups for severe disease include neonates, older persons and those with co-morbidities. We describe the epidemiology and clinical manifestations of laboratory confirmed CHIK in a naïve population and compare outcomes by age, sex and previous health status. Data was collected from patients with acute febrile illness (AFI) enrolled in the Sentinel Enhanced Dengue Surveillance System (SEDSS) project who presented to St. Luke's Episcopal Hospitals in Ponce and Guayama, Puerto Rico from May to September 2014. Blood, urine, nasal and oropharyngeal specimens were collected and RT-PCR and immunodiagnostic testing was performed for 21 pathogens, which included dengue and chikungunya viruses, influenza and other respiratory viral pathogens. Demographic and clinical information was collected on enrollment. Of 2,262 AFI patients enrolled, 663 (29%) had laboratory confirmed CHIK. Fifty-two percent were female, the mean age was 34 (SD±23). Nine percent of cases were admitted. The highest proportion of admission was among infants (75%) and adults over 60 (14%), 1 death was reported. Clinical manifestations included: arthralgia (86%), headache (77%), back pain (68%), rash (67%), conjunctivitis (64%), and arthritis (47%). Eight percent had mucosal, intestinal or urinary tract bleeding manifestations. Diabetic cases were more likely to be admitted than non-diabetics (OR= 3.0 CI 95%: 1.5, 5.99) and hypertensive cases more than non-hypertensive (OR= 2.9 CI 95%: 1.45, 5.9). Chikungunya presented in all age groups with the highest proportion of hospital admissions among infants and older adults. Adults with co-morbidities had a higher risk of admission. The study will continue to the end of the first epidemic in Puerto Rico and should provide useful information for health professionals in the clinical management of CHIK.

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SEQUENCING OF CHIKUNGUNYA VIRUS STRAINS CIRCULATING IN NICARAGUA, 2014-2015

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Chikungunya is a re-emerging infectious disease caused by a mosquito-borne arthrogenic alphavirus, chikungunya virus (CHIKV). The 12-kb positive-sense RNA genome contains a 5'UTR, non-structural protein genes (NS1-4), structural protein genes (C-E3-E2-6K-E1), and a 3'UTR. The disease involves sudden onset fever, intense pain and inflammation in joints, and muscles and an impaired ability to ambulate that lasts for months or years. Endemic areas include Africa and Asia. Since 2004, CHIKV has expanded into Europe and the Pacific region, and since the end of 2013, into the Caribbean and Central America. Viral sequences from St. Martin, the point of introduction in the Americas, belonged to the CHIKV Asian genotype. In Nicaragua, the first imported case was described in July 2014 and the first autochthonous case in September. Here, we sequenced CHIKV strains circulating in Nicaragua using samples from national surveillance and 2 ongoing pediatric studies in Managua: a community-based cohort and a hospital-based study. The initial sample set included 5 imported cases from August 2014 and 16 autochthonous cases from October 2014 to February 2015; sequencing of additional strains is underway. Whole genome amplification of nucleic acids isolated from serum samples, combined with Nextera technology, was used to generate libraries for deep sequencing on the HiSeq2000 platform (Illumina). Complete full-length sequence was obtained from one individual and partial genome sequence was obtained from 2 other samples. We also designed primers to amplify and sequence the E1 gene via Sanger methodology, yielding sequence from 5 imported and 14 autochthonous cases. Results to date indicate that the Nicaraguan strains belong to the Asian genotype and are similar to those in the Caribbean and Panama. Some of the autochthonous strains have silent mutations and some have non-synonymous mutations (e.g., E1-I173V or E1-V302I). All imported and autochthonous cases thus far contain E1-A226. Additional genome regions and samples are being analyzed to better understand the evolutionary dynamics of CHIKV during its introduction and dissemination in Nicaragua.

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A ROLE FOR SYNDECAN PROTEOGLYCANS IN ALPHAVIRUS ENTRY

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Eastern equine encephalitis virus (EEEV) is unique among encephalitic alphaviruses in both its high rate of neurovirulence and its natural ability to bind cell surface heparan sulfate (HS). Among non-neurovirulent alphaviruses, efficient HS binding usually accompanies positive-charge mutation in the E2 attachment glycoprotein resulting from passage in cell culture. This type of cell culture adaptation typically renders the virus less virulent but for EEEV, HS binding is essential to its neurovirulence in adult mice and may facilitate mosquito infection. Thus, HS-binding residues in E2 are maintained in naturally circulating EEEV. Similarly, HS binding is critical for the neurovirulence of Sindbis viruses (SINV) containing a mutation at E2 position 55 selected for adult mouse virulence. We hypothesize that the connection between neurovirulence and HS binding for EEEV and neurovirulent SINV lies in their specific receptor usage. Using a Raji cell system that exhibits a receptor-entry defect and minimal infectivity for alphaviruses yet expresses forms of HS capable of non-productively binding HS-dependent alphaviruses, we have determined that syndecan proteoglycans can facilitate entry and productive infection of these cells by HS-dependent alphaviruses. Syndecans are a four-membered family of transmembrane glycosaminoglycans, each modified with multiple, distinct HS moieties. Notably, the capacity for infection facilitation by individual syndecans was different between different viruses suggesting qualitative differences in virus-HS receptor interactions. Effects of syndecan receptor utilization on replication and cellular responses to infection are currently being investigated.

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INDUCTION OF HOST TRANSLATION SHUTOFF CONTRIBUTES TO THE ANTIVIRAL STATE RESISTANCE OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS

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Alphavirus antagonism of induction or effector phases of the IFN response is poorly understood. We recently demonstrated that eastern equine encephalitis virus (EEEV) avoids IFN-α/β and antiviral effector induction via microRNA-mediated suppression of virus replication in myeloid cells. Arthritogenic alphaviruses such as Sindbis virus (SINV) or chikungunya virus (CHIKV) cause a limited, non-fatal infection in adult mice suggesting limited antagonism of the IFN response. In contrast, Venezuelan equine encephalitis virus (VEEV) mouse infection is rapidly fatal, associated with systemic replication, widespread myeloid cell infection and rapid induction of high levels of serum IFN-α/β. In cell culture, VEEV replication is more resistant to the established antiviral state than SINV, CHIKV or EEEV. VEEV resistance is temporally associated with host macromolecular synthesis shutoff (transcription, translation or both) and STAT1 signaling blockade. In the current studies we found that increased resistance of VEEV was first evident after initial translation of viral genomes, and production of nonstructural proteins (nsPs). Using a plasmid expression system, we observed that expression of VEEV, SINV, CHIKV or EEEV nonstructural protein 2 (nsP2) alone each blocked STAT1 signaling. VEEV, SINV or CHIKV nsP2 or VEEV capsid, but not EEEV nsP2 inhibited cellular translation, while SINV and CHIKV nsP2 and VEEV or EEEV capsid, also inhibited cellular transcription. Importantly, VEEV nsP2 significantly reduced host translation in IFN-α/β primed cells while SINV nsP2 was less effective. Finally, VEEV nsP2 reduced the efficacy of the antiviral state versus other viruses in IFN-primed cells. Our results suggest that nsP2-mediated translation shutoff is an important factor in the antiviral state resistance of VEEV, and that closely related viruses such as VEEV and EEEV have evolved very different strategies to overcome innate antiviral responses.

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EVIDENCE OF MICROSCALE HUMAN MOVEMENT DRIVING CHIKUNGUNYA SPREAD IN BANGLADESH

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Human movement has been implicated in pathogen spread. However, evidence to support this role is limited as it requires good data on human movement and also a sound characterization of the spatial spread of pathogens, which is challenging where chains of transmission are unobserved. To address this knowledge gap, we collected nationally representative movement data from Bangladesh. We also collected detailed epidemiological data from an outbreak of chikungunya and developed models to characterize pathogen spread. We visited 70

randomly selected communities and gave ten individuals a GPS device that recorded their location every minute for up to four days and calculated the distance to their home. The chikungunya outbreak occurred in Tangail district in 2012. An investigation team visited every home in the outbreak village and collected information on disease symptoms from all individuals (N=1970). From this data, we fit transmission models using the time and location of symptom onset. In the movement study, we found that children (those under 16 years) were 0.9 as likely to be at home than adults (95% confidence interval: 0.7-1.1) at any time point. In addition females were 1.5 times more likely to be at home than males (1.2-1.8). When outside the home, individuals often still remained nearby, with a median distance between their location and home of 88m (77m-101m). These findings were virtually identical to our estimates of transmission risk in the chikungunya outbreak: we estimated the relative risk of children being infected was 0.9 compared to adults (0.7-1.2) and the relative risk of being infected for females was 1.5 times that for males (1.2-1.8). The median distance for transmission events outside the home was 95m (74-124). Basic mechanistic models demonstrated that these findings were consistent with infections happening within homes and that despite the presence of an intermediary vector, chikungunya spread is highly correlated with human movement (and lack of movement). Interventions to prevent chikungunya transmission, such as insecticides and removal of ovipositioning sites, should be targeted at small spatial scales.

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DEVELOPMENT OF A CHIKUNGUNYA VACCINE CANDIDATE USING EILAT VIRUS AS A PLATFORM

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In December of 2013, chikungunya virus (CHIKV), an alphavirus in the family ***Togaviridae***, was introduced into the island of Saint Martin in the Caribbean, resulting in the first autochthonous cases reported in the Americas. As of April 2015, CHIKV has been reported in 50 American countries with over 1.3 million suspected cases. CHIKV causes a severe arthralgic disease for which there are no approved vaccines or therapeutics. We developed a "pseudoinactivated" vaccine for the disease using Eilat virus (EILV) as a platform. EILV is an alphavirus isolated from a pool of mosquitoes collected in Israel. It replicates efficiently in insect cells but is unable to replicate in vertebrate cells. EILV is host-restricted in at least two points in its replication cycle: 1) attachment/entry, and 2) viral RNA replication. Our central hypothesis was that a chimeric alphavirus containing the non-structural protein genes of EILV and the structural protein genes of CHIKV will retain the vertebrate host restriction of EILV and provide safe, effective protection against CHIKV challenge. To test this hypothesis, we generated chimeric EILV/CHIK infectious cDNA using standard cloning techniques, and rescued the virus in insect cells. We then performed immunogenicity and safety experiments in mice. After a single vaccination, EILV/CHIK protected mice from disease following challenge with CHIKV, induced higher neutralizing antibody titers, and resulted in higher CD4+ and CD8+ T cell responses when compared to live-attenuated and inactivated vaccine strains of CHIKV. Additionally, EILV/CHIKV showed no neurovirulence in immunocompromised infant mice after intracranial inoculation. These results suggest that chimeric EILV/CHIKV can elicit a protective immune response against CHIKV challenge following a single dose in mice while maintaining an ideal safety profile, warranting its further development as a potential CHIK vaccine candidate.

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CHARACTERIZATION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS NON-STRUCTURAL PROTEIN 3 WITH HOST FACTORS IN INFECTED CELLS

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The mosquito-borne virus Venezuelan Equine Encephalitis Virus (VEEV) belongs to the ***Togaviridae*** family and is considered an important biodefense pathogen and select agent. There are currently no approved vaccines or therapies to treat the disease, therefore it is imperative to identify novel targets for therapeutic development. The VEEV genome encodes for 4 nonstructural proteins (nsP1-4) and 5 structural proteins (capsid, envelope 3 (E3), E2, 6K and E1). Apart from its role in viral RNA synthesis, nsP3 has not yet been fully characterized. Viral replication is facilitated by interaction of the nsPs with host factors involved in replication, translation and signaling, notably host kinases that are modulated by VEEV during infection. We have previously reported that VEEV-nsP3 interacts with the host protein Inhibitor of nuclear factor kappa-B kinase subunit beta. In our current study, we aim to investigate the effects of additional host-nsP3 interactions on the phosphorylation status of nsP3 and the consequences of these interactions on viral replication. An HA tagged nsP3 infectious clone (rTC-83-nsP3-HA) and expression plasmid was constructed. Replication kinetics and protein expression of rTC-83-nsP3-HA was compared to rTC-83 to ensure that the presence of the tag did not interfere with viral kinetics or viral protein production. It has been reported that there are several possible phosphorylation residues on nsP3 rendering it a highly phosphorylated protein and our mass spectrometry analysis corroborated those reports. Ongoing studies involve identifying host proteins that interact with nsP3 and investigating the effects of the identified interactions on the phosphorylation status of nsP3. This study will aid future investigations in identifying host proteins as potential broad spectrum therapeutic targets for treating VEEV infections.

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A STOCHASTIC FRAMEWORK TO MODEL THE IMPORTATION OF CHIKUNGUNYA-INFECTED TRAVELLERS INTO THE US

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Chikungunya virus (CHIKV) is transmitted by the bite of infected mosquitoes of certain species, including ***Aedes aegypti*** and ***Aedes*** albopictus which are quite common in the American continent. Though rarely fatal, Chickungunya disease often produces severe symptoms in those infected and can persist for several months or even years after the virus is cleared from the human host. From the month of December 2013 to February of 2015, the Pan-American Health Organization (PAHO) reported more than 1.25 million cases in the continent, most of them concentrated in South and Central America and the Caribbean. In the absence of vaccines and treatments specific to Chikungunya, a good understanding of the mechanisms of CHIKV transmission is critical to guide policies aimed at limiting further propagation of the disease. In this work, we developed a stochastic modeling framework in order to estimate the number of monthly arrivals of CHIKV-infected individuals at each state of the US. Our framework is built by incorporating PAHO prevalence data from the affected countries in the American continent together with detailed data for airline travel from these same source countries into the US. A comparison of our importation estimates with US surveillance data at the state level shows reasonable agreement and suggests significant under-reporting at many of the source countries. Our framework, coupled with country-level forecasts, should prove useful to local public health officials for obtaining estimates of the expected number of imported Chikungunya cases during outbreaks abroad.

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MOLECULAR CHARACTERIZATION OF CIRCULATING CHIKUNGUNYA VIRUS IN SUCRE - COLOMBIA

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Chikungunya virus (CHIKV) is an Alphavirus from the Togaviridae family transmitted by mosquitoes. With an approximately 12 Kb single-stranded genome, two open reading frames code two polyproteins: structural (C, E3, E2, 6K y E1) y nonstructural (nsP1, nsP2, nsP3 y nsP4) proteins. It have been identified three genotypes related to their geographic origin: West African, East/Central/South African (ECSA) and Asian. The virus cause a disease with signs and symptoms very similar to other prevalent diseases in tropical areas, but the main clinical symptom is a painful and invalidating poly-arthralgia. The first case in America was reported in December 2013 in Saint Martin´s Island and in September 2014 an endemic outbreak started in Colombia. Until February 2015 in Colombia 189.959 cases have been reported and 19.365 in the department of Sucre. In this study we describe the molecular detection of CHIKV in febrile patients and corresponding genotype present in Sucre - Colombia, during the 2014 - 2015 outbreak in the country. There were collected serum samples and clinical information from Chikungunya fever compatible participants, during acute phase. Molecular detection of CHIKV was performed by RT-PCR with specific primers (nsP1). Positive samples were passed in C6/36 cells to obtain viral isolates and supernatants were used to amplify and sequence E1 gene for further phylogenetic analysis (Bayesian inferences). A total of 128 participants were included in the study from November 2014 to February 2015, been the arthralgia and rash the more frequent clinical findings. Forty-two (32.8%) participants were positive for molecular detection with an average age of 20 years old. There were identified 19 isolates causing cytopathic effect to cell monolayers. Phylogenetic analysis with 1044 nt complete E1 gene sequences revealed that all isolates belonged to the Asian genotype, with genetic distances below 1% within them. The Asian genotype with recent introduction in the Americas was circulating in the department of Sucre during 2014-2015 outbreak and with no significant genetic changes to those previously reported isolates in other geographic areas.

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EILAT VIRUS HOST RANGE RESTRICTION IS PRESENT AT MULTIPLE LEVELS OF THE VIRUS LIFE CYCLE

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Most alphaviruses are mosquito-borne and exhibit a broad host range, infecting many different vertebrates, including birds, rodents, equids, humans, and nonhuman primates. This ability of most alphaviruses to infect arthropods and vertebrates is essential for their maintenance in nature. Recently, a new alphavirus, Eilat virus (EILV), was described, and in contrast to all other mosquito-borne viruses, it is unable to replicate in vertebrate cell lines. Investigations into the nature of its host range restriction showed the inability of genomic EILV RNA to replicate in vertebrate cells. Here, we investigated whether the EILV host range restriction is present at the entry level and further explored the viral factors responsible for the lack of genomic RNA replication. Utilizing Sindbis virus (SINV) and EILV chimeras, we show that the EILV vertebrate host range restriction is also manifested at the entry level. Furthermore, the EILV RNA replication restriction is independent of the 3′ untranslated genome region (UTR). Complementation experiments with SINV suggested that RNA replication is restricted by the inability of the EILV nonstructural proteins to form functional replicative complexes. These data demonstrate that the EILV host range restriction is multigenic, involving at least one gene from both nonstructural protein (nsP) and structural protein (sP) open reading frames (ORFs). As EILV groups phylogenetically within the mosquito-borne virus clade of pathogenic alphaviruses, our findings have important evolutionary implications for arboviruses.

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A NOVEL METHOD TO IDENTIFY THE MOST POTENT HUMAN MONOCLONAL ANTIBODIES AGAINST DENGUE VIRUSES

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We have developed an ***ex vivo*** viremic blood neutralization assay (ViBNA) that uses viremic blood from hospitalized dengue patients, human mAbs and ***Aedes aegypti*** mosquitoes. The ViBNA allowed us to rank a panel of human mAbs for their potency in neutralizing the infectiousness of dengue virions for ***Ae. aegypti*** mosquitoes. Our data identifies human mAbs that bind quaternary epitopes within or between DENV homodimers as the most potent class of antibodies elicited by natural DENV infection. Other classes of mAbs, such as those that bind the fusion loop or domain III, were less potent or not potent at all in blocking transmission of DENV. Our results set a new benchmark for evaluating the potency and relevance of human mAbs and have implications for identifying correlates of immunity and for mAb-based therapeutic strategies.

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SCREENING OF DENGUE VIRUSES IN HUMAN SERA AND ANALYSIS OF SPECIFIC SEROTYPES FROM LAHORE PAKISTAN

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Dengue is a vector borne viral infection which poses a serious threat to public health in most of the tropical and subtropical countries around the world, including Pakistan. Dengue has been occurring as an annual epidemic since 2006 in Pakistan. More than fifteen thousand cases were listed in 2011 from Punjab with about > 250 deaths. Dengue situation is alarming with high risk of epidemics in future. About four antigenically varying dengue viruses are reported for dengue infection. Current study was designed to detect Dengue viruses with molecular detection of dengue serotype using RT-PCR in infected human sera. Dengue infected human sera (n=100) were collected during July 2013 to January 2014 for screening of DENV serotypes using dengue NS1 AG specific ELISA kit. DENV positive samples (n=40) were used for molecular detection of dengue viruses serotypes by reverse transcriptase PCR (RT-PCR) using universal and type specific primers for dengue viruses nucleotide sequencing targeting the C-prM gene junction. Among forty dengue NSI AG ELISA positive samples, 12 sera (30%) were found positive with type specific nested PCR. Out of 12 PCR +ve samples, five samples (41.6%) were positive for each DEN-2 and DEN-3. Whereas, two samples (16.6%) revealed the simultaneous presence of DEN-2 and DEN-3 serotypes. In conclusion, current study documented for the first time the detection of dengue viruses serotypes in human sera with DEN-2 and DEN-3 prevailing serotypes during the study period in Lahore, Pakistan. Detection of particular prevailing serotype will be useful to control the spread of dengue disease in Pakistan.

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EVALUATION OF A DENGUE DECISION SUPPORT TOOL

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In order to prepare for disease epidemics, decision support systems, which take into account multiple risk factors, are required to implement timely control measures. Seasonal climate forecasts and strong disease surveillance systems provide an opportunity to anticipate epidemics several months in advance. A prototype decision support tool was developed for dengue fever and tested ahead of the FIFA football World Cup, June 12-July 13, 2014, in Brazil. Probabilistic dengue forecasts for June 2014 were generated using a spatio-temporal modelling framework. The model was driven by seasonal climate forecasts and the observed epidemiological situation in Brazil at the forecast issue date. The forecasts were made available three months ahead of the games. Here, we evaluate the ability of the model framework to correctly determine the occurrence of low-, medium- and high-risk of dengue for the 12 host cities and all 553 microregions in Brazil, by comparing the probabilistic predictions to the observed dengue incidence rates for June 2014. For the 12 microregions of interest, the forecast achieved a hit score of 75%. For all 553 microregions across Brazil the forecast achieved a score of 70%. This decision support model framework may be useful, not only ahead of mass gatherings, but also before the peak dengue season each year, to control or contain potentially explosive dengue epidemics. It is hoped that this prototype will serve as an example for scientists, international health surveillance teams and decision makers of the data and tools required to produce and communicate timely predictions of climate-sensitive disease risk.

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COMPARISON OF HEAMAGGLUTINATION INHIBITION ASSAY (HAI) AND ANTI-IGG MONOCLONAL ANTIBODY ELISA (IGG-MAB ELISA) BY USING 4G2 (FLAVIVIRUS MAB), 2H2 (DENGUE COMPLEX MAB), AND J93 (JE MAB) FOR DETECTION OF ANTI-DENV/JEV IMMUNOGLOBULIN G

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Hemagglutination Inhibition assay (HAI) is commonly used for serology diagnosis of DENV and JEV infections. However, HAI is time- and resource-intensive, because of the multiple steps required for serum processing. The purpose of this study was to evaluate if a monoclonal antibody-based capture enzyme-linked immunosorbent assay (mAb ELISA), is equally effective for IgG screening of DENV/JEV infections as the more traditional HAI. The IgG-mAb ELISA originally developed by Johnson, et al. uses the 4G2 monoclonal antibody (Flavivirus mAb), which targets the envelope protein on the surface of dengue virus (DENV). We selected 170 pairs of acute and convalescent serum specimens collected during routine DENV surveillance in Kamphaeng Phet, Thailand, in 2004-2005. These specimen pairs underwent testing by HAI to measure changes in neutralizing titers, followed by plaque reduction neutralization test (PRNT50) as confirmatory testing. Comparison of HAI and IgG-mAb ELISA showed that the specificity of the IgG-mAb ELISA was 100.0% when using the 4G2 mAb or when using the alternative 2H2/J93 mAbs. The sensitivity was 92.9% and 97.6% for 4G2 and 2H2/J93 mAbs, respectively. Our study supported that the results of both IgG-4G2 and IgG-2H2/J93 mAbs ELISAs correlated highly with the DENV/JEV HAI assay. Therefore, the anti-DENV/JEV IgG-mAbs ELISA is a potential assay for serological screening of DENV/JEV infections.

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THE PREVALENCE OF DENGUE IN GRENADA: A FIVE-YEAR RETROSPECTIVE STUDY

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Dengue has been endemic in Grenada for decades and ranks high among re-emerging pathogens that have increased globally. The goal of this study was to determine the recent prevalence of dengue and its serotypes (DENV 1-4) in Grenada. Our target population included symptomatic persons who sought care at the St. George's University (SGU), University Health Services (UHS) during 2009 - 2013. Individuals from our target population completed an Investigation Form for Suspected Dengue Infection, which included general patient data, questions about travel history and possible signs and symptoms associated with dengue. Dengue seropositivity was determined for all 298 samples taken over the five-year period; 90 were confirmed to be positive (30.2%). The annual prevalence of dengue from 2009 to 2013, based on serology, was found to be 34.38%, 36.96%, 26.79%, 16.21% and 29.27%, respectively. The CDC DENV-1-4 Real-Time RT-PCR Assay (Multiplex) was used for the detection and serotype identification of the dengue virus in seropositive samples taken during the acute phase. Sixty-five (65) of the 90 serologically confirmed dengue samples and 11 of the serologically negative samples were processed by qPCR. Serology data was compared to dengue qPCR data from the target population. The qPCR results showed that DENV-1 and DENV-2 were present in 2010, DENV-1 was present in both 2011 and 2012 and DENV-1 and DENV-4 were present in 2013. From the data gathered, it appears that dengue cases peak between August to November, which coincides with our rainy season. The highest prevalence was seen in 2010, and the lowest prevalence was seen in 2012. This study provides novel data on the prevalence of currently circulating dengue serotypes in Grenada. Our data will provide critical information for the formation of public health policies that will be developed for the control of mosquito-borne diseases such as dengue in Grenada. Future studies will include the sequencing of the detected DENV serotypes to determine the predominantly circulating strains.

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INFLUENCE OF MATERNAL IGG PROFILE ON PLACENTAL TRANSFER OF DENGUE VIRUS-SPECIFIC ANTIBODIES TO NEONATES

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Maternally transferred dengue IgG antibodies are likely to play an essential role in immunity and pathogenesis of dengue infection in infants. In order to investigate the kinetics of dengue-specific maternal antibodies transferred to children in the first years of life, a birth cohort of children living in an area of intense circulation of dengue virus in the northeast of Brazil has been established. Here, we carried out the analysis of 376 mother-newborn pairs to investigate the transference of antigen-

specific antibodies via placenta. Maternal and umbilical cord samples were obtained during the time of delivery. Serotype-specific antibody profile was determined by PRNT, while in-house ELISA was used to both measure DENV-specific IgG titers (total and subclasses) and quantify IgG in the sera. Antibody titers were log-transformed and placental transfer calculated as ratio (value infant/value mother). In maternal sera, 202 out of 376 (53.7%) showed a monotypic profile against DENV3, 30.6% to the combination of DENV3/DENV4 and 5.3% had detectable neutralizing antibodies against others serotypes combinations. Dengue-specific IgG titers were significantly higher in cord blood than in maternal samples ($p<0.05$), which is consistent with an active transport mechanism across the placenta. Same pattern was also observed when comparing serotype-specific antibodies titers to DENV3 and DENV4 in infants and mothers. DENV-specific IgG1 were more efficiently transferred to the neonate than IgG4 antibodies. More importantly, higher levels of maternal total IgG antibodies were associated with reduced transference of total IgG (R-squared=0.3015, $p<0.05$) and DENV3 antibodies to the neonate (R-squared=0.0129, $p=0.021$). Additionally, placental transference of DENV-specific IgG was reduced in mothers who experienced multitypic previous infections. These results suggest that maternal IgG levels directly influence placental transfer of DENV-specific antibodies and, thus, may contribute for dengue immunophatology on neonates.

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ACUTE FEBRILE ILLNESS DUE TO DENGUE VIRUS INFECTIONS AMONG CHILDREN IN WESTERN KENYA

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Although dengue virus (DENV) is the most common arthropod-borne viral disease, little is known about DENV burden and disease between epidemics. Recent outbreaks of DENV have occurred in coastal Kenya; therefore, there is a need to confirm the presence of dengue virus infections and disease elsewhere in Kenya. Febrile children, aged 2-17 years, presenting at Chulaimbo (rural site) and Obama Children's (urban site) hospitals in western Kenya had RNA extracted from whole blood and standard PCR was performed in a two-step protocol (pan-DENV followed by DENV serotyping). Malaria testing by microscopy was performed on all samples. Of 113 children tested, 31 (27%) were positive, and 82 (73%) were negative. Those from the rural site were more likely to be positive ($p<0.05$): 12% (4/33) were positive from the urban site vs. 34% (27/80) from the rural site. There was no statistical difference between genders. Older children were more likely to be acutely infected (mean age 5.3 vs. 3.6 years; $p<0.01$). All positive samples were DENV 1. For acute DENV infections, mean days of illness were 2.3, mean temperature was 38.9°C and was statistically higher in the DENV positive group ($p<0.05$). No children with acute DENV were hospitalized. DENV positives were more likely to be from households with more residents ($p<0.05$). DENV cases from the rural area occurred from July 11 to November 14, 2014, after the long rains. Sixty-one percent of samples were blood smear positive for malaria and 78% of DENV positives were blood smear positive. Testing is ongoing and positive PCR results will be confirmed by sequencing. In conclusion, preliminary findings of this study confirm the existence of acute dengue virus infections with resultant febrile disease in childhood from serotype DENV1 in both rural and urban villages in western Kenya. The rural village site was more likely to have acute dengue cases than the urban site, and they occurred toward the end of the rainy season. Those with acute DENV were malaria blood smear positive in most cases, therefore on site diagnosis needs to be available for accurate classification of febrile disease etiology.

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AN IMMUNOGENICITY OF E-DOMAIN III IS BOOSTED BY TRIMETHYL CHITOSAN (TMC) NANOPARTICLES DELIVERY SYSTEM

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Dengue virus infection is a major public health problem due to its high prevalence, rapid transmission, and serious complication. Many attempts were made to establish the preventable vaccine. One of great interest is a subunit vaccine due to its safety, ease of dose adjustment, the large scale in manufacturing. Unfortunately, subunit vaccine is poor in immunogenicity. To overcome this limitation, nanoparticle delivering system is applied to achieve an accurate immunization. A key feature of nanoparticle-delivered vaccine is its ability to simultaneous deliver antigen with adjuvanticity to specialized immune cells. In this study, the potential use of trimethyl chitosan (TMC) nanoparticles (NPs) as an adjuvant and delivery system for E-domain III of dengue virus type 3 (EDIII) was investigated. Recombinant soluble EDIII was produced using ***Pichia pastoris*** expression system. Western blotting with EDIII-specific antibody demonstrated that antigenicity of EDIII was preserved. Purified EDIII was reacted with TMC under ionotropic gelation method to form EDIII-TMC NPs. The EDIII-TMC NPs possess mean particles size of 255 nm with narrow size distribution and positive charge. Immunoblot analysis revealed that the integrity of entrapped EDIII was preserved. A release study showed that within 24 h more than 70% of EDIII disassociated from TMC NPs at 37°C, pH 5. The immunogenicity of EDIII-TMC NPs was then evaluated using an ***ex vivo*** model, primary human dendritic cells (DCs). The EDIII-TMC NPs-treated DCs exhibited a viability exceeding 90% at 48 h of treatment. Flow cytometric analysis showed the strong up-regulation of maturation markers on treated DCs. The EDIII-TMC NPs-treated DCs cultures increased production of various cytokines including proinflammatory cytokines (IL-1β, IL-6, TNF-α), Th1-inducing cytokines (IFN-γ, IL-2, IL-12p70), Th2-inducing cytokine (IL-10), chemokines (MIP-1β, MCP-1), growth factors (G-CSF, GM-CSF). In conclusion, the EDIII-TMC NPs was successfully developed and was shown to exert strong immunogenicity. These findings highlight the potential of using TMC NPs as an adjuvant delivery system for dengue vaccine.

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ANALYSIS OF VIRAL QUASISPECIES AND DENGUE DISEASE SEVERITY BASED ON NEXT GENERATION SEQUENCING DATA

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The error-prone nature of RNA polymerase and the intra-serotypic recombination due to rapid geographical spread of dengue cause high mutation rates in the viral genome, resulting in a genetically diverse population of viruses known as quasispecies. Although quasispecies have been well studied in the pathogenesis of chronic infections, its role in acute infections such as dengue remains to be elucidated. The analysis of dengue quasispecies is imperative especially in geographically-isolated countries, such as the Philippines, where there is a notable persistence of a single genotype yet dengue hemorrhagic fever epidemic cycles and severe forms of the disease are exhibited. In this study, next generation sequencing was used to analyze the sequences and estimate the frequencies of quasispecies isolated from 20 acute dengue serum samples, and correlate the genomic variations to disease progression and severity. Viral RNA was extracted from confirmed dengue sera with well-defined clinical profiles and prepared for whole genome sequencing with the Illumina MiSeq. Software packages ShoRAH and ViQuas were used in parallel for the quasispecies inference and reconstruction pipeline. The diversity and estimated frequencies of reconstructed haplotypes were

subsequently compared to the samples' clinical profiles. The sequences are currently undergoing quasispecies analysis. Once completed, the diversity and frequency of the quasispecies will be compared to the severity of disease corresponding to the sample. The target date of completion for this study is on June 30, 2015.

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CELLULAR ANTIVIRAL RESPONSE AGAINST DENGUE VIRUS REPLICATION

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The intrinsic antiviral defense is based on cellular restriction factors that are constitutively expressed and, thus, active even before a pathogen enters the cell. The promyelocytic leukemia (PML) nuclear bodies (NBs) are discrete nuclear ***foci*** that contain several cellular proteins involved in intrinsic antiviral responses against a number of viruses, but little information is available regarding the antiviral role of PML against RNA viruses. Dengue virus (DENV) is an RNA emerging mosquito-borne human pathogen affecting millions of individuals each year by causing severe and potentially fatal syndromes. Since no licensed antiviral drug against DENV infection is currently available, it is of great importance to understand the factors mediating intrinsic immunity which may lead to the development of new pharmacological agents. In the present study, we investigated the ***in vitro*** antiviral role of PML in DENV-2 A549 infected cells. First, we evaluated the impact of PML silencing and overexpression on DENV-2 replication. The silencing of all PML isoforms caused about 0.76 log increment in DENV-2 titre. On the other hand, the PMLIV isoform overexpression reduced significantly the extracellular DENV-2 production. These results were in accordance with the viral antigen expression observed by immunofluorescence. Moreover, we analyzed the intracellular localization of PML-NBs during DENV-2 replication. Confocal microscopy images showed that the typical punctuate nuclear staining pattern of PML-NBs was lost during DENV-2 infection. Furthermore, it was observed a weak viral protein signal in neighboring cells, which also displayed increased number and size of PML-NBs. The pattern of PML did not change during the early stages of infection, and only after the new progeny of DENV-2 was released, a reduction in PML-NBs staining was observed. Altogether these results strongly suggest that a viral protein might be responsible of the PML-NBs disruption. These host- virus interactions may serve as targets for antiviral intervention.

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POST CHIKUNGUNYA EPIDEMIC CLUSTER OF DENGUE-1 VIRUS INFECTION AMONG SCHOOL CHILDREN IN GRESSIER REGION, OUEST DEPARTMENT OF HAITI

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Dengue is the most common tropical and subtropical mosquito-borne viral infection caused by four distinct serotypes (1-4). In May 2014 an outbreak of chikungunya virus (CHIKV) started in the Ouest department in Haiti and the epidemic spread throughout the country. As the epidemic waned in the fall of 2014; febrile illnesses among a cohort of school children in the Gressier region remained relatively high. 177 febrile cases suspected for CHIKV infection reported from September 2014 to February 2015were tested for CHIKV and all four DENG serotypes using RT-PCR. Fourteen percent (25/177) were positive for Dengue-1 virus by RT-PCR while none were positive for CHIKV. The results indicate a common misdiagnosis of CHIKV, and active back to back transmission of DENG-1 virus in this region of Haiti.

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EVIDENCE OF TRANSMISSION OF DENGUE AND CHIKUNGUNYA VIRUSES IN WESTERN KENYA

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Dengue virus (DENV) and chikungunya virus (CHIKV) are important re-emerging mosquito-borne pathogens that have been spreading rapidly, causing endemic and epidemic disease in tropical and sub-tropical regions. For many African countries, limited resources and lack of national surveillance systems make evaluating the true burden of DENV and CHIKV disease difficult. In 2014, we initiated a prospective study to measure DENV and CHIKV incidence in children who develop febrile illnesses, and seroprevalence among healthy children in western and coastal Kenya. Testing of serum samples for IgG and IgM to DENV and CHIKV by ELISA is ongoing, and neutralizing antibodies will be measured in a subset of samples. Preliminary results from IgG assays of samples from children residing in western Kenya confirm active transmission of both DENV and CHIKV. Specifically, among children who presented at the local health center with undetermined febrile illness from whom paired acute and 1-month convalescent serum samples were obtained, 1 of 221 (0.5%) paired sera from children who resided in the rural village of Chulaimbo demonstrated seroconversion for DENV IgG vs. 3 of 117 (2.6%) paired sera from children residing in Kisumu, a large urban center (p=0.12). For CHIKV IgG, 6 of 209 (2.9%) sera from Chulaimbo children seroconverted vs. none of 115 in Kisumu children (p=0.09). Further, we found that among healthy children, 11.5% of Chulaimbo children were positive for serum DENV IgG vs. 2.8% of Kisumu children (p=0.0003). CHIKV IgG seroprevalence was similar between rural and urban centers (6.8% in Chulaimbo vs. 6.3% in Kisumu, p=1.0). No difference in seroprevalence was noted based on gender, overall or by location, with 39% of DENV- and 41% of CHIKV-seropositive children female. Children who were seropositive for either virus were older than seronegative children (mean age 8.6 vs. 7.1 years, p≤0.0001). These data provide evidence that DENV and CHIKV transmission is presently occurring in western Kenya and underscore the need for surveillance of these rapidly re-emerging infections to monitor developing outbreaks and allocate limited public health resources.

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GENETICALLY MODIFIED *AEDES AEGYPTI*: THE SOLUTION TO OUR PROBLEM?

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This presentation intends to analyze the workings of the ongoing genetically modified ***Aedes aegypti*** initiative and its possible elimination of the spread of dengue fever, yellow fever and chikungunya. The diseases associated with ***A. aegypti*** are public health burdens with high incident and prevalent rates in Africa, Asia, Europe and Americas leading to morbidity and mortality. This exposition addresses the following question: What are the breeding mechanisms of genetically modified ***A. aegypti***? How can these modified arthropods play a role in the elimination of dengue fever, yellow fever and chikungunya? What are the possible

adverse effects in humans? To answer these questions, this study critically examines ongoing research and experimental results pertaining to this innovation. Moreover, it will offer recommendations for continuous eradication of dengue fever, yellow fever and chikungunya.

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DENGUE AND MALARIA: ETIOLOGIES OF ACUTE FEBRILE ILLNESS IN ABIDJAN, IVORY COAST, 2011-2012

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Dengue disease is endemic in most tropical areas; however, dengue burden is uncertain in Africa. A prospective study was conducted in two hospital settings in Abidjan, Ivory Coast, from December 2011 to December 2012 to estimate the proportion of dengue and malaria cases among febrile patients and to describe the clinical and virological features of confirmed dengue cases. One week per month, blood samples were taken from patients of all ages who presented to outpatients clinics with fever (≥38°C). Patients with fever for more than 7 days, or a fever of known origin, and patients with jaundice were excluded. Thick blood films were examined and anti-DENV IgM and reverse transcription-polymerase chain reaction (RT-PCR) were performed. Eight hundred and twelve (812) subjects were studied (48.3 % women and 51.7% men) with 46.4 % of patients aged less than 9 years old. Seven hundred ninety six subjects (796; 98%) were tested for anti-dengue virus IgM and RT-PCR and thick blood film tests were performed on 807 samples. Four hundred and nine subjects (409; 50.4%) were clinically diagnosed as malaria, and no dengue case was reported based on clinical diagnosis. Three febrile patients (0.4%) had laboratory-confirmed dengue with one sample positive for DENV-3 and 234 patients (29%) had laboratory-confirmed malaria. This study confirmed the presence of dengue virus in Abidjan outside of an epidemic. These results continue to question dengue transmission in Africa and stress the importance of laboratory capacity to ascertain dengue burden in Africa.

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STATISTICAL FITS OF MINIMAL WITHIN-HOST MODELS PROVIDE INSIGHTS INTO VIROLOGICAL DIFFERENCES BETWEEN DENGUE SEROTYPES

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Dengue infections range in severity from asymptomatic infection to life-threatening dengue hemorrhagic fever and dengue shock syndrome. Though dengue pathogenesis is still poorly understood, epidemiological studies have shown that a heterologous secondary infection and the infecting serotype are important risk factors. Here, we present mathematical within-host models of primary and secondary dengue infections that are the first to quantitatively describe how the interaction of the immune system with dengue virus leads to high cytokine production that impacts the probability of manifesting severe disease. Statistical fits of these models to viral load data from a clinical cohort of patients infected with dengue serotypes 1, 2, or 3 yield two hypotheses for important virological differences between dengue serotypes: (1) serotypes differ in their infectivity of host cells; and (2) serotypes differ in their ability to subvert viral clearance mechanisms. We show that dengue disease risk critically depends on which of these two hypotheses are at play. This work highlights the complex relationship between serotype-specific dengue viral load patterns and the risk of developing disease, and the critical need for viral load data early on in infection to discriminate between these hypotheses.

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DESIGNING MULTIFACETED DENGUE SURVEILLANCE SYSTEMS

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Dengue is a mosquito-borne viral disease that affects millions of people every year. Timely and accurate surveillance of dengue at multiple geopolitical scales is critical to prevention and control. Despite their clear importance, surveillance systems are often shaped by historical, logistical and economic constraints rather than optimized to address specific objectives. Here, we designed and evaluated a sentinel surveillance system to monitor regional, island-wide, and serotype-specific dengue incidence in Puerto Rico. Using 15 years of historical clinic-level dengue data, we identified the subset of clinics that best achieves these diverse surveillance objectives. The optimal group of 22 clinics identified by our methodology is expected to be almost as informative as the entire system of 105 clinics, and more informative than subsets of clinics chosen using alternative criteria such as patient volume and geographic diversity of clinics or patients. In out-of-sample validation, the optimized system captured more than 78% of the spatiotemporal variation for each objective: 86% for serotype-specific incidence, 78% for regional incidence, and 97% for island-wide incidence. In general, our data-driven selection method can identify sentinel surveillance sites that robustly achieve diverse public health objectives.

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TEMPERATURE ALTERS DENGUE VIRUS BLOCKING BY *WOLBACHIA* IN MOSQUITOES

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Up to half of the world's population is at risk of contracting dengue, a disease caused by the dengue virus (DENV) and transmitted by mosquitoes. ***Wolbachia pipientis***, an obligate intracellular bacterium, is being developed as a biocontrol strategy against dengue because it limits replication of the virus in the mosquito. The ***Wolbachia*** strain ***w***Mel, which has been stably introduced into the mosquito vector, ***Aedes aegypti,*** has been shown to invade natural mosquito populations and spread to near fixation in field releases. However, conditions in the field can differ substantially from those in the laboratory and environmental factors such as temperature affect the infection and transmission of DENV in mosquitoes. Recently, the diurnal temperature range (DTR), which reflects the variation and fluctuation in temperature that occurs from the highs and lows during the day was found to significantly change the outcome of infection of the mosquitoes as compared to a constant temperature that is normally used in laboratories. Here, we studied the dissemination and transmission rate of DENV in ***A. aegypti*** under three temperature regimes (25±0°C constant, 25±4°C diurnal and 28±4°C diurnal). Firstly, we found that the constant temperature of 25±0°C overestimates the dissemination rates and transmission potential of the virus as compared to a diurnal temperature of 25±4°C. Raising the baseline of the diurnal temperature from 25±4°C to 28±4°C enhances the dissemination rates and transmission potential of the virus in the mosquitoes. Secondly, ***Wolbachia*** mediated virus blocking in terms of DENV infection rate in mosquito head and head DENV titer were affected by temperature (ie. temperature x ***Wolbachia*** effect). More specifically, a higher temperature resulted in a greater reduction of ***Wolbachia***-infected mosquitoes achieving dissemination. Lastly, higher temperature also significantly reduced ***Wolbachia*** density in the head of the mosquitoes. Our study raises the importance of not confining the study of vector-pathogen interactions to a constant rearing temperature of 25°C.

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SUBSET DISTRIBUTION AND PARTIAL MATURATION OF DENDRITIC CELLS DURING ACUTE DENGUE INFECTION

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Dendritic cell (DC) is considered as a cellular target for dengue virus. Dengue viral infection may block full phenotypic and functional maturation of DC resulting in change in the ratio of DC subsets which correlates with disease severity and might be the cause of the failure to induce protective adaptive immunity. The study to investigate distribution of DC subsets, maturation of DC during acute dengue infection and its potential function during the course of infection are warranted. In this study, flow cytometric analysis of the frequency and maturation stage of myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) were conducted. To study the change in DC subsets in the peripheral blood of dengue infected patients, the patient blood samples were determined for phenotypic characterization of mDCs and pDCs. mDCs were identified as cells that are lin-/DR+/CD11c+/CD123- whereas pDCs were identified as a gated population of cells that are lin-/DR+/CD11c-/CD123+. Results showed that while a transient increase in the frequency of pDCs were observed in some patients, mDCs were a major population presented during acute infection. To determine the maturation of mDCs and pDCs, a panel of monoclonal antibodies against CD86/CD83/CD40/CD80 was used. While no significant expression of maturation marker was observed for pDCs, the result showed high expression frequency of maturation markers including CD40 and CD86 for mDCs. In contrast, the expression of CD80 and CD83 could not be observed. To identify subpopulations of mDCs, a panel of monoclonal antibodies against CD1b/CD141/CD16 was used. While CD1b, CD141 and CD16 were used to identify different mDCs subsets, only CD16+ mDCs and CD141+ mDCs were observed at high frequency. Taken together, the results showed the presence of specific subpopulations of mDCs and a partial maturation of mDCs were induced during acute dengue infection. More importantly, the data obtained in this study suggested for a rationale design of a novel dengue vaccine with an aim to enhance DC maturation in order to induce a protective immune response against dengue viral infection.

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PHASE 1 STUDY OF A TETRAVALENT DENGUE PURIFIED INACTIVATED VACCINE (DPIV) IN HEALTHY U.S. ADULTS: SAFETY AND IMMUNOGENICITY RESULTS THROUGH MONTH 13 AND AFTER A BOOSTER DOSE IN A SUBSET OF SUBJECTS

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We report safety and immunogenicity of an investigational tetravalent DPIV up to Month 13 (M13) and after a booster dose in Year 2 (Y2). In this Phase 1, observer-blind study (NCT01666652), 100 healthy adults in the continental United States were randomized 1:1:1:1:1 to receive saline placebo or 1 of 4 DPIV formulations (1µg of each dengue virus [DENV] type adjuvanted with either aluminum hydroxide [Alum], $AS01_E$ or $AS03_B$, or 4µg of each DENV type adjuvanted with Alum) at Day (D) 0 and D28. A subset of 4µg+Alum (n=3) and 1µg+$AS01_E$ (n=6) recipients received a booster dose (same formulation) 15–21M post-dose 2. Subjects were followed up for serious adverse events (SAEs), potential immune-mediated diseases (pIMDs) and medically-attended AEs (MAEs). Solicited and unsolicited AEs were monitored respectively for 7D and 28D post-booster. Neutralizing antibody titers were determined by microneutralization assay (MN50). Four SAEs were observed through M13 (3 in 1µg+$AS03_B$; 1 in 1µg+$AS01_E$); none were related to vaccination. No pIMDs and 11 MAEs were reported. In the 9 booster recipients, 1 grade 3 solicited AE was reported (muscle aches, 1µg+$AS01_E$); no unsolicited AEs, pIMDs or SAEs were reported. The M13 per-protocol cohort for immunogenicity included 84 dengue-naïve subjects; the dose 3 cohort included all 9 subjects. Geometric mean antibody titers (GMTs) waned from D56 to M7 then stabilized through M13 in all DPIV groups. M13 GMTs against DENV-1, -2, -3, -4 ranged from 5.3–7.9 (1µg+Alum), 7.3–13.2 (4µg+Alum), 9.3–36.7 (1µg+$AS01_E$), 8.2–24.9 (1µg+$AS03_B$), and 5.0 (placebo). Booster recipients had rapid rises in MN50 titers: 1M post-booster, median MN50 titers against DENV-1, -2, -3, -4 were, respectively, 4421, 3909, 5662, 20370 (1µg+$AS01_E$), and 7570, 3989, 3747, 20390 (4µg+Alum); 6M post-booster, these were 1115, 806, 1290, 792 (1µg+$AS01_E$), and 436, 625, 936, 700 (4µg+Alum). All DPIV formulations at D0 and D28 were well tolerated with favorable safety profiles up to M13, and induced balanced immune responses. GMTs stabilized from M7 through M13, and a booster in Y2 led to strong anamnestic responses. Whether a booster is required remains to be determined.

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REGULATORY EFFECTS ON THE SYNTHESIS OF DENGUE VIRAL E PROTEIN DURING THE UNFOLDED PROTEIN RESPONSES (UPR) IN MOSQUITO CELLS

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Dengue fever and dengue hemorrhagic fever/dengue shock syndromes are increasing in their importance as a life-threating infectious disease in the world, particularly in tropical and subtropical areas. Dengue virus is its etiological agent and is naturally transmitted by ***Aedes*** mosquitoes between humans. As a result, the virus is able to replicate in both mammalian and mosquito cells. However, unlike in mammalian cells which usually end up with apoptosis in response to dengue virus infection, mosquito cells usually survive the infection with trivial damage to the infected cells. It facilitates that the invaded virus forms a huge number of progeny virions in mosquito cells; in the meantime, virus-induced endoplasmic reticulum (ER) stress is usually induced due to the unfolded protein responses (UPR) in infected cells. We have demonstrated that BiP/Grp78 is upregulated and eventually involved in viral E protein folding during dengue virus infection in mosquito cells. In addition, spicing of X-box-binding protein-1 (XBP1) is found to be activated, leading to promotion of protein disulfide isomerase (PDI) expression and thus appropriate synthesis of viral E protein. This study provides evidence to elucidate how disulfide bond-containing viral proteins may be formed via a collaborative modulation of chaperones and transcription factors.

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HYPOXIA FAVORS ANTIBODY DEPENDENT DENGUE

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Dengue virus (DENV) enters humans through the infective saliva of a blood-feeding ***Aedes*** mosquito. This virus is thought to rapidly infect dendritic and Langerhans cells, which then migrate to draining lymph nodes. There, DENV is amplified before spread to other target organs, such as the spleen and liver. Lymph nodes, spleen and liver are hypoxic under homeostatic conditions. Previous studies have shown that hypoxia can modify the transcriptome of human monocytes affecting its immunoregulatory responses and could therefore have profound but yet unexplored effects on DENV infection. We report here that, compared to cells cultured at 20% O2 (normoxia), infection of THP1 and primary

monocytes at 3% O2 (hypoxia), which is the reported O2 levels in lymph nodes, produced 2-fold more infectious DENV. Interestingly, the protein levels of FcγRIIA but not FcγRIIB is up-regulated under hypoxic conditions. The differential expression of these FcγR explains our observed increased uptake of DENV immune complexes and requirement for higher antibody concentration to fully neutralize DENV in monocytic cells cultured under hypoxic compared to normoxic conditions. These findings suggest that FcγRIIA expression is under the control of hypoxia, for which hypoxia-inducible-factor 1a (HIF1α) plays a major role in regulating the cellular response to hypoxia. Indeed, treatment of THP1 cells with desferrioxamine (DFX) to inhibit the degradation of transcription factor HIF1α under normoxic conditions resulted in increased FcγRIIA expression and DENV immune complex uptake. Finally, using high resolution microscopy, we show that FcγRIIA directly mediates internalization of DENV immune complexes. Collectively our data indicates that the host response to antibody dependent DENV infection in lymphoid organs that are physiologically under hypoxic conditions is fundamentally different from those observed ***in vitro*** under normoxia.

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SERUM ANTIBODY AVIDITY AND SUBCLASS IN SUBCLINICAL AND SYMPTOMATIC DENGUE VIRAL INFECTIONS

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Existing assays for dengue virus infection, including virus neutralization, are poorly predictive of clinical protection from infection and disease. The objective of this study was to evaluate the utility of serotype-specific serum antibody avidity and IgG subclass in predicting protection from clinical illness following DENV infection. Serum samples were collected from participants in longitudinal cohort and community-based febrile surveillance studies in Iquitos, Peru. We compared serotype-specific neutralization titers, IgG avidity (as measured by immunoassay), and IgG subclass titers (as measured by immunoassay) in pre- and post-infection samples from individuals with symptomatic or subclinical DENV infection. Based on preliminary analysis of DENV-3 and DENV-4 infections, there was a modest but statistically significant correlation between post-infection neutralization titers and IgG avidity. In pre-infection samples, IgG avidity was increased among subclinical infections compared with symptomatic infections. For IgG subclasses, IgG1 was the dominant subclass of antibodies, followed by IgG4; however, we did not observe clear relationship between titers of specific IgG antibody subclass and outcome from infection (i.e., subclinical or symptomatic). Based on our preliminary analysis, IgG avidity warrants further evaluation as a marker of protection. Potential implications for dengue epidemiological studies and vaccine candidate evaluation will be discussed.

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A COMPARATIVE STUDY ON ACTIVE AND PASSIVE EPIDEMIOLOGICAL SURVEILLANCE IN FIVE LATIN AMERICA COUNTRIES

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Dengue is a public health problem that concerns more than 100 countries in the world. Usually the disease burden estimates come from the National Epidemiological Surveillance Systems (NESS), but the depth and breadth of the system, as well as the quality of the data are often criticized. We aim to describe main characteristics of NESS in five countries in Latin America (Brazil, Colombia, Honduras, México and Puerto Rico) where a clinical trial was conducted, and contrast the NESS data with the placebo arm of the trial data to better understand differences in dengue burden. NESS data on incidence of suspected and/or confirmed dengue cases by age group and by different geographical levels, if available, were extracted for the period between 2011 and 2014. Incidence rate of confirmed dengue fever (DF) cases ranged from 0.01% per year in Honduras to 0.31% in Brazil. The incidence rate of DHF had a great variability and ranged from 35 per 100,000 population in Honduras to less than 1 per 100,000 population in Puerto Rico and Brazil. The Phase III randomized, placebo-controlled dengue vaccine trial (CYD15) prospectively collected data from children aged 9-16 years and followed up between June 2011 and April 2014. 6,939 children from the placebo arm were included in the analysis. There were 389 virologically confirmed DF cases from 3,617 febrile episodes (2.84 per 100 person-year), ranging between 1.5 in Puerto Rico and 4.1 in Honduras. Ten DHF cases were found, for an incidence of 0.07 per 100 person-year. Rate difference in the similar age groups between CYD15 placebo group and NESS at the national level ranged between 16x in Brazil and 61x in México. At lower geographical level (state) were 6.9x in Brazil and 17.6x in Mexico, and in city (site) level were 3.9x in Brazil and 12.6x in Mexico . The rate differences highlight that dengue burden data depends heavily on case definitions and clinical assessment. Our results help to better understand the clinical burden of dengue disease in these 5 countries and contribute to the WHO objective to estimate the true burden of dengue disease by 2015.

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T CELL IMMUNITY TO VACCINATION WITH A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE CANDIDATE IN NON HUMAN PRIMATES

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Dengue virus (DENV) causes a rapidly spreading mosquito-borne human viral disease that has major impact on global health and economics. Currently, there is no licensed vaccine against DENV. We have developed a live attenuated tetravalent dengue vaccine candidate (TDV) based on an attenuated dengue 2 virus (TDV-2) and three chimeric viruses containing the pre-membrane and envelope genes of DENV-1, -3 and -4 expressed in the context of the attenuated DENV-2 genome (TDV-1, 3, and -4, respectively). In this study we sought to characterize the cellular responses elicited by the TDV backbone in non-human primates (NHPs), identify the target proteins of this response, and determine their multifunctional and cross-reactive nature. Using peptide arrays and intracellular cytokine staining, we demonstrated that the vaccine elicits $CD4^+$ and $CD8^+$ T cell responses targeting the non-structural NS1, NS3 and NS5 proteins of DENV-2. Both T cell subsets produced IL-2, IFN-γ, and TNF-α, and were multifunctional in nature. In addition, $CD8^+$ T cells expressed the CD107a marker, and exhibited cross-reactivity with the NS proteins of the other three DENV serotypes. Overall, these findings highlight the immunogenic profile of TDV vaccine candidate and support the further evaluation of clinical samples from ongoing phase II clinical trials.

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DENGUE IGG/IGM IN FEBRILE PATIENTS SUSPECTED TO HAVE MALARIA IN LAGOS, NIGERIA

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Malaria is the most suspected cause of febrile illness in Nigeria not dengue as there are dearth of information on dengue in Nigeria though dengue exists in West Africa especially in Côte d'Ivoire. Malaria and dengue are the most common arthropod-borne diseases in humans exhibiting

similar geographic distribution and clinical presentation. The objective of this study was to screen suspected patients who presented with fever at Badagry general hospital, Regina Mundi hospital, Mushin, Randle general hospital, Suru-lere, and Igando general hospital facilities in Lagos, southwest, Nigeria, for malaria using microscopy and dengue by using the ELISA IgG and IgM. A total of 247 children and adult patients were screened at presentation: The patients presented with the following symptoms: temperature ≥37°C [(13.9%)], history of fever in the last 48hour 34 (52.5%), chills (43.1%), loss of appetite (42%), headache (48%), and weight loss (28.2%). 14 (5.7%) were positive for malaria and 9 (3.70%) for dengue IgG while 3 (1.2%) were positive for dengue IgM. Of the dengue IgG positives, only 1 patient showed positivity with further IgM capture ELISA test (MAC-ELISA). There were no cases of malaria and dengue co-infections. Both malaria and dengue showed statistical association with fever but not with sex or age. In order to further compare the relationship between malaria and dengue IgG and IgM using chi-square test, no statistical significant relationship existed between the two infections. Similarly, there was also no statistically significant relationship between malaria and dengue IgM infections. Dengue fever which is regarded as one of the most important mosquito-borne viral disease is clinically difficult to diagnose at the early stage especially in developing countries and could be mistaken for malaria. Attention is currently not focused on dengue at the moment in Nigeria. An expanded study is suggested to document possible dengue as well as confirmatory tests on capture IgM ELISAs so that cases could be detected early when they present as well as encouraging proper surveillance.

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LONG TERM DENGUE DISEASE PATTERNS IN BANGKOK, THAILAND: 1973 TO 2012

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Since 1962, Queen Sirikit National Institute of Child Health (QSNICH) and the Armed Forces Research Institute of Medical Sciences (Armed Forces Research Institute of Medical Sciences) have engaged in collaborative studies of dengue. Here, we analyze 40 continuous years of dengue surveillance from 1973 to 2012. We describe long-term trends in dengue disease including serotype predominance, age distributions, relative proportions of primary and secondary infections, and disease severity. Data were analyzed from 25,715 patients with laboratory-confirmed DENV infection admitted to QSNICH. DENV-1 and DENV-2 predominated for long periods, while DENV-4 has never reached the same peaks. In recent years, all four serotypes have more consistently circulated at the same time. The mean age of dengue cases increased from 7.12 to 8.14 years old in our study consistent with a decrease in force of infection. There was also an increase after 1990 in the proportion of cases that were secondary for DENV-1 and DENV-3. An overall decrease in disease severity occurred during the 40 year study period. Concurrently, a decrease in the proportion of dengue cases that were DHF and DSS in secondary infections with increasing age was observed after nine years old. We shed light on how this dengue epidemiology has interacted with other changes in the population such as demography and healthcare changes. Our findings may inform similar changes that may only now be occurring in other countries.

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MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUS CIRCULATING IN BANGKOK, THAILAND, 2003-2013

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Dengue virus (DENV) is the most prevalent arbovirus globally and is hyperendemic in Southeast Asia (SEA). Thailand is considered a potential epicenter for transmission of all four DENV serotypes (DENV-1 to 4) throughout SEA. Previous studies of the molecular epidemiology and evolution of DENV in Thailand were conducted by phylogenetic analyses using samples collected from 1973-2002. In this study we use recent and current DENV strains circulating in Thailand to update our DENV genetic diversity phylogenetic analyses. Envelope gene sequences from 287 DENV isolates obtained from Bangkok in 2003-2013 were evaluated. Phylogenetic analysis revealed genotype I (DENV-1), Asian I (DENV-2), genotype II (DENV-3), and genotype I (DENV-4) to be the major circulating genotypes during the study period. Clade extinction and replacement events were found for all serotypes. The highest viral diversity was found in DENV-3 with three genotypes detected: I, II, and III. The re-emergence of DENV-3 genotype III was identified in 2009 and this genotype has been co-circulating with genotype I in recent years. Only one DENV-3 genotype II strain was detected in 2012 and was likely imported from a neighboring country. Our DENV genotypic analysis provides the necessary baseline to help monitor the arrival of emerging new strains.

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HUMAN PLASMABLAST RESPONSES TO SECONDARY DENGUE INFECTION

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Despite the massive disease burden and pressing need for antivirals and vaccines against dengue, the immunology of dengue virus (DENV) infections remains poorly understood. In this study, we describe B cell responses generated during the acute phase of DENV infection. Our lab has previously shown that a large population of DENV-specific plasmablasts appears in the blood of dengue patients around the time of fever subsidence. To understand the functional properties of these acute-phase cells and what role the antibodies they make play in an immune response, we isolated plasmablasts from 4 Thai patients experiencing secondary DENV infection and generated 53 monoclonal antibodies (mAbs) by single-cell RT-PCR and cloning of the plasmablast VDJ genes. We determined that a large majority of the DENV envelope-specific mAbs in our panel was either fully (4 serotypes) or partially (2-3 serotypes) cross-reactive, with a large majority also exhibiting cross-neutralizing activity ***in vitro***. Serotype-specific neutralizing mAbs represented <20% of the entire mAb panel. Interestingly, more than half of the mAbs generated from two patients displayed stronger neutralization of DENV1 than DENV2 even though they were diagnosed with DENV2 at the time of sample collection. This is reminiscent of original antigenic sin, given all patients had prior DENV exposures. Further, a majority of serotype-specific neutralizing mAbs either moderately or potently enhanced DENV infection of U937 cells indicating that the potential for ADE is not limited to cross-reactive mAbs.

These initial characterizations of plasmablast-derived mAbs give insight into the specificity and function of early antibody responses in dengue infection at a single-cell level.

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EFFECT OF REPEAT HUMAN BLOOD FEEDING ON *WOLBACHIA* DENSITY AND DENGUE VIRUS INFECTION IN *AEDES AEGYPTI*

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Wolbachia is an endosymbiotic bacterium that has been introduced into ***Aedes aegypti*** to limit the replication of dengue virus (DENV) in the vector. In both mosquito cell lines and in whole mosquitoes higher ***Wolbachia*** infections show increased DENV blocking. ***Wolbachia*** density is known to be affected by several factors including host nutrition. Since ***Ae. aegypti*** are "sip" feeders returning often to obtain blood meals there was a need to assess whether a relationship exists between ***Wolbachia*** densities and human blood feeding as this could lead to greater DENV inhibition over the life of the mosquito. The ***w***Mel ***Wolbachia*** infected ***Ae. aegypti*** line and the Wildtype ***Ae. aegypti*** mosquito which is not infected with ***Wolbachia*** were concurrently reared for this study. There were three treatment groups for each mosquito line; a control group which was not fed human bloodmeal, a second group which was given one human bloodmeal and a third group which was given two successive human bloodmeals two weeks apart. Apart from the controls which were not blood fed, all mosquitoes were first given human bloodmeal 5 days post eclosion. The mosquitoes in the third treatment group were given a second human blood meal 12 days post eclosion. All the three treatment groups were orally infected with DENV simultaneously 19 days post eclosion. The midguts and salivary glands which are the tissues necessary for infection and transmission of DENV in the mosquito, were dissected from each individual mosquito 10-11 days post infection. RNA/DNA was then simultaneously extracted from each dissected tissue and carcass/remainder of the mosquito body for DENV RNA copies and ***Wolbachia*** density quantification respectively. We found no clear evidence that ***Wolbachia*** density increased with feeding and therefore saw no corresponding improvements in DENV blocking. Hence ***Wolbachia***-based DENV blocking should be stable with respect to mosquito feeding cycle.

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KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING DENGUE AND ITS SOCIODEMOGRAPHIC DETERMINANTS IN COLOMBIA: A MULTIPLE CORRESPONDENCE ANALYSIS

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During the last decades, a number of studies have been analyzed the knowledge, attitudes and practices (KAP) of the population regarding dengue. However, none of them have applied multivariate geometric data analytic techniques to generate indexes from KAP domains. Likewise, results of such analysis have not been used in order to determine the potential effects of sociodemographic variables on the levels of KAP. The objective was to determine the sociodemographic factors related to different levels of KAP regarding dengue in two hyper-endemic cities of Colombia using a multiple correspondence analysis (MCA). In the context of a Cluster Randomized Trial, 3998 households were surveyed in two Colombian cities between 2012 and 2013. To generate indexes of KAP we performed a MCA followed by a hierarchical cluster analysis to classify each score in different groups (from less to more score). A quantile regression analysis for each score group was conducted considering fixed effects. Indexes explained 56%, 79% and 83% of the variance of knowledge, attitudes and practices domains with means 4.2, 1.4 and 3.2, respectively. The highest values of the index denoted higher levels of knowledge and practices while the attitudes index did not show the same relationship and was excluded from the analysis. In the quantile regression, age 0.06 (IC95% 0.038, 0.076), years of education 0.15 (IC95% 0.09, 0.2), and history of dengue in the family 0.21 (IC95% 0.15, 0.27) were positive related to lower levels of knowledge. However, the effect of such factors gradually decreases or disappears when knowledge was higher. Only decision-making about family health care increased knowledge score in the higher quantile. Practices indexes did not evidence correlation with sociodemographic variables. Multiple Correspondence Analysis is a new useful tool for the analysis of knowledge and practices regarding dengue from KAP questionnaires because allows for the transformation of several categorical variables into a single coherent index. Moreover, the magnitude of the effect of sociodemographic variables in the knowledge scores varies according to the levels of knowledge

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HEALTH SEEKING BEHAVIOR AND TREATMENT INTENTIONS OF DENGUE AND FEVER: A HOUSEHOLD SURVEY OF CHILDREN AND ADULTS IN VENEZUELA

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Dengue in Venezuela is a major public health problem with an increasing incidence of severe cases. Early diagnosis and treatment influences the outcome of dengue illness, as delay in care-seeking is associated with severe dengue. We aimed to understand patterns of health seeking behaviour (HSB) in individuals exposed to high dengue transmission in order to improve early attendance to health centres. Between September 2013 and February 2014 a cross-sectional household survey was performed in Maracay, Venezuela. Intended HSB of adults and parents/guardians was assessed. Data was collected through structured questionnaires from 105 individuals. In the case of suspected dengue, most people (60%) would choose to first seek medical help versus first treating at home, in contrast to 11% in the case of fever . Amongst those who decided to visit a doctor, a suspected dengue infection would prompt them to search medical help earlier than if having fever ($p<0.001$). Multivariate analysis of the determinants associated with the intention to firstly visit a doctor versus treating at home in the case of dengue showed that feeling at risk prompted people to first seek medical help (OR=3.29; p=0.042). Determinants of first treating a dengue infection at home were: deciding in the conduct of a child (as opposed to an adult) (OR=0.30, p=0.021), reporting a previous dengue infection (OR=0.29; p=0.031) and living in the neighbourhood Caña de Azúcar (OR=0.28, p=0.038). Understanding the patterns of HSB helps target dengue control interventions. Improving awareness and dengue disease recognition may enhance early attendance to medical care of affected populations and thereby reduce mortality and the development of severe illness. Especially for those with a previous dengue infection, efforts have to be made to promote prompt health centre attendance.

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ANALYSIS OF CLONAL LINEAGES OF DENGUE VIRUS ENVELOPE PROTEIN SPECIFIC ANTIBODIES FROM A SINGLE PATIENT

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The humoral immune response to dengue infection plays a key role in homotypic immunity and, theoretically, in the antibody dependent enhancement of infection during heterotypic, secondary infections. Our understanding of the human anti-dengue antibody repertoire is limited. The goal of this study was to determine the genetic diversity of anti-dengue envelope proteins produced by a single patient. Human monoclonal antibodies (HMAb) targeting the DENV envelope protein were generated by molecular cloning and characterized. All antibodies where tested for neutralizing and enhancing activity. Epitope mapping was done using shotgun mutagenesis. The heavy and light chain variable regions of each HMAb were then sequenced and analyzed using IMGT/V-QUEST and Cloanalyst software programs. All hMAbs bound to the E protein of one or more DENV serotype. Epitope mapping studies revealed that all of the hMAbs targeted epitopes located on Domain II (DII) of the E protein. Functional characterization of these hMAbs revealed extensive cross-reactivity among the four DENV serotypes as well as marked heterogeneity with regards to relative binding affinity and neutralization and enhancement potential. Categorizing individual hMAbs into three distinct epitope classes revealed that hMAbs within a particular epitope class shared similar functional characteristics. Analysis of VDJ genes encoding the heavy and light chain variable regions revealed three separate lineages that closely matched the groupings based on functional characteristics. Clonal analysis of hMAbs from a single patient revealed distinct lineages of broadly neutralizing, weakly neutralizing and non-reactive hMAbs that bind to adjacent regions of the E protein. These results could impact vaccine design, ie development of sub-unit vaccine that will stimulate distinct population of memory B cells.

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EBOLA PREPAREDNESS IN MALAWI: NATIONWIDE HEALTH EDUCATION USING A SHORT MESSAGE SERVICE (SMS) ON MOBILE PHONES

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Novel approaches are needed to rapidly educate whole populations on Ebola prevention in the face of the epidemic. Since September 2014, an SMS (Short Message Service) health information delivery platform called Moyo Wanga was introduced across Malawi to disseminate information on Ebola and other diseases in English and the local language, Chichewa. Malawi has a population of 14million with 5 million cellphone users. A database of over 300 SMS texts covering Ebola, HIV/Aids, Tuberculosis and other Tropical diseases were placed on a server. The server was connected to the three major cellphone networks in Malawi using an internet VPN (virtual private network). Using any type of cellphone, a user dials one shared code for any of the three networks to access the Moyo Wanga database and selects an SMS using drop down menus. The platform also has a dialogue facility that allows a user to send back questions and a physician to offer solutions. Beginning with the initial 7,700 SMS's downloaded in the first 2 weeks, the service continues to show ever increasing downloads. With people sharing SMS's within and across networks health information is disseminated rapidly across the country. Mobile technology using SMS's is being used effectively to rapidly disseminate crucial preventive information about Ebola and other diseases in Malawi. Knowledge of the transmission and preventive steps are critical in stopping Ebola spread to Malawi and other countries where the disease has not reached.

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INFLUENZA SEROCONVERSION RATES IN A COHORT OF YOUNG CHILDREN, BANGKOK, THAILAND

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Influenza causes a substantial burden of disease in young children. In 2011, we began enrolling a cohort of children aged <36 months to estimate the burden of influenza in Bangkok, Thailand. Children with and without underlying conditions (e.g., low birth weight, respiratory, cardiopulmonary and neurological disease) who sought care at Queen Sirikit National Institute of Child Health were followed for 2 years. Serum samples were collected every 6 months from children enrolled within the first 6 months of birth and tested by hemagglutination inhibition (HI) assay using representative of influenza viruses circulating at the time of the study (A/California/07/2009 (H1N1), A/Victoria/361/2011 (H3N2), B/Brisbane/60/2008, and B/Wisconsin/1/2010). Seroconversion was defined as >4-fold rise in HI titers in 2 consecutively collected serum samples. We excluded vaccinated children and reported preliminary finding of seroconversion rates by high-risk and healthy children. Between August 2011 and September 2013, 63 (34 healthy and 29 high-risk) of the 299 enrolled children have been tested to date. The median age at baseline blood collection was 3.9 months (range, 0.5-6.3) and it did not differ between 2 groups (p=0.76). No children had influenza seroconversion during the first 6 month of age. Over 2 years, 16 healthy and 11 high-risk children seroconverted (2.6 vs. 2.0/100 person-months [PM]; p=0.50). Seroconversion rates were nonsignificantly higher in healthy than in high-risk children during >6-12 months (2.1 vs. 0.6/100 PM; p=0.26), >18-24 months (3.1 vs. 2.7/100 PM; p=0.86) and >24-30 months (7.3 vs. 2.4/100 PM; p=0.36) periods. The rates were nonsignificantly lower in healthy than in high-risk children during >12-18 months period (3.0 vs. 4.0/100 PM; p=0.65). Six (37%) healthy and one (9%) high-risk children who seroconverted reported no respiratory symptoms within 1 month before seroconversion. Influenza seroconversion within the first 2 years of life was detected. There was no difference in the rates of seroconversion between high-risk and healthy children; however, the sample size was small. Asymptomatic infection was observed.

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RAPID DETECTION OF ALL KNOWN EBOLA VIRUS SPECIES BY REVERSE TRANSCRIPTION LOOP MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) ASSAYS

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Ebola virus disease (EVD) is a highly virulent infectious disease caused by ***Ebola virus*** and with a case fatality rate ranging from 25-90%. Since the first outbreak in 1976, EVD has been characterized by sporadic outbreaks in different parts of Africa with the current outbreak in West Africa being the latest. Limitation of spread of outbreaks therefore relies on accurate diagnosis and quarantine of cases. Reverse transcription-loop mediated isothermal amplification (RT-LAMP) is a nucleic acid amplification method which amplifies nucleic material using DNA polymerase with

strand displacement activity under isothermal conditions. Five sets of six oligonucleotide primers were designed for specific identification of each of the five species of ***Ebolavirus*** using PrimerExplorerV4, a LAMP primer design software. The limits of detection of the ***Ebolavirus*** species-specific primer sets were evaluated using ***in vitro*** transcribed RNAs. Comparison between each ***Ebolavirus*** species-specific RT-LAMP assays and RT-PCR and qRT-PCR was done using viral RNA of each species. The lowest detection limit of species-specific RT-LAMP assays for Zaire (EBOV), Sudan (SUDV), Tai Forest (TAFV), and Reston (RESTV) ebolavirus was 410, 320, 140, or 62 copies/reaction, respectively, and the detection time (measured in minutes and given as a mean ± standard deviation of 3 different experiments) for each of the species-specific RT-LAMP assays was 16.8 ± 1.2, 13.9 ± 0.9, 16.5 ± 1.4, or 19.0 ± 1.1, respectively. EBOV species specific RT-LAMP assay had a better sensitivity than qRT-PCR using ENZ-FP, ENZ-RP, and ENZ-P, while it had a similar sensitivity with qRT-PCR using enp-F, enp-R, and enp-P. EBOV RT-LAMP assay was also more sensitive than the nested RT-PCR assay by a factor of 10. SUDV RT-LAMP assay had a similar sensitivity with qRT-PCR. TAFV and RESTV RT-LAMP assays were more sensitive than the conventional RT-PCR assay by a factor of 100 and 10, respectively. ***Ebolavirus*** species-specific RT-LAMP assays show promise and could become an important diagnostic tool for the detection of EVD.

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BOOSTING THE NOVEL EBOLA VACCINE CANDIDATE CHAD-EBOV Z WITH MODIFIED VACCINIA VIRUS ANKARA (MVA) SIGNIFICANTLY ENHANCES EBOLA-SPECIFIC ANTIBODY RESPONSES

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The ongoing Ebola virus outbreak in West Africa is the largest and most complicated to date with over 23,000 cases and almost 10,000 deaths. There are currently no licensed vaccines for Ebola, although several candidates are in clinical trials. Despite falling incidences, an effective vaccine may still be necessary to contain the current Ebola outbreak and would be a significant component of the response effort to future outbreaks. The viral vector vaccine Chimpanzee Adenovirus 3 (ChAd3) encoding Zaire Ebolavirus surface glycoprotein (EBOV GP) has shown efficacy in non-human primate studies and was selected for rapid assessment in clinical trials. We previously conducted a Phase 1 clinical study of this vaccine in 60 healthy UK adults to assess safety and immunogenicity. The single-dose vaccine induced only moderate anti-EBOV GP antibody titres that were significantly lower than those previously seen in protected macaques. We conducted a Phase 1 clinical trial to assess the impact of a heterologous viral vector boost on the immune response primed with ChAd3-EBOV Z. In this study 30 individuals were boosted with an MVA encoding Zaire GP and 3 additional filovirus antigens. Anti-EBOV GP titres peaked 2 weeks post-boost with geomean titres 7-fold higher than the peak post-prime. This is around the level that was previously shown to be protective in macaques after vaccination with rAd5 expressing Ebola Glycoprotein. At 2 weeks post-boost 100% of individuals had neutralising titres against the Mayinga strain of Ebola, compared to just 43% at the peak post-prime. Ebola-specific antibody titres and neutralising activity induced by ChAd3-EBOV Z can be significantly enhanced by boosting with a heterologous viral vector vaccine. Assessments of these vaccines are ongoing and a large-scale efficacy trial of this prime-boost regimen is planned to begin in West Africa shortly.

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EVALUATION AND INTRODUCTION OF REAL TIME PCR NEW ASSAYS FOR THE DIAGNOSTIC AND SURVEILLANCE OF HUMAN RESPIRATORY VIRUS

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This study proposed the evaluation of four multiplex real time PCR for the diagnosis of 15 human respiratory viruses causing acute respiratory infection, as well as the introduction of the optimized system for the diagnosis and laboratory surveillance. For the optimization of multiplex real time PCR 352 nasopharyngeal swabs from July-August 2013 were used, multiplex RT-PCR was used as ¨gold standard¨ technique. Sensitivity, specificity, positive and negative predictive values and kappa index of multiplex real time PCR was determined. Efficiency of simple and multiplex real time PCR was calculated by calibration curve and slope determination. In addition, laboratory diagnosis in the period September 2013 to May 2014 with the optimized multiplex real time PCR was performed. Of the total of samples processed for optimization, 162 were positive by multiplex real time PCR and 112 by gold standard technique. Sensitivity, specificity, positive predictive value, negative predictive value and average kappa of the evaluation assays was 100%, 98.4%, 67%, 100% and 0.8, respectively. Furthermore, efficiency values for multiplex real time PCR systems were in the range 90.30 % to 103.09 % similar to those obtained by the simple system. Introduction of optimized assays allowed the detection of 1290 clinical samples positive for respiratory viruses, with the highest positivity percentage for human respiratory syncytial virus, 47.83%. In summary, multiplex real time PCR was more sensitive than multiplex RT-PCR and the efficiency values were similar to the simple real time PCR. These optimized systems allowed to update the algorithm for the diagnosis and surveillance of respiratory viruses in Cuba.

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IMPACT ON IMMUNOGENICITY OF VARYING THE INTERVAL BETWEEN THE PRIME AND BOOST OF A CANDIDATE EBOLA VACCINE CHAD3-EBO Z AND MVA-BN FILO IN HEALTHY UK ADULTS

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The current Ebola epidemic is the largest in history, resulting in more deaths than all previous outbreaks combined. Development of an effective vaccine would maximise safety of those at greatest risk of disease during outbreaks. A leading candidate vaccine strategy currently under development involves heterologous prime-boost immunisation with a chimpanzee Adenovirus (ChAd3) followed by a Modified Vaccinia Ankara virus (MVA), both containing a Zaire strain of the Ebola glycoprotein. Previous trials with viral vectored malaria vaccines have been administered in a prime-boost sequence with an interval of between 4 and 8 weeks, and resulted in very high T cell responses in addition to moderate antibody levels. There is no previous data showing the effect on T cell or antibody immunogenicity of reducing this interval between prime and boost

vaccinations to less than 4 weeks. We undertook a phase I study to assess the safety and immunogenicity of such a vaccination strategy involving healthy adults in the UK. 62 volunteers received ChAd3 followed by MVA, both containing a Zaire strain of the Ebola glycoprotein, at intervals between 1-10 weeks. IFN-γ production by T-cells was measured by ELISpot at various points throughout the trial. Intracellular cytokine staining was used to determine the relative proportions of CD4+ and CD8+ T-cells secreting IL-2, IFN-γ and/or TNFα in response to peptide stimulation. IgG responses were also measured by ELISA. While this trial is ongoing, preliminary data showed that a shorter interval was associated with an enhanced T-cell response, while there was no significant difference in antibody levels.

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EFFECTIVENESS OF A BRIEF, INTENSIVE, PHYLOGENETICS-FOCUSED, BIOINFORMATICS WORKSHOP IN A MIDDLE INCOME COUNTRY

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There is an increasing role for bioinformatic and phylogenetic applications in tropical medicine research. However, scientists often lack training to utilize these methods, with a paucity of accessible courses available in this field. To help address this training gap, we offered a brief, intensive bioinformatics workshop in Lima, Peru, in January 2015. To improve future workshops, we objectively measured participants' baseline knowledge in pathogen-applied bioinformatics and assessed workshop efficacy in improving such knowledge. We also sought to identify baseline and residual bioinformatic training needs. A 20-point written questionnaire was administered to all participants at the beginning and end of the five-day hands-on workshop covering knowledge domains of sequence quality control, alignment/formatting database retrieval, models of nucleotide evolution, sequence statistics, tree building, and results interpretation. Changes in median questionnaire scores and associations of score changes with previous bioinformatic experience were analysed using non-parametric tests. All 21 workshop participants were Peruvian and 52% had prior phylogenetic analysis experience. The mean years of scientific work/training were 4.9 (SD 3.4). Models of evolution/tree-building methods was the lowest scoring domain at baseline (median score 1/5, 20%) and after the workshop (median score 3/5, 60%). The greatest score gains were in results interpretation and models of evolution/tree-building methods ($p < 0.001$). There was considerable median gain in total knowledge scores (increase of 30%, 6 point gain, $p < 0.001$) with gains as high as 55% (11 point gain). Higher baseline median scores were seen in those with previous phylogenetic experience as compared to those without ($p = 0.04$). Despite the small sample size, the knowledge gained from the workshop was sufficiently large to be detected in this study. An intensive five-day workshop model appears to be effective in improving pathogen-applied bioinformatics knowledge of scientists working in a middle income country setting.

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STUDY THE EFFECTS OF INFLUENZA VACCINATION ON CHILDREN IN THAILAND BY USING NEXT GENERATION SEQUENCING

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Influenza vaccination has been practiced to prevent seasonal influenza infections in high risk groups including children and elderly. The effects of the influenza vaccines in the influenza virus (IFV) subpopulations have not yet well understood. Nasopharyngeal swaps collected from 18 children diagnosed with influenza infections by serology and molecular assays (with and without vaccination) were collected: 6 cases of influenza A H3N2, 6 cases of influenza A pdm H1N1/09 and 6 cases of influenza B. High-throughput sequencing was utilized to study the IFV subpopulations. A total of 22.3 millions of passed-filter sequence reads were identified IFV. Approximately 80.3%-95.9% had ≥Q30 quality score. Diverse depth of coverage (DOC) was observed with sequence alignment analysis against their corresponding influenza reference strains: A/California/07/2009, B/Wisconsin/01/2010 and B/Brisbane/11/2010. The DOC is found to be parallel to the amount of the IFV in the specimens. The nucleotide heterogeneity (measured numbers of variances or numbers of mixed bases in the genome), is utilized to determine population diversity. Nearly all genomic fragments of the 18 clinical specimens contain variances compared to the vaccine reference strains. Slightly less nucleotide heterogeneity were observed in the vaccinated group compared to the unvaccinated group for influenza A H3N2 infection (DOC = 13.4-1191.7). On the contrary, the breakthrough influenza A pdm H1N1/09 and influenza B infections contains less DOC that nucleotide heterogeneity cannot be determined.

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DESCRIPTIVE EPIDEMIOLOGY OF THE EBOLA VIRUS DISEASE OUTBREAK IN NIGERIA, JULY TO SEPTEMBER 2014

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The current epidemic of Ebola virus disease (EVD) was first reported in March 2014 in West Africa which is the largest ever reported globally. Nigeria had its first imported case on the 23rd of July 2014; we investigated and described the epidemiological profile of this outbreak that affected two megacities in the Nigeria in terms of person, place and time characteristics of the cases identified. Using field investigation techniques, cases were identified through contact tracing, line-listed and described. We adopted the WHO case definitions for EVD. A suspected case was defined as any person with axillary temperature ≥38oC, who has visited an affected area within past 21days or has had contact with a confirmed or probable case and has two or more cardinal symptoms of EVD. A confirmed case was a suspected case with positive reverse transcription (RT)-PCR laboratory result and a probable case was a suspected case evaluated by a clinician or any deceased suspected case with an epidemiological link with a confirmed EVD case. A total of 20 cases were identified (19 laboratory-confirmed Ebola cases and one probable case); 16 (80%) in Lagos State and 4 (20%) in Rivers State. The mean age of cases was 39.5±12.4 years with over 75% within the age group 20-39 years. There were more females 11 (55%) than males 9 (45%). The most frequent exposure type was direct physical contact 14 (73.7%) and median incubation period was 11days. The overall case-fatality ratio (CFR)

was 40%; CFR was higher among healthcare workers (46%) compared with non-healthcare workers (22%). The epidemic curve initially shows a typical common source, followed by a propagated pattern and duration of epidemics was 43 days. Investigation revealed the size and spread of the outbreak and provided information on the characteristics of persons, time and place. Enhanced surveillance measures, including contact tracing and follow-up proved very useful in early case detection and containment of the outbreak.

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CONTRIBUTION OF LOCAL COMMUNITY MEMBERS IN THE DETECTION OF NEW PATHOGENS OF ZOONOTIC POTENTIAL IN THE DEMOCRATIC REPUBLIC OF CONGO

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In the past decade, 60 % of emerging infectious disease (EID) events were zoonosis and of those 72% of the pathogens involved were of wildlife origin. Activities like subsistence hunting, butchering and trading of wild animals are key factors for the risk of zoonotic infections in humans. The United States of America Aid for International Development (United States Agency for International Development) Emerging Pandemic Threat (EPT) PREDICT project, in collaboration with the "Institut National de Recherche Biomedicale" and the Kinshasa School of Public Health, implemented a surveillance system for zoonotic pathogens at the Human-Wildlife interface in geographic hot spots. Local community members, especially hunters were recruited, sensitized and trained in prevention technics of zoonosis. They participated to the surveillance effort by collecting dry blood spots (DBS) from wild hunted animals, under the supervision of trained field staffs. From December 2010 to September 2012, a total of 14,779 samples were collected from wild animals under the PREDICT project. Of these samples, hunters and other community members collected 5,395 (36,5%) on DBS. From those DBS, 285 tested positive for known or new viral pathogens of zoonotic potential using PREDICT protocols. Community members who participated to the PREDICT project collected good quality samples that were used to identify known and new zoonotic pathogens. Their inclusion on a national base can improve disease surveillance for EID.

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RAPID DIAGNOSIS AND FIRST CHARACTERIZATION OF THE VIRUSES RESPONSIBLE FOR THE 2014 EBOLA VIRUSES OUTBREAK IN THE DEMOCRATIC REPUBLIC OF CONGO

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Following a request from the Ministry of Health (MoH), we made a rapid diagnosis of the etiologic agent responsible for an outbreak of hemorrhagic fever in Boendé, within the Equateur Province of DRC. On 11 August 2014, a woman died in Ikanamongo village following symptoms of hemorrhagic fever. On the following day, additional suspected cases were reported in nearby villages. Epidemics caused by Ebola virus often occur following contact with a sick or dead animal. Congruently, the putative index case had butchered a wild animal of unknown species. Blood samples from the first 8 subjects, individuals who had close contact with the index case, were collected and transported to the INRB laboratory by a MoH team on August 22nd. The presence of the Zaire Ebola virus was confirmed in these specimens by conventional PCR that amplifies a 550 bp section of the filovirus L-gene. Analysis of the sequences obtained confirmed that the outbreak in DRC was caused by a different strain of the Ebola virus than is associated with the large outbreak in West Africa, and thus these concurrent outbreaks are derived from independent origins. Subsequent next generation sequencing of the patient specimens enable full-genome sequencing of two isolates and nearly full-genome sequence of a third. All sequences were immediately publically released via Genbank and ProMED. The swift laboratory diagnosis allowed the DRC government to implement effective control measures, including quarantines, installation of mobile diagnostic laboratory, intensive monitoring of cases, and contact tracing, which prevented a broader geographic spread of the outbreak and led to its relatively rapid conclusion.

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NOVEL VIRUSES DETECTED IN *ANOPHELES GAMBIAE* IN WEST AFRICA

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Adult bloodfed ***Anopheles gambiae*** mosquitoes were collected during a field study conducted in West Africa in order to monitor pathogens infecting humans in the area. Mosquitoes were pooled and RNA-Seq was performed. Within the data set two putative insect specific viruses new to ***Anopheles gambiae*** were discovered: Phasi Cheron Like Virus (PCLV), a Phlebovirus originally described from ***Aedes aegypti*** in Thailand, and a novel insect specific flavivirus, provisionally designated ***Anopheles*** flavivirus (AnFV). In this study we report genetic characterization of these viruses. In particular we (1) compared the reads to PCLV in our dataset to the published genome, (2) assembled the AnFV genome, and (3) reconstructed phylogenies of both viruses. In addition, we determined the field prevalence of both viruses. We also discovered what appears to be a new virus in the order Mononegavirales. Our data demonstrated that PCLV from ***Anopheles*** is genetically similar to the previously published genome, and has a relatively low field prevalence in our study area. Also, AnFV forms a new clade within insect specific flaviviruses and has a similar field prevalence to other insect specific flaviviruses. The results of this study add knowledge to the understudied field of insect specific viruses, including the first ***Anopheles*** specific flavivirus.

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POTENTIAL DISTRIBUTION OF EBOLA, MARBURG, AND LASSA VIRUSES IN AFRICA

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Numerous recent studies have illuminated the current distribution of Ebola, Marburg, and Lassa viruses. Hitherto, the potential distributions of key reservoir species have not been incorporated integrally into the efforts of the risk mapping of these deadly viruses. With the most complex and worse outbreaks in West Africa, it became highly desirable to understand the current and future surprises of the distribution of these viruses in Africa. This will shed the lights to the future strategy for both diagnostic and control programs. Here, we identified the potential distribution of the three viruses, and tested their niche equivalence based on time-specific data from the NASA's Terra Satellite. We also provided the rich maps for the regions where two or more of these viruses occur. These rich maps were based on the maximum entropy approach that estimate the probability of virus or animal reservoir occurrences from independent

disease events as well as environmental and demographic factors. All maps was hosted as digital rich pictures into mobile system to be accessed by researchers and health professionals in the field. This study took the advantage of including time-specific data for both the recent outbreaks in West Africa and the environmental factors. Our results assess the most recent situation of the distribution of hemorrhagic fever viruses, and offer the possibility to be translated into action in the national and international control programs of hemorrhagic fevers in Africa.

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ROBUST EXPRESSION OF EARLY INNATE IMMUNITY IMMUNITY GENES IN DROMEDARY CAMEL PLASMACYTOID DENDRITIC CELLS

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Middle Eastern respiratory syndrome coronavirus (MERS-CoV) has caused more than 1000 confirmed cases of disease with a 36% fatality rate. ***In vitro*** studies demonstrated that MERS-CoV is sensitive to type I and type III interferons (IFN) and the virus down regulates the interferon response in human respiratory cells, suggesting that innate immunity plays a critical role in the outcome of infection. Plasmacytoid dendritic cells (pDC) produce high amounts of IFN in response to viral infection and are particularly important in controlling viral infections. Human DCs secrete large amounts of type I and type III IFN when cultured with MERS-CoV. Furthermore, while studies are conflicting, MERS-CoV does not appear to replicate in human DCs. Dromedary camels (Camelus dromedarius) are thought to be reservoir hosts of MERS-CoV. A large number of domesticated camels in the Middle East have antibody to the virus, suggesting a potential for spillover to humans. Experimental infection of camels with MERS-CoV showed that camels were susceptible to MERS-CoV infection with subclinical to mild disease that is confined to the upper respiratory tract, including regional lymph nodes. We sought to characterize the innate immune response to MERS-CoV in camel pDCs to delineate differences between humans and camels. Flt3L-derived pDCs were established from camel bone marrow and cultured with MERS-CoV. No virus replication occurred and the cells remained healthy upon microscopic examination. Many genes involved in viral sensing were elevated after 8 hours of exposure to MERS-CoV, including TLR7, STAT1, MDA5, RIG-I, IKKE and TBK1. Expression of genes late in the IFN signaling pathway appeared less sensitive to MERS-CoV, or viral accessory proteins inhibit their expression. TNF expression was substantially elevated at 2 and 8 hours; however, it had subsided by 24 hours. These data indicate that camel pDCs are responsive to MERS-CoV, are not productively infected, and may control virus early after cellular entry.

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PREVALENCE OF THE RUBELLA IN CHILDREN FROM SIX MONTHS TO FIVE YEARS IN DEMOCRATIC REPUBLIC OF THE CONGO

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Background Rubella can be a very contagious and severe disease for the fetus, but preventable by a safe and effective vaccine. Epidemiological data based on measles laboratory surveillance introduced by the DRC Ministry of Health confirm circulation of rubella virus in Democratic Republic of Congo. Furthermore, studies among pregnant woman in Kinshasa found circulation of the virus in 90 % of screened women, however no study has estimated virus circulation among children at the national level. To date, the Expanded Programme of Immunization (EPI) has not yet introduced the rubella vaccine in the routine immunization schedule. During the second Demographic and Health Survey (DHS) held by the government in 2013, a serological evaluation of exposure to Rubella virus was conducted among children 6 months to 5 years old. Material and methods A total of 8,116 of dried blood spots were collected during the second DHS from 540 locations in DRC and forwarded to University of California Los Angeles-DRC Research program Laboratory located at the National Institute of Biomedical research (INRB) in Kinshasa. Serologic testing was made by ELISA technique using Dynex M2 Multiplex rubella IgG detection. Results Overall prevalence was 34.4 %. However, we observed a progression of prevalence within age groups; 14% among children 6 to 8 months and 47.6% among children 48 months to 59 months. Conclusions: Current data support circulation of Rubella virus in the whole country with an increase of exposure with the age. In DRC, there is has need to re-evaluate immunization strategies for introduction of Rubella Vaccine in Routine Immunization.

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RIFT VALLEY FEVER EMERGENCE OF UNPRECEDENT MAGNITUDE IN SENEGAL IN 2013-2014

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Rift Valley fever (RVF) is an acute viral anthropozoonosis causing epizootics/epidemics associated with high toll of morbidity and mortality among human and livestock populations in Africa. In Senegal, RVFV has been repeatedly detected among humans, livestock, and mosquitoes especially in the Northern and the Southern regions. In 2013, multiple RVFV epizootics ***foci*** and 5 RVF human confirmed cases were identified countrywide including in the capital city. This paper reports multidisciplinary field investigations and laboratory findings of this outbreak. Human suspected cases and their contacts, ruminants and arthropods in contact with confirmed and/or suspected cases were sampled in affected areas including Linguere (Northern), Mbour (Central) and Kedougou (SouthEastern) regions. Human and animal sera were tested by ELISA (IgM, IgG) and RT-PCR for RVFV. Mosquitoes were sorted in monospecific pools and tested for RVFV RT-PCR detection and isolation. During the human investigation, 535 patients were sampled from which 2.05% (11/535) were tested positive for RVFV (10 IgM, 1 RT-PCR) including 8 in Mbour, 2 in Kedougou and 1 in Linguere and 4.48%(24/535) had evidence of RVFV past infection (IgG). In term of clinic signs, it was the first time that RVF severe case with encephalitis and retinitis notified in Senegal. Fifty two animals (12.06%) were tested positive by RT-PCR for RVFV only in Northern regions. Although no animal's evidence of RVFV recent infection was found in Central and SouthEastern regions, IgG antibodies were significantly higher in Mbour (75%) than in Kedougou (25.8%)($p<0.0001$). Concerning entomologic investigation, 645 arthropods were collected and RVF was detected in one pool of mosquitoes in Linguere. Phylogenetic analyses showed that the strains from human and mosquito clustered together. In conclueion, it was the largest spreading of RVFV touching urbanized areas including the capital city. Regarding the potential risk of reemergence through familial breeding, RVF surveillance should be implemented in order to provide promptly suitable and effective preventing and control measures.

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RAPID AND SENSITIVE DETECTION OF BAT INFLUENZA VIRUSES BY REAL TIME RT-PCR ASSAYS

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Influenza viruses are important human and livestock pathogens and new reassortants of zoonotic origin have potential to cause pandemics. Aquatic birds harbor diverse influenza A viruses and are recognized as major influenza virus reservoirs in nature. However the recent discovery of influenza viruses of a new H17N10 subtype in Guatemalan fruit bats and a new H18N11 subtype in Peruvian fruit bats, along with preliminary seroprevalence studies suggest that New World bats may carry divergent influenza viruses and could be an unrecognized reservoir in nature. To understand how influenza viruses are maintained in bat populations, systematic and global prevalence studies using more sensitive and efficient screening methods are needed. In this study, we developed two real time RT-PCRs targeting conserved regions within the NS and M gene segments of known bat influenza viruses to enable sensitive and efficient detection from bat clinical samples. These assays are simple, rapid and at least 4X more sensitive for detection of bat influenza viruses compared to the generic pan-flu PCR used previously. These assays were used to screen collections of bat swabs previously screened. A total of 803 rectal and 95 oral swabs from Guatemala (2009-2011) and Peru (2010) were tested by generic pan-flu PCR and rescreened with the bat influenza M and NS real time RT-PCRs. In addition to the previously tested flu-positive bat samples, one additional rectal swab sample (***Carollia perspicillata)*** from Guatemala (2010) was positive for bat influenza virus by real time PCRs, but missed by generic flu PCR. Full genome sequencing was performed by Sanger and NGS methods. Phylogenetic analysis showed that this latest bat flu virus is more closely related to H18N11 (82.5%-96.3% nt identity to 8 orf segments of A/bat/Peru/10) rather than to H17N10 (53.5%-81.0% nt identity to 8 orf segments of A/bat/Guat/09). These new assays provide a rapid and sensitive tool to screen bat populations to better understand the ecology and evolution of bat influenza viruses.

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ERYTHROCYTE INVASION MECHANISMS OF *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES FROM THREE ENDEMIC AREAS IN GHANA

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Plasmodium falciparum invades human erythrocytes using an array of ligands which interact with several receptors including sialic acid (SA), complement receptor 1 (CR1) and basigin. Naturally acquired immunity against several blood stage ligands have been shown to effectively block invasion and parasite growth ***in vitro***, making these antigens potential vaccine candidates. Based on this, we hypothesized that in malaria-endemic areas, parasites vary invasion pathways under immune pressure. Furthermore, parasites from areas with varying endemicity would differ in the receptor-ligand interactions used for erythrocyte invasion. Therefore, invasion mechanisms of clinical isolates collected from three zones of Ghana with different levels of endemicity (Accra<Navrongo<Kintampo) were compared using standardized methods. Blood samples were collected from children aged 2-14 years diagnosed with malaria. Erythrocyte invasion phenotypes were determined using enzymes, which selectively cleave receptors from the erythrocyte surface. In addition, antibodies against CR1 and basigin were used to determine the contributions of these receptors to invasion. Gene expression levels of ***P. falciparum*** invasion ligands were compared against parasitemia levels and age. The parasites generally expressed SA-independent invasion phenotypes across the endemic areas, with parasites from Kintampo showing the highest invasion rates in neuraminidase-treated erythrocytes. CR1 was a major mediator of SA-independent invasion while basigin was essential for both SA-dependent and SA-independent invasion mechanisms. Relational analyses between ligand gene expression levels with age and parasitemia of donors at enrolment showed that PfRh5 had the strongest correlation with parasitemia. In conclusion, erythrocyte invasion phenotypes expressed by ***P. falciparum*** are influenced by endemicity levels. The PfRh5-basigin pathway is a potential vaccine target.

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THE ENDOTHELIAL PROTEIN C RECEPTOR (EPCR) RS867186-GG GENOTYPE IS ASSOCIATED WITH INCREASED LEVELS OF SOLUBLE EPCR AND PROTECTION FROM CEREBRAL MALARIA IN UGANDAN CHILDREN

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Endothelial protein C receptor (EPCR) expression on brain microvasculature endothelium may be an important determinant of disease severity in malaria. Plasma soluble EPCR (sEPCR) levels are higher in individuals with the rs867186-G allele, and in Thai adults the rs867186-GG genotype was associated with protection from severe malaria. In the present study, we performed rs867186 genotyping in Ugandan children with cerebral malaria (CM, n=326), severe malarial anemia (SMA, n=227), uncomplicated malaria (UM, n=71) and healthy community children who lived in the same extended household or neighborhood as children with CM or SMA but had no history of CM or SMA and did not develop either during 12-month follow-up (CC, n=262). Plasma and CSF sEPCR levels were assessed in children with adequate sample volume (Asserachrom® sEPCR immunoassay). The rs867186-GG genotype was more common in CC (3.0%) than CM (0.3%, p=0.007). The presence of rs867186-G was associated with increased plasma sEPCR levels in each disease group ($p<0.0001$ for all). Plasma sEPCR levels were significantly higher in CC or UM than in CM or SMA ($p<0.0001$ for trend). In children with CM, plasma sEPCR correlated positively with TNF-α (p=0.02) and parasite biomass (***Plasmodium falciparum*** histidine-rich protein-2 level, p=0.006) but not IL-1β or IFN-γ. Plasma sEPCR levels were not associated with differences in mortality or neurocognitive morbidity. CSF sEPCR levels were elevated in children with CM as compared to North American controls ($p<0.0001$), but were not associated with mortality or morbidity. In Ugandan children, the presence of rs867186-G correlates with increased plasma sEPCR levels, increased plasma sEPCR levels correlate with decreased malaria disease severity, and the rs867186-GG genotype is associated with protection from cerebral malaria. The results suggest that the rs867186-GG genotype may decrease risk of cerebral malaria in part through effects on bound and soluble EPCR.

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A MODIFIED CONCEPT OF MALARIAL RELAPSE

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The idea that the hypnozoite (a term coined by me decades ago) is the origin of relapse in ***Plasmodium vivax*** (and ***P. ovale***) malaria, something for which there is as yet no formal proof, has become dogma.

For particular reasons which will be explained, it would be surprising to discover that hypnozoites are not the source of malarial relapse. Nevertheless, it is now apparent from various recent research findings that hypnozoites are not necessarily the origin of all relapse-like recurrences of malaria caused by ***P. vivax***. We could be missing the elephant in the room; and indirect evidence from several publications will be provided to support this novel, genetically based concept that nonhypnozoite parasite stages might give rise to relapse-like, recurrent human malaria. Re-evaluation of the hypnozoite theory of relapse is timely because of the renewed focus on ***P. vivax*** and liver stages of ***Plasmodium***. Hypnozoites have also assumed a new significance because they are seen as a threat to the current (post-2007) goal of eradicating malaria worldwide.

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DEVELOPMENT OF SPLENOMEGALY DURING RODENT MALARIA BY MYELOID-RELATED PROTEIN (MRP)

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Splenomegaly is one of the typical symptoms of malaria. However, the pathogenesis of splenic enlargement still remains unclear. Myeloid-related protein (MRP) 8 and MRP14 are expressed by inflammatory macrophages and secreted upon activation. Previous studies have demonstrated that the accumulation of MRP-expressing macrophages is associated with the pathological changes in various inflammatory diseases. In order to elucidate whether MRP-expressing macrophages are also involved in splenomegaly during malaria, we investigated expression of MRP in the spleens of mice infected with ***Plasmodium berghei***. Enlargement of the spleen was prominent on day 7 post-infection, and histological analyses of the spleens demonstrated deposition of malaria pigments and accumulation of macrophages. Immunohistochemical staining of the tissue revealed the accumulation of macrophages expressing MRP. In these infected mice, MRP levels in the plasma were higher than those of uninfected controls. In order to verify whether plasma MRP is involved in the splenomegaly during malaria, we intravenously administrated recombinant MRP8 and MRP14 to ***P. berghei***-infected mice. The administration of MRP did not affect parasite number in the peripheral blood or hematocrit. On the other hand, the splenomegaly was exacerbated in MRP-treated mice, and their spleen weight increased significantly more than PBS-treated controls. Immunohistochemical staining of the spleen showed that more MRP-expressing macrophages accumulated in MRP-treated mice than PBS-treated controls after infection. Also, even in the absence of ***Plasmodium*** infection, administration of MRP could induce enlargement of spleen along with the accumulation of MRP-expressing macrophages in naïve mice. These data indicates that elevated MRP during malaria is one of the key molecules for development of splenomegaly.

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LIVER FUNCTION TESTS IN PRESCHOOL NIGERIAN CHILDREN WITH SYMPTOMATIC UNCOMPLICATED *PLASMODIUM FALCIPARUM* INFECTION

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Malaria remains the leading cause of childhood morbidity and mortality in Nigeria. The liver is involved in the pathophysiology of malaria, and severe falciparum malaria may affect liver function. In order to evaluate the status of liver function in uncomplicated malaria, the authors analysed baseline liver function test results of preschool children with parasitologically confirmed symptomatic uncomplicated ***Plasmodium falciparum*** infection. Study was conducted in Calabar in Southeast Nigeria, an area with perennial stable malaria transmission. Children aged 6-59 months with fever were included if they had asexual ***P. falciparum*** parasite density ≥ 2000/mm3, no feature of severe malaria; and written parental confirmed consent. Thick blood smear was stained with 3% Giemsa for microscopic detection and quantification of malaria parasites. Assay of plasma levels of liver enzymes (alanine aminotransferase - ALT and aspartate aminotransferase - AST), bilirubin and creatinine were performed using standard biochemical laboratory methods. Results showed mild elevation of plasma levels of ALT (i.e. ALT > 45 U/L) and AST (i.e. AST >55 U/L) respectively in 8.5% and 21.1% subjects. Moderate elevation of ALT (i.e. ALT >90U/L) and AST (i.e. > 110 U/L) was observed in 1.5% and 3.2% subjects respectively. Creatinine was normal in all subjects except 0.6% with mild elevation (>62mmol/L). Those with bilirubin level higher than double the group mean were 7.18%. Malaria parasite density was marginally positively correlated with elevation of AST (correlation coefficient= 0.02; p<0.001) and ALT (correlation coefficient= 0.01; p=0.67). Researchers conclude that in children with uncomplicated ***Plasmodium falciparum*** infection, liver function is essentially normal; with only mild elevation of liver enzyme in a few.

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ONE OF THE *PLASMODIUM* RHOPTRY PROTEINS IS ESSENTIAL FOR MALARIA SPOROZOITE GLIDING MOTILITY, WHICH IS REQUIRED FOR MOSQUITO SALIVARY GLAND INVASION MACHINERY

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Mosquito salivary gland invasion of ***Plasmodium*** sporozoite is an essential step for malaria transmission. Sporozoites, the malaria infective stages are formed inside oocyst on mosquito midgut then released into hemocoel. Sporozoites then migrated via hemolymph circulation and finally invade salivary glands. To initiate the salivary gland invasion, a parasite locomotion called gliding motility is required for this invasion step. To date, micronemal protein, thrombospondin-related anonymous protein (TRAP) has been shown to be involved in gliding motility. However, the molecular mechanisms for mosquito salivary gland invasion are still remaining unclear. To determine the mechanisms of sporozoite invasion machinery, we focus on rhoptry proteins because it has been suggested that they are involved in host cell invasion by merozoite. Since it is well known that most of rhoptry proteins could not be disrupted, sporozoite stage specific gene silencing system had been estrablished. Recently, we found that rhoptry neck protein 2 (RON2) is also involved in salivary gland invasion by sporozoite. We intend to elucidate the roles of all possible rhoptry proteins during sporozoite invasion using gene silencing method. We generate sporozoite stage specific gene silencing transgenic parasites, replaced the endogenous promoter to the merozoite specific promoter using rodent malaria parasite, ***P. berghei***. It was shown that our target rhoptry protein expression in mutant sporozoites was reduced approximately 10 times less than those in control parasite by western blotting. The mutant sporozoites were formed in oocyst and released into hemocoel normally. Whereas, the number of invaded mutant sporozoites collected from salivary gland were about 100 times less than those of control parasites. Furthermore, these mutant sporozoites display a severe defect in gliding motility. These results demonstrated that this target rhoptry protein plays an important role in sporozoite gliding and involved in salivary gland invasion.

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THE FUNCTIONAL ANALYSES OF A *PLASMODIUM* ALVEOLIN PROTEIN DURING MOSQUITO STAGE PARASITE DEVELOPMENT

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Plasmodium spp. has unique structure named inner membrane complex (IMC) beneath the plasma membrane in all invasive stages. IMC is composed of the membranous sacs which connect motor complex and microtubule cytoskeleton, and might has important roles during invasion. It has been reported that some proteins, containing the conserved peptides, are localized to IMC named as Alveolin family. Until now, 12 ALVs are listed in ***Plasmodium*** by genomic sequencing. Some of them are mosquito stage specific, while the others are expressed in all invasive forms. The diversity of alveolins might be required for differentiation, membrane stability, motility or invasive ability of different stage parasites to proceed the complicated life cycle efficiently. To elucidate the functions of conserved ALVs, we have developed the mosquito stage-specific gene silencing system by promotor exchange. Using this system, we previously reported that ALV5 is essential for ookinete initial elongation and motility. To understand the comprehensive functions of ALV family proteins during malaria life cycle, we selected another ALV family protein which is also commonly expressed in all three invasive stages. We produced transgenic parasites (ALV-cKD) that repress the new ALV gene expression during ookinete and sporozoite stage. Target ALV protein amount in ALV-cKD cultured ookinetes was decreased by 90% of that in wild type using western blotting analysis. Approximately only 6% of ALV-cKD ookinete had morphologically normal mature shape inside mosquito midguts whereas 49% showed mature ookinete shape in wildtype. In accordance with this result, oocyst number of ALV-cKD on midgut was reduced 9 times less than that of wild type. These results demonstrated that our target ALV is required for ookinete normal structure, which might be related to their cell transversal ability. In addition, only few ALV-cKD sporozoites were collected from salivary glands. It suggested that our target ALV is also involved in sporozoite formation and/or salivary gland invasive ability, which is different from the ALV5 function.

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HUMANIZED MOUSE MODEL OF *PLASMODIUM FALCIPARUM* INFECTION IN BLOOD STAGE

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Malaria is a devastating mosquito-borne infectious parasitic disease resulting in substantial disease burden and morbidity. ***Plasmodium falciparum*** is the most deadly of the species causing human malaria. Plans to eliminate malaria as an important disease is hampered by the lack of an effective vaccine and widespread resistance to antimalarial drugs. An important limitation in developing new therapies for this human pathogen is the lack of accessible ***in vivo*** model systems for research studies. In this study we have analyzed a humanized mouse model for studying blood-stage infections of ***P. falciparum.*** The commercially available NOD scid gamma (NSG) mouse (Taconic) were engrafted with human red blood cells (huRBC) in conjunction with clodronate treatments (O^+ huRBC every 3 days IV with 100µl clodronate liposome IP). After 3 cycles of engraftment, NSG mice supported high level of huRBC (approximately 25%) in circulation. This huRBC-engrafted NSG model supported robust growth ***P. falciparum*** parasite line-PfKF7G4, which constitutively expresses luciferase and mCherry, achieving parasitemias of >10% in circulating human RBCs. By Giemsa-stained blood smear all stages of ***P. falciparum*** asexual development were observed in circulation. Mature gametocytes produced ***in vivo*** were infective for mosquitoes leading to oocyst formation in the mosquito midgut and salivary gland sporozoites. The huRBC-engrafted NSG model was modified for support of ***P. vivax*** blood stage studies by engrafting with adult blood enriched for reticulocytes are going on. This humanized model will help to accelerate the development of novel drug and vaccine study in malaria research.

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IDENTIFICATION OF RED CELL AND METABOLIC ENZYME VARIATION ASSOCIATED WITH PRIMAQUINE SAFETY AND EFFECTIVENESS AGAINST *PLASMODIUM VIVAX* MALARIA

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Plasmodium vivax (Pv) presents challenges to malaria elimination because it produces hypnozoites, dormant liver-stages that cause relapse infections from weeks to years without mosquito transmission. If untreated, hypnozoites are a disease reservoir whose extent is unknown. Primaquine (PQ) is the only WHO-recommended drug that kills Pv hypnozoites to achieve radical cure but causes life-threatening hemolytic anemia in G6PD deficient (G6PDdef) people. Thus, PQ usage has been limited in many malaria-endemic countries and undermines Pv elimination efforts globally. Also, polymorphic expression of the drug-metabolizing enzyme Cytochrome P450 2D6 (CYP2D6) have been associated with PQ failure through observing Pv relapses in people who have received standard PQ treatment (30 mg/14 days). These findings suggest that optimizing PQ usage requires an understanding of G6PD and CYP2D6 genetic variation. Here, G6PD and CYP2D6 gene sequences were amplified using long-range PCR strategies. Combined post-PCR SNP, deletion and duplication genotyping and Illumina sequencing was used to assess variation. Study participants included n=7 subjects from the USA (multiple ethnicities) and n=22 Madagascar. G6PD and CYP2D6 Illumina sequencing results were compared to reported reference sequences (X55448, G6PD normal; AY545216, CYP2D6*1). G6PD sequence analysis identified known allelic variants and showed concordance with predicted G6PD normal and G6PDdef enzyme activity phenotypes. CYP2D6 alleles predictive of low, intermediate and extensive metabolism were observed. Illumina sequence and CYP2D6 genotyping results were concordant. For the Malagasy study participants, sequences of both African and Southeast Asian origins were observed suggesting that the Malagasy population is rather unique. Our results indicate that genetic variation in G6PD and CYP2D6 genes may both confound safe and effective PQ use. This intersection of human genetic variation must be better understood to develop safe and effective PQ usage strategies to achieve elimination of Pv in endemic regions of the world.

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BIOENERGETICS STUDIES OF *PLASMODIUM FALCIPARUM* MITOCHONDRION USING SEAHORSE FLUX ANALYZER

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In recent years, the malaria parasite's mitochondrion has drawn attention as a validated drug development target largely based on its molecular and functional divergence to human mitochondrion and successful development and clinical use of a mitochondrial electron transport chain (ETC) inhibitor, atovaquone. However, in contrast to the deep molecular and biochemical understanding of the mitochondrion in mammalian cells,

many aspects of ***Plasmodium*** mitochondrial function, bioenergetics, and associated metabolomics still remain unclear. In order to conduct bioenergetic studies of ***P. falciparum*** parasites, we developed a novel assay protocol utilizing the Seahorse flux analyzer, which allows us to assess the parasite's respiration and glycolytic activities in real-time and simultaneously with a readout of an oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Using schizont stage parasites that were isolated from red blood cells by saponin lysis, we successfully monitored the kinetics of various metabolic substrates and products of the tricarboxylic acid cycle and ETC. As results, we found that glutamate, but not pyruvate, was able to increase OCR, and that glycerol-3-phosphate dehydrogenase had the largest potential as an electron donor among tested mitochondrial dehydrogenases. We also observed oligomycin-sensitive OCR elevation by ADP with the presence of glucose, providing supportive evidence for the existence of oxidative phosphorylation in ***Plasmodium***. Furthermore, we tested various mitochondrial inhibitors to see how these small molecules affect OCR. Cytochrome bc1 inhibitors, such as antimycin A, decreased OCR when any dehydrogenases of ETC were activated, while a dihydroorotate dehydrogenases (DHOD) inhibitor, genz669178, only decreased the OCR induced by dihydroorotate. This result demonstrated that our assay system provides a novel method for not only target identification but also mode of action study of mitochondria targeting antimalarials. Further studies including bioenergetic profiling of developmental stages and drug resistant lines will be discussed.

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THE EFFECT OF IRON SUPPLEMENTS ON HEME IN PREGNANT WOMEN WITH MALARIA AND WITHOUT MALARIA

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Plasmodium falciparum malaria threatens about 200 million people worldwide resulting in 655,000-1000000 deaths annually with pregnant women and children at high risk. Although current anti-malarial treatment are effective in targeting parasites, recent studies have shown that the pathogenesis of severe malaria is not only due to parasitemia but also by parasite derived factors and host factors such as heme and heme oxgenase-1 (HO-1) as a result of hemolysis. Pregnant women in general are routinely recommended to take iron during pregnancy with the aim of meeting the increased iron demands during pregnancy. The objective of this study was to assess the effect of iron supplements on Heme in pregnant women with malaria and without malaria. We hypothesized that pregnant women with malaria who take iron supplements will have higher levels of Heme than pregnant women without malaria who do not take iron supplements. A cross-sectional study of women presenting for delivery at two hospitals in Kumasi, the Komfo Anokye Teaching Hospital and the Manhyia Polyclinic, and one hospital in Accra the Korle-Bu teaching hospital. The preliminary results showed that pregnant women with malaria who took iron supplements had significant higher median levels of heme 59.301 (43.08060.4) than pregnant women without malaria who did not take iron supplements 35.714 (33.03662.202), p = 0.026. In conclusion, malaria in pregnancy is associated with increased Heme reflecting the degree of hemolysis induced by parasites (sequestered or systemic) and pregnancy outcomes. Findings from this study may provide insight on the effect of iron supplements on malaria derived heme in pregnancy which may result in development of preventive chemotherapy that target both parasites and hemolysis.

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PFEMP-1 EXPRESSION AND ANTIBODY IMMUNITY IN MALAWIAN PEDIATRIC CEREBRAL MALARIA PATIENTS

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PfEMP-1, a family of variant antigens expressed on the surface of ***Plasmodium falciparum*** infected erythrocytes, has been implicated in parasite evasion of the host immune system. PfEMP-1 is encoded by a family of 60 var genes that encode protein domains that enable infected erythrocytes to adhere to vascular endothelium and other erythrocytes. The var genes are classified on the basis of promoter sequence, chromosome position and protein domains. Recent studies suggest that parasites expressing a subset of PfEMP-1 variants are associated with more severe malarial disease, including cerebral malaria (CM). As CM remains a major cause of death amongst pediatric patients in Malawi, we employed qRT-PCR to determine the PfEMP-1 types expressed by parasites in the blood of pediatric patients admitted with stringently defined CM. Despite the wide range of variability in the ***P. falciparum*** isolates and the associated var genes, we were able to amplify and characterize the var repertoire of Malawian parasites using a panel of PCR primers initially used on parasites isolated from pediatric malaria patients in Tanzania. From this cross-sectional study, we report the diversity and types of PfEMP-1 antigens isolated from Malawian pediatric CM patients. Our results will be discussed in the context of other clinical (retinopathy status, MRI features, outcome) and immune parameters.

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PLASMODIUM FALCIPARUM K13 PROPELLER GENE MUTATION FROM NORTHWEST ETHIOPIA ASSOCIATED WITH DAY-3 POSITIVITY

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Artemisinin combination therapy (ACT) is considered first-line to treat uncomplicated falciparum malaria worldwide. Recently, artemisinin resistance has emerged in Southeast Asia. Resistance to artemisinin has been shown to be highly associated with mutations on the propeller domain of ***Plasmodium falciparum*** K13 gene. The mutations identified in Southeast Asia have not been observed in Africa to-date. In this study, we show a unique mutation in the K13 propeller domain of ***P. falciparum*** strains in Northwest Ethiopia that has not been previously reported in Asia and Africa. Confirmed falciparum malaria patients (n=148) in five districts in Northwest Ethiopia were enrolled in a 28-day ACT trial. Nested PCR for K13 propeller gene was performed on DNA samples extracted from filter paper blood spots. The PCR product was sequenced bi-directionally and the sequences were compared with the reference sequence of K13 gene (PF3D7_1343700).***P. falciparum*** K13 propeller gene was amplified from genomic DNA isolated from 125 out of 148 blood samples collected from the five sites. We have found a unique mutation in K13 propeller domain (R622I) in 3/125 (2.4%) samples. The three isolates with R622I mutation came from Negade-Bahir and Aykel districts close to the Ethiopia-Sudan border. One of the three patients infected with the mutant strain had a day-3 positive result by microscopy. Homology modeling of the mutant protein indicates that the mutation is highly likely to disrupt the function of the protein. The study has shown the emergence of a novel mutation on the propeller domain of the ***P. falciparum*** K13 gene in Northwest Ethiopia with possible association to day-3 positivity.

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MUTATIONS IN K13, PFCRT AND PFMDR1 GENES AND EFFICACY OF ARTEMETHER-LUMEFANTRINE IN RELATION TO TREATMENT OUTCOMES IN KENYAN CHILDREN WITH UNCOMPLICATED FALCIPARUM MALARIA

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Artemisinin-based combination therapies (ACTs) remain highly efficacious in sub-Saharan Africa (SSA) but resistance is found in Southeast Asia (SEA). Mutations in the ***Plasmodium falciparum*** K13-propeller domain are important determinants of ACTs resistance in SEA but there is no evidence of such in SSA. However, ACTs have been shown to select for K76 in pfcrt gene and N86, 184F and D1246 (NFD) in pfmdr1 gene (K+NFD haplotype) in SSA. An open-label randomized study was conducted to investigate selection of K76, N86, 184F and D1246 genotypes, and K-13 mutation in recurrent parasites in western Kenya. 454 children with uncomplicated falciparum malaria were enrolled in the study and followed up for 42 days. Parasite clearance rates were calculated following WHO recommendation. Parasites collected on day 0 and subsequent days were genotyped by direct sequencing or by PCR-based single-base extension on Sequenom MassARRAY platform. Pfmdr1 copy numbers were determined by real-time PCR. The median slope half-life was 2.18 (range: 0.94, 6.94) with a 90% parasite clearance achieved in 40 hours. Day 0 (129) and subsequent days parasites (135 re-infections and 17 recrudescence) were successfully genotyped. On day 0, the prevalence of K76, N86, 184F and D1246 was 50% and 71.2%, 34.9% and 67.2% respectively. There was no significance difference in prevalence of genotypes for day 0 vs. re-infection parasites. However, there was statistically significant difference for day 0 vs. recrudescence parasites in K76, N86 and D1246 loci; K76 and N86 were significantly associated with recrudescence. Recurring parasites harbored statistically higher K+NFD haplotype compared to day 0. There was no variation in pfmdr1 copy number. Analysis of K13 mutations is underway. ACTs remain highly efficacious in western Kenya. However, a few parasites had high half-lives. These parasites are of interest and more detailed genetic analysis is underway. In line with previous studies, we showed selection of K76 and N86 in recurring parasites. There is need for considerations of new policies for management of sustained ACTs efficacy in SSA.

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GENETICALLY DETERMINED RESPONSE TO ARTEMISININ BASED COMBINATION THERAPY IN WESTERN KENYA *PLASMODIUM FALCIPARUM* PARASITES

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In 2006, artemether-lumefantrine (AL) became the first-line treatment of uncomplicated malaria in Kenya due to widespread SP resistance. AL remains highly efficacious but there are heightened concerns because ACTs resistance is now well documented in Southeast Asia (SEA). SNPs in K13-propeller gene have been identified as the determinants of ACTs resistance in SEA though they are not present in Kenyan parasites. Genetically determined artemisinin resistance in ***Plasmodium falciparum*** has been described in SEA in association with slow parasite clearance rates (CRs). This study attempted to elucidate whether parasite genetics can provide basis for discovering genetic markers associated with ACTs resistance in Kenya. A randomized open labeled trial was conducted to evaluate whether genetic factors play a role in CRs in patients treated with ACTs from western Kenya. In addition, the genetic profiles of these parasites were compared to those collected before the introduction of AL (pre-ACTs). 118 subjects were enrolled in the study and randomized to receive either AL or Artesunate Mefloquine. A panel of 12 microsatellites (MS) and 91 SNPs distributed across the ***P. falciparum*** genome were genotyped. Parasite CRs were calculated using the WWARN online parasite clearance estimator tool. All subjects achieved parasite clearance within 42 hours of treatment with a median clearance half-life of 2.55 hours (1.19-5.05). The 12 MS showed high polymorphism with post-ACTs parasites being significantly more diverse compared to pre-ACTs ($p < 0.0001$). Based on SNP analysis, 15 of 90 post-ACTs parasites successfully analyzed were single-clone infections. Analysis revealed 3 SNPs in chromosome 12 and 14 were significantly associated with delayed parasite CRs and might be useful in tracking artemisinin resistance in Kenya. Further, genetic analysis using Bayesian tree revealed parasites with similar parasite clearance as more closely related. Therefore, we have described parasites with genetically determined response to artemisinin treatment which can provide basis for discovering genetic markers associated with ACTs resistance in Kenya.

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GENETIC CHARACTERISTICS OF *PLASMODIUM FALCIPARUM* FOUND IN SUBJECTS RANDOMIZED TO DISCONTINUATION VERSUS CONTINUATION OF COTRIMOXAZOLE PROPHYLAXIS

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The WHO recommends cotrimoxazole (CTX) prophylaxis for HIV-1 infected individuals in regions with high prevalence of infectious diseases. However, with scale-up of antiretroviral therapy (ART), the usefulness of CTX is not well defined especially since it is thought its usage might increase risk of developing cross-resistance to closely related drugs such as sulfadoxine-pyrimethamine (SP). We conducted a non-blinded non-inferiority randomized controlled trial in Homabay, western Kenya to assess CTX prophylaxis discontinuation (DIS) vs. continuation (CON) among HIV-1 infected adults. The subjects had to be on ART for >18 months with CD4 >350 cells/mm3. 500 subjects were enrolled; 250 in DIS arm and 250 in CON arm. Blood samples were collected every 3 months, at time-points in months 0, 3, 6, 9 and 12. Malaria prevalence and mutations associated with SP resistance in pfdhfr and pfdhps genes were assessed by direct sequencing. The prevalence (overall) of ***Plasmodium*** was 3.8%, with 3.2% in DIS and 0.6% in CON. The prevalence of mutant haplotype for each arm at each time-point was calculated and compared. Pfdhfr 51I/59R/108N haplotype was present only in DIS arm in all the 5 time-points (prevalence 16.7% - 66.7%) except for month 9 in CON arm. Pfdhfr 51I/108N/164L was present in months 0, 9 and 12 in both DIS and CON arms. In pfdhps gene, 437G/540E haplotype appeared in both arms at all time-points whereas 437G/540E/581G was present only in month 6 in DIS arm only. Combined 51I/59R/108N/437G/540E appeared only in DIS arm in all time-points (prevalence 16.7%-50%) whereas N51I/C59R/108N/437G/540E appeared only in CON arm in month 9 (prevalence 33.3%). Homabay has malaria prevalence of over 40%. In this study, both arms had overall malaria prevalence of less than 4%, with CON arm having less than 1%. Our data does not show evidence of selection of mutations associated with SP resistance. Given high mortality and morbidity caused by malaria, CTX demonstrates usefulness and eliminates the need for use of SP as intermittent preventive treatment in pregnant women and infants.

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SELECTIVE SWEEPS AND GENETIC LINEAGES OF *PLASMODIUM FALCIPARUM* MULTI-DRUG RESISTANCE (PFMDR1) GENE IN KENYA

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Artemether-lumefantrine (AL) has been the first-line treatment for uncomplicated falciparum malaria in Kenya since 2006. AL selects for K76 in pfcrt and N86, 184F and D1246 in pfmdr1 genes in recurring parasites compared to the baseline infections. Microsatellite (MS) analysis of loci flanking genes associated with antimalarial drug resistance has been used in defining the geographic origins and dissemination of resistant parasite. Kenya has diverse malaria transmission intensities with varying malaria endemicities. This study investigated evidence of selective sweep and genetic lineages in pfmdr1 genotypes selected for by AL in treatment of malaria infections in Kenya. Parasites (247) from different regions in Kenya (Kisumu, Kisii, Kericho and Malindi) were analyzed for polymorphisms at codons 86, 184 and 1246 in pfmdr1. Samples were typed for 8 NMS and 13 MS loci flanking pfmdr1. Full data set was obtained in 79% (186) of the samples. Overall, prevalence of N86 and D1246 was highest at 85.1% and 90.5% respectively. The most prevalent haplotype was NFD at 53.2% whereas the least prevalent was YFY at 1.1%. Per site, N86 was highest in Kisumu at 92.6% and lowest in Malindi at 65.1%. Kericho had the lowest prevalence of mutant alleles in all the loci whereas Malindi had the highest. Kisumu had the highest prevalence of NFD (63.4%) whereas Malindi had the lowest (29.7%). The mean HE for NMS was 0.96 vs. 0.627 for the 13 MS indicating selection. Parasites carrying mutant alleles had reduced HE compared to the wild type NYD except for NFD. Analysis of parasite genetic lineages is underway. Data show high prevalence of NFD and NYD, difference in genetic diversity between sites and evidence of selection in pfmdr1 gene that is statistically different between sites. Data indicate parasites are evolving differently in response to AL drug pressure from one region to another suggesting rate at which AL tolerance will develop in different regions of Kenya might vary.

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ARTEMISININ-BASED COMBINATION THERAPY EFFICACY IN KISUMU, WESTERN KENYA: *IN VIVO* AND *IN VITRO* EFFICACY FINDINGS

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Plasmodium falciparum (Pf) resistance to artemisinin is prevalent in Southeast Asia (SEA) and is a threat to malaria control efforts. Africa is currently spared, but this observation is evocative of the emergence of chloroquine and sulphadoxine-pyrimethamine resistance that was first observed in SEA and later in Africa. More comprehensive monitoring is required in malaria endemic areas. In 2013-2014, we conducted an efficacy study of artemether lumefantrine (AL) and artesunate mefloquine (ASMQ) for the treatment of uncomplicated Pf malaria in Kisumu, Western Kenya - an area with high malaria transmission. A total of 118 subjects were randomized in a 1:1 ratio to receive either AL or ASMQ. Treatment was directly observed. Blood draws for malaria tests were performed at hours 0, 4, 8, 12, 18, 24 and 6 hourly thereafter until 2 consecutive negative malaria blood films (MBFs) were obtained. Blood samples for MBFs were also collected during weekly follow-up visits from day 7 to 42. Hour 0 samples were tested for ***ex vivo*** sensitivity to antimalarial drugs. Findings from the AL arm are presented here. The geometric mean parasitemia at presentation was 37892.5 parasites/µL (95% CI 25294.2, 56765.6). There were no cases of early treatment failure. Before PCR correction, 54.2% (32/59) had 28 day adequate clinical and parasitological response (ACPR) and 35.6% (21/59) had 42 day ACPR. After PCR correction, 100% had 28 and 42 day ACPR. The median time to clear 99% of parasitemia (PC99) was 21.19 hours (range 10.40 - 32.25), while the median time to clear 50% of parasitemia (PC50) was 7.42 hours (range 0.83 - 15.54). The median parasite clearance slope half-life was 2.45 hours (range 1.56 - 4.02). The influence of age on the parasite clearance parameters was not statistically significant. Hour 0 drug sensitivity IC50 median values for artemether, dihydroartemisinin and lumefantrine were 4.13 nmol (IQR 1.68, 10.75), 8.34 nmol (IQR 1.84, 35.21) and 31.69 nmol (IQR 3.40, 111.49) respectively. AL efficacy for the treatment of uncomplicated malaria in Kisumu is still high. This study provides baseline malaria parasite clearance profiles that must continuously be monitored.

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HIGH LEVEL *PLASMODIUM FALCIPARUM* SULFADOXINE-PYRIMETHAMINE RESISTANCE WITH THE CONCOMITANT OCCURRENCE OF THE SEPTUPLE HAPLOTYPE IN TANZANIA

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Sulphadoxine-pyrimethamine (SP) was abandoned as the first-line treatment; however, it is still being used for intermittent preventive treatment during pregnancy (IPTp-SP). Here, we assessed the pattern of ***Plasmodium falciparum*** dhps and dhfr haplotypes in areas with different transmission intensities in Tanzania. A total of 264 samples were collected during cross-sectional survey in three districts of Muheza, Muleba and. The haplotypes were amplified by PCR and then detected by SSOP-ELISA. Results: The triple Pfdhfr mutant haplotypes (CIRNI) were predominant in all sites with significantly higher frequencies at Muheza district (93.9%) when compared to Muleba (73%) and Nachingwea (65.15%), ($p<0.001$). In contrast, the prevalence of triple Pfdhps SGEGA haplotype was significantly higher at Muheza (38.8%) as compared to Muleba (1.5%) and none at Nachingwea ($p<0.001$). The combinations of Pfdhfr-Pfdhps as quintuple CIRNI-SGEAA (n=25), sextuple CIRNI-SGEGA (n=24) and CIRNI-AGEGA (n=53) haplotypes were detected including the emergence of a septuple mutant haplotype CIRNI-AGEGA (n=9) predominantly at Muheza. In conclusion, the high prevalence of Pfdhfr-Pfdhps mutant haplotypes could undermine the efficacy of IPTp-SP leading to poor pregnancy outcomes.

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PREVALENCE OF ANTIMALARIAL DRUG RESISTANT ALLELES ACROSS VARIABLE TRANSMISSION ZONES IN THE GAMBIA

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Exacerbation of interventions and the introduction of artemisinin combination therapies (ACTs) are thought to have accounted for the decline in Malaria transmission across sub-Saharan African (sSA). However emergence of artemisinin resistance in South-East Asia and fears of spread to Africa calls for vigorous monitoring of ACT efficacy and anti-malarial drug resistance markers. In parts of sSA approaching malaria elimination, low transmission and loss of immunity can lead to epidemics and establishment of imported or emerging drug resistant strains. This study therefore sought to map the prevalence of drug resistance markers across hot and low transmission areas across the Gambia which in the last decade has shown declining malaria prevalence approaching pre-elimination levels. Polymorphic markers for kelch gene (K13 SNP 580, 543, 539), ***Plasmodium falciparum*** multi-drug resistant protein-1 (***Pf***mdr1 SNP N86Y, D1246Y,) ***Pf***ATPase402 and the ***Pf***crtK76T SNPs were typed in 335 parasite isolates using Taqman allelic discrimination assays or Sanger

sequencing. Samples were collected from cross-sectional surveys and passively detected infections. Preliminary results of genotype proportions in the coastal regions of the Gambia indicate that there were 10.5% mutant alleles for *Pf*ATPase402, increasing to 20% in the central river flood plains and 14% in rural settlements in the East. *Pf*mdr1 N86Y mutant allele proportion was highest in the coastal regions (14%), 3% in the central regions and 10% in the upper river regions. *Pf*mdr1 SNP D1246Y attained 1.6% in the coastal region. Only reference alleles of the K13 propeller polymorphisms were identified. This study shows the persistence of MDR mutations in the Gambia despite the discontinuation of Chloroquine. These markers are indirectly associated with delayed clearance in ACT treatment and will require continuous monitoring.

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MAINTAINED EFFICACY A DECADE AFTER THE INTRODUCTION OF ARTESUNATE PLUS SULPHADOXINE-PYRIMETHAMINE FOR *PLASMODIUM FALCIPARUM* MALARIA IN AFGHANISTAN

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Combination therapy with artesunate plus sulphadoxine-pyrimethamine was adopted as recommended treatment for ***Plasmodium falciparum*** infection in Afghanistan in 2003. We have performed a series of efficacy studies to examine the efficacy of AS+SP against ***P. falciparum*** in sentinel sites in Afghanistan from 2007 to 2014, accompanied by relevant molecular studies. Initial work (n=120) involved randomising patients to artesunate plus sulphadoxine-pyrimethamine or dihydroartemisinin-piperaquine, while subsequent studies were therapeutic efficacy studies of artesunate plus sulphadoxine-pyrimethamine. The studies enrolled 303 patients across four provinces in the north and east of the country. Efficacy was high in all the trials, with an adequate clinical and parasitological response (ACPR) of more than 95% in all groups and trial stages. Genotyping for drug-resistance alleles at dhfr indicated fixation of the S108N mutation and a prevalence of the C59R mutation of approximately 95%. Other mutations in dhfr and dhps were generally rare or absent entirely. The prevalence of the dhps K540E mutation fell over the course of the study (5/60 samples to 0/135; p = 0.0024) suggesting that despite the ongoing use of sulphadoxine-pyrimethamine, there is no evidence of worsening resistance to it components. For the study undertaken in 2012-2014, only two samples of 60 successfully sequenced carried a K13-propeller mutation.These data confirm maintained efficacy of the artesunate plus sulphadoxine-pyrimethamine combination against ***P. falciparum*** and suggest that the extent of sulphadoxine-pyrimethamine resistance has not worsened and may be improving.

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PHARMACOKINETICS OF TRANSFER OF PIPERAQUINE INTO THE BREAST MILK OF PAPUA NEW GUINEAN MOTHERS

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Women living in malaria-endemic areas, such as coast Papua New Guinea (PNG), are at high risk of malaria infection during pregnancy. Currently recommended treatment strategies include prompt treatment of symptomatic malaria and intermittent presumptive treatment in pregnancy (IPTp). A promising candidate for IPTp is dihydroartemisinin-piperaquine (DHA-PQ) which has been assessed in a number of safety, efficacy and pharmacokinetic studies. Whilst available data suggests this combination is safe and effective for use in pregnancy, there are no published pharmacokinetic studies of the transfer of PQ into breast milk and its subsequent ingestion by the infant. The transfer of PQ into breast milk was investigated in 27 pregnant PNG women who received a 3-day course of DHA-PQ or sulfadoxine-pyrimethamine-PQ during the second/third trimester. Breast milk samples were collected 1, 2, 3-5, 7-11 and 14-17 days post-delivery with a maternal blood sample also collected at time of delivery. Milk and plasma PQ was assayed using high performance liquid chromatography. A population-based approach was used to model log e (plasma) and milk concentration-time data. PQ breast milk transfer was found to be best described by a sigmoid Emax model. A milk:plasma ratio was found to be 0.58 (population average) with a peak of 2.5 found at delivery. The median estimated absolute and relative cumulative infant PQ doses were 22 µg and 0.07%, respectively, corresponding to absolute and relative daily doses of 0.41µg/kg and 0.004%. Model-based simulations for PQ treatment given at birth, 1 week post-delivery and 6 weeks post-delivery showed that the highest median estimated relative total infant dose (0.36%, or median absolute total dose 101 µg/kg) was seen after maternal PQ treatment 6 weeks postpartum. The maximum simulated relative total and daily dose from any scenario were 4.3% and 2.5%, respectively, lower than the recommended 10% upper limit. Therefore, it was demonstrated that PQ is transferred into breast milk after maternal treatment but the level of infantile exposure appears safe.

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A SURVEY OF THE RESERVOIR OF MOLECULAR MARKERS OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL DRUG RESISTANCE FROM A HIGH TRANSMISSION SETTING IN BONGO DISTRICT, GHANA

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Despite significant strides made to decrease the burden of malaria globally, billions of infections still persist for months in the human population. These chronic infections constitute the reservoir of infection and if left untreated, serve to fuel continued transmission. ***Plasmodium falciparum*** resistance to anti-malarial drug treatments threatens malaria control and elimination activities worldwide. To eliminate malaria, it is essential that parasite populations be monitored so that genetic diversity, including drug resistance, is examined before, during and after interventions. A study based on a panel of 4 drug resistant genes; Pfcrt, Pfmdr1, Pfdhfr, and Pfdhps, was completed on 242 slide positive ***P. falciparum*** isolates collected from a cross-sectional survey of asymptomatic participants (>1 year) in two villages at the end of the 2012 dry season in Bongo District (BD), Ghana. The loci investigated include codons 72, 73, 74, 75 and 76 of the Pfcrt gene; 86, 184, 1034, 1042, and 1246 of the Pfmdr1 gene; 51, 59, 108, and 164 of the Pfdhfr gene; 436, 437, 540, 581, and 613 of the Pfdhps gene. Mutations in key codons associated with resistance were detected (MEGA V6) following sequencing of the positive PCR amplicons. Over 15% (n=139), 81% (n=207), 90% (n=186), and 80% (n=228) of samples had at least one of the mutations in Pfcrt, Pfmdr1, Pfdhfr, and Pfdhps, respectively. The prevalence of Pfcrt mutation K76T was 7% and 12% for Pfmdr1 mutation N186Y. The prevalence of Pfdhfr S108N mutant was 87%, and the Pfdhps mutation A437G was 80%. C72V73I74E75T76 haplotype had a prevalence of 3.6%. In addition, 15 atypical/novel non-synonymous mutations that have not been previously reported in Ghana, were observed among 21% of samples in Pfdhps and Pfdhfr. This data also predicted ≈35 ***P. falciparum*** clades within BD using a neighbor-joining

phylogeny. Our data portray a highly diverse ***P. falciparum*** population circulating in BD with possible resistance to CQ and SP. This data will be useful for improving malaria surveillance and for determining the specific parameters that need to be utilized when monitoring the effects of interventions on ***P. falciparum*** diversity.

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PLASMODIUM FALCIPARUM PFCRT-RESISTANT HAPLOTYPES IN CHILDREN WITH UNCOMPLICATED MALARIA FOUR YEARS AFTER CHANGE IN POLICY FROM CHLOROQUINE AS FIRST-LINE ANTIMALARIAL MEDICINE IN LAGOS, NIGERIA

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Chloroquine (CQ) was widely used for the treatment of ***Plasmodium falciparum*** for several decades. Despite the change in National malaria drug policy to artemisinin combination therapy (ACT) in Nigeria in 2005 due to CQ resistance to ***P. falciparum***, CQ is still widely used in the treatment of malaria because it is cheap, affordable and accessible. Genetic markers to predict ***Plasmodium*** parasites' resistance especially for single nucleotide polymorphisms (SNPs) have the potential to provide information on P falciparum resistance to antimalarial. This study determined the prevalence of Pfcrt haplotypes and point mutations in Pfmdr1 genes four years after the change in antimalarial treatment policy to the ACTs in Lagos, a commercial city in Nigeria.It was a cross sectional study of uncomplicated malaria in children less than 12 years that presented with fever and other symptoms suggestive of malaria. Parasites DNA were extracted from 119 patients out of 251 children that were positive for ***P. falciparum*** by microscopy and amplified. The occurrence of haplotypes was investigated in Pfcrt gene using probe-based qPCR and nested PCR for SNPs in Pfmdr1 gene.The majority of the children (91.6%) harboured parasites with the mutant Pfcrt haplotype (CVIET). Five of the isolates (4.2%) had a mixture of genotypes encoding CVMNK and CVIET,while 4.2% had the wild type (CVMNK). SVMNT was not seen in this population. Furthermore, the frequency of point mutations in Pfmdr1 was 62.2% and 69.0% for codons Y86 and F184 respectively. There were no mutations at codons 1034, 1042 and 1246 of the Pfmdr1 genes. The high frequency of the CQ-resistant haplotypes (CVIET) and mutations in the Pfmdr1 known to be associated with CQ failure seen in this study suggest that CQ resistance ***P. falciparum*** parasites are still in circulation. Continuous use of CQ may increase the level of resistant Pfcrt haplotypes and point mutations in Pfmdr1 genes and could threaten the efficacy of current ACTs. There is need to strengthen current case management efforts at promoting ACT and restricting access to CQ and other antimalarial monotherapy by the drug regulatory Agency.

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MOLECULAR SURVEILLANCE OF POLYMORPHISMS IN THE K13 PROPELLER DOMAIN OF *PLASMODIUM FALCIPARUM* MALARIA FROM THIES, SENEGAL, 2011-2014

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In Senegal, artemisinin combination therapy (ACT) has been adopted in 2006 as the first-line treatment for uncomplicated ***Plasmodium falciparum*** malaria. Recent reports have provided evidence for the evolution of artemisinin resistance in the Greater Mekong Subregion, threatening current malaria control and elimination efforts. Monitoring of the recently identified artemisinin resistant K13 propeller mutations in the context of therapeutic efficacy studies has now been adopted as a way to monitor changes in the sensitivity patterns of parasites to artemisinin drugs. Some of the mutations in the K13 propeller domain have been found to be associated with delayed parasite clearance and ring stage parasite survival. The goal of this study was to determine the presence or absence of K13 propeller mutations from ACT therapeutic efficacy study samples collected in Thies, Senegal, during 2011-2014. We performed Sanger sequencing of the K13 propeller gene using protocols established in our laboratory. A total of 251 samples were analyzed for mutations in the K13 propeller domain using the Geneious Pro R8 software. An automated single nucleotide polymorphism (SNP) calling workflow developed in our laboratory using Geneious Pro R8 was used for this analysis. Briefly, by selecting a user defined sequence list and reference sequence as an input, the workflow automatically mapped the input sequences to the reference sequence, identified all SNPs, and exported the final SNP calls. Each step created a sub-folder allowing the user to check the results. SNPs were only called if both the forward and reverse strands had the mutation. No mutations were detected in the K13 propeller domain; all 251 samples from Thies, Senegal, were wild type. Overall, the K13 molecular data is consistent with the therapeutic efficacy study results which showed ACT remains efficacious in Thies, Senegal.

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DYNAMICS OF MALARIA DRUG RESISTANCE IN THE GAMBIA

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Chemotherapy has been one of the most effective malaria control measures, but has always been limited by repeated appearance and spread of resistance to almost all antimalarial drugs in use. Recent reports of uprising resistance to Artemisinin in South East Asia calls for the surveillance of its development and spread. This current study is based in a rural setting in eastern part of The Gambia, where participants (n=120) diagnosed with uncomplicated malaria were recruited from a health facility. Samples were collected before and after treatment, with participants followed for 42 days. Parasites were tested ***ex vivo*** against 11 popular anti malarial drugs including artemisinin and its derivatives. Median IC_{50} values were generated and for n=50 (Piperaquine = 30.3nM; Artemisinin = 7.4nM; Dihydroartemisinin = 3.15nM; Lumefantrine = 82.43nM; Amodiaquine = 11.4nm; Quinine = 69.4nm; Chloroquine = 61.3nM; Pyremethamine = 8410nM; Artesunate = 4.6nM; Mefloquine = 27.2nM and Artemisinin = 10.3nM). Analysis is being conducted to compare ***ex vivo*** data with ***in vivo*** clinical response, which reported 30% (n=120) recurrence of parasites, primarily in the last 2 weeks of follow up visit. Genotyping by both SNP barcode and MSP are being used to investigate the parasite populations at initial infection and subsequent recrudescence or re-infection. In addition, molecular analysis of drug resistant mutations (pfcrt, pfmdr1, dhps, dhfr) will be correlated with the ***ex vivo*** response and the observed ***in vivo*** clinical outcome. This analysis will help provide a picture of the efficacy of the current line of treatment (Artemisinin based combination Therapy) in a sub-Saharan setting as well the distribution of drug resistance markers in a parasite population.

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RECRUDESCENT *PLASMODIUM FALCIPARUM* ISOLATES FROM DHA-PIPERAQUINE FAILURES IN CAMBODIA: *IN VITRO* SUSCEPTIBILITY TO NEWER ANTIMALARIAL DRUGS

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Dihydroartemisinin-piperaquine (DHA-PPQ) is the frontline artemisinin combination therapy (ACT) for falciparum malaria in Cambodia, but recent treatment failures - caused by resistance to both DHA and PPQ - are now prevalent in this country's western provinces. Alternative treatments, including ACT partner drug replacements, are urgently needed. To investigate potential treatments, we culture-adapted ***Plasmodium falciparum*** clinical isolates that recrudesced following treatment with DHA-PPQ in Cambodia in 2012-2013, and measured their in-vitro susceptibilities to two novel potent antimalarial compounds (NITD609, OZ439), various alternative ACT partner drugs [lumefantrine (LUM), pyronaridine (PYN), ferroquine (FQ), naphthoquine (NQ)], and PPQ. Using a SYBR Green I fluorescence assay, we calculated the in-vitro IC_{50} values of these drugs for 36 recrudescent parasites - both the initial isolate at the time of clinical presentation and the recrudescent isolate at the time of treatment failure. The geometric mean IC_{50} values (GMIC50s) for initial isolates were: 0.9 nM for NITD609, 2.4 nM for OZ439, 21.8 nM for LUM, 6.8 nM for PYN, 33.7 nM for FQ, 12.9 nM for NQ, and 51.5 nM for PPQ. The $GMIC_{50}$s for all seven drugs were not significantly different between initial and recrudescent isolates. Additionally, there were no positive correlations between GMIC50s between drugs, except for NQ versus PYN. These data indicate that contemporary Cambodian isolates causing DHA-PPQ failures are highly susceptible to newer antimalarials, and suggest that these drugs might be useful components of alternative combination therapies for multidrug-resistant malaria in Southeast Asia. These data also provide valuable baseline GMIC50s for these drugs should they become routinely used in Cambodia in the near future.

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ASSESSMENT OF THE EFFICACY OF ARTESUNATE-AMODIAQUINE: RECOMMENDED THERAPY BY THE NATIONAL MALARIA CONTROL PROGRAM (NMCP) IN WESTERN MADAGASCAR

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Madagascar remains malaria endemic, but there are plans to shift from malaria control policies towards achieving pre-elimination status. Although there is evidence that four malaria species are transmitted across Madagascar, ***Plasmodium falciparum*** is the predominant species nationally, with clusters of ***P. vivax*** transmission particularly in the highland fringe regions of western Madagascar. In order to assess the efficacy of 3-day artesunate-amodiaquine (ASAQ) therapy in Madagascar, drug efficacy was evaluated in children presenting with uncomplicated malaria. Children between six months and 5 years with uncomplicated ***P. falciparum*** and ***P. vivax*** malaria were enrolled in May 2012 to September 2012, in Tsiroanomandidy, in a western, endemic area of Madagascar. The day-28 treatment failure rate assessed by conventional microscopy was compared to molecular diagnostic evaluation by a ligase detection reaction-fluorescent microsphere assay (LDR-FMA). Risks of clinical and parasitological treatment failure after adjustment by molecular diagnosis were estimated using Kaplan-Meier survival analysis. Secondary outcomes included fever clearance, parasite clearance, change in hemoglobin levels between Day 0 and the last day of follow-up, and the incidence of adverse events. Comparison of microscopy and LDR-FMA data was performed for eight individuals for whom data from day 0 to day 28, one person was missing the day 21 samples. Among these 8 individuals parasitemia cleared by day 2 post-treatment, however, parasitemia was observed to return on day 28 for one individual. The molecular diagnostic signal lingered beyond day 2 for all individuals for up to 21 days post-treatment. Return of molecular diagnostic signal was observed to return for 3 individuals. In the context of AS-AQ effectiveness studies, it will be important to monitor clearance of malaria by microscopy and molecular diagnostic strategies.

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THE INFLUENCE OF THE 581G MALARIA PARASITE MUTATION ON INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP): A SYSTEMATIC REVIEW AND META-ANALYSIS

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The World Health Organization (WHO) recommends the provision of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) to pregnant women resident in areas of moderate (stable) or high malarial transmission to reduce the incidence of low birthweight (LBW) and other adverse birth outcomes attributable to malaria. However, the protective effect of IPTp-SP has been compromised due to parasite mutation. Of particular concern is the ***Plasmodium falciparum*** dihydropteroate synthetase (***Pfdhps***) resistance mutation at codon 581G which appears to render falciparum malaria parasites 'super resistant' to SP. We conducted a systematic review and meta-analysis of the protection against the incidence of LBW conferred by > 2 doses of IPTp-SP. Two or more doses of IPTp-SP versus placebo or no IPTp-SP cut the odds in half of delivering a LBW newborn among primi- and secundigravidae (odds ratio [OR] = 0.54; 95% Confidence Intervals [CI]: 0.35, 0.84; $P < 0.00$). Among multigravidae, the odds were reduced by 30% (OR = 0.70; 95% CI: 0.51, 0.95; $P = 0.04$). We then used a geographical database of biomarkers to obtain point prevalence estimates of the Pfdhps 581G mutation among parasites from the same locations where IPTp-SP studies had been conducted. Using these estimates, we carried out sensitivity analyses and found that IPTp-SP protected primi- and secundigravidae against the incidence of LBW where the prevalence of the parasite mutation 581G was < 10.1% (OR = 0.49; 95% CI: 0.29, 0.81; $P = <0.01$) and where the prevalence of 581G was > 10.1% (OR = 0.73; 95% CI: 0.29, 1.81; $P = 0.03$). In contrast, among multigravidae there was only borderline protection against LBW conferred by IPTp-SP in areas where the prevalence of 581G was < 10.1% (OR = 0.56; 95% CI: 0.37, 0.86; $P = 0.07$) and no evidence of protection in settings where the prevalence of 581G was > 10.1% (OR = 0.96; 95% CI: 0.70, 1.34; P = 0.47). This suggests that there may be a threshold that could be used to guide IPTp-SP policy change.

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PLASMODIUM FALCIPARUM RECRUDESCENCE IN CULTURE FOLLOWING PULSE TREATMENTS WITH LUMEFANTRINE

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Multidrug-resistant ***Plasmodium falciparum*** is a major threat to global malaria control. Currently there is world-wide recommendation for the use of artemisinin-combination therapy (ACT). Artemether-lumefantrine is the recommended treatment for falciparum malaria in

51 countries. The lumefantrine component is a synthetic aryl-amino alcohol that mainly targets the mature asexual stages of the parasite. While there are no confirmed characterizations of parasite resistance to lumefantrine, there is substantial evidence for treatment failure in patients treated with artemether-lumefantrine. A risk for resistance is that the relatively long half-life of lumefantrine (3-5 days) exposes a proportion of parasites that have not yet been cleared to sub therapeutic levels of lumefantrine after artemether leaves the circulation. In this study, parasites from a Cambodian clone of ***P. falciparum*** (CP-803) were subjected to pulse treatment with lumefantrine at incremental concentrations. These treatment pulses reduced parasitemia to subpatent levels before recrudescent parasite populations were obtained. Interestingly the selected parasites were completely eliminated by continuous exposure for 28-days at <10% of the drug concentration used for the pulse treatments, and the lumefantrine IC_{50} levels of the selected line remained little changed from that of the unselected parasites.

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POLYMORPHISMS IN K13, PFCRT, PFMDR1, PFDHFR AND PFDHPS IN PARASITES ISOLATED FROM SYMPTOMATIC MALARIA PATIENTS IN BOBO-DIOULASSO, BURKINA FASO

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The emergence of resistance to artemisinin derivatives in western Cambodia could jeopardize the control and elimination of malaria. Known resistance-mediating polymorphisms in the K13, pfcrt, pfmdr1, pfdhfr and pfdhps are of greatest importance to monitor the spread of resistance. Samples for this study were collected in 228 malaria patients randomized to receive artemether-lumefantrine or artesunate-amodiaquine for the treatment of uncomplicated malaria in Colsama and Sakaby health centers, Bobo-Dioulasso, Burkina Faso. Blood sample were collected on filter paper on day 0,1,2,3,7 14,21,28 and on any day the patients felt ill. We evaluated the prevalence of polymorphisms in K13, pfcrt K76T, pfmdr1 (N86Y, Y184F) and pfdhps (A437G, K540E) in parasites collected prior treatment. We reported 1.8% (5/221) of K13 synonymous mutant alleles (two C469C, one Y493Y, one G496G, and one V589V), 24.5%, 19.5% and 70.0% respectively for mutant pfcrt 76T, pfmdr1-86Y and pfmdr1-184F. Sulfadoxine-pyrimethamine (SP) resistance associated pfdhfr 51I, 59R and 108N were found in 141/228 (61.8%), 124/228 (54.4%) and 146/228 (64.0%) samples and pfdhps 437G in 145/228 (63.5%) samples. These data provide baseline prevalence of key antimalarial drug-resistance polymorphisms in Bobo-Dioulasso, Burkina Faso. e results suggest that artemisinin combination therapies and SP may retain good efficacy respectively in the treatment and prevention of malaria in Bobo-Dioulasso, Burkina Faso.

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THE ROLE OF THE MALARIA PARASITE SUGAR PHOSPHATASE, PFHAD1, IN FOSMIDOMYCIN RESISTANCE AND METHYLERYTHRITOL PHOSPHATE (MEP) PATHWAY REGULATION

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The methylerythritol phosphate (MEP) pathway for isoprenoid precursor biosynthesis is an attractive target for novel anti-malarial drug development, as compounds that target this pathway lack toxicity concerns for humans. The small molecule compound fosmidomycin inhibits the MEP pathway enzyme deoxyxylulose 5-phosphate (DXR) and is in clinical trials for combination therapy with other anti-malarial compounds. Fosmidomycin-resistant ***Plasmodium falciparum*** strains were generated ***in vitro***. Genetic analysis of these parasites revealed that they are highly enriched for mutations in PfHAD1. The crystal structure of PfHAD1 was solved in order to determine the effects of the mutations on PfHAD1 structure and function, which revealed that these mutations cause loss of PfHAD1 function via protein misfolding or interference with substrate binding. We found PfHAD1 to be a sugar phosphatase member of the haloacid dehalogenase (HAD) superfamily with catalytic activity towards a variety of sugar phosphate compounds, including intermediates of glycolysis - which feed into the MEP pathway. Metabolic profiling revealed that fosmidomycin-resistant parasite strains lacking PfHAD1 have substantial increases in MEP pathway metabolites. Together, these results demonstrate that PfHAD1 regulates substrate availability to the MEP pathway and that loss of PfHAD1 function confers fosmidomycin resistance in ***P. falciparum***. While the metabolic effects and a biological phenotype of PfHAD1 have been elucidated, the substrate specificity and mechanism of catalysis for PfHAD1, or HAD superfamily members generally, have not been well defined. Crystal structures of PfHAD1 in complex with three different upstream MEP pathway precursors reveal how domain movement in PfHAD1 enables diverse substrate recognition. These studies further inform the molecular role of HAD enzymes, in regulation of an ancient, evolutionarily conserved, and essential metabolic pathway.

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MOLECULAR SURVEILLANCE FOR K13 GENE AND OTHER *PLASMODIUM FALCIPARUM* MOLECULAR MARKERS ASSOCIATED WITH ANTIMALARIAL RESISTANCE IN SURINAME

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The emergence and spread of antimalarial resistance in ***Plasmodium falciparum*** has the potential to severely limit the efficacy of antimalarial regimens. In Suriname, the current ***P.falciparum*** first-line treatment, artemisinin and lumefantrine (AL), is monitored every three years. One of the indicators for suspected resistance associated with artemisinin drugs is delayed parasite clearance (persistence of >10% of parasites on Day 3 after treatment initiation). An antimalarial trial using 3 days of artesunate monotherapy followed by mefloquine and primaquine was conducted during 2013-2014. Forty ***P. falciparum*** samples obtained at the time of enrollment were tested for the artemisinin resistance-associated K13 gene and for other known drug resistance markers, ***pfcrt, pfmdr1, pfdhfr,*** and ***pfdhps***. The K13 propeller domain and drug resistant genes were PCR amplified using established laboratory protocols and the amplicons were sequenced using Sanger method. Our results showed that all 40 samples contained only the wild type K13 sequence. In addition, all isolates carried the chloroquine-resistant ***pfcrt*** genotype SVMNT (codons 72-76), triple mutant pyrimethamine resistant ***dhfr*** genotype (50R/51I/108N), triple mutant sulphadoxine resistant ***dhps*** genotype (437G/540E/581G) and ***pfmdr1*** mutant genotype. Only a single isolate out of the 40 tested had two copies of ***pfmdr1*** gene, previously associated with mefloquine resistance. In addition, when analyzing neutral microsatellite data, the haplotypes found in Suriname were similar to those previously reported in Guyana, Venezuela, and Brazil, which indicates active migration in this area. In summary, this study found no evidence for the presence of artemisinin-resistant K13 alleles. Continued monitoring of antimalarial efficacy, using ***in vivo*** trials and molecular markers, are necessary to detect changes in treatment response and prevalence of ***P. falciparum*** resistant alleles.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH DIHYDROARTEMISININ-PIPERAQUINE ON *PLASMODIUM FALCIPARUM* POLYMORPHISMS THAT MODULATE DRUG SENSITIVITY IN A TRIAL OF UGANDAN SCHOOLCHILDREN

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Dihydroartemisinin-piperaquine (DP) offers prolonged protection against malaria, due to the long half-life of piperaquine, but its impact on polymorphisms mediating ***Plasmodium falciparum*** resistance is uncertain. In a trial undertaken in Tororo, Uganda in 2011-12, monthly treatment with DP for one year in schoolchildren aged 6-14 years decreased the incidence of malaria by 96% and the incidence of asymptomatic parasitemia by 94% compared to children receiving placebo. To assess the impact of DP on parasite resistance-mediating polymorphisms in this trial, we assessed the prevalence of key polymorphisms between isolates that emerged at different intervals after treatment with DP. Blood was obtained during each episode of fever and monthly in asymptomatic children. Samples collected within 14 days of treatment for malaria (with artemether/lumefantrine) were excluded. 810 samples from symptomatic (160) and asymptomatic (650) episodes of parasitemia were assessed at 4 loci that modulate sensitivity to aminoquinoline antimalarials (N86Y, Y184F, D1246Y in pfmdr1 and K76T in pfcrt) utilizing a ligase detection reaction fluorescent microsphere assay. For pfmdr1 N86Y and pfcrt K76T, the prevalences of mutant genotypes, compared to wild type/mixed genotypes, were significantly greater in children who had received DP within 30 days of the episode of parasitemia (18% for N86Y; 96% for K76T) compared to those not treated within 60 days (8.3%, p=0.035 for N86Y; 86.1%, p=0.049 for K76T) or within 90 days (6.9%, p=0.012 for N86Y; 84.5%, p=0.031 for K76T). Associations were not seen between time since treatment and other SNPs. DP offered potent preventive efficacy against malaria, but parasites that emerged soon after treatment were more likely than parasites not under drug pressure to harbor pfmdr1 and pfcrt polymorphisms associated with decreased sensitivity to aminoquinoline antimalarials. DP offers promising preventive efficacy but additional studies of its potential selection of drug resistant parasites are warranted.

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THE EFFICACY, SAFETY AND TOLERABILITY OF REPEAT DOSING WITH DIHYDROARTEMISININ-PIPERAQUINE FOR THE PREVENTION AND TREATMENT OF MALARIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Intermittent preventive treatment (IPT) of malaria is a potential strategy for the control of malaria in infants, children, adults and pregnant women. Dihydroartemisinin-piperaquine (DP) is an effective and well tolerated antimalarial. The long half-life of piperaquine (~22 days) makes it an attractive choice for IPT. We conducted a systematic review and meta-analysis to determine the efficacy and safety of repeated exposures to 3-day courses of DP. We searched MEDLINE, EMBASE, Web of Science, Scopus, CINAHL Plus, the Cochrane Library databases, WHO Global Health Library and the Malaria in Pregnancy Consortium Library. Studies were eligible if they included prospective data on participants that received more than one dose of DP for IPT or case-management. Random effects models were used. Our search identified 745 citations; after title review 365 abstracts were reviewed. Nine unique patient populations were included: two repeat treatment studies (1 in children <5y [N=312] and 1 in pregnant women [N=5192]) and seven randomized, controlled IPT trials (5 in children <5y [N=5394], 1 in school children [N=740], 1 in adults [N=961]). In total, there were 12, 435 participants; 3099 were exposed to DP, including 2180 <5 years of age and 485 pregnant women. Comparator interventions included placebo, artemether lumefantrine, sulfadoxine-pyrimethamine (SP), SP-amodiaquine, SP-piperaquine, SP-chloroquine, and trimethoprim-sulfamethoxazole. The range of doses of DP was 3-18. Overall, monthly IPT-DP provided greater protective efficacy (PE) against any parasitemia than placebo (pooled PE: 88%, 95% confidence interval 84-93%). In total, 282 serious adverse events (SAEs) were reported, 62 among those exposed to DP; no study reported a disproportionate number of SAEs in DP recipients. Electrocardiogram results were reported from 19 participants from one study; all QTc intervals were reported within normal limits. The limited data on repeat DP exposures suggest that 3-day course of DP is safe and effective and a good option for IPT. Additional data are needed on the potential for QT prolongation and the safety of repeat exposures in pregnancy.

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HEALTH WORKERS' KNOWLEDGE ON ADMINISTRATION OF INJECTABLE ARTESUNATE FOR TREATMENT OF SEVERE MALARIA IN OROMIA AND SNNPR REGION, ETHIOPIA

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The World Health Organization (WHO) recommends injectable artesunate as the first drug of choice for the treatment of severe malaria. Although Ethiopia adopted this recommendation and revised the malaria case management guidelines in 2012, no official training of health workers was performed on the new guidelines for management of severe malaria. We assessed the health workers knowledge on appropriate use of injectable artesunate for treatment of severe malaria cases. The study was conducted among 320 health workers randomly selected from 1,498 health facilities of malarious district of Oromia and Southern Nation Nationality and People Regional State (SNNPR). Self-administered questionnaires were used to assess the knowledge on proper administration of injectable Artesunate. Of the total participants, 66.3% were from Oromia state and 15.7% were doctors, 54.7% were nurses, 12.25% were health officers and the rest pharmacists. Majority (94.1 %) correctly responded that injectable artesunate is recommended for treatment of severe malaria but only 11 (3.4%) could demonstrate the steps for proper preparation and administration of the drug. Only 2% said that it is safe to administer injectable artesunate during all trimesters of pregnancy and 63% cited that proper administration sites for injectable artesunate. Although most health workers know that injectable artesunate is the first drug of choice for treatment of severe malaria, the study demonstrated that there is a lack of knowledge on preparation and administration of drug. Intensive training with practical sessions is required for health workers for proper managing severe malaria cases using injectable artesunate. Distribution of injectable artesunate, without building the capacity of health workers may result in risk of misuse of and development of resistance to artesunate.

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AN ASSESSMENT OF INTERMITTENT PREVENTATIVE TREATMENT IN PREGNANCY COVERAGE ACROSS SUB-SAHARAN AFRICA FROM 2000-2015

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Malaria in pregnancy has been shown to cause various poor health outcomes for mothers and their children. Intermittent Preventative Treatment of pregnant women (IPTp) with sulfadoxine-pyrimethamine (SP), the administration of one full treatment course of SP at routine second and third trimester prenatal visits, has been shown to reduce severe maternal anemia, low birthweight, and perinatal mortality with minimal adverse effects. Data from country reports and nationally-representative household surveys were used to assess the coverage levels of IPTp since the year 2000. Among the 37 countries with national IPTp policies, 30 reported on the number of women who attended ANC at least once and 31 reported on the number of doses of IPTp administered in 2013, more countries than in previous years. These reports were compared to the estimated number of pregnant women for each country, derived from UN population estimates. For 9 countries reporting on receipt of 3 or more doses of IPTp, a median of 17% of all pregnant women received 3 or more doses of IPTp; a median of 43% of pregnant women received 2 doses of IPTp in 31 countries, and 57% received at least one dose as reported by 30 countries. In 2013, a median 89% of pregnant women in reporting countries attended ANC, which, when compared to the proportion receiving at least one dose of IPTp, suggests a number of missed opportunities for delivery at ANC. The combination of yearly NMCP-reported IPTp distribution data with estimates of pregnant women and household survey data to assess trends over time shows an impressive increase in the uptake of all doses of IPTp through 2007, no substantial change during 2007-2010, followed by a modest upward trend projected through 2015. Given the importance of IPTp as a malaria intervention for a high risk population, continued efforts should be made to quantify the coverage levels.

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IMPACT OF RAPID DIAGNOSTIC TESTS FOR THE DIAGNOSIS AND TREATMENT OF MALARIA AT A PERIPHERAL HEALTH FACILITY IN WESTERN UGANDA: AN INTERRUPTED TIME SERIES ANALYSIS

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The WHO recommends that all suspected malaria cases receive a parasitological diagnosis prior to treatment with artemisinin-based combination therapy. A recent meta-analysis of clinical trials evaluating RDTs for the management of patients with fever found substantial reductions in antimalarial prescriptions when health workers adhered to treatment protocols based on test results. However few studies have reported on the impact of RDTs on health systems outside research settings. We conducted a retrospective interrupted time series, comparing rates of malaria diagnosis, treatment, and resource utilization before and after introduction of RDTs at a peripheral health facility in rural Western Uganda. We graphically depicted the use of malaria diagnostic tests throughout the study period and fit regression models to identify correlates of three outcomes of interest: (1) length of stay (2) the proportion of patients referred to a higher-level health facility, and (3) administration of antibiotics. Over the course of the study period, 14,357 individuals underwent diagnostic testing for malaria with either a RDT (9,807) or microscopy (4,550). The proportion of patients with parasite-based diagnoses more than tripled to 34% after the introduction of RDTs. RDTs largely replaced microscopy as the diagnostic method of choice. Compared to patients admitted during the pre-RDT period, patients admitted to the health center with malaria in the post-RDT period had significantly reduced odds of being referred to another health center (AOR=0.49, P=0.038), receiving antibiotics (AOR=0.42, P<0.001), and a significantly shorter mean length of stay (β= -0.32, 95%CI -0.52 to -0.13). Our study is one of the few to demonstrate significant improvement in clinical outcomes and process measures following the introduction of RDTs for the diagnosis of malaria at a rural health facility in Uganda. We observed a reduction in referrals and shorter mean inpatient LOS even as antibiotics were prescribed less frequently. This change greatly increased laboratory throughput and the resultant proportion of patients receiving a parasite-based diagnosis.

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DIAGNOSTIC PERFORMANCE OF A NOVEL MALARIA LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY IN A FIELD SETTING

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In endemic settings, WHO recommends confirmation of malaria prior to treatment either by microscopic examination of blood films or the use of Rapid diagnostic tests (RDTs). The renewed interest in global eradication of malaria however, calls for more sensitive and high throughput diagnostic tools for the end game. Although RDTs have a faster turnaround time, there is a high rate of false positivity due to persistent circulation of antigen after infection; thus, molecular tools such as polymerase chain reaction (PCR) that amplify parasite DNA are being developed. Deployment of PCR in field settings or peripheral centers where they are most needed is not feasible therefore isothermal amplification methods such as Loop mediated isothermal amplification (LAMP) are being developed. In this study, we report the diagnostic performance of a novel highly sensitive LAMP assay targeting the apicoplast genome, in a field setting. The study was carried out in the screening stage of an ongoing trial comparing the effect of dihydroartemisinin-piperaquine (DHA-PPQ) alone or with single doses of Primaquine (PQ) on gametocyte carriage among individuals with asymptomatic malaria. Samples were collected from consenting individuals in the study villages around Basse and Walikunda in The Gambia from October to December 2014. From a single finger prick, samples were collected from 495 participants for microscopy, RDT and dried blood spots (DBS). DNA was extracted from the DBS by a simple methanol extraction method and the LAMP assay was performed in a field site the same day. A mean of 35 samples were collected daily and turnaround time for the LAMP assay was approximately two hundred and seventy minutes. Preliminary results show malaria prevalence of 38% by RDT and 34% by LAMP. Using RDT as the reference method, sensitivity of the LAMP assay was 77% (95%CI 63 - 88%) and specificity was 91% (95%CI 83 - 96%). Positive and negative predictive value of the LAMP assay was 84% and 87% respectively. As it becomes more feasible to deploy molecular tools for diagnosis of malaria at peripheral levels, global eradication of malaria can gradually become a reality.

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SPREAD OF PFHRP2- AND PFHRP3-NEGATIVE *PLASMODIUM FALCIPARUM* PARASITES IN RURAL COMMUNITIES FROM THE PERUVIAN AMAZON REGION: IMPLICATIONS FOR RAPID DIAGNOSTIC TESTS

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The ***Plasmodium falciparum*** Histidine-Rich-Proteins 2 and 3 (HRP2/3) is a parasite antigen that is a key target of rapid tests to diagnose malaria. Clinical field samples lacking pfhrp2, pfhrp3 and their flanking genes were recently reported in peri-urban communities of the Peruvian Amazon. Little is known about the geographical expansion of the deletion in rural far away communities from Iquitos city, and resulting implications for malaria diagnosis. The aim of this study was to estimate the frequency of the deletion in pfhrp2, pfhrp3 and their flanking genes from clinical samples in three communities far from Iquitos. 146 samples were collected in San José de Lupuna (13 in 2012, 102 in 2013, and 31 in 2014) a rural community crossing Nanay river, 10km from Iquitos city; 219 samples were collected in Cahuide (14 in 2012, 163 in 2013, and 42 in 2014), a rural community located 60 km from Iquitos and 178 samples came from Santa Emilia (March 2013 -June 2013), a remote community located 150 km from Iquitos. qPCR was used to confirm and quantify ***P. falciparum*** infections. Parasitemia levels were classified in 3 groups: high (>200 molecules/µl), medium (20-200 molecules/µl), and low (<20 molecules/µl). High and medium parasitemia levels were tested for the amplification of pfhrp2-3 and their flanking genes by PCR, with detection limit of 10 molecules/µl. Of 227 samples, 122 (54%) were negative for pfhrp2; 47 from Cahuide, 31 from Lupuna and 44 from Santa Emilia. Out of 122 of pfhrp2 negative samples, 71 also lack the PF3D7_0831900 gene and only 23 lack pfhrp2 and both flanking genes. For pfhrp3 gene, 110 (48%) were negative; 34 from Cahuide, 36 from Lupuna and 40 from Santa Emilia. Out of 110 of pfhrp3 negative samples, 52 also lack the PF3D7_1372100 gene and only 14 lack pfhrp3 and its flanking genes. This is the first report of the pfhrp2 and pfhrp3 gene deletion in remote and rural communities of the northeast Peruvian Amazon where the vast majority of malaria is located in Peru. These data suggest a high frequency of the pfhrp2-3 gene and flanking gene deletions. This parasite population is becoming fixed in the region, with implications for diagnosis and potentially pathogenesis.

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EVALUATION OF MALARIA MICROSCOPY DIAGNOSIS FOLLOWING IMPLEMENTATION OF A QUALITY ASSURANCE PROGRAM IN LOW-TRANSMISSION AREAS, KENYA - 2014

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Malaria accounts for 9 million outpatient visits in Kenya annually. Prompt diagnosis of malaria is critical for early treatment, but microscopy services are often of poor quality or not available in health facilities. Kenya implemented a national malaria laboratory quality assurance (QA) program in 2013 to improve malaria diagnosis starting with low-transmission areas. We evaluated the performance of microscopy diagnosis after 8 months of QA implementation using blood slides archived in January and February 2014. From March to April 2014, we visited 21 health facilities in low-transmission areas implementing QA and 21 that had not started the QA program (control) matched on level of services provided and geographic location and randomly collected a total of 720 blood slides; 360 in each branch. The slides were re-examined by certified independent expert microscopists; results were used as the reference for validity and reliability. Eighty-four (23%) blood slides were malaria positive in each branch. Twenty-two (26%) slides were falsely positive in QA compared to 43 (51%) in control facilities. Sensitivity was 95% (95% CI: 87-99%) in QA facilities compared to 62% (95% CI: 49-74%) in control facilities. Specificity was 93% (95% CI: 89-95%) in QA compared to 85% (95% CI: 81-89%) in controls. The positive predictive and negative predictive values were 74% (95% CI: 63-83%) and 99% (95% CI: 97_100%), respectively, for QA facilities compared to 49% (95% CI: 38-60%) and 91% (95% CI: 87_94%), respectively, for controls. Primary county hospitals recorded the largest inter-observer agreement differences; kappa (κ) for QA facilities was 0.90 (95% CI: 0.81-0.99) and κ for controls was 0.38 (95% CI: 0.19-0.56). Overall, significant differences between inter-observer agreements were observed between QA facilities (κ=0.80; 95% CI: 0.72-0.88) and control facilities (κ=0.43; 95% CI: 0.32-0.54). Facilities implementing the malaria laboratory QA program in low-transmission areas performed unsatisfactorily in our evaluation of the validity and reliability of malaria microscopy diagnosis, although they outperformed matched control facilities.

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NINA-LAMP COMPARED TO MICROSCOPY AND RDT FOR THE DETECTION OF MALARIA

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LAMP based method have shown potential to detect sub-microscopic infections. However, the requirement of electrical instrumentation has limited the use of LAMP in resource poor environments. PATH has introduced a new heating system that is called NINA for simple operation of LAMP. In the current study, we have evaluated the efficacy of NINA-LAMP for detection of traveler's malaria in comparison with Microscopy, nested PCR and the only FDA approved RDT (BINAX NOW Malaria). In total, 69 (38 falciparum and 31 non-falciparum) microscopy positive and 71 negative samples were selected retrospectively for this study. Samples were collected in different times at Calgary Laboratory Service from returning travelers with fever. LAMP was performed using a commercial kit from Eiken Chemical Company, Japan by both NINA and PCR machine. We did not find any consistent difference in assay between LAMP conducted in NINA versus PCR machine. LAMP was 100 %(95% CI, 93.43-100) sensitive and 95.77% (95% CI, 87.33-98.90) specific in comparison with microscopy whereas sensitivity and specificity were 100% (95% CI, 93.60-100) and 98.55% (95% CI, 91.11-99.92) when compared to nested PCR for detection of all malaria cases. We also demonstrate high accuracy for ***P. falciparum*** (sensitivity 100% and 97.61%; Specificity- 97.06% and 100%; Microscopy and nested PCR) and non-falciparum (sensitivity-100% and 100% Specificity- 100% and 99.08%; microscopy and nested PCR) detection. On the other hand, the RDT was overall 85.50 %(95% CI, 74.5-92.4) and 85.91% (95% CI, 75.16-92.68) sensitive and 97.18% (95% CI, 89.28-99.51) and 98.55% (95% CI, 91.11-99.92) specific compared to microscopy and nested PCR, respectively. Although the RDT was accurate in ***P. falciparum*** diagnosis (sensitivity-94.74% and 90.48%), poor performance was observed for non-falciparum malaria detection (sensitivity-74.19% and 70.97%) compared to microscopy and nested PCR. We conclude that LAMP assay is highly sensitive and specific for symptomatic malaria diagnosis. As NINA is a non-instrumented system, it has the potential to replace RDTs at both field site and point of care in all settings.

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EVALUATION OF LAMP AS A MALARIA DIAGNOSTIC TOOL IN REACTIVE CASE DETECTION IN NAMIBIA WITH RDTS AS THE SOURCE OF DNA

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The number of sub patent infections increases up to 70% of the infected population as malaria decreases. Therefore, in low prevalence settings, malaria cases could be going undetected due to difficulty in detection of low parasite density infections with RDTs. Namibia is moving towards elimination and in order to eliminate malaria, all asymptomatic and symptomatic reservoirs of malaria that could perpetuate the spread of malaria need to be traced by reactive case detection in combination with LAMP. Consequently, the study evaluated the use of LAMP with nPCR as a reference in reactive case detection. All reported malaria cases from the Engela health district were followed up at their households, the reported case and all the individuals in the same household and 4 surrounding households were tested for malaria with RDTs and these RDTs were collected from all 2790 individuals that were tested. There were 1658 RDT samples from case neighbourhoods and 1132 samples from controls. DNA was extracted from the RDT samples for malaria diagnosis with LAMP. Nested PCR was performed on all LAMP positive samples and 10% of the negative samples as a reference standard. Species determination was done with RDTs, LAMP kits and cytochrome B digestion. RDTs detected 37 malaria infections with a sensitivity of 56.06%. LAMP detected 66 malaria infections with a sensitivity of 100%. A total of 64 of the LAMP positive samples were also nPCR positive and all LAMP negative samples were also nPCR negative. Both RDTs and LAMP determined that all the malaria infections were caused by P.falciparum and this was confirmed by n-PCR. The number of malaria infections detected doubled with the use of LAMP as compared to RDTs, in addition LAMP detected 4 times more secondary cases than RDTs. The majority of the malaria infections, 97%, were from case neighbourhoods. This indicates that individuals in proximity to malaria infections are more likely to be infected by malaria. Reactive case detection is an important surveillance tool in combination with LAMP and not RDTS to detect all cases around reported cases that are usually asymptomatic.

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AN ADVANCED COMPUTER VISION PLATFORM FOR CLINICAL DIAGNOSIS OF MALARIA

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Accurate malaria diagnosis is necessary to prevent unnecessary deaths, curb malaria drug resistance related to unnecessary treatment and uncover asymptomatic malaria patients. While numerous diagnostic assays exist, the need for a low-cost, rapid and highly accurate malaria test remains. We have merged inventions in sample preparation, machine design and software algorithms to create the first complete computer vision platform for blood analysis and malaria diagnosis. A blood sample is stained using a proprietary fluorescent dye and scanned in the automated microscopy system in a process that takes 4 minutes. The device then produces a malaria diagnosis, speciation and parasitemia per red blood cells. Clinical trials on the platform performed in Europe, Africa and India show a sensitivity of ~97% and a specificity of ~98% with speciation for ***Plasmodium vivax*** at 95.5% and ***Plasmodium falciparum*** at 99%, showing superior accuracy when compared with RDTs and microscopists. The device is currently commercially available and has achieved sales in Africa and India with projected device sales reaching 100 in 2015.

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UNDERESTIMATION OF ADJUSTED ODDS-RATIO DUE TO IMPERFECT DIAGNOSTIC RESULTS AND A PRACTICAL CORRECTION APPROACH

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Logistic regression is a statistical model widely used in epidemiology to identify and quantify the effect of potential disease risk factors. Although it is acknowledged that imperfect diagnostic tests distort disease prevalence estimates, little is known about the impact of imperfect tests on adjusted odds-ratios. We derive a first order approximation that reveals that imperfect diagnostic test results can lead to substantially underestimated effect sizes and overly narrow 95% confidence intervals, with important implications regarding the identification of risk factors. To overcome this bias, we propose a Bayesian model that explicitly accounts for imperfect detection. Using simulations, we show that this method can substantially improve upon the results from the standard logistic regression. Using malaria data from Bangladesh, we demonstrate how the proposed method leads to point estimates that are 1.1 - 23.0 fold larger than the corresponding point estimates from the standard logistic regression. Our method has the potential for widespread adoption by researchers and offer substantial improvements to current modeling practice in epidemiology.

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EMBRACING THE USE OF RAPID DIAGNOSTIC TESTING IN MALARIA DIAGNOSIS: EXPANDING ACCESS TO ACCURATE DIAGNOSIS

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Malaria has remained a major public health problem in Kenya despite being treatable. Proper diagnosis before treatment is recommended as it allows for appropriate patient management. The main diagnostic methods available are microscopic examination of stained blood smears and Rapid diagnostic tests (RDTs). RDTs have been shown to be effective in expanding access to malaria diagnosis to populations with limited access to good quality microscopy due to their ease of use and interpretation, lower training requirements, and lack of requirements for electricity, among others. RDTs have also been shown to have higher sensitivity and specificity as compared to routine clinical microscopy. Despite the overwhelming support for the use of RDT, there is anecdotal evidence of people mistrusting their negative results after an RDT test and thus lack of compliance with their results. There are also widespread reports from community members and healthcare workers of RDT negative patients being found positive by microscopy. Mistrust of negative RDT results could be a result of limited prior experience with RDT as well as discrepancies between results of microscopy and RDTs and hence the uncertainty about the accuracy of results. In Kenya, there have been no reports of the perceptions of RDTs and adherence to RDTs since their roll-out in 2011. Here we will present baseline data from 1300 households in two malaria-endemic areas in western Kenya collected in preparation for the roll out of Community level subsidized RDT and ACTs. We will report the uptake of diagnostic testing for fevers, and people's self-reported confidence in the results of their test, stratified by type of test (microscopy vs. RDT). We will also compare reported confidence in the test to actual treatment decisions taken amongst malaria-positive and malaria-negative patients. The findings will enable us to evaluate the need to delve further into understanding the level of knowledge, experiences, acceptance and opinions about

the use of RDTs for malaria diagnosis in the population. These findings have important consequences for the success of community-based case management for malaria.

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ASSESSING THE PERFORMANCE OF THREE HRP2 BASED RAPID DIAGNOSTIC TESTS FOR THE DIAGNOSIS OF MALARIA IN CENTRAL GHANA

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Rapid Diagnostic Tests (RDTs) could give False-negative results which present a greater challenge which may lead to delay in the initiation of treatment. We evaluated the diagnostic accuracy of hrp2 based RDTs with pLDH using microscopy as gold standard and to possibly detect parasites with hrp2 deleted genes. The cross-sectional study randomly consented and enrolled 754 participants from the two major public hospitals in the middle-belt of Ghana. Blood samples obtained were screened for malaria using the three types of hrp2 based RDT from CareStart and SD Bioline. Ethical clearance was given by KHRC IEC. A prevalence of 39.1% of malaria using microscopy was recorded. There were 28.6% (215/752) males and 71.4% (537/752) females with the Mean(SD) age of 21.4(17.8) years. Compared to microscopy the Sensitivity, Specificity, Positive-Predictive-Value, Negative-Predictive-Value and the ROC were 98.2%, 66.5%, 82.6%, 95.6% and 0.82 for CareStart Hrp2, 98.2%, 66.5%, 82.6%, 95.6% and 0.82 for CareStart Hrp2/pLDH and 98.2%, 69.2%, 84.2%, 96.0% and 0.84 for SD Bioline RDTs. All three RDTs used recorded 1.8% (5/281) false negative results. In conclusion, the diagnostic performance of the three HRP2 based RDTs according to the WHO criteria was excellent. There have been extensive report of malaria parasite infections caused by strains with hrp2 deleted gene. The brands of RDTs which target pLDH of malaria parasites could hopefully enable practitioners identify patients infected with parasites strains with hrp2 gene deletion; kits which are only hrp2 based will miss. The kits with the added pLDH could identify infections which are current and also those infections which persist as a result of treatment failure. There is however a decrease in specificity which could result from the persistence of hrp2/hrp3 proteins even weeks after effective treatment of malaria.

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USING THE EMERGENCY TRIAGE ASSESSMENT AND TREATMENT (ETAT) APPROACH TO REDUCE MORBIDITY FROM SEVERE MALARIA IN CHILDREN UNDER 5 IN HOSPITALS IN BENIN

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The ARM3 consortium, led by Medical Care Development International (MCDI) and funded by United States Agency for International Development/PMI, supports the Government of Benin (GOB) in improving malaria health outcomes in accordance with the National Malaria Control Plan (NMCP), including the reduction of malaria-associated mortality by 70%. In Benin, most hospital deaths among children <5 occur within 24 hours of admission because the waiting period before consultation. In an effort to reduce severe malaria case fatality rate of under 5 children arriving at reference hospitals, ARM3 rolled-out the Emergency Triage Assessment and Treatment (ETAT) approach, developed by the World Health Organization, in 25 hospitals (beginning with 12 in July 2013, Phase 1, and 13 additional hospitals in April 2014, Phase 2). Through learning sessions based on the "Collaborative Approach to Health Care Improvement" methodology, the 25 hospitals trained health workers on ETAT implementation including: (1) conducting a review of ETAT indicators; (2) evaluating implementation of ETAT at each site; (3) identifying best practices and lessons learned for dissemination; (4) updating databases; and (5) developing a 3-month action plan for each site. ARM3, the DSME, and national/departmental NMCP conducted monthly data quality validation of the targeted indicators in order to assess each hospital's performance. After one year of ETAT implementation, for hospitals in Phase 1, the % of children under 5 evaluated upon arrival at hospitals, increased from 3.4% to 95%, and the adherence rate to ETAT standards rose from 10% to 78%. For hospitals in Phase 2, after 6 months of implementation, the ratio of adherence to severe malaria guidelines rose from 50.2% to 82.2 % and the case fatality rate of severe malaria declined from 5.9% to 4.8%. The ETAT approach has shown positive results in 25 hospitals and the ARM3 project will continue to work with the MOH and the NMCP to train additional health workers on ETAT in Benin. ARM3 will also work with the GOB to ensure that sufficient ETAT supplies are available at health facilities and that HR policies are designed to reduce turnover of health workers trained on ETAT.

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MONITORING OF QUALITY OF RAPID DIAGNOSTIC TESTS IN SENEGAL, 2007-2014

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In 2007, the Senegal National Malaria Control Program introduced rapid diagnostic tests (RDTs) to improve the quality of malaria case management. RDTs have been deployed in all health facilities in the country and at the community level. To ensure the quality and reliability of RDTs used in Senegal, a quality control system was implemented with the support of WHO / FIND / TDR. The Parasitology Laboratory at Universite Cheikh Anta Diop achieved accreditation as a reference laboratory for RDT quality control in 2009. Upon receipt of RDTs in country, lot samples are collected systematically and sent to the Parasitology Department of UCAD. Following the WHO protocol for the quality control of RDTs, a visual inspection is carried out and the sensitivity of the RDTs assessed according to results with samples of known parasite densities of 200 parasites /µl, 2000 parasites /µl, and negative control. From 2007 to 2014, 800 RDTs from 100 lots of two brands of HRP-2 based RDTs were tested, 100 RDTs annually upon reception in country. Sensitivity against both at 2000 parasites /µl and 200 parasites /µl was 100%, and specificity against negative controls was 100%. During these years, 500 RDTs from 60 lots were collected from health facilities around Senegal after variable time under field storage conditions, and also exhibited 100% sensitivity against 2000 parasites /µl and 200 parasites /µl knowns, and 100% specificity against negative controls. In 2014, problems were noted at the operational level with evaporation of solvent (buffer) in the individual bulbs in unit kits of RDTs after 18 months in storage conditions in the field, despite the confirmation of the quality of the RDTs at reception. This phenomenon has not been observed with the buffer bottles in kits of 25 tests. After this quality control, it is recommended that a systematic control of RDT lots at the reception at central level and every 6 months for the lots at the peripheral level as temperature and storage conditions vary from one zone to another. Finally inspections at the peripheral level should be strengthened to ensure good storage conditions for RDTs.

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FIELD-BASED QUALITY MONITORING OF MALARIA RAPID DIAGNOSTIC TESTS IN RESOURCE-LIMITED SETTINGS: EXPERIENCE FROM UGANDA

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Rapid Diagnostic Tests were introduced in Uganda as alternative diagnostic tool for malaria. Quality of Rapid Diagnostic Tests can deteriorate and requires monitoring under field setting. Positive control wells are in in advanced field trials but there are early concerns about their feasibility, application across varying endemicity and potential to increase the unit cost of Rapid Diagnostic Tests. In this high transmission remote setting, we piloted the use of locally prepared control blood samples and the use of sentinel sites expert microscopy for field monitoring of malaria Rapid Diagnostic Tests. Fresh blood samples were collected, prepared and characterized into high and low positive parasitemia and clear negative. The known blood controls were used to check the quality of sampled RDTs directly on site at the selected health facilities and communities across five districts. Additional testing was done with expert microscopy at sentinel sites. Monitoring of RDTs was done quarterly and integrated into the district-based routine supervision. Data on RDT performance, sensitivity and specificity was analyzed using SPSS version 12. Rapid Diagnostic Tests maintained an average accuracy of 98.8 % against standard known control blood samples. Sensitivity and specificity of RDTs against expert microscopy of blood smears at sentinel-sites was 94.5% and 84.3% respectively. The use of locally prepared known control blood samples and comparison with expert microscopy of blood smears at sentinel-sites is feasible and could potentially provide simple, effective, low-cost and sustainable alternative Quality monitoring system for Rapid Diagnostic Tests in resource limited and remote field settings.

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FIELD ASSESSMENT OF *PLASMODIUM FALCIPARUM* DRIED TUBE SPECIMENS UNDER AMBIENT TEMPERATURE CONDITIONS IN BENIN

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Quality control of malaria rapid diagnostic tests (RDTs) remains a challenge due to the lack of positive and negative control standards to monitor test quality and health worker performance. Dried ***Plasmodium falciparum*** parasite samples prepared as dried tube specimens (DTS) have been shown to be suitable as quality control samples for RDTs and are stable under refrigeration (4°C) and ambient temperature conditions in Ethiopia. A field assessment to test the stability of DTS under temperature conditions typical of tropical Africa, and to assess the utility of DTS for proficiency testing in Benin was conducted. Briefly, reactivity of replicate DTS samples containing 0, 500 and 1000 parasites/µl stored at 4°C at a reference laboratory (RL); Laboratoire du service des explorations diagnostique, Cotonou, were compared to reactivity of aliquots of the same samples stored at ambient temperatures at two health facilities (HFs); St. Michel health Center, Cotonou 5 and Ayelawadje Health Center Cotonou 2/3, where maximum and minimum temperatures were recorded once daily during July 2014 to January 2015. DTS testing was performed at 0, 4, 8, 12, 16, 20 and 24 weeks with each DTS tested on duplicate RDTs stored under manufacturer recommended temperatures at the RL and on RDTs stored under site-specific conditions at the two HFs. DTS were reactive at all time points irrespective of storage conditions. However, at 20 and 24 weeks, DTS stored under ambient conditions at the two HFs rehydrated poorly and relative band intensities observed by eye on RDTs were about 4-fold less compared to similar DTS stored at 4°C in the RL. Health worker testing of 4 vials of DTS with reactivity blinded to the health workers provided an opportunity to observe and address incorrect testing procedures. These data suggest that in sub-Saharan climates such as Benin's, DTS should be stored under refrigeration for optimal, prolonged stability and DTS is a useful tool for improving health worker performance of malaria RDTs in Benin.

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DO ANTIBODIES TO *PLASMODIUM FALCIPARUM* HISTIDINE-RICH PROTEIN 2 (HRP2) INFLUENCE THE RESULTS OF HRP2-BASED DIAGNOSTIC ASSAYS?

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Plasmodium falciparum Histidine-Rich Protein 2 (HRP2)-based assays have become valuable tools in the diagnosis and control of malaria. Although these assays have high sensitivity and specificity, false-negative and -positive results do occur. One speculation for misdiagnosis is that antibodies (Ab) to HRP2 might interfere with assay results. Surprisingly, no comprehensive study on Ab to HRP2 has been reported. This study sought to identify Ab to HRP2 and assess their prevalence, isotype and avidity. We reasoned that Ab to HRP2 would be 1) present in exposed, but not non-exposed, individuals, and ii) increase in titer with exposure (age). A bead-based multiplex assay was used to measure Ab to recombinant HRP2, and MSP1, MSP2 & MSP3 for comparison. Monoclonal Ab 2G12 that recognizes the central repeat sequence DAHHAADAHH of HRP2 (present in all isolates) and 1E1-A9 (to YAHHAHHA in 99.3% of isolates) were used to optimize the HRP2 assay. Titers of 1:100,000 and 1:1,000 were obtained, showing rHRP2 was appropriately coupled to the beads. As expected, high levels of IgG to the 3 merozoite antigens were detected in plasma from 100 Cameroonian adults living in a high transmission area, but not in 100 US unexposed controls. Unexpectedly, the frequency distribution curves were essentially identical for exposed and unexposed adults for IgG to HRP2. Further results showed no increase in IgG Ab to HRP2 with age using 120 plasma samples of Cameroonians living in a high transmission area aged 5 to 80 years. Avidity of IgG to HRP2 was equivalent in exposed and unexposed individuals. To detect IgM to HRP2, 81 samples from slide-positive children & adults were screened. A small difference in IgM reactivity to HRP2 in plasma from malaria-infected and healthy US controls was observed, but it was unclear if the increase was due to high background or IgM to HRP2. Overall, these results failed to conclusively detect Ab to HRP2. Theories as to why Ab to HRP2 might not be present in malaria-infected individuals will be discussed.

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USE OF MALACHITE GREEN-LAMP FOR THE DETECTION OF MALARIA PARASITES IN FIELD STUDIES

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Molecular tools are increasingly being considered for detecting sub-microscopic infection of malaria parasites in active surveillance programs and research studies. The current molecular diagnostic tools include the nested PCR, real-time PCR and LAMP isothermal assay. The LAMP assays are more amenable for use in limited laboratory settings than other PCR methods as they do not require a thermal cycler to run the test. Different

groups have described the LAMP assay performed using specialized equipment platforms which, unfortunately, reduces the versatility of the LAMP technique for large scale field application. In order to overcome this limitation, we describe the use of the malachite green (MG) dye as a visual endpoint read out for the LAMP assay. Three primers sets to detect *Plasmodium* (pan species), ***P. falciparum*** and ***P. vivax*** were tested. The MG-LAMP assay was performed in a 40-well mini-heat block (<$300) and 0.04% MG dye was added to the amplification master mix prior to the amplification. Parasite DNA obtained by QIAGEN extraction method was used. The assay was run for 1 hour after which the tubes were removed and kept at room temperature for 10 minutes before being scored. Positive reaction was indicated by the retention of the light blue/green color and negative reaction was indicated by colorless reaction mixture. Samples were scored by three independent readers. The sensitivity and specificity of the MG-LAMP was determined using 397 clinical samples. The MG-LAMP assay results were compared to a real-time PCR assay (PET-PCR) as a reference test. The sensitivity/specificity of the genus MG-LAMP assay was shown to be 99.2%/88% and that of ***P. falciparum*** and ***P. vivax*** assays was shown to be 98.8%/99.2% and 94.7%/99.1% respectively. The inter-rater agreements for the three primers were 0.896 for genus, 0.969 for ***P. falciparum*** and 0.956 for ***P. vivax***. This report describes the MG-LAMP assay as an affordable, sensitive and scalable field colorimetric molecular assay for malaria parasite detection that can be further evaluated as a high-throughput tool for the assessment of infections in endemic countries.

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QUANTITATIVE GLUCOSE-6-PHOSPHATE DEHYDROGENASE(G6PD) BIOSENSOR ANALYZER FOR THE POINT-OF-CARE TEST WITH CAPILLARY BLOOD

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Accurate G6PD activity measurements are required to identify and treat vivax or ovale malaria patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. This is because if such patients take 8-aminoquinoline antimalarials like primaquine or tafenoquine, they may experience acute hemolysis anemia by oxidative stress from the drugs. To this end, we have developed the CareStartTM G6PD biosensor analyzer for point-of-care test of the quantitative measurement of G6PD enzyme activity in the whole blood using an electrochemical method. In our recent clinical evaluation in Ethiopia, capillary blood samples (n=60) were collected from adults to compare the performance of the biosensor analyzer to that of a quantitative spectrophotometric method. The result showed no significant difference between these two methods (paired test-test, t=0.02, p=0.9855). The total enzyme activities ranged from 25.2 to 157.8 and 21.1 to 134.5 (U/dL blood) for spectrophotometric method and biosensor analyzer, respectively. In the meantime, the average of the total G6PD enzyme activity was 65.6 (U/dL blood) for both methods. The distribution of the G6PD activity was similar in two methods when two cut-offs (35 and 70 U/dL blood) of the G6PD activity were applied. For example, 5 persons (8.3%) were blow 35 in both methods; 35 (58.3%) and 37 persons (61.6%) were between 35 to 70; 20 (33.3%) and 18 persons (30.0%) were above 70 U/dL blood of G6PD activity in spectrophotometric and bioanalyzer method, respectively. The dimension of the system is 62x118x30(mm) with 98g of weight without two AAA alkaline batteries for the operation. The range of G6PD activity measured by the biosensor is 0 - 300 U/dL using 5µl blood. Up to 1,000 test results can be stored in the analyzer and the data is downloadable to a PC. The analyzer can be operated between 10 - 40 °C and 10 - 90 % relative humidity. The strip for the system can be stored at 2 - 40 °C for 2 years. The CareStartTM G6PD biosensor analyzer is an accurate and convenient point-of-care alternative to the spectrophotometric method for quantitative measurement of G6PD activity in whole blood.

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WHAT IS AN OPTIMAL TREATMENT DEFINITION FOR QUANTITATIVE MOLECULAR DIAGNOSIS OF ERYTHROCYTE-STAGE *PLASMODIUM* INFECTION IN CONTROLLED HUMAN MALARIA INFECTION CLINICAL TRIALS?

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Controlled human malaria infection (CHMI) studies allow for safe evaluation of the tolerability and efficacy of experimental drug and vaccine candidates. Subjects are monitored through clinical and laboratory follow-up, including traditional use of Giemsa-stained thick blood smears (TBS) and more recent use of nucleic acid tests (NATs) like PCR and reverse transcription PCR (RT-PCR). NATs allow the detection and quantitative measurement of peripheral parasitemia 2-6 days earlier than microscopy. With increased sensitivity, NATs afford CHMI studies the opportunity to forego the traditional approach of domiciling subjects during the malaria-associated symptom stage (typically 8-18 days post-CHMI) and follow subjects on an outpatient basis. We sought to develop a NAT-based definition of positive infections warranting curative treatment following CHMI that would (a) minimize both malaria-associated symptoms and any associated medical risk to subjects and (b) ensure that study efficacy data could be depended on to make go/no go decisions about the experimental products under investigation. We retrospectively analyzed TBS, RT-PCR and clinical records from several previous CHMI studies. The historical difference between time-to-positivity (TTP) for RT-PCR (analytical sensitivity 20 para/mL) vs. TBS was 3.9 days (95%CI 3.4-4.4 d). Based on this data, we evaluated how well various 'treatment thresholds' in asymptomatic patients would have performed. Using a threshold of two positive RT-PCR results (one per day) including 1 or more results of >250 parasites/mL, subjects would have been treated for breakthrough infection 2.8 days (95%CI 2.4-3.2 d) earlier than when using a traditional TBS-based threshold. With this threshold, most subjects would be expected to be treated before developing malaria-related symptoms. We have now conducted prospective CHMI studies guided by this treatment threshold that corroborate these predictions. We advocate that CHMI centers develop consensus guidelines for NAT-based treatment thresholds and for monitoring the adequacy of treatment.

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ASYMPTOMATIC MALARIA DETECTION BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION DURING THE DRY SEASON IN KEUR SOCÉ, SENEGAL

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Submicroscopic malaria infections in asymptomatic carriers are considered to be an increasing source of malaria transmission in areas of decreasing endemicity and are, by nature, extremely difficult to identify and treat. Senegal has achieved a drastic reduction in malaria prevalence over the past 10 years, moving from more than 1.5 million cases in 2006 down to less than 300'000 in 2014 (80% reduction), and is likely to be facing significant residual transmission caused by such asymptomatic infections before elimination can be achieved. We evaluated the prevalence of asymptomatic malaria infections in the area of Keur Socé (Sudano-Sahelian region of Senegal, low transmission) during the dry season (March 2015). To that end, we screened a large population (n=1'250) of asymptomatic

adults for the presence of ***Plasmodium*** parasites using loop-mediated isothermal amplification (LAMP) reactions performed on-site. Around of 4% of the screened individuals were found to be asymptomatic ***Plasmodium*** carriers (n=49, prevalence=3.9%) using Loopamp™ MALARIA Pan Detection Kits. Positive samples were further tested using ***P. falciparum*** specific LAMP reactions (Loopamp™ MALARIA Pf Detection Kit) and 30 (65.3%) of these were found to be positive for ***P. falciparum***, suggesting that a large fraction (34.7%) of the asymptomatic infections detected are caused by non- ***P. falciparum*** species. Confirmation of LAMP results by nested PCR (nPCR) and parasitemia determination by quantitative PCR are pending. Based on previous studies, it is expected that the limit of detection, sensitivity and specificity of LAMP reactions will be close to that of nPCR. Importantly, the ability to perform LAMP reactions on-site allowed us to inform study participants of their infection status within 36 hours after sample collection and to ensure the adequate provision of antimalarial treatments to the identified asymptomatic carries. These results suggest that LAMP is a sensible approach to overcome the limited sensitivity of microscopy and rapid diagnostic tests for screen-and-treat interventions.

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QUALITY ASSESSMENT OF MALARIA DIAGNOSTICS IN VIETNAM WITH POTENTIAL IMPACT ON MALARIA ELIMINATION OPERATIONS

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Accurate malaria diagnosis is essential for malaria elimination operations. In Vietnam, microscopy is the standard of care for malaria diagnosis, with front-line microscopists conducting initial testing at commune-based clinics and rechecks conducted two or three times at higher levels. Rapid diagnostic tests (RDTs) are now being rolled out to areas where microscopy is not available, where presumptive treatment was previously the standard of care. Quality assurance programs are in place in Vietnam for malaria microscopy to promote accuracy, quality and validity. We conducted assessments of malaria diagnostics in Phu Yen and Quang Tri Provinces, Vietnam. We randomly selected slides that had been cross checked to be reread again by WHO-qualified readers. Proficiency tests were conducted to assess the competency of microscopists working at each level. Laboratory performance was assessed using a WHO checklist. Results: The rereading of 654 negative slides revealed that in the routine malaria microscopy there were 14 (2.14%) false negative slides (3 ***Plasmodium falciparum***, 10 ***P. vivax***, 1 both species). Results from rereading of additional negative and all the positive slides, as well as RDT assessments, are on-going. The assessment of proficiency of 21 cross checker microscopists was 94.0% and 97.9% for sensitivity and specificity, respectively, while their species accuracy was 77.6%, ***P. falciparum*** sensitivity was 88.8%, and counting accuracy was 58.1%. The sensitivity and specificity for 13 front-line microscopists were 85.6% and 95.6% respectively, while their species accuracy was 68.5%. On-site visits revealed that laboratories assessed had no standard operating procedures or bench aids. Conclusions: Vietnam has a fully functioning malaria microscopy cross checking system with quality output. Still, false negative diagnostic results may hamper malaria elimination operations. In addition, mathematical modeling tools will be employed to investigate the benefit of malaria microscopy and RDTs, as well as estimate the potential danger of false negative diagnostic results in elimination settings.

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INTENSIFIED CASE FINDING IN RESOURCE LIMITED SETTINGS USING VILLAGE-BASED STRATIFICATION: LESSONS FROM ANN TOWNSHIP, RAKHINE STATE, MYANMAR

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In 2013, Myanmar reported more than 333,800 confirmed malaria cases, however, the estimated burden of malaria is closer to 2.6 million suspected cases annually. Lack of a strong surveillance system and poor public health infrastructure contribute to the large discrepancy between the estimated number of cases and the number of reported cases. Malaria case burden has not been systematically used in Myanmar for planning or prioritizing malaria control activities in resource limited settings. Active case detection or temporary screening points by mobile outreach teams have been included in malaria case management activities in an effort to identify hidden malaria burden and provide malaria services in remote areas far from formal health facilities. These activities have incurred significant costs, in part due to the remote locations. The Control and Prevention of Malaria (CAP-Malaria) Project has implemented a village-based strategy (VBS) for intensified case detection (ICD) in an effort to identify and prioritize hidden malaria hotspots while trying to control costs. By introducing the VBS-ICD in Ann Township, CAP-Malaria was able to reallocate resources and better target malaria hotspots resulting in the identification of almost double the number of positive cases (October 2014 - March 2015) compared to the previous year. In Rakhine State, total contribution of malaria case burden from Ann Township increased from 55% to 75% among identified positive malaria cases (2,542 cases), compared to the previous year. The national program uses village micro-stratification which takes into account ecological information (e.g. topology and vegetation) and distance from health facilities, however, this information is not used as part of evidence-based programming and implementation. CAP-Malaria's experience has shown other factors, including malaria burden, can help to improve village malaria stratification allowing for improved results from intensive case detection efforts.

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ANTIMALARIAL ACTIVITY AND INHIBITION OF THE HEMOZOIN FORMATION BY CHLOROQUINE-ANALOGUES

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Several studies have shown the high resistance of ***Plasmodium falciparum*** to chloroquine and other aminoquinolines used as traditional antimalarials, making the search for new drugs urgent. The aim of this study was to evaluate the ***in vitro*** activity of six chloroquine analogs (4-aminoquinoline derivatives) against ***P. falciparum***, using resistant (W2 clone) and sensitive parasites (3d7 strain) by Sybr green method. The citotoxicity was determined against monkey kidney cells (BGM) by neutral red assay, and the selectivity index (SI) calculated (a ration between MDL50 and IC50). The possible mechanism of action of chloroquine analogues was also studied ***in vitro*** using hemozoin formation assay. All of the evaluated compounds were obtained in good yields and were active ***in vitro*** against resistant (IC50 of 7 to 212 ng/mL) and sensitive parasites (IC50 of 16 to 373 ng/mL) not presenting cross resistance, besides that they were not toxic with SI up to 7652. Two of the 4-aminoquinoline derivatives, named CEQ and DAQ, received spatial atencion. CEQ is an aminoquinoline containing just a simple -(CH2)2- spacer and a NH2 final group. DAQ has a C≡C group in lateral chain and NEt2 as final group. Both compounds were tested ***in vivo*** against ***P. berghei*** and were active, reducing the parasitemia around 90% until the day 11 after the infection and increasing significantly the animal survival in comparison with untreated control. Four chloroquine analogs inhibited significantly the ***in vitro*** hemozoin formation, in a dose-response manner, at doses lower

than CQ. The results suggest that these aminoquinolines seem represent promising alternatives for the treatment of chloroquine-resistant malaria, and act on a crucial point of the parasite.

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AKB9785 PRESERVES TIE2 PHOSPHORYLATION AND DECREASES ACUTE LUNG INJURY IN AN EXPERIMENTAL MALARIA MODEL

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Malaria-induced acute lung injury (ALI) carries a high fatality rate despite the use of potent antimalarial therapies and optimal supportive care. Therapies targeting the underlying pathophysiology of ALI in malaria may be required to further improve clinical outcome. The angiopoietin-tyrosine kinase 2 (Ang-Tie2) signalling pathway is a key regulator of vascular integrity and emerging evidence indicates that disruption of Ang-Tie2 axis contributes to the development of ALI. AKB9785 is a phosphatase inhibitor that selectively inhibits vascular endothelial-phosphotyrosine phosphatase (VE-PTP)/HPTPβ, a receptor tyrosine phosphatase expressed in endothelial cells that negatively regulates Tie2 activation. We hypothesise that the use of AKB9785 as an adjunctive therapy will significantly improve survival, reduce vascular leak and decrease ALI in the ***Plasmodium berghei*** ANKA (PbA) model. C57BL/6 mice infected with $1x10^6$ PbA-infected erythrocytes received either 25 mg/kg AKB9785 or vehicle control q8h subcutaneously starting three days post-infection until sacrifice. IgM and total protein concentration were measured in bronchoalveolar lavage fluid (BALF) as a marker of vascular integrity, and Evans blue assay (EBA) in the lungs as a marker of vascular leak at baseline and day 6/7 post-infection. Biomarkers of endothelial activation/dysfunction were determined in the lung tissue and plasma. IgM was significantly reduced ($p=0.0194$, Mann-Whitney U test) and survival improved ($p=0.0027$, Log-rank test) in AKB9785 treated mice compared to controls. We will report additional measures of ALI in this model and in an additional murine model using angiopoietin-1 deficient mice, to test the hypothesis that Ang-1-Tie2 activation contributes to vascular integrity and ALI prevention. In conclusion, AKB9785 significantly improved survival and shows evidence of reduced pathological vascular leak associated with ALI in PbA-infected C57BL/6 mice. These findings suggest that targeting the Tie2 pathway with selective VE-PTP inhibitors could be used as adjunctive therapy for SM/ALI.

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ASSESSMENT OF ACIDOSIS PROFILE IN PATIENTS WITH SEVERE MALARIA USING INNOVATIVE TECHNIQUE

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Acidosis is an important cause of mortality in severe falciparum malaria. A simultaneous bio-analytical method for qualitative and quantitative assessment in plasma of eight small organic acids potentially contributing to acidosis in severe malaria was developed and validated. High-throughput strong anion exchange solid-phase extraction in a 96-well plate format was used for sample preparation. Hydrophilic interaction liquid chromatography (HILIC) coupled to negative mass spectroscopy was utilized for separation, detection and quantification. Eight possible small organic acids; L-lactic acid (LA),α-hydroxybutyric acid (aHBA), β-hydroxybutyric acid (bHBA), p-hydroxyphenyllactic acid (pHPLA), malonic acid (MA), methylmalonic acid (MMA), ethylmalonic acid (EMA) and α-ketoglutaric acid (aKGA) were analyzed simultaneously using a ZIC-HILIC column. This method was validated according to U.S. Food and Drug Administration guidelines with additional validation procedures for endogenous substances. LC-MS acid concentration profiles in relation to clinical parameters of three groups; severe malaria (n=141), uncomplicated malaria (n=87) and healthy (n=68) were analyzed by pattern recognition analysis to compare, classify and predict unknown samples. The results of principal component analysis (PCA) showed that four acids (LA, aHBA, bHBA and pHPLA) have more significant discriminant power than other four, thus they all considered. In addition, PCA result showed that healthy could be classified from malaria completely with variance of three first PCs (73.11, 15.41 and 7.84%, respectively), however severe could not classify from uncomplicated completely. Linear discriminant analysis (LDA) model indicated excellent sensitivity and specificity for identification of malaria and healthy which are both (100%) in cross validated prediction. However, the result indicated fair sensitivity (65%) and good specificity (91%) for identification of severe and uncomplicated in cross validated prediction. This innovative technique could be useful tool for the assessment of acidosis in patients with severe malaria.

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DRUG-DRUG INTERACTIONS BETWEEN PRIMAQUINE AND SSRI/SNRI ANTIDEPRESSANTS: IMPLICATIONS FOR PRIMAQUINE CO-ADMINISTRATION WITH CYP 2D6 INHIBITORS

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The antimalarial activities of primaquine and other 8-aminoqinoline molecules are directly dependent upon bio-activation through CYP 2D6 metabolism. Factors that would reduce an individual's ability to metabolize primaquine through the CPY 2D6 pathway such as CYP 2D6 poor metabolizer status and/or co-administration of drugs that inhibit the CYP 2D6 enzyme activity could reduce anti-malarial activity and exacerbate drug-related toxicities. In the present study, the inhibitory potential of the selective serotonin reuptake inhibitor (SSRI) and serotonin norepinephrine reuptake inhibitor (SNRI) classes of antidepressants for CYP 2D6 mediated primaquine metabolism was assessed using ***in vitro*** and ***in vivo*** drug metabolism and pharmacokinetic assays. The SSRI/SNRI classes of drugs displayed a range of inhibitory activities on CYP 2D6 mediated metabolism of primaquine ***in vitro*** (IC_{50} 1-94 μM). Fluoxetine and paroxetine were the most potent inhibitors (IC_{50} ~ 1μM) of CYP 2D6 mediated primaquine metabolism, while desvenlafaxine was the least potent (IC_{50} ~ 94 μM). The ***in vivo*** inhibition of CYP 2D6 mediated metabolism of primaquine was also assessed ***in vivo*** using a primaquine pharmacokinetic study. The pharmacokinetic profile of primaquine was assessed alone or after co-administration of paroxetine. Co-administration of paroxetine with primaquine significantly increased liver concentrations, and area under the curve values for primaquine. This can likely be attributed to decreased CYP 2D metabolism as reflected by the decreased clearance (CL/F) for primaquine when co-administered with paroxetine. The results indicate that caution should be exercised with concomitant use of primaquine with SSRI/SNRI antidepressants and/or other CYP 2D6 inhibitors as the clinical implications in the context of anti-malarial activity for these interactions are unknown.

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HIT TO LEAD OPTIMIZATION OF THE APICOPLAST-TARGETING ANTIMALARIAL AGENT MMV008138

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Compounds that target isoprenoid biosynthesis in ***Plasmodium falciparum*** could be a welcome addition to malaria chemotherapy, since the methylerythritol phosphate (MEP) pathway used by the parasite is not present in humans. Through a phenotypic rescue screen of 400 compounds in the publicly available Malaria Box, we determined that only one of the compounds (MMV008138) is toxic to ***P. falciparum*** by inhibiting isoprenoid biosynthesis. Since the relative and absolute stereochemistry of this compound was not known, we prepared all four stereoisomers. The active stereoisomer of MMV008138 was found to be (1R,3S)-; none of the other stereoisomers had significant growth inhibitory activity. Structure variation was then carried out to interrogate the effect of D-ring substitution and isosteric replacement of the carboxylic acid group, resulting in compounds possessing improved drug-like character.

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PHARMACOKINETICS AND PROPHYLACTIC EFFICACY OF NANO- AND MICRO-PARTICLE DECOQUINATE SUSPENSION FOLLOWING SINGLE INTRAMUSCULAR INJECTION IN MICE

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Prophylactic efficacy and pharmacokinetics (PK) were examined following single intramuscular (IM) depot formulation of decoquinate (DQ) suspension injected into mice infected with ***Plasmodium berghei*** sporozoites. DQ nano- and micro-particles suspended in an oily vehicle to retard drug release is suitable for long-term malaria prophylaxis. PK studies in normal animals and antimalarial efficacy in liver-stage malaria mice were conducted at various IM-DQ doses for 2, 4, 6, or 8 weeks prior to infection with ***P. berghei*** sporozoites. The liver stage efficacy evaluation was monitored by using an ***in vivo*** imaging system (IVIS). Full causal prophylaxis was shown in mice with a single IM dose of nanoparticle DQ (0.42 µm) at 120 mg/kg for 2-3 weeks and with microparticle DQ (8.31 µm) at 120 mg/kg lasted 8 weeks prior to inoculation. The 120 mg/kg IM dose with the two formulations was shown to be the minimal prophylactic dose required to provide full causal prophylaxis of malaria sufficient for a period of 2-8 weeks. A significant increase in the elimination half-life of the microparticle DQ formulation (1,447 hrs.) was achieved compared to that of the nanoparticle DQ (524 hrs.). Similarly, the AUC of the microparticle IM-DQ formulation in plasma was observed to be 17,609 ng·h/ml, which is double the AUC observed for the nanoparticle IM-DQ (8,465 ng·h/ml) at the same single 120 mg/kg dose administered to both animal groups. Body clearance results indicated that the CL/F in the animals treated with nanoparticle DQ was14.28 L/hr/kg, which is twice as fast as the clearance observed in animals treated with the microparticle DQ formulation (6.82 L/hr/kg). PK/PD evaluations have demonstrated the minimal inhibitory concentration (MIC) of DQ to provide full causal prophylaxis in mice infected with ***P. berghei*** sporozoites is 5.12 ng/mL. The microparticle IM-DQ formulation provided a longer and more constant DQ release in the plasma, which resulted in a 2.4 fold longer drug exposure time above MIC. The prophylactic effect of the microparticle formulation observed in mice was shown to be 3-4 times longer than the nanoparticle DQ.

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PLASMODIUM FALCIPARUM EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 INHIBITORS KILL MALARIA PARASITES IN CULTURE

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The ***Plasmodium falciparum*** Equilibrative Nucleoside Transporter Type 1 (PfENT1) was hypothesized to be a potential target for novel antimalarial drugs. Malaria parasites are purine auxotrophs, incapable of ***de novo*** purine biosynthesis. They import purine precursors from the host and modify them through the purine salvage pathway to generate the purine nucleotides needed for RNA and DNA synthesis and other cellular metabolic processes. PfENT1 is the primary purine import transporter. Previous studies showed that PfENT1-knockout parasites are not viable in culture at purine concentrations found in human blood (< 10 µM). Based on these results, we and others had hypothesized that PfENT1 inhibitors might represent therapeutic leads for the development of novel antimalarial drugs. We developed a robust yeast-based high throughput screen to identify PfENT1 inhibitors. We screened a 64,500 compound library and identified 171 hits. Nine of the best hits, representing five distinct chemotypes, inhibited [^{3}H]adenosine uptake by PfENT1-expressing yeast and by red blood cell free trophozoite stage parasites with IC_{50} values in the 5-50 nM concentration range. These nine compounds inhibited parasite proliferation in culture in the 5-50 µM concentration range, but were not cytotoxic for yeast (Frame, Deniskin et al., (2015) ACS Chem Biol. 10(3):775-83). We now show that these nine compounds are highly selective for PfENT1 compared to the human ENT1 and human facilitated nucleobase transporter. The compounds are parasitocidal after 24 hours of exposure in culture. The compounds also inhibit the ***P. vivax*** ENT1 transporter and known non-synonomous single nucleotide polymorphisms from field isolates of PfENT1 and PvENT1. These results support the hypothesis that PfENT1 is a promising potential target for the development of novel antimalarial medicines. The compounds we have identified are potential therapeutic leads for antimalarial drug development.

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TRANSLATIONAL PLATFORMS TO INVESTIGATE ANTIMALARIAL DRUG COMBINATIONS

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During recent years there has been considerable success in the identification and progression through to early clinical testing, of novel antimalarial drug candidates. Accordingly there is an increasing need to evaluate clinically a burgeoning portfolio of potential drugs that could ultimately be used to eradicate malaria. Given that historically most new treatments sooner or later are overcome by the ability of the parasite to develop resistance, the concept of a combination therapy of complementary drugs as a treatment regimen is becoming increasingly attractive. Such an approach affords a new antimalarial, protection against the emergence of resistance thereby ensuring continued utility in the broader population potentially for many more years. The strategic direction proposed here is the alignment of the readouts from ***in vitro*** and ***ex vivo*** assays with correlative data from our mouse model of ***Plasmodium falciparum*** malaria, which already has proven clinical translational ability. To that end we are establishing quantitative readouts of drug-treated parasites to monitor accurately effects on both the viability of parasites and clearance by the host. This system can be implemented with different ***P. falciparum*** strains to study precisely the effect of combinations, not only in standard strains but also in parasites with well-characterized resistance to parent drugs. We anticipate that information generated across these platforms will create the foundations upon which future antimalarial combination regimens will be based, and provide an

opportunity for the evaluation of clinically-relevant combination regimens as alternative route for clinical progression. Details on the strategy as well as preliminary results of initial stages will be presented.

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COMPARATIVE EMBRYOTOXICITY OF DIVERSE ANTI-MALARIAL COMPOUNDS USING RAT WHOLE EMBRYO CULTURE AND THE APPLICATION OF THE SCREEN IN LEAD IDENTIFICATION FOR DISEASES OF THE DEVELOPING WORLD MOLECULES

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Malaria is a major health problem and a serious global health threat. In 2008, there were 247 million cases of malaria and nearly 1 million deaths (World Health Organization, 2011). The frequency and severity of infection is greatest in pregnant women. The increased susceptibility creates a problem as many of the anti-malarial drugs available on the market today are teratogenic. TB is second only to HIV as the leading infectious killer of adults worldwide. It is among the three greatest causes of death of women aged 15-44 and is the leading infectious cause of death among people with HIV/AIDS. Kinetoplastid diseases are a group of infections caused by different parasites: sleeping sickness (caused by Trypanosoma brucei), leishmaniasis (caused by Leishmania spp) and Chagas (caused by Trypanosoma cruzi). Over 30 million people get infected resulting in over 120,000 deaths world-wide annually.GSK is striving to develop new medicines for Diseases of the Developing World with reduced risk for adverse embryo-foetal outcomes. The early screening of potential candidate molecules using rat whole embryo culture (rWEC) will allow for an informed selection of compounds for further development. To support this strategy six marketed anti-malarials were assessed for their potential to induce teratogenicity. The results demonstrated that the rWEC can detect development defects induced by marketed anti-malarials and is a suitable platform for screening compounds in discovery. It has subsequently been used to support the candidate selection of molecules across the disease areas and clarified if the findings were related to target or chemical structure and supported the preclinical study plan to support inclusion of WCBP in clinical studies.

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CHARACTERIZATION OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTE ADHESION TO HOST RECEPTORS AND IDENTIFICATION OF EFFECTIVE ANTI-ADHESION MOLECULES TO PREVENT THIS INTERACTION USING ATOMIC FORCE MICROSCOPY

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Anti-adhesion adjunct therapy aimed at preventing ***P. falciparum***-infected erythrocyte (IE) adhesion to host receptors might be of great benefit in the effective treatment of severe malaria. Currently, adhesion of IE to host receptors ***in vitro*** is often characterized using static plate adhesion assays. While useful at identifying interactions, these assays do not provide insight into the biophysical properties that underlie the IE/host receptor interactions. These can differ significantly among host receptors, thus their characterization is integral toward the development of tailored anti-adhesion therapy. We demonstrate here that single cell force spectroscopy (SCFS) can be used to measure detachment force and work required for de-adhesion between individual living IE cells and host receptors. In addition, this approach can be utilized to characterize various anti-adhesion molecules, including antibodies and small molecules, which effectively disrupt IE/host receptor interactions. We have recently identified a number of anti-adhesion molecules using a two-step high throughput approach , as reported previously. In this work we confirmed and characterized their anti-adhesion activity using SCFS. Our results demonstrate that SCFS can be effectively used for detailed biophysical characterization of the IE-host receptor interactions including effects of anti-adhesion molecules at the single-cell level.

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IN VIVO EFFICACY OF JPC-3210 AND PARTNER DRUGS FOR MALARIA ERADICATION USING THE RODENT-*PLASMODIUM BERGHEI* MODIFIED THOMPSON TEST WITH AN EXTENDED FOLLOW-UP PERIOD

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The aminomethylphenol, JPC-3210 is being developed as a potent long acting schizontocide to be used in combination with other antimalarial agents for the treatment of malaria infections and possibly to support malaria elimination programs. In the modified Thompson test using a three day twice daily administration, the lowest dose required to obtain 100% cure at day 30 was 2 mg/kg/day for JPC-3210, 4 mg/kg/day for pyronaridine and 16 mg/kg/day for piperaquine. The blood elimination half-lives of JPC-3210, pyronaridine and piperaquine in mice are relatively lengthy at 125 h, 79 h and 197 h, respectively. When the modified Thompson test was extended by an additional 30 days, parasites reappeared in both JPC-3210 and piperaquine treated groups. Most of the parasites observed were male or female gametes. The pyronaridine group remained slide negative out to day 60. Subinoculation studies with blood from all three treatment groups at day 60 into naïve mice are currently ongoing to evaluate the development of recrudescent infections. When JPC-3210 was co-administered with the gametocytocidal drug primaquine, the reappearance of parasites out to day 60 day was prevented despite the short elimination half-life of primaquine (1.8 h) in mice. Additionally, we plan to evaluate whether artesunate, dihydroartemisinin, or methylene blue will influence the development of late parasitemia. The 60 day test systems will also be extended to include lumefantrine and mefloquine. Our conclusion is that the modified Thompson test with a 60 day follow-up period should be used to assist in the selection of new antimalarial drug combinations for the treatment and elimination of malaria.

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RICE FARMERS' WILLINGNESS TO PAY FOR MALARIAL VECTOR LARVAL SOURCE MANAGEMENT: THE CASE OF RUHUHA COMMUNITY IN EASTERN RWANDA

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This study is part of an action research project that aims to eliminate malaria in the community of Ruhuha in southeastern Rwanda. A study component has recently established that rice farming in the area creates significant malaria risk, which is in line with earlier work that documents this link in various settings. So far, none of the interventions that have been proven to be effective in tackling rice farming-induced mosquito breeding sites have been implemented in Ruhuha. Therefore, the project

has decided to use external funds to supports a larviciding intervention with Bacillus thuringiensis israelensis (Bti), but for just one rice cultivation season (semester). Future interventions will thus depend on co-payment of rice farmers and the wider community. The present study, conducted prior to Bti application, aims to assess the willingness to pay (WTP) for larviciding. Out of 1,914 rice farmers organized into four cooperatives, 320 farmers were randomly selected to participate in a cross-sectional study conducted in January 2015. The maximum WTP was elicitated through a contingent valuation exercise using the bidding game method. Focus group discussions were held with farmers. The mean WTP was US$ 2.2 and US$ 0.5 per farmer per season for lumpsum and per are, respectively. The median WTP revelead that 50% of the participants were willing to pay at least US$ 1.4 and US$ 0.3 for lumpsum deduction and per are, respectively. A multivariate analysis showed that more income from rice, a higher starting bid and being cued first on a lumpsum rather than a progressive deduction, were significantly associated with higher WTP (p<0,05). While acceptability of the intervention appears generally high, as witnessed for instance by a stated willingness to invest (non-compensated) labor time in applying Bti once it is established that it is effective in reducing malaria incidence, the WTP levels reported can only cover one fourth (1/4) of the full intervention cost (US$ 9 for lumpsum and US $ 1,8 per are). To fill this gap, financing models need to be developed to support rice farmers and their communities to increase the sustainability of the intervention.

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MOLECULAR EPIDEMIOLOGY OF MALARIA TRANSMISSION IN VHEMBE DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA

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Malaria remains one of the most devastating parasitic infections, contributing to mortality and morbidity on the African continent. However, for the past decade, massive international efforts have resulted in reducing the number of annual malaria deaths globally. Certain countries have moved from malaria control to attempt to achieve elimination of the disease within their borders. Malaria transmission in South Africa is seasonal with low transmission densities (<1/1000 risk population). This, in addition to well-managed malaria control programmes, led to the shift in focus towards implementation of malaria elimination strategies, aimed at achieving zero cases of malaria transmission by 2018. However, currently, only malaria incidence data in terms of blood stage parasite load is readily available for South Africa, with data on the transmissible gametocyte forms of these parasites and its carriage in the population limited. Whilst the first is important in malaria control strategies, in the situation of aiming towards malaria elimination, it is imperative that data on transmittable forms of the parasite is available. This will guide, monitor and assess the success of elimination strategies. This study is therefore quantifying the ***Plasmodium*** species and gametocyte carriage in the population through cross sectional surveys at health facilities, communities and farms using sensitive molecular technologies. The study has a four-tiered analysis profile, including molecular detection of parasite infections (and simultaneous speciation thereof), analysis of molecular markers for drug resistance of all positive samples followed by genetic barcoding as an indication of clonality and origin of infection. The findings of this study will help in identifying the source of persistent malaria transmissions and informing evidence based policy changes involving the deployment of e.g. primaquine and targeted interventions in South Africa in support of the malaria elimination agenda.

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COMPARATIVELY ASSESSMENT OF THE EFFICACY OF DIFFERENT FORMATS OF DISPENSING THE IFAKARA SYNTHETIC MOSQUITO LURE AGAINST MALARIA VECTORS IN THE SEMI FIELD SYSTEM

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The Ifakara odour blend was originally developed and tested by Okumu et al. The odour mixture was formulated from various synthetic chemicals that mimic the odour from human body emanations including sweat and breath that elicit mosquito host-seeking behavior. The Ifakara lure has been proven more attractive than actual human odour when placed apart but when a human is placed next to the lure, the human remain more or squarely attractive to mosquitoes. The initial dispensing mechanism of Ifakara blend involved the use of nylon strips (made of 15 denier microfibers) that are commonly available. The present study involved a semi-field experimental evaluation of different formats of dispensing Ifakara synthetic lure against host-seeking mosquitoes. The new Ifakara synthetic lure in pellets format, packaged in sachets by Biogents (BG) Ltd (Germany), was comparatively evaluated with the nylon strips as a means of dispensing the mosquito chemical attractants. The impregnated pellets were packed at the required concentrations, either with 4 group of compounds (4C) combined or the 9 compounds of separate pellets (9C). While the lure in nylon strips was formulated following the original procedures by Okumu et al. Carbon dioxide gas generated from yeast and molasses fermentation was used to augment the attractiveness of the synthetic lure. The evaluation was done using BG-sentinel traps developed by (Biogents, Germany). When the BG sentinel trap was baited with impregnated pellets (4C), it caught significantly higher number of Anopheles arabiensis (RR = 333.15 [149.57-742.08], P < 0.001) than un-baited BG trap. Similarly, the significantly number An. arabiensis (RR = 329.84 [148.08-734.70], P <0.001) were caught from the BG baited with pellets in 9C than un-baited trap. In addition, the BG-trap baited with nylon strips caught significantly more number of An. arabiensis (RR = 292.66 [131.36-652.00]. We conclude that the new formulation of Ifakara lure in pellets (4C/9C formats) may offer a simple and long-lasting dispensing mechanism of mosquito odorants to be used in attracting and killing, mass trapping or surveillance of mosquito vectors.

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DEVELOPMENT OF RECYCLE-BASED HOMEMADE SUGAR BAITS AGAINST *ANOPHELES ARABIENSIS*

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Innovative vector control approaches are needed to reach malaria elimination. Ivermectin (IVM) has been proven to kill mosquitoes post blood-feeding on treated humans. In this study IVM was incorporated into an attractive sugar bait (ASB) made from household waste and other recycled materials. The KD90 of IVM in 10% sugar solution against ***Anopheles arabiensis*** was determined as well as the most attractive fruit bait concoction. Floral baits, different prototype designs as well as best deployment site for the ASB within a household are under study in Bagamoyo, Tanzania. Dose response experiments to determine KD90 of IVM against Anopheles arabiensis were done in laboratory conditions.

Serial dilutions of IVM in sugar solution were investigated in separate cages (30x30x30 cm) . Mosqutoes mortality was observed after 3, 6, 24 and 48 hours post introduction of the treatments. Fruit bait attractivity of 7 different fresh fruit juices were investigated inside 6 large cages (1,20x1,20x1,20 cm) placed inside a tunnel. Solutions were marked with specific food colouring and the occurence of sugar feeding was recorded after 24 hours by squeezing the mosquito abdomens and observing the presence or absence of food colouring. Ivermectin proved to be toxic against malaria vectors in very low concentrations. Over 90% of ***An. arabiensis*** were knocked down 48 hours post sugar feeding on sucrose solutions containing at least 1‰ IVM. Results from sugar feeding preference on different fruits show that An. arabiensis will feed on a variety of fruit solutions as well as just water and sugar. No fruit concotion proved to be particulary more attractant with mosquitoes prefering orange, watermelon and guava over papaya, tomato, mango or banana. Further research is on-going with aim of designing a homemade recycled-based ASB using the knowledge gathered so far.

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ESTABLISHING NEW LINE OF *PLASMODIUM FALCIPARUM* EXPERIMENTAL CHALLENGE INFECTION CLONAL STRAINS IN SUPPORT CONTROLLED HUMAN MALARIA INFECTION STUDIES

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Malaria remains a significant global health threat to the world's population. With resistance having developed for all classes of drugs and no licensed vaccine, there are efforts to develop new and/or improved antimalarial drugs and vaccines. The controlled human malaria infection (CHMI) model, which is alternate to Phase IIb field studies, is used for testing safety and efficacy of new antimalarial drugs and vaccines. However, these experimental infections and challenges are mostly done using limited number of clones obtained > 30 years ago. This limits data interpretation because correlation of experimental and natural infections might not be obvious because field parasites are highly genetically and phenotypically diverse. This study set out to expand the genetic and phenotypic diversity of ***Plasmodium falciparum*** available for CHMI by developing clonal strains from recently obtained field isolates. Forty field isolates from different regions in Kenya underwent limiting dilution to generate single clones. For each of the 40 parent parasite, 3-10 clones were obtained generating a total of 212 clones. For genetic characterization, 12 microsatellites and 300 SNPs distributed across the ***P. falciparum*** genome were analyzed. Of the 212 clones, 80 were true single clones based on MS and SNPs analysis. Phylogenetic analysis revealed close kinship within multiple-genotype from each parasite infection. Multi-locus analysis showed matching genotypes within individuals up to seven loci combinations, indicating that although clones from the same parent have close kinship, they are not identical but have diverse genetics. To determine the parasite phenotypic characteristics, establishment of IC50 for 18 antimalarials is underway. The infectiousness of each clonal line will be established by assessing gametogenesis and oocyst/sporozoites production in mosquito. A CHMI study will be conducted to assess the ability of these clones to infect human. Highly characterized (genetic, ***in vitro*** and ***in vivo***) parasites will be deposited with Malaria Research and Reference Reagent Resource Center.

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SEVEN YEAR TRENDS OF MALARIA IN ZANZIBAR, 2008-2014: A PRE-ELIMINATION SETTING

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Over the last decade, Zanzibar has experienced substantial declines in malaria burden. Surveillance of malaria cases can assist with programmatic decision making by helping to monitor seasonality, detect hotspots of transmission, and identify outbreaks of malaria. This study reports seven-year trends of passively detected malaria cases in Zanzibar. Weekly malaria data were collected through the malaria early epidemic detection system (MEEDS) which transmits aggregate malaria case data via mobile phone from public health facilities to a web-based server. Data for patient attendance and malaria diagnostic testing results from 2008 to 2014 were analysed to investigate trends in malaria burden in a pre-elimination area. Between 2008 and 2013, incidence of cases had dropped by 29% in Unguja (2,238 vs.1,598) and 50% in Pemba (918 vs. 419), with the largest reduction seen in children under 5 years (70% in both Unguja and Pemba). Case reports indicate that transmission has changed from perennial to highly seasonal, with peak case numbers in May and June. Rainfall was associated with increases in malaria cases in Unguja ($p<0.001$), although not Pemba. In Unguja, several stable hotspots of transmission were detected in 2010, 2011 and 2013. These hotspots appeared only following the long rains. Rainfall was associated with increases in malaria cases in Unguja ($p<0.001$), although not Pemba. Surveillance systems have identified substantial decline in malaria burden, changing transmission patterns and stable hotspots of transmission, which can now be more specifically targeted with intensified interventions.

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STRATIFYING AND MAPPING MALARIA RISK TO INFORM SUB-NATIONAL MALARIA ELIMINATION IN ETHIOPIA

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Although much of Ethiopia remains at risk of malaria, routine surveillance data from the last decade have noted declining malaria outpatient morbidity and inpatient mortality. Based on this progress, Ethiopia has set a new strategic goal of eliminating malaria in select low transmission areas by 2020. The annual micro-planning exercise conducted by district malaria health officers compiles malaria data from nearly 100% of the 16,013

public health facilities. The creation of large numbers of additional primary health care facilities, including 13,000 health posts in rural communities, was temporally associated with improved access to prompt malaria case management, including parasitological confirmation and improved surveillance system completeness between 2005 and 2014. Of the 11,950,186 fever cases reported nationally from July 2012 to June 2013, 93% underwent laboratory testing. Of the 5,011,418 total malaria cases, 84% were parasitologically confirmed. Of the confirmed cases, 70% were due to ***Plasmodium larviciding intervention falciparum*** and 30% due to ***P. vivax***. District level annual parasite incidence per 1000 population (API) from 835 districts was used to stratify the country into four distinct API strata: <1, 1-4.99, 5-99.99, and ≥100. Of the total 84.2 million Ethiopians, 33.6 million live in areas considered malaria free (API <1) and are not targeted for malaria vector control measures. About 50 million (60%) live in malaria transmission risk areas (API>1), generally located at elevations below 2,000 meters. Of those living in malaria risk areas, 14.3 million people (29%) live in high transmission areas (API≥100), 26.5 million (53%) live in moderate transmission areas (API 5-99.99) and 9.2 million (18%) in low transmission areas (API 1-4.99). High transmission areas were largely on the western border with South Sudan and Sudan, whereas large clusters of low transmission areas were concentrated in Somali and Oromia Regions. Identification of districts with API between 1-4.99 and mapping of these districts will inform the selection of low transmission districts or clusters of districts appropriate for additional pre-elimination and elimination activities.

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A CLUSTER-RANDOMIZED TRIAL OF TARGETED CONTROL TO ELIMINATE MALARIA IN CENTRAL SENEGAL: MAIN RESULTS IN YEAR 2

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Targetting ***foci*** of malaria transmission may be more effective in reducing transmission than if the same effort is expended in blanket control measures, and has the potential advantage of limiting selection for resistance. The purpose of this trial was to evaluate the extent to which a targetted malaria control strategy combining vector control with indoor residual spraying (IRS) and chemotherapy, delivered by district health staff to hotspot villages, can reduce the transmission of malaria in low endemic areas. The trial will also determine whether, as part of this strategy, chemotherapy should be delivered to all members of targetted communities (MDA, Mass Drug Administration) or only those who have been tested and are known to be infected (MSAT, Mass Screening and Treatment). In 30 clusters, all households in hotspot villages were targetted to receive IRS with Actellic 300CS in July, followed, in 15 clusters, by MDA with dihydroartemisinin-piperaquine (DHA-PQ) administered to all persons in the household in September and again in October. In the other 15 clusters, instead of MDA, all persons in the household were screened using a malaria RDT and those who tested positive treated with DHA-PQ. 10 clusters served as controls. Interventions were delivered over two years (2013 and 2014), and the primary outcomes were the incidence of malaria, and the prevalence of parasitaemia just after the main peak period of transmission, in year 2. In each intervention arm, about 80,000 persons were enrolled each month in each year. In 2014, parasite prevalence was 1% in September (cluster range: 0.05% to 5%), and 0.99% in October (cluster range: 0.03% to 5.2%). A survey was done four days after MDA and MSAT to assess adherence and to ask about side-effects. Side-effects were reported by 20% (117/599) in September and 15% (91/598) in October, but with excellent adherence to the regimen. Indirect effects of the interventions on transmission will be assessed by comparing between the trial arms the incidence of malaria in non-targetted areas in each cluster. Total effects (direct + indirect) will be evaluated by comparing overall incidence.

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THE COSTS AND COST-EFFECTIVENESS OF TWO SPATIALLY TARGETED, MULTI-COMPONENT MALARIA ELIMINATION STRATEGIES: RESULTS OF A LARGE THREE-ARM CLUSTER-RANDOMIZED TRIAL IN RURAL SENEGAL

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In areas of low and patchy transmission, it is hypothesized that targeting residual hotspots can cost-effectively eliminate malaria. We conducted a three-arm, cluster-randomized controlled trial in an area of low, patchy, and highly seasonal transmission in rural Senegal over two malaria seasons in 2013-14. Health posts (n=46) serving approximately 320,000 people were randomized into 40 clusters: one of two multi-component hotspot strategies (n=15 clusters each) or control (n=10 clusters). In both intervention strategies, hotspot villages were identified and community health workers (CHWs) offered residents indoor residual spraying (IRS) in July each year. In September and October, CHWs again conducted door-to-door visits in hotspot villages in the intervention arms; in one arm, they offered mass screening and treatment (MSAT) with rapid diagnostic tests (RDTs) and dihydroartemisinin-piperaquine (DHA-PQ) and in the second arm, they offered mass drug administration (MDA) with DHA-PQ. In all three arms, health promotion encouraged care seeking for fever and health posts provided enhanced case management, including RDT testing and for positive cases, treatment with antimalarials and provision of a long-lasting insecticide-treated bed net. Based on detailed micro-costing, we report the incremental financial and economic cost per recipient of each of the 6 intervention components: hotspot identification, promotion of care-seeking, IRS, MDA, MSAT, and enhanced case management. We use a decision analytical model to assess the cost-effectiveness of each of the three strategies from a societal perspective based on intention-to-treat including hotspot and non-hotspot villages and present the incremental cost per malaria case averted and per disability-adjusted life-year averted. We explore uncertainty with univariate and probabilistic sensitivity analysis illustrated with cost-effectiveness acceptability curves and the cost-effectiveness plane. The relative costs of the six malaria interventions and the efficiency of alternative elimination strategies constitute important considerations for policy makers.

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EFFICACY OF ACTELLIC® 300 CS (PIRIMIPHOS-METHYL) AFTER TWO YEARS OF INDOOR RESIDUAL SPRAYING IN SENEGAL

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In central-western Senegal, scaling-up of control measures has been effective in reducing malaria incidence, but additional measures are now required to eliminate the disease. However, widespread resistance to currently used insecticides threatens the effectiveness of bednet and IRS (Indoor Residual Spraying) programmes. In 2013 and 2014, as part of a large-scale cluster randomized trial of a targetted control strategy, we evaluated the duration of efficacy of Actellic® 300CS, a capsular

formulation of pirimiphos methyl, when used for IRS. IRS with pirimiphos methyl was delivered to all households in malaria hotspot villages by spray-teams of community health workers (CHWs) who were trained and supervised by staff of the Public Health department. Each year, efficacy was assessed over 7 months in 5 villages. In each village, 5 treated rooms were selected and untreated rooms used as controls. Bioassays were performed on each of 3 walls in each room, to measure knock-down and 24-hour mortality of lab-reared ***Anopheles gambiae*** and of locally-caught wild strains. In 2014 steps were taken to improve the training and supervision of CHW sprayers and the duration of efficacy measured in the same way as in 2013. In 2013, 24-hour mortality (adjusted for control mortality using Abbott's formula), two months after spraying, was 92% , decreasing to 72% after 3 months, and 37% after 4 months. Mortality at 7 months was 48%. In 2014, the 24-hour mortality was 97%, 97%, 82%, 76% after one, two, four and seven months respectively. In all villages, vectors were highly sensitive to carbamates (bendiocarb) and organophosphates (pirimiphos-methyl) in both years (24-hour mortality rates 98% and 100%). These results showed that IRS with Actellic® 300CS was highly effective but the duration of efficacy depends on the quality of spraying.

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SOCIAL AND CULTURAL FACTORS INFLUENCING PREGNANT WOMEN'S ADHERENCE TO ANTIMALARIAL TREATMENT IN RURAL GAMBIA

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Non-adherence to antimalarial treatment in pregnancy has been identified as a major barrier to malaria control efforts. There is limited evidence on the cultural and contextual factors that influence adherence to anti-malarial treatments in pregnancy. This study aims to understand these factors by exploring community perceptions on pregnant women's adherence to their antimalarial treatment in a rural area of The Gambia.A qualitative ethnographic study was conducted in three villages in the Upper River Region of The Gambia from June to July 2014. Data collection included semi-structured interviews and participant observation, which included informal conversations . Interview transcripts and field notes were entered, coded and analysed using NVivo Version10. In-depth semi-structured interviews were conducted with 30 participants, which included women of reproductive age (n=15), mothers-in-law (n=5), husbands (n=4) and health workers (n=6). Adolescent girls and older women were more likely to delay revealing their pregnancies to health workers. For adolescents, delayed disclosure of pregnancy was linked to feelings of awkwardness and social shyness. For older mothers, delayed disclosure of pregnancy was associated with their social role and position. Delayed disclosure of pregnancy was indicated as an influence on women's views regarding the importance of treatment adherence. In general, women indicated having insufficient information on treatment efficacy and the possible side effects of antimalarial medication. Mothers in-laws were identified as influencing pregnant women's non-adherence to malaria medication. Husbands were regarded as potential reinforcers to pregnant women complying with antimalarial treatment regimens. Culturally adapted community-based health information and treatment should be targeted at adolescent and older pregnant women who are at risk of being isolated from health facility-based education and treatment for malaria in pregnancy. Additionally, mothers-in-law and husbands should be included in facility and community based health promotion programs targeted at pregnant women.

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THE BIOPHYSICAL CHARACTERIZATION OF HRP2 REVEALS INSIGHTS TOWARD IMPROVED DIAGNOSTICS FOR *PLASMODIUM FALCIPARUM* MALARIA ELIMINATION

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Malaria programs aimed at eliminating ***Plasmodium falciparum*** (***Pf***) seek to incorporate active infection detection strategies to target low density infections that could drive low prevalence transmission. Currently available immunoassays lack the sensitivity required for active infection detection strategies; the limit of detection (LOD) of these rapid diagnostic tests (RDTs) is too high to identify all transmissible infections. Malaria infection detection tests (IDTs) with a significantly improved LOD would enable more effective elimination interventions while retaining the critical advantages of low cost, ease of use, and rural deployment. The ***Pf*** specific histidine-rich protein 2 (HRP2) is a useful biomarker for clinical diagnostics because it is specific to current or recent ***Pf*** infection, and may be equally effective even in the absence of circulating parasites. Thus, improved HRP2 tests are of particular significance for stratification of focal mass drug administration (FMDA) and focal test and treat (FTAT) malaria elimination tactics. However, further characterization of the HRP2 protein is necessary before we can fully exploit its potential. Our aim is to facilitate the development of improved malaria HRP2 IDTs by thoroughly investigating HRP2 structure-function relationships and their impact on epitope-antibody interactions. The highly polymorphic, low amino acid complexity, and unstructured nature of the HRP2 protein makes quantification, optimization, and standardization of RDTs challenging. Current HRP2-detecting RDT immunoreagents primarily target the Type 2 (AHHAHHAAD) and Type 7 (AHHAAD) tandem repeat motifs that comprise the most prevalent antigen epitope, AHHAADAHHA. Amino acid sequence analysis and biophysical characterization of native and recombinant HRP2 constructs including quantitative ELISA, biolayer interferometry, and circular dichroism under a variety of physiological and clinical test media reveal the impact of sequence variations on immunoreagent binding and are useful to guide design parameters of highly sensitive ***Pf*** malaria HRP2-based IDTs under development.

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UNNEEDED DEATHS: ABOUT MALARIA IN MADAGASCAR

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Since 2005, the Malagasy Government has planned to achieve malaria elimination in Madagascar. ACT is recommended for treating uncomplicated malaria; and insecticide treated bed nets for prevention purposes. Financial support from international organizations such as the Global Fund to Fight AIDS, Tuberculosis and Malaria; the President Malaria Initiatives, UNITAID; UNICEF have given some hope to insuring availability and affordability of ACTs. But that has been illusory. The whole strategy to combat malaria was never implemented properly in a continuous manner. Above all, the Global Fund episodically interrupted funding likely following the proven misuse of the funds. In the last five years, tens of devastating tropical cyclones hit the country. Political turmoil and instability do not allow good management of the public health policies. Fatal malaria epidemics occur. We carried out the investigation in the dry South; the wetter North-West region both in May 2012 and the rainy South-Western in June 2011. Malaria prevalence among villagers was 25% to 55%. The epidemiological data clearly shows that the number of malaria cases is still increasing up to today although some health officials

have questioned this increase at that period. Surprisingly, many people believe that malaria (locally called tazo) does not kill; but that it is actually unknown pathologies with high fever and convulsion that occur and kill. So they seek "spiritual care" from traditional healers. Therefore, people die of malaria; and also of other infectious diseases. Actually, antimalarial drugs are not available in several health centers in rural area. Fighting malaria means fighting mortality and morbidity. The number of death related to malaria in Madagascar is underreported for many reasons. The government support in funding of malaria control is necessary in order to increase patients' access to life-saving intervention to lower the rates of malaria and the unneeded deaths related to malaria. We will present more data to the alarmingly increasing malaria epidemics in Madagascar, as the aftermath due to the failure of the system in this combat against malaria.

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NON-CHEMICAL TECHNOLOGY FOR INSTANTLY KILLING SUSCEPTIBLE AND RESISTANT MALARIA VECTORS THAT BITE HUMANS OUTDOORS IN TANZANIA

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Long lasting insecticidal net, indoor residual spraying and larval source management have been the successful frontline interventions against malaria for decades with a major reduction in malaria mortality by 47% globally and 54% in Africa. Unfortunately, the major malaria vectors have now developed biochemical, physiological and behavioral resistance towards most of insecticide related interventions, thus, posing a skeptical query on the human protection from the existing insecticidal interventions in the near future. A 3 X 3 Latin square comparative study was conducted against wild mosquitoes in the Lupiro village located in the south eastern part of the rural Tanzania. Three solar-powered mosquito landing boxes (MLB) were fitted with modified low-cost commercially available mosquito zappers as electrocuting grids. One MLB was fitted with one electrocuting grid on one side, second MLB was fitted with two electrocuting grids on two sides and third MLB was fitted with three electrocuting grids on three sides. The MLBs were baited with synthetic human lure (Ifakara lure) and the entire electric system was controlled by water proof light sensor, in which the system turns on at dusk and shut off at dawn, thus improving the battery life span and providing minimal human supervision. A significantly number of mosquitoes were killed by the MLB with two or three grids, relative to MLB with one grid ($P<0.05$). The malaria vector An.arabiensis were killed in higher number compared to any other species, though the non malaria vectors (i.e. Culex and Mansonia species) number also increased as the grid number increase. The non blood fed mosquitoes were 99% thus suggesting host seeking status. These findings suggest that the biochemical and physiological resistance of outdoor malaria vectors can now be tackled in a way that does not augment environmental concerns and chances of resistance development. Moreover, a mosquito landing box fitted with electrocuting grids can effectively complement the existing front line interventions against malaria

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DEVELOPMENT ASSISTANCE AND GOVERNMENT EXPENDITURE FOR MALARIA ELIMINATION

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In 2010, malaria was the fifth-largest cause of years of life lost globally, with approximately 1.2 million deaths attributed to the disease. In parts of sub-Saharan Africa, where malaria burden is highest, it is the leading cause of death. Despite this ongoing impact, remarkable progress is being made towards reducing the incidence and prevalence of malaria. To date, 111 countries have eliminated malaria, while another 34 countries, including China, South Africa, and Argentina, are realistically moving toward elimination. However, little is known about how much governments and donors spend on the prevention and treatment of malaria in these countries. We tracked development assistance for health (DAH) and government health expenditure (GHE) for the prevention and treatment of malaria. For DAH, we tracked resources from source to channel to recipient country or region, focusing on 34 malaria elimination countries from 1990 to 2012 and generated projections of malaria DAH from 2013 to 2017. For GHE, we use macro-economic, socioeconomic, and epidemiological data, as well as expenditure information from the World Malaria Reports to estimate the share of domestic government health budgets spent on malaria for 1995 through 2012 for 34 countries. Analyzing these trends in expenditure exposes which countries and donors are prioritizing the elimination of malaria, as well as how funding for elimination has evolved over time. A strategic input for ongoing efforts to eliminate malaria in the 34 elimination countries, these funding trends form the basis for understanding how these and other countries can realistically move towards elimination.

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THE IMPACT OF TARGETED MASS DRUG ADMINISTRATION USING DIHYDROARTEMISININ-PIPERAQUINE IN SOUTHERN PROVINCE ZAMBIA: INITIAL FINDINGS

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With the recent call for malaria elimination, there is renewed interest in mass treatment with highly effective antimalarials to clear parasites from the human reservoir to break malaria transmission. A community randomized controlled trial of 60 health facility catchment areas was used to quantify the effectiveness of 2 rounds of mass drug administration (MDA) or focal MDA (fMDA) using dihydroartemisinin + piperaquine (DHAp), versus a control of routine prevention and care but no mass treatment, in Southern Province, Zambia, an area of endemic malaria transmission with high vector control coverage. The study was stratified by malaria transmission above and below 10% parasite prevalence. All eligible participants in the intervention rounds were tested for ***P. falciparum*** using an HRP2 rapid diagnostic test (RDT). MDA consisted of treating all eligible residents with DHAp in each round (Dec 2014 just prior to the rains and Feb 2015 at the beginning of the rainy season), irrespective of RDT result. fMDA, conducted simultaneously to the MDA rounds, consisted of treating all eligible household members with DHAp where anyone in the house tested positive by RDT. Primary outcomes included malaria parasite prevalence measured by pre and post cross-sectional surveys at the end of high transmission season (April-May), and malaria infection incidence measured in a cohort of 2,100 individuals followed monthly for 12 months; infection status was determined by RDT and PCR. The study was powered to detect a 50% reduction in these outcomes compared to the control group. The baseline survey showed malaria prevalence in children <6 years old to be 8.9% (95% CI: 5.6 - 12.2%) and 49.9% (95% CI: 40.3 - 59.5%) in low and high transmission areas, respectively. The 2 MDA and fMDA rounds reached 287,145 people, combined, over both rounds (coverage of eligible respondents was approximately 80-85%); parasite prevalence decreased from 8.3% to 4.6% between rounds at a time when transmission normally rises dramatically. The impact of the MDA/fMDA rounds will be presented using data from the follow-up survey and from the first 6 months of the infection incidence cohort study.

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SCHOOL-BASED TREATMENT WITH ACT TO REDUCE TRANSMISSION' (START-IPT): EVALUATION OF THE COMMUNITY IMPACT OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN UGANDAN CHILDREN

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Intermittent preventive treatment (IPT) for malaria in schoolchildren has been shown to benefit individual children, and has the potential to decrease malaria transmission at the community level. This cluster-randomized trial assessed the impact of IPT of malaria in schoolchildren with dihydroartemisinin-piperaquine (DP) on community-level clinical outcomes and malaria transmission in Jinja, Uganda. A total of 84 clusters were randomised equally between intervention and control. A total of 10,746 children were enrolled in the intervention. Monthly IPT with DP was delivered from June-Dec 2014, and up to 6 rounds of DP (dosed by weight, 3-day course) were delivered to participants. The impact of the intervention was measured by comparing parasitological outcomes in community and school surveys pre- and post-intervention, and from continuous entomology surveillance. Safety monitoring was also conducted. The baseline community survey was carried out from Feb-June 2014; 10,033 participants from randomly selected households in the 84 clusters were enrolled. Parasite prevalence by microscopy was high in community residents (25%), particularly in school-aged children (35%), which was confirmed by the baseline school survey (43%). The final community survey was conducted in Jan-April 2015, and microscopy of samples is ongoing at the time of abstract submission. The final school survey was conducted in Nov-Dec 2014; 1092 students were enrolled, including 13 students randomly selected from each of the 84 schools, and preliminary microscopy results suggest that parasite prevalence was much lower in the intervention arm (9%) than in the control (44%), crude odds ratio 0.09 (95% CI:0.05-0.17). The entomology survey includes 200 households, 5 randomly selected from 40 clusters, and was conducted for one year, ending in April 2014. Mosquitoes were collected from each house monthly using CDC light traps, and will be analysed to estimate sporozoite rate for an effect of the intervention on transmission. Full results of the study will be presented and discussed in light of implementation challenges to inform chemoprevention and control of malaria.

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QUANTITATIVE G6PD TESTING FOR SAFE TREATMENT OF *PLASMODIUM VIVAX* MALARIA WITH 8-AMINOQUINOLINES: A POINT-OF-CARE ELECTROCHEMICAL DEVICE

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Currently the only class of drugs that can completely cure a patient of ***Plasmodium vivax*** parasites (radical cure), thus reducing the risk of relapse, are the 8 aminoquinoquinolines such as the registered drug primaquine. This class of drugs presents a safety risk to subjects with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency is an X-linked disorder that affects more than 400 million people worldwide. Moderate to severe, life-threatening hemolytic anemia episodes can develop if G6PD-deficient individuals are treated with 8-aminoquinoline drugs. This risk represents a major barrier to wide-scale adoption of radical cure. Therefore, determination of a malaria patient's G6PD activity level is critically important before 8-aminoquinoline drug therapy. The G6DX Diagnostic System uses an electrochemical sensor to make a simultaneous quantitative measurement of red blood cell G6PD and hemoglobin in whole blood in a point-of-care setting with results available in a minute. We describe the proof-of-feasibility of such a test, presenting analytical performance data over the critical G6PD activity dynamic range, usability, and acceptability data collected in several ***P. vivax*** endemic countries. The G6DX Diagnostic System is in the research prototype stage and is not yet commercially available.

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RECOMBINANT HUMAN G6PD FOR QUALITY CONTROL AND QUALITY ASSURANCE: RESOURCE FOR ROBUST G6PD TESTING IN *PLASMODIUM VIVAX* RADICAL CURE

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Primaquine is an 8-aminoquinoline based drug that has been available for malaria radical cure since the 1950s and currently is used with or without glucose-6-phosphate dehydrogenase (G6PD) testing. Good medical practice requires that people know their G6PD status prior to receiving an 8-aminoquinoline drug. Robust G6PD tests are currently under development to meet the needs for radical cure of ***Plasmodium vivax*** with 8-aminoquionlines. A large gap for the support of point-of-care G6PD testing is the availability of reagents to support quality control (QC) of G6PD products along the supply chain from the manufacturer to the end user. While there are reagents and systems to support QC of laboratory screening tests, they are not configured in terms of shelf life and volumes to support quality assurance (QA) programs for point-of-care G6PD tests. Feasibility to lyophilize recombinant human G6PD as a QC reagent is demonstrated. For calibration of G6PD assays, a standard reagent of G6PD was used to create a panel of normal, intermediate and severe deficiency, representing 100%, 30% and 10% activity respectively, as well as a no-enzyme control. Recombinant G6PD was expressed in ***E.coli*** and purified and stored at -80°C. Aliquots were thawed and combined with mannitol and sucrose in single use tubes and lyophilized. After lyophilization, enzyme activity was not altered. Results are presented from real time and accelerated stability studies of the lyophilized G6PD enzyme. These reagents could support a framework for a sustainable QC/QA system to support robust point-of-care G6PD testing for ***Plasmodium vivax*** radical cure.

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FACTORS THAT ARE ASSOCIATED WITH THE RISK OF ACQUIRING *PLASMODIUM KNOWLESI* MALARIA IN SABAH, MALAYSIA: A CASE-CONTROL STUDY

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Plasmodium knowlesi has long been present in Malaysia, and is now an emerging cause of zoonotic human malaria. Cases have been confirmed throughout South-East Asia where the ranges of its natural macaque hosts and Anopheles leucosphyrus group vectors overlap. The majority of cases are from Eastern Malaysia, with increasing total public health notifications despite a concurrent reduction in ***P. falciparum*** and ***P. vivax***

malaria. The public health implications are concerning given ***P. knowlesi*** has the highest risk of severe and fatal disease of all ***Plasmodium spp*** in Malaysia. Current patterns of risk and disease vary based on vector type and competence, with individual exposure risks related to forest and forest-edge activities still poorly defined. Clustering of cases has not yet been systematically evaluated despite reports of peri-domestic transmission and known vector competence for human-to-human transmission. A population-based case–control study was conducted from December 2012 to January 2015 at two adjacent districts in north-west Sabah, Malaysia. 228 ***P. knowlesi*** PCR-confirmed malaria cases presenting to the district hospital sites meeting relevant inclusion criteria were enrolled, with three community controls matched to the same village as the case selected randomly. Study procedures included blood sampling and administration of household and individual questionnaires to evaluate potential exposure risks associated with acquisition of ***P. knowlesi*** malaria. Results from the primary per protocol analysis will be presented, with adjusted ORs for exposure risks between cases and controls calculated using conditional multiple logistic regression models. Secondary outcomes will include differences in exposure variables between ***P. knowlesi*** and other ***Plasmodium*** species, risk of severe ***P. knowlesi*** malaria, and evaluation of ***P. knowlesi*** case clustering.

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MEASURING SOCIOECONOMIC INEQUALITIES IN MALARIA RISK IN RURAL UGANDA

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Relative wealth is an important risk factor for malaria. However, there is little consensus on how to measure wealth in malaria studies in rural African communities. We evaluated the agreement between six indicators of socioeconomic position and assessed their relative performance in detecting socioeconomic inequalities in malaria in Nagongera, eastern Uganda. Socioeconomic information was collected for all children aged six months to ten years living in 100 households, who were followed for 36 months. Parasite prevalence was measured every three months and malaria incidence was determined by passive case detection. Mosquito density was measured using monthly light trap collection. Socioeconomic position was determined using (1) two wealth indices derived from Principal Component Analysis, (2) income, (3) occupation, (4) food security, (5) vulnerability and (6) education. Indicators were assessed in terms of (1) relative agreement and (2) sensitivity to malaria inequalities. We will present the relative agreement between indicators and the association between each indicator and human biting rate, malaria infection and clinical malaria. We will discuss the most appropriate indicators for measuring relative wealth in this setting and the implications for future study design.

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PREVALENCE OF MALARIAL INFECTION AMONG PRIMARY SCHOOL CHILDREN IN AN URBAN SETTING IN UGANDA

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This study was undertaken to estimate the prevalence and distribution of ***Plasmodium*** infection and identify local risk factors among school children in Kampala the main urban setting in Uganda in order to contribute to the national updating of the malaria epidemiology profile. Urban malaria is still a problem in Sub-Saharan Africa, contributing up to 25% of the global burden of malaria among urban dwellers. In Uganda, urban malaria is still predicted with least certainty. The prevalence of malaria parasitemia in Kampala the biggest urban setting in Uganda was 5% in 2009. There has been a scale up in malaria control interventions since however information is lacking to capture the transitioning epidemiology. We conducted school based surveys in Kampala between June and August 2014 to estimate the prevalence and distribution of ***Plasmodium*** infection and identify local risk factors among school children in Kampala the capital city of Uganda. A finger-prick blood sample was collected for a thick and thin blood smear and children with fever had a malaria rapid diagnostic test performed using Para Check-Pf device. Participating schools were mapped using hand-held GPS receivers. The survey was conducted in 20 primary schools and a total of 2,069 children aged 5 to 16 years were randomly enrolled. Malarial parasite prevalence was 3.04% (95% CI: 0.962-0.977) with ***P. falciparum*** as the main species. About 42.10% of the children slept under an ITN the previous night and 8.36% lived in houses sprayed with IRS in the last twelve months. There were no significant predictors of malaria parasitemia identified. In conclusion, malarial parasite prevalence has remained low in this urban setting. Inexpensive school surveys can be used to monitor malaria prevalence to inform transitioning malaria epidemiology.

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ADAPTIVE GEOSTATISTICAL DESIGNS: OPTIMIZING SAMPLING IN DISEASE PREVALENCE MAPPING TO SUPPORT TARGETED INTERVENTION STRATEGIES

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Geostatistical methods are being used increasingly to support disease control efforts and analyse disease prevalence. Adaptive geostatistical designs (AGD) allow collection of exposure and outcome data over time to depend on information obtained from previously collected data to optimise data collection towards the analysis objective. AGDs are especially useful in poor resource settings where uniformly precise mapping may be unrealistically costly and the priority is often to identify critical areas where interventions can have the most health impact. If successfully implemented, AGDs should outperform current standard sampling by improving on predictive performance and hotspots identification. AGDs are timely and could be instrumental in monitoring/accelerating disease transmission reduction. We developed two main classes: singleton and batch sampling. In singleton sampling, locations xi are chosen one at a time, so that each new location xk+1 can depend on data obtained at previously sampled locations x1,...,xk. In batch sampling, locations are

chosen in batches of size b>1, allowing new batch, {x(kb+1),...,x(k+1) b}, to depend on data obtained at locations x1,...,xkb. Batch sampling can't be more efficient theoretically than singleton, but is more realistic in practice. Using simulated data, we have evaluated batch sampling designs and assessed their efficiency relative to their singleton adaptive and non-adaptive counterparts by comparing their average prediction variance. We will present simulation results and describe an application to a multi-year rolling cross-sectional Malaria Indicator Survey that is being conducted within a 5-year malaria transmission reduction project in communities living around Majete Game Reserve, Malawi. Aims of this application are to: describe local variation in malaria infection in children below 5 years; identify hotspots that could guide more targeted disease control efforts; and investigate association of prevalence with environmental and social risk-factors, using a combination of survey data and publicly available, remotely sensed climate and environmental information.

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THE GEOGRAPHY OF IMPORTED MALARIA TO NON-ENDEMIC COUNTRIES

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Despite over fifty countries having achieved malaria elimination over the past century, the disease remains a problem to many 'malaria-free' countries through cases imported from endemic regions each year. Imported cases to non-endemic countries remain difficult to diagnose, expensive to treat and can occasionally spark secondary local transmission, while the movement of malaria between endemic countries is driving the spread of drug resistance. Quantifying the international movements of malaria can aid in improving our understanding of these phenomena and facilitate the design of mitigation strategies, providing insights into the epidemiology of malaria in regions where reliable surveillance data are lacking. We describe the assembly of a database of all publicly-available nationally reported statistics on imported malaria over the past 15 years, covering over 50,000 individual cases, and assessments of the geographical variations seen were undertaken. Results highlight the clear geographical differences that exist between non-endemic countries and regions in terms of imported malaria case numbers, origins and species composition, as well as the variations in composition for the countries where cases originate. Infection movements are strongly skewed towards a small number of high traffic routes, with the geographical distribution of cases correlating strongly with existing data on transmission intensities. The mapping of communities of countries linked strongly by imported case movements reveals clear groupings that are a result of historical, language and travel ties. Finally, examination of the species composition of origin cases provides a unique insight into the distribution, prevalence and acquisition risk of each of the malaria parasites.

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RISK FACTORS ASSOCIATED WITH OCCURRENCE OF PLACENTA MALARIA IN A POPULATION OF PARTURIENTS IN ABEOKUTA OGUN STATE, NIGERIA

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Placental malaria has long been acknowledged as a complication of malaria in pregnancy and has been associated with poor pregnancy outcome in malaria endemic areas. This study was conducted to determine the risk factors associated with occurrence of placenta malaria in a population of parturients in Abeokuta Ogun State. Maternal and placenta blood and relevant maternal demographic information were obtained from 211 parturients. Chi-square tests and regression model were computed to measure risk using SPSS version 16.0. Overall, 40.1% (86 of 211) of the parturients had malaria at delivery, with 19.0% (40 of 211) having placenta malaria. Age range 18-22 years (OR= 4.4, 95% CI = (1.1 - 17.4), p= 0.046), primigravidae (OR= 2.1, 95% CI = (0.9 - 5.1), p= 0.028) and living in a congested apartment (OR= 1.6, 95% CI = (0.4 - 6.0), p= 0.029) as a significant risk factor for placenta malaria. Non usage of Intermittent Preventive Treatment (IPT) (OR= 2.6, 95% CI = (1.2 - 5.4), p= 0.018), Long Lasting Insecticidal Nets (LLINs) (OR= 2.7, 95% CI = (1.3 - 5.5), p= 0.005) were also risk factors for placenta malaria. In Abeokuta, approximately one of every five parturients had placenta malaria at delivery, with 55% having parasite densities between (501-5000 parasites/µl of blood). Proper use of LLIN and IPT for pregnant women is hereby recommended.

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TRANSMISSION OF MALARIA AMONG INTRAVENOUS DRUG USERS IN THE UNITED STATES: A SHIFT FROM ENDOGENOUS TO EXOGENOUS CASES, 1929-1975

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Malaria remains a major health issue in developing countries. It is often overlooked in the industrial world unless there is a history of travel to a malarial zone. An especially overlooked issue, however, is malaria transmission between intravenous drug addicts. The sharing of needles among heroin users who are malaria-positive may well spark cases of this disease. Analysis of peer-reviewed literature in PubMed and PubMed Central from 1900 to 1975 was undertakebn. . The spread of malaria through shared equipment for injecting intravenous drugs was first reported in 1929 in Egypt. Since that time, several cases of induced malaria among heroin users have been identified. Beginning in the early 1930s, reports of malaria transmitted between IV drug users began to appear in the eastern United States, typically among individuals who reported no history of foreign travel. This trend continued until the late 1960s and early 1970s, the years of the Vietnam War, during which time cases were imported to California and New York after the Vietnam War. In each of these cases, transmission of the malaria parasite was due to sharing of needles among addicts. Usually, the index case had recently traveled to a malaria zone such as Southeast Asia and had contracted malaria. There has been a shift from domestic cases of malaria to imported cases during the latter part of the 20th century. Conclusions: Malaria among intravenous drug users remains an important, if not overlooked, public health issue. The number of heroin users is believed to be growing, and the potential for more cases of induced malaria remains high in the United States. Malaria among drug addicts remains a clinical problem of which physicians should be aware. The diagnosis of malaria should be considered in all intravenous drug users with fever and chills. This issue is hardly a new one and remains a potential public health issue in the early 21st century.

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IMPACT OF TARGETED MALARIA TREATMENT ON THE TRANSMISSION OF *PLASMODIUM FALCIPARUM* ALONG THE THAI-MYANMAR BORDER

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The emergence and spread of artemisinin resitance in ***Plasmodium falciparum*** (Pf) is challenging the efforts of malaria control and

elimination in South East Asia. The Shoklo Malaria Research Unit through the support of the Welcome Trust and the Bill & Melinda Gates Foundation has implemented a pilot study in four villages along the Thai Myanmar border to assess whether Targeted Malaria Treatment (TMT) can eliminate the parasite reservoir and contain artemisinin-resistance. Entomological surveys using human landing catch technique were conducted in parallel to parasitological surveys to address the Pf-malaria transmission before and after intervention. One thousand two hundred and eighty two malaria vectors belonging to the Minimus, Maculatus and Dirus Groups were collected during baseline surveys (before TMT, during the rainy season). Bites of malaria vectors occur all night long but An. maculatus s.l. and An. dirus s.l. exhibit a peak in their biting behaviour during the early evening and a higher tendency to exophagy. An average of 267 bites of malaria vectors was received per person and per month (95% CI 226-309). Pf-sporozoitic index was 2.2 ‰ (95% CI 0.0-4.4, n=1,782 mosquitoes inspected) and we estimated that each person received an average of 0.6 (95% CI 0.02-1.18) Pf-infective bites per month. Half of the transmission occurred outside the premise (2 on 4 infective bites) and half of the transmission occurred between 5:00 and 06:00 a.m. (2 on 4 infective bites). Data on the impact of TMT on Pf transmission will be presented during the meeting. In conclusion, malaria transmission in the studied area involves early feeding and exophagic vectors that could maintain residual transmission (i.e. transmission that is not controlled by full coverage of the population with long lasting insecticide-treated bed-nets) after TMT. Therefore the development and evaluation of vector control tools adapted to malaria transmission settings in South-East Asia are needed in order to act in synergy with TMT and achieve artemisinin-resistance containment.

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HIGH PREVALENCE OF FALCIPARUM MALARIA IN ASYMPTOMATIC INDIVIDUALS AND NO PFMDR1 AMPLIFICATION IDENTIFIED IN DEMOCRATIC REPUBLIC OF CONGO

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Malaria remains a major public health problem in Democratic Republic of Congo (DRC) with 14 million cases reported by the WHO malaria report in 2014. These figures only include patent malaria cases that were detectable by microscopy or by RDT. Asymptomatic malaria cases are known to be prevalent in endemic areas and are generally untreated, resulting in a significant source of gametocytes that may serve as reservoir of disease transmission. Considering that microscopy certainly underestimates the prevalence of ***Plasmodium*** infections within asymptomatic carriers and that PCR assays are currently recognized as the most sensitive methods for ***Plasmodium*** identification, this study was conducted to weigh the asymptomatic carriage in DRC by a molecular method. We additionally assessed the pfmdr1 gene amplification, that is related to many antimalarial drugs' resistance. Globally, almost half of the samples collected in the 6 provinces on the asymptomatic individuals (280/600; 46.6%) had ***Plasmodium*** infections and the most species identified was ***P. falciparum*** (97.8%) alone or combined with P. malariae. The lesser prevalence was found in Nord-Kivu province (22%) nearly at 1800 meter altitude. No pfmdr1 amplification > 2 copies was found. The high prevalence reported in our study should interpellate the bodies involved in malaria control in DRC to take in account asymptomatic carriers in actions taken and consider asymptomatic malaria as a major hurdle for malaria elimination. This study was the first to assess pfmdr1 amplification in DRC.

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DOES STRESS PROVOKE *PLASMODIUM FALCIPARUM* RECRUDESCENCE?

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Plasmodium falciparum, unlike ***P. vivax***, must maintain infection in the blood/bone marrow over many months/years in order to bridge periods between transmission periods. Asymptomatic parasitemia at very low concentrations is now known to be quite common due to molecular detection methods. Old tropical medicine texts commonly list many stressful events stated to provoke recrudescent falciparum parasitemia such as fatigue, heat/chill, trauma/surgery, famine/war, transit between areas and other febrile illness. The older literature is reviewed to discover the factual basis of such varied reports since they have not been recently confirmed. Surgery / trauma studies have variably shown falciparum recrudescence in areas with high rates of infection and drug suppression. Travel particularly during times of war or famine has been noted to induce recrudescence likely contributing to complex public health emergencies. Provocative tests such as infections of epinephrine or endotoxin to aid diagnosis of cryptic infections could not be shown to induce falciparum recrudescence. It seems likely that human stress sometimes induces falciparum recrudescence of an otherwise asymptomatic infection. Reproducing such observations today has been radically altered as malaria chemotherapy has evolved from suppressive quinine to curative artemisinin combinations. Host stress provoked recrudescence may be part of ***P. falciparum's*** survival strategy.

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HOST FACTORS IMPACTING UPON THE FUTURE USE OF PRIMAQUINE IN MALARIA-ENDEMIC SOUTHWESTERN UGANDA

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As malaria transmission continues to decline in southwestern Uganda, aggressive strategies, such as the addition of primaquine (PQ) to artemisinin-combination therapies (ACTs), are being considered. Despite the potential benefit of PQ in reducing transmission, concerns over its safety and efficacy have hampered its deployment. In particular, those with glucose-6-phosphate dehydrogenase (G6PD) deficiency are at a higher risk of hemolytic toxicity, and recent metabolic variants of CYP2D6 have the potential to impact upon PQ efficacy. To better assess the prevalence of host factors that may impact PQ use in southwestern Uganda, we conducted a stratified, two-stage cluster sampling cross sectional survey among 631 children under five years of age. Blood samples were collected to determine the following: (1) quantitative G6PD deficiency by spectrophotometric assay (Trinity Biotech®) and (2) qualitative G6PD deficiency assay by rapid diagnostic test (CareStart™ G6PD RDT). In addition, DNA was isolated to conduct (1) genotyping of the G6PD A- allele (RFLP analysis to detect the 202A/376G mutation), and (2) CYP2D6 genotyping to identify poor and ultrarapid metabolizers. Using the spectrophotometric assay as the gold-standard, the prevalence of mild G6PD deficiency (defined as 10-60% of normal activity) was 13.8% (95% CI: 11.1-16.5) as compared with 8.6% (95% CI: 6.4-10.8) by RDT. No children in our study were classified as being severely deficient (<10% enzyme activity). Of the 577/631 children with normal G6PD status by RDT, 37 were mildly deficient by quantitative assay. Of the 54 children found to be G6PD deficient by RDT, 4 were quantitatively normal. Performance

characteristics of the CareStart™ G6PD RDT as compared with the Trinity Biotech® spectrophotometric assay revealed low/moderate sensitivity and high specificity (57.5% and 99.3%, respectively). Further comparison of G6PD qualitative and quantitative assays to molecular results is underway and will be presented. In addition, CYP2D6 prevalence estimates will also be presented. Our preliminary results suggest the need for improved point-of-care G6PD screening methods.

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RISK FACTORS FOR MALARIA INFECTIONS IN FEVER HOTSPOTS AND COLDSPOTS IN A HIGH TRANSMISSION REGION IN WESTERN KENYA

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Identifying and understanding the risk factors for malaria infections in regions with high transmission is important for targeting control measures to the local situation. We sought to determine malaria indices in fever hotspots and coldspots. We conducted a prospective cohort study in Bungoma East sub-County, a region with persistently high malaria. A total of 400 participants in randomly selected households in six sentinel villages were followed up longitudinally and tested for malaria using malaria rapid diagnostic tests at quarterly intervals for a period of one year. Multivariate logistic regression analysis with generalized estimating equations was used to estimate the risk of malaria infections. A total of 870 malaria vectors were captured out of which 73.6% (no=640) were identified as members of ***Anopheles gambiae*** group. The member species of ***An. gambiae*** group were identified by polymerase chain reaction as follows; 24.5% (no=117) were ***An. arabiensis*** while ***An. gambiae*** ss consisted of 75.5% (no=483) of the total collection. Parasite prevalence by RDT in the fever cold spot was 19.2%, 12.3%, 7.5%, 9.4% and 17.4% per survey respectively. In the fever hotspot, parasite prevalence was 22%, 8.5%, 5%.4%, 9.7% and 32.4% per survey respectively. The person-time incidence rate of malaria was not significantly different between the two regions. However, incidence significantly varied between the villages and was significantly correlated with entomological risk factors in some of the villages. Risk factors for malaria infections are; children below five years, (O.R 3.7; P: 0.02), Not sleeping under the net the previous night (O.R. 1.7; P: 0.01), Asymptomatic infected individuals (O.R. 9.5; P: 0.004), the type of wall for the house (O.R 0.3, $P < 0.001$), the village one lives (OR 0.3; $P < 0.001$).There is a slightly higher risk of malaria infection for individuals living in the fever hotspots although this does not reach significance level. There is heterogeneity in malaria transmission among the villages. This study has identified factors defining the local situation in Western Kenya and should be targeted if malaria is to be eliminated in this region.

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LARGE RESERVOIR OF ASYMPTOMATIC *PLASMODIUM VIVAX* IN A MESOENDEMIC AREA OF BELU REGENCY, WEST TIMOR

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With the call for malaria elimination in Indonesia, ***Plasmodium vivax*** and the asymptomatic infection resevoir are considered particularly important as submicroscopic infections escape diagnostic tools, thereby confounding elimination efforts. Microscopy was compared to Real-Time PCR to identify submicroscopic infections from subjects (n=1013) living in Kabupaten Belu, West Timor. Microscopy detected parasites in 10.6% (107/1013) subjects, while PCR resulted in a 29.8% (302/1013) infection rate. Of the PCR diagnosed samples, 65.2% (197/302) were submicroscopic (negative by microscopy). Eighty four percent (166/197) of submicroscopic infections were ***P. vivax*** with lower number of ***P. falciparum*** (25/197) and P. malariae (1/197). Submicroscopic ***P. vivax*** infection predominated all age groups. Few mixed submicroscopic infections were found (3 Pf + Pv; 2 Pv + Pm). In conclusion, the presence of 3x more malaria infections detected by PCR (29.8% prevalence) when compared to microscopy demonstrates the large number of submicroscopic infections, of which 84% were ***P. vivax***, indicating that this area will be challenging for malaria elimination. This more sensitive diagnostic mechanism (Real-Time PCR), combined with other tools will have to be implemented if elimination is to be considered in this area.

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IN VITRO ANTIPLASMODIAL ACTIVITY OF BIOSYNTHESIZED SILVER NANOPARTICLES USING *PSYCHOTRIA NILGIRIENSIS* LEAF EXTRACT AGAINST *PLASMODIUM FALCIPARUM*

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The utilization of various plant resources for the biosynthesis of metallic nanoparticles is called green nanotechnology, and it does not utilize any harmful chemical protocols. The present study reports the plant mediated synthesis of silver nanoparticles using the plant leaf extract of ***Psychotria nilgiriensis***, which acts as a reducing and capping agent. The obtained nanoparticles were characterized using UV-visible spectroscopy; EDX (energy-dispersive X-ray), SEM (Scanning electron microscope), XRD (X-ray diffraction) and Fourier transform infrared (FTIR) analysis. The efficacy of green synthesized AgNPs at different concentrations (25, 50, 75 and 100µg/ml) were tested on ***Plasmodium falciparum***. Synthesized AgNPs particles were confirmed by analysing the excitation of surface plasmon resonance (SPR) using UV-vis spectrophotometer at 422 nm.The scanning electron micrograph showed structures of spherical, cubic shape, and the size range was found to be 40-60 nm. The EDX spectra showed the purity of the material and the complete chemical composition of the synthesized AgNPs. XRD study shows that the particles are crystalline in nature with face centered cubic geometry. The FTIR analysis of the nanoparticles indicated the presence of proteins, which may be acting as capping agents around the nanoparticles. Biosynthesis of nanoparticles may be triggered by several compounds such as carbonyl groups, terpenoids, phenolics, flavonones, amides, proteins, alkaloids and other reducing agents present in the biological extract. The parasitic inhibition was dose-dependent. The synthesized AgNPs showed significant anti-plasmodial activity when compared to aqueous leaf extract of P. nilgiriensis. The maximum efficacy was observed in synthesized AgNPs against ***P. falciparum*** (IC_{50}=100 µg/ml; 100%) respectively. This method is considered as a new approach to control the malarial parasite, ***P. falciparum***. Therefore, this study provides first report on the anti-plasmodial activity of synthesized AgNPs using ***P. nilgiriensis*** against ***P. falciparum.***

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ESTIMATING THE GLOBAL CLINICAL BURDEN OF *PLASMODIUM VIVAX* MALARIA

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Accurate burden of disease estimates are essential to assess the relative impact of a disease, generate targets for control and measure progress towards elimination, and have been identified as a key knowledge gap in ***Plasmodium vivax*** research. Annual clinical incidence must be measured to estimate the burden of ***P. vivax*** malaria. However, accurate incidence data is relatively rare. Incidence must be obtained through longitudinal studies, making it costly and time consuming to collect. Prevalence data, on the other hand, is widely available from cross-sectional surveys. A model defining the relationship between ***P. vivax*** infection prevalence and incidence of clinical disease was developed to address this disparity in data availability. The model accommodated the unique biology and epidemiology of ***P. vivax*** and provided separate prevalence-incidence

relationships for areas known to have different patterns of relapse. The model, combined with an updated map of ***P. vivax*** endemicity based on prevalence survey data through 2014, provided global annual case numbers with associated measures of uncertainty. These results highlight regions where ***P. vivax*** morbidity burden is greatest and where improved survey coverage is needed to increase the certainty of outputs generated.

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MALARIA PARASITEMIA AMONG RESIDENTS SEEKING CLINICAL CARE IN AN URBAN INFORMAL SETTLEMENT AREA IN NAIROBI, KENYA

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Although Nairobi, Kenya--with an altitude of approximately 1,600 meters and low seasonal temperatures--is considered low-risk for malaria transmission, malaria is frequently diagnosed throughout Nairobi. We investigated the epidemiology of malaria among patients presenting to a study clinic in a population-based surveillance system located in the densely-populated, informal settlement of Kibera, Nairobi, over a 5-year period (01 January 2007 to 31 December 2011). We describe the epidemiological and clinical characteristics of febrile patients and malaria cases in Kibera, Nairobi. During the 5-year study period, 105,960 patient visits occurred at the study clinic, and 17% (n=18,183) had a measured temperature of ≥37.4°C. A total of 11,825 (65%) febrile patients had a microscopy test performed for malaria; 2,630 (22%) were positive. Malaria parasitemia was detected throughout the year with peaks generally in January, May and September. Children ages 5-14 years old had the highest proportion (29%) of positive malaria blood slide results followed by children ages 1-4 years (23%) old. Sixty-three percent (3% unknown travel status) of malaria cases reported travelling outside of Nairobi in the previous month; 79% reported travel to just three counties (7%, 3/46) in western Kenya. History of recent travel was strongly associated with malaria parasitemia (OR: 8.9; 95% CI: 8.0-9.9). Malaria parasitemia was frequently observed among febrile patients at a health facility in Kibera, Nairobi. The majority of patients had travelled to counties in western Kenya, which have the highest rates of parasitemia in the country. However, over one-third 34% reported no travel history, which raises the possibility of local transmission of malaria in this densely-populated, urban setting. Reducing/eliminating malaria transmission in western Kenya and communicating and implementing effective malaria prevention strategies to travelers is likely to reduce the malaria burden in Kibera, Nairobi.

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NEW STRATEGIES FOR ESTIMATING MALARIA TRANSMISSION: USING SEROLOGY TO ESTIMATE INDIVIDUAL LEVEL EXPOSURE

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Current metrics of malaria transmission are restricted to population level estimates limiting their utility for understanding the more granular, individual level heterogeneities inherent in ***Plasmodium falciparum*** epidemiology. Age specific serological profiles in a population, and particularly the seroconversion rate, are useful proxy metrics for transmission intensity. However, assuming a homogenous population, a person of the same age with comparable exposure history should have a similar antibody profile suggesting that obtaining an analogous individual level metric for exposure is possible. To test this hypothesis we extended the standard reverse catalytic model to incorporate determinants of exposure including elevation and use of mosquito control using a Bayesian framework to predict each individuals' probability of being seropositive, an analogous measure to the force of infection. Data from a large cross-sectional survey in the western Kenyan highlands was used to train the model, and was subsequently validated on available datasets from the same study site as well as other sites with a range of transmission intensities. Initial results suggest that the predicted probability of being seropositive per year of age were strongly correlated (r=0.96) to the true seroprevalence and showed similar spatial patterns. When applying the model to other data from the same site, there was good discriminatory capacity (AUC: 0.76) for seropositivity. The predicted values resulted in a slight underestimation of the true seroprevalence per year of age however there was still a good correlation (r=0.79). These findings show that obtaining individual estimates of malaria exposure are possible and have important implications for understanding and controlling for intrinsic heterogeneity in malaria exposure.

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AN INVESTIGATION INTO THE RISK FACTORS OF MALARIA IN A PRE-ELIMINATION RURAL AND PERI-URBAN SETTING OF NORTHERN NAMIBIA

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A remarkable over 75% reduction of cases has been achieved, with case numbers dropping as low as 1,546 in August of 2013, from 537 115. With the reduction of malaria transmission, Namibia has moved from a control phase to a pre-elimination phase. Although malaria cases have been on the decline, a plateau has been reached since 2008. This plateau provides direct evidence that, the existing tools used in the control phase cannot be used to achieve the elimination phase. This also suggest that there are perhaps other risk factors that are not known and thus poses challenges to elimination. This risk factors may be the presence of breeding sites within households, net ownership and usage, Indoor Residual Spraying, whether people slept inside or outside, and their travel history. There is a gap in knowledge on what intervention tools should be used in order to achieve the elimination phase. Malaria risk factors therefore need to be established by conducting surveillance, and thus provide evidence on how to mitigate against any failing interventions. Re-Active Case Detection (RACD) was used to investigate cases reported at health facilities. Control Houses were randomly selected from the National Census Enumeration points for inclusion in the study. A combination of an open and closed ended questionnaire was administered to all members living in the same house as a reported case or a chosen control. A total of 944 individuals were recruited. There were 408 and 536 individuals from 51 case and 87 control households respectively. It was found that 71% of the case households are found close to breeding sites, whilst only 58% of the control households are found close to breeding sites. Individuals in case households also slept outside more than those in control households. Unexpectedly, net ownership and usage was higher in control households by a factor of 1.13. Spraying was also higher in control households, with only 12% of the case households spray and 18% of the control households. Reactive case detection can be implemented as a tool to identify common behavioral risk factors in individuals within households.

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PREVALENCE OF MALARIA BY AGE AND GENDER IN KABERAMAIDO, LWALA AND DOKOLO DISTRICTS IN THE EASTERN UGANDA

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Malaria is a major health concern in affected developing countries. It is endemic in Uganda with 34 million people at high risk accounting for

7,277 malaria attributed death in 2013. There are limited data on gender and age distribution of malaria cases in hospital and outpatients' settings. This study assessed malaria case records between January 2006 and April 2013 in the Kaberamaido, Lwala and Dokolo health districts in Northern Uganda. A total of 346,769 outpatients and 75,955 inpatient children were examined for malaria. Of these, 183,849 (53.05 %) outpatients and 31,459 (41.42 %) inpatients were clinically diagnosed with malaria with 28,954 (70.52%) testing positive on 41,058 (19.07%) blood smears examined in both groups. Covering both genders, 56.02% and 46.32% clinically diagnosed malaria cases were below and above 5 years of age, respectively. Females were the most affected. The proportions of positive malaria cases per health district were 47.9%, 46.1% and 51.8% for Kaberamaido, Lwala and Dokolo, respectively. In 2009, over 8000 malaria cases principally in children above age 5 were recorded in Dokolo. Overall, Dokolo was the most affected malaria district, followed by Kaberamaido. In conclusion, malaria prevalence remains very high in study sites in Northern Uganda. Children under the age of 5 were the most affected with girls more susceptible than boys. Due to the shortage of microscopes and qualified microscopists, malaria remains poorly diagnosed in Uganda with the majority of diagnoses still made clinically.

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THE ASYMPTOMATIC MALARIA RESERVOIR IN A REGION OF DECLINING MALARIA TRANSMISSION IN SOUTHERN PROVINCE, ZAMBIA: DEMOGRAPHIC CHARACTERISTICS AND ASSOCIATED RISK FACTORS

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Asymptomatic malaria has been reported in areas of declining transmission among individuals with partial immunity acquired from previous exposure. Undetected and untreated asymptomatic individuals can act as parasite reservoirs posing a threat to further control and their active detection and characterization is important to achieve elimination. To better understand this threat, demographic characteristics as well as seasonal and spatial distributions of asymptomatic malaria infections were assessed in an area of declining transmission in Southern Province, Zambia. Cross-sectional surveys were conducted between 2009 and 2013 when annual parasite prevalence was ≤ 1.5% by rapid diagnostic test (RDT). Households were randomly selected based on satellite imagery and all residents were eligible for enrollment. Blood was collected by finger prick for microscopy, RDT and as dried blood spots for nested PCR for cytochrome b gene of human ***Plasmodium*** species. Questionnaires were administered to collect information on age, sex, recent history of malaria symptoms and antimalarial use. Asymptomatic cases among positive individuals were determined based on absence of fever, chills and headache both within 48hrs and a fortnight prior to screening and without a recent history of malaria medication. Seasonal and spatial distributions of asymptomatic individuals were based on sample collection dates and geo-coordinates of households respectively. Of 3,555 participants tested by all three methods, 53 were positive for ***Plasmodium*** by nested PCR. Fifty-two participants had complete survey data available, 19 (36.5%) of whom were asymptomatic. Participants aged between 5 and 20 years were more likely to have asymptomatic parasitemia identified by PCR during both rainy and dry seasons. Identifying asymptomatic parasitemia among school-aged children in both wet and dry seasons suggests the potential for school-based strategies to eliminate the parasite reservoir. Submicroscopic asymptomatic parasite reservoir detected by PCR highlights the need for more sensitive diagnostic tools in low transmission settings moving towards elimination.

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THE EPIDEMIOLOGY OF RELAPSING *PLASMODIUM VIVAX* MALARIA IN WESTERN MADAGASCAR

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Malaria is a major health problem in Madagascar. While cases have dropped since 2003, malaria was still the second leading cause of death among children under five years in 2011. The epidemiology varies considerably between different regions of the country, but the entire population is considered to be at risk of infection by four ***Plasmodium*** species, with ***P. falciparum*** responsible for 90% of cases. Prevalence of ***P. vivax*** is highest in the western highlands, where the climate is dry and hot. The dormant hypnozoites of the ***P. vivax*** life-cycle make it more challenging to control than ***P. falciparum***. As a first step towards assessing the importance of relapse infections and the significance of the invisible reservoir of ***P. vivax*** hypnozoites, it is important to determine the relapse periodicity of the ***P. vivax*** strains causing infection in Madagascar. Molecular analysis can also allow insight into the population structure of the parasites and help estimate the relative contribution of hypnozoites to the burden of clinical cases. To investigate these objectives, a longitudinal study was initiated in an area of Madagascar endemic with ***P. vivax*** where all individuals reporting to clinics with fevers were tested for infection with rapid tests, microscopy and molecular diagnostics. All ***P. vivax*** positive patients were monitored with active monthly follow-up and PCR-based diagnosis of blood-stage infection. Up for four repeated clinical ***P. vivax*** episodes were observed in patients monitored during the first year of the study, and genetic SNP analysis provides insight into the evolution of the parasite strains during the course of the follow-up. Madagascar plans to advance from malaria control to pre-elimination status. However, the complexities and heavy contribution of apparent relapses to the ***P. vivax*** burden will continue to represent an important barrier to successful elimination unless access to safe radical cure therapy is made available.

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HETEROGENEITY IN MALARIA TRANSMISSION IN THE PERUVIAN AMAZON: RAPID ASSESSMENT THROUGH A PARASITOLOGICAL AND SEROLOGICAL SURVEY

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Where malaria endemicity is low and markedly seasonal, sensitive tools are needed for better stratifying risk of infection and targeting interventions. A cross-sectional survey was conducted in 3 endemic sites of the Peruvian Amazon to characterize the current malaria transmission patterns, identify hotspots, and detect recent changes in transmission using parasitological and serological measures. After full census of the study population in Nov 2012, a total of 651 survey participants were examined and a blood sample taken for the detection of malaria parasites (microscopy, PCR) and antibodies to ***Plasmodium vivax*** (PvMSP119,PvAMA1) and P.falciparum (PfGLURP,PfAMA1) antigens by ELISA. Risk factors malaria infection (species-specific positive PCR) and malaria exposure (seropositivity to any of two species-specific antigens) by species were assessed by survey logistic regression models. Age-specific seroprevalence was analyzed using a reversible catalytic conversion model for generating seroconversion

rates(SCR). SaTScan using a Bernoulli model was used to detect spatial clusters of serology-positive individuals within each site. The overall parasite prevalence by PCR was low, i.e. 3.9% for ***P. vivax*** and 6.7% for ***P. falciparum***, while the seroprevalence was much higher, 33.6% for ***P. vivax*** and 22.0% for ***P. falciparum***, with substantial differences between study sites. Age and location were significantly associated with ***P. vivax*** exposure; while location, age and outdoor occupation were associated with ***P. falciparum*** exposure. ***P. falciparum*** seroprevalence curves showed a stable transmission throughout time, while for ***P. vivax*** transmission curves were better described by a model with two SCRs. The spatial analysis identified well-defined clusters of ***P. falciparum*** seropositive individuals in two sites, while it detected only a very small cluster of ***P. vivax*** exposure. The use of a single parasitological and serological malaria survey has proven to be an efficient and accurate method to characterize the species specific heterogeneity in malaria transmission at micro-geographical level as well as to identify recent changes in transmission.

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ESTIMATING THE PROPORTION THE DISTRIBUTION OF *PLASMODIUM FALCIPARUM* MALARIA-ATTRIBUTABLE FEVERS THE ASYMPTOMATIC INFECTION IN AFRICA

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In order to accurately assess the infectivity of populations endemic with ***P. falciparum*** malaria, asymptomatic infections must be accounted for, as these are not detected by passive case detection but still contribute to onwards transmission. The effect that geographic heterogeneity of malaria endemicity and environmental confounders have on the proportion of infections that are asymptomatic has not yet been well characterised. Additionally, the importance of the asymptomatic reservoir in terms of prospects for elimination is not yet fully understood. In areas where ***P. falciparum*** remains highly endemic, most individuals host parasites at a diagnostic detectable level throughout the year. As such, nearly all fevers in these populations will be accompanied by a parasite infection, but malaria may rarely be the fever's causal mechanism. Modelling the spatiotemporal distribution of this malaria-attributable fraction of fevers will allow insight into the true contribution of malaria compared to other causes of febrile illness. Here, we fit observed field data from large-scale routine surveillance of diagnostic outcome and individual fever history to established mechanistic models of malaria infection, in order to estimate the proportion of asymptomatic infection and malaria-attributable fevers geographically through time, and investigate a number of environmental and sociodemographic factors that affect these proportions.

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MODELED COUNTERFACTUAL OF PREDICTED MALARIA CASE INCIDENCE USING A DIFFERENCE-IN-DIFFERENCE ANALYSIS, UNGUJA AND PEMBA ISLANDS, ZANZIBAR, 1999-2010

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Malaria morbidity and mortality fell in Zanzibar (Unguja and Pemba islands) following introduction of artemisinin-based combination therapy for uncomplicated malaria in late 2003 and long-lasting insecticidal nets and indoor residual spraying in 2006. The impact of such public health interventions, scaled-up in short successions, is challenging to quantify without a contemporaneous counterfactual to understand what would have happened in the absence of these interventions. We modeled the impact of malaria control interventions scale-up using a counterfactual of confirmed malaria case incidence per 1,000 population (cMCI) predicted from a generalized linear autoregressive model fitting the relationship between cMCI, climate and other relevant pre-scale-up (1999-2003) data. The full prediction model included district, month, rainfall, and minimum and maximum temperature, standardized by district population estimates over time. The full model then predicted a counterfactual with malaria control interventions scale-up between 2004-2010. The impact of malaria control interventions scale-up on cMCI was then estimated using the difference-in-difference estimator, while controlling for district malaria diagnostic testing rate. We estimated 0.61 (95%CI, 0.43-0.79; p<0.001) lower monthly cMCI in the scale-up period in Zanzibar. The impact was larger in Pemba (0.73, 95%CI 0.39-1.08; p<0.001) than in Unguja (0.52, 95%CI, 0.33-0.72; P<0.001). A modeled counterfactual of predicted cMCI using a difference-in-difference analysis showed malaria control interventions scale-up significantly reduced cMCI from what it would have been in the absence of interventions scale-up in Zanzibar islands.

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THE EFFECTIVENESS OF NON-PYRETHROID INSECTICIDE-TREATED DURABLE WALL LINERS AS A METHOD FOR MALARIA CONTROL IN ENDEMIC RURAL TANZANIA: A CLUSTER RANDOMIZED TRIAL IN NORTHERN EASTERN TANZANIA: RESULTS OF THE BASELINE SURVEY

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Non-pyrethroid insecticide-treated durable wall lining (DL) is a new method of vector control that releases insecticides and kill vectors that rest on its surface, and are expected to be efficacious for 3-4 years. To determine whether the combined use of LLINs and DL provides additional benefit over LLINs alone, a two arm cluster randomized controlled trial is underway in Muheza district, Tanzania. We present baseline epidemiological and entomological parameters from the study site prior to study implementation. After mapping and doing a census of the area, 60 clusters were created, and 15-20 houses from the core areas were randomly selected for a survey conducted in January-February 2015. All consenting household members completed household and demographic questionnaires, and blood samples were drawn for malaria diagnosis, anemia testing, and immunochromographic testing (ICT) for ***Wuchereria bancrofti***. A total of 2,513 people from 932 households were sampled. Malaria parasitemia by mRDT was 23.6 % (95% CI: 20.4 -26.9%); 4.2% (95% CI: 2.9-5.5%) had positive ICTs. Malaria parasitemia was more common in children 5-12 years (34.5%; 95% CI 28.5- 40.6%) compared with children 12 years, (19.0%; 95% CI: 16.4-21.6%). Anemia (haemoglobin <8g/dL) prevalence in children <5 years was 5.1% (95% CI: 1.5-8.7%). To determine the biting rate Indoor host-seeking mosquitoes were sampled using CDC light traps in 8 randomly selected houses with open eaves and children under five years of age in the core sampling area of all sixty clusters between May and September 2014. Pooled estimates of human biting rate/person/night for the two malaria vectors (An. gambiae s.l and An. funestus) during the wet and dry seasons were 24 and 22, respectively. The biting rate for ***W. bancrofti*** vector ***Culex***

quinquefasciatus during the two seasons was 18 and 6, respectively. The DL and LLIN interventions are scheduled to begin in July 2015. The results of this study will provide important information to help guide vector control strategies within national malaria control programs.

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ADHERENCE TO NATIONAL GUIDELINES FOR THE DIAGNOSIS AND MANAGEMENT OF SEVERE MALARIA, MALAWI 2012

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Severe malaria has a case fatality rate of 10-20%. Given the complexity of evaluating multi-faceted components of health systems, few studies have addressed the quality of severe malaria case management. In July-August 2012, a nationwide, cross-sectional survey of severe malaria management was conducted in 36 health facilities (HFs) selected with equal probability from a list of all public sector HFs in Malawi that admit patients with severe malaria. Patient care records from all admissions during October 2011 (low season) and April 2012 (high season) with an admission diagnosis of malaria or prescription of an antimalarial were eligible. Up to 8 charts were randomly sampled within each age (<5 and ≥5 years) and month stratum. Severe malaria was defined by a) admission diagnosis or b) documentation of any signs of severe malaria. Treatment with at least one dose of an intravenous antimalarial was considered correct. A total of 906 records with complete data from 35 HFs were included; one HF had no patient records. Patients were 3 months to 81 years old; 55% were female. Overall, 387 patients (42%) had an admission diagnosis of severe malaria. A severe sign was documented in 464 records (51%). Patients <5 years were significantly more likely to be diagnosed with severe malaria than those ≥5 years either by admission diagnosis or documented severe sign; risk ratio (RR) 1.3 (95% confidence interval [CI], 1.1-1.6) and 1.6 (95% CI, 1.4-1.8), respectively. Correct treatment was more common for patients identified by admission diagnosis (79%) than by documented severe sign (68%) (p <0.001). Notably, 14% of severe malaria patients by admission diagnosis and 20% with a documented severe sign were treated exclusively with an oral antimalarial and 8% and 13%, respectively, did not receive any effective antimalarial, with no significant differences by age. Case management of severe malaria remains a challenge. Despite the high proportion of severe malaria patients receiving recommended treatment, it is concerning that 8-13% received no effective therapy. Challenges to adhering to national guidelines should be identified and addressed to improve quality of care.

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MALARIA AND ILLEGAL GOLD MINES IN A HIGH INCOME COUNTRY: FIRST DESCRIPTION OF THE DISEASE BURDEN

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Malaria is endemic on the Guiana Shield. In French Guiana, a French overseas territory, although the number of cases has decreased since 2005, ***foci*** of infection still remain, particularly within illegal gold mines. There, malaria patients often self-medicate, resulting in a risk of resistance to anti-malarial treatments, notably to artemisinine. The mobility of gold miners increases the risk of spreading both malaria and the resistance to antimalarials, and puts the population at risk of new outbreaks despite the great efforts put into anti-malarial policy in this region. This study aims to estimate and map the prevalence of ***Plasmodium*** carriers and to determine knowledge attitudes and practices concerning malaria in illegal gold miners. Inclusions were carried out from January to March 2015 (continued until June 2015) at the resting sites along the Maroni river. Illegal gold mine workers periodically go to these sites for rest, supplies, or medical care. People working on gold mining sites in French Guiana and being on the resting site for less than 7 days were included. A malaria-RDT, thick and thin blood smear, molecular diagnosis by PCR were performed. Persons also answered a questionnaire and had a medical examination. Informed consent was obtained. On the 03/01/2015, 128 persons were included of the 360 expected for the complete study duration. On PCR, 25 (19.5%) were positive for malaria, with 11 ***P. vivax*** (44%), 10 ***P. falciparum*** (40%), 3 mixed faciparum+vivax (12%) and 1 P. malariae (4%). Seventeen (65.4%) were asymptomatic. The last time they had malaria, 37% did a test, and 64% declared regularly using self-medication containing artemisinine. This first study about the epidemiology of malaria in illegal gold miners in French Guiana shows a very high prevalence of malaria along with a high proportion of asymptomatic carriers. Therefore control of malaria in French Guiana must take into account illegal gold miners, the main but hidden reservoir of malaria endemicity. These results should help French Health Agencies to implement adapted measures to deal with malaria in this population and hope to avoid the emergence of artemisinine resistance.

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THE RISE OF VECTOR RESISTANCE AND INSECTICIDE COSTS: AN ASSESSMENT OF INSECTICIDE CHANGE FOR INDOOR RESIDUAL SPRAYING AND MALARIA BURDEN IN ZIMBABWE

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Indoor residual spraying (IRS) has been implemented since the 1940s in Zimbabwe and has protected millions of people from malaria. However, long-term insecticide exposure leads to vector resistance. While entomological studies have shown increasing resistance to PYs in mosquitoes, the population-level effects of changing insecticides is not well understood. In November 2014 the President's Malaria Initiative-supported (PMI) Africa Indoor Residual Spraying (AIRS) project in Zimbabwe started using organophosphate-based insecticides (OPs) in

four high malaria burden districts (Chimanimani, Mutare, Mutasa, and Nyanga) in Manicaland Province. Previously, these areas were sprayed with pyrethroid-based insecticides (PYs). OPs are substantially more expensive than PYs; they cost approximately ten times more per area sprayed than PYs, according to a 2013 AIRS Costing Study. Thus, as programs expand and require more expensive insecticide, the number of beneficiaries an IRS campaign can protect is dependent on the type of insecticide used. With Health Management Information System (HMIS) and entomological data through transmission season (May-June 2015), we will: 1) compare the number of confirmed malaria cases in health facilities in four districts in 2011-2013 (under PYs) to the same districts in 2014-2015 (under OPs); and (2) compare the number of confirmed malaria cases in health facilities in the four OP districts in 2014-2015 to four comparison PY districts in the same time period. We will strengthen our analysis by comparing mosquito densities from our routine entomological monitoring before and after using OPs for IRS. Although not randomized-controlled, the analysis provides a relatively inexpensive method to suggest the most effective pesticide for reducing malaria burden in these districts, while considering rising insecticide costs and program budget limitations. Preliminary results are not available at the time of abstract submission as malaria season in the target districts continues through May-June 2015. We will present our methodology and the results of our analysis, if selected.

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SHOULD INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY BE MAINTAINED IN AREAS OF LOW TRANSMISSION: ANALYSIS OF BIRTHWEIGHT, INTERVENTION COVERAGE, AND EPIDEMIOLOGIC STRATUM IN SENEGAL

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Intermittent preventive treatment during pregnancy (IPTp) with at least two doses of sulfadoxine-pyrimethamine (SP) is recommended for prevention of malaria during pregnancy, along with use of insecticide treated nets (ITNs), to reduce low birthweight and miscarriages. In 2006, Senegal adopted IPTp with SP free of charge for pregnant women, along with training and point of delivery equipment for directly observed therapy. The prevalence of markers of resistance to SP is monitored. Introduction of IPTp coincided with the intensification of other malaria control interventions including ITNs and artemisinin-based combination therapy, and parasite prevalence among children under 5 years fell from 6% in 2008 to 1.2% in 2014, with near zero prevalence in the north. Many question the applicability of continuing IPTp in the north of Senegal. We analyzed data from Senegal's continuous Demographic and Health Survey from 2013 to examine the benefit of the coverage of two doses of SP on low birth weight, by epidemiologic stratum. We compared IPTp coverage and prevalence of low birthweight in the zones of low, moderate, and high transmission in Senegal to test the hypothesis that IPTp would have a decreased effect in zones of low transmission, given the Senegalese context of intense seasonality and epidemiologic stratification. Nationally, IPTp coverage is 41.3% and the rate of low birth weight (LBW) is 13.0%. The zone of moderate transmission has the lowest rate of LBW at 10.2%, while having the lowest coverage of IPTp (36%). The rate of LBW is similar in the low transmission zone (14.7%) and the high transmission zone (13.7%), despite the highest IPTp coverage in the low transmission zone (48%) compared to 40% in the high transmission zone. As there was no clear relationship between IPTp coverage and rate of low birthweight when stratified by epidemiologic strata, further analysis is ongoing to consider factors such as number of doses, use of LLINs, season, parity, nutrition, and socio-economic status. While national survey data may shed light on this question, a prospective study should be conducted to ascertain the contribution of IPTp in northern Senegal.

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TRACKING THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON MORBIDITY AND MORTALITY OF CHILDREN IN SENEGAL THROUGH THE ROUTINE HEALTH INFORMATION SYSTEM

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The World Health Organization recommends seasonal malaria chemoprevention (SMC), a monthly treatment course of anti-malarial, for up to four months during malaria transmission season for children 3-60 months of age, in regions with highly seasonal transmission. In 2014, the Senegal National Malaria Control Program (NMCP) implemented SMC in the four regions of Senegal that meet WHO criteria, with a target of 624,139 children 3 - 120 months, increasing the age range given data showing a displacement of burden of malaria toward older children. SMC campaigns were conducted in August, September, and October, with administrative coverage rates of 98.6% 97.9%, and 98.0%, respectively. We examined routine information collected from malaria sentinel sites, the public health system, and reference hospitals to determine the impact of SMC on morbidity and mortality. Twenty sentinel sites, four in regions targeted for SMC, report the weekly number of total consultations, suspected malaria cases, patients tested, and confirmed malaria cases. Morbidity data from the sentinel sites were examined during the period covered by the campaign (epi week 35-48) in 2013 (baseline) and 2014. The number of confirmed cases among children < 5 and among children 5-10 at the four sites decreased 50% and 60%, respectively, from 2013 to 2014. The proportion of confirmed malaria cases among children 0-120 months among all confirmed malaria cases declined from 39% in 2013 to 23% in 2014. The incidence of all confirmed malaria cases reported by all the public health facilities in the SMC regions decreased from 81/1000 in 2013 to 60/1000 in 2014, a 26% reduction. The number of hospitalized malaria cases among children < 5 years recorded at health facilities in the four regions decreased 50% (1,384 in 2013 to 688 in 2014). The number of deaths due to confirmed malaria among children < 5 years recorded at reference hospitals in the four regions decreased 61% (135 in 2013 to 53 in 2014). Data from sentinel sites confirmed data from the routine health system, and enabled understanding of burden reduction by age group. With these very encouraging results the NMCP will continue SMC in 2015 and 2016.

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MALARIA: BURDEN, PREVENTION, AND TREATMENT SEEKING PRACTICES AMONG NOMADIC PASTORALISTS IN SENEGAL

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Senegal has had remarkable declines in malaria in the last decade. In the north, annual incidence is now less than 5 confirmed cases per 1000 people. Senegal is home to nomadic pastoralists who travel south to high malaria transmission zones during dry season, returning north when the rains start. There was concern that the migration of pastoralists at the start of the rains might sustain malaria transmission in the north. Nomadic and migrant populations present a challenge for public health, as they

may be located in remote areas, have less access to prevention and care, suffer stigmatization, and be more at risk for disease. We conducted a modified snowball sampling survey of nomadic pastoralists at 6 sites in northern Senegal. We enrolled 1800 people 6 months and older, and collected data regarding demographics, access to care and preventive measures, and a blood sample for rapid diagnostic test (RDT), blood smear, and PCR. Of these, 31.5% were under 15 years, and 38.7% were female. Literacy among adults was 12%, and 16% had heard messages about malaria in the last three months, 94% of these by radio. Of the 55% who knew at least one preventive measure against malaria, 84% cited bednets. Though 64% had a net, only 29% had received a net from a mass distribution campaign, and 26% reported using a net every night. Of the 21% with a family member with fever in the last month, 23% sought care. The primary reason for not seeking care (26%) was distance. However, parasite prevalence by PCR was 0.5%, similar to the remainder of the north. Sensitivity and specificity of RDT compared to PCR was 100% and 99%, respectively. All of the molecular barcodes identified among the migrants were novel in Senegal, providing no evidence that these strains had originated in the south, though 96% had been in the district two weeks or less at the time of the survey. While nomadic pastoralists have poor access to prevention and care of malaria, parasite prevalence is low, and they do not appear to be a source of ongoing transmission. Efforts are being made to include them for insecticidal net distribution and to train health volunteers among them to provide diagnosis, treatment, and health messages.

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MEASURING THE PREVALENCE OF MALARIA PARASITEMIA USING LAMP IN A HIGH-TRANSMISSION COMMUNITY IN UGANDA

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The epidemiology of malaria parasitemia, particularly in older children, adults, and HIV-infected persons, is poorly understood. Knowledge gaps regarding parasitemia hinder elimination efforts in high transmission areas, where large segments of the population are asymptomatic carriers of malaria parasites, but retain the ability to transmit disease. We performed a cross-sectional analysis of community residents (n=10,875) in Nankoma, Uganda, in which we collected 9,629 dried blood spots (DBS), representing 89% sampling of the population. In order to describe population-level parasitemia, we tested a random subset of 4,000 HIV-negative samples and all 147 HIV-positive samples for malaria parasitemia using loop mediated isothermal amplification (LAMP). We estimated population-level prevalence using a weighted proportion, and fit a multivariate logistic regression model to identify independent predictors of malaria parasitemia. We found an overall prevalence of parasitemia in the community of 82.1% (95% CI, 81.3 - 83.0%). Age-specific prevalence increased steadily among children until age 9, peaking at 92.3% in children under 10 years old, then decreased until age 35, with a prevalence of 61.3% in those older than 35 years. When controlling for age, we found male sex to be independently associated with malaria parasitemia (aOR =1.27, 95% CI, 1.05 - 1.53, p=0.01), while self-reported insecticide-treated bed net (ITN) use the prior evening was not. HIV-infected individuals had a lower odds of parasitemia when compared to uninfected persons (aOR=0.09, 95% CI 0.06-0.15, p=<0.01), likely representing this group's use of trimethoprim-sulfamethoxazole prophylaxis. Among HIV-infected adults, a CD4 count of less than 200 was associated with parasitemia (aOR = 7.00, 95% CI 1.18 - 41.39, p=0.03), while antiretroviral use was not. These data show extremely high levels of parasitemia at all ages in a highly endemic community in Uganda, including an overall prevalence of 82.1% and a greater than 60% prevalence in adults. Successful malaria elimination efforts need to address high-level parasitemia across all age strata.

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EPIDEMIOLOGY OF MALARIA IN NON-AMAZONIAN COLOMBIAN REGIONS: IMPORTANCE OF ASYMPTOMATIC SUBJECTS AS TRANSMISSION *FOCI*

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Despite major progress towards Malaria Control in Colombia and Latin America, it still remains as an important public health problem. However, control and elimination programs still rely on methods incapable of detecting most of asymptomatic subjects, which endure untreated as potential reservoirs for transmission. Here we describe the epidemiology of malaria in non-Amazonian Colombian endemic regions throughout a three year follow-up, and discuss the importance of asymptomatic subjects and their persistence for malaria elimination. We conducted a series of cross-sectional surveys in eleven sentinel sites (SS) of Buenaventura, Tierralta and Tumaco, Colombia in 2011, 2013 and 2014, in which a census was taken and a random sample of houses drawn from each SS. People from the selected houses were asked to complete a questionnaire about clinical, epidemiological and demographic information and provide a blood sample for diagnosis of malaria by thick blood smear microscopy (TBS) and real time polymerase chain reaction (qPCR). Additionally, cases were georeferenced. A total of 3,046 samples were taken from all the SS whereof 58% were women. A total prevalence of 9.7, 7.3 and 3.5% was found in 2011, 2013 and 2014 respectively by qPCR. Only 2% of the cases detected by qPCR were detectable by TBS and 73% of all infected subjects were asymptomatic. Whereas Buenaventura and Tierralta presented a consistent prevalence decrease along these years, Tumaco had a rise of malaria cases in 2013 but then decreased in 2014. ***P. vivax*** accounted for the majority of cases in Tierralta and Buenaventura but only 33-50% of the cases in Tumaco. During two consecutive cross-sectional surveys conducted, two people remained positive and asymptomatic for malaria. In this study we found an important prevalence of malaria in endemic regions considered to be of low and moderate transmission. The fact that only 2% of the cases were detectable by TBS, which is the most widely used diagnostic method by National Malaria Control Programs, highlights the importance of considering the introduction of molecular methods for the diagnosis of malaria as a public health tool.

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PREVALENCE AND FACTORS ASSOCIATED WITH MALARIA IN PREGNANCY IN RURAL RWANDAN HEALTH FACILITIES: A CROSS-SECTIONAL STUDY

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Malaria in pregnancy (MIP) is a serious health risk for the pregnant woman and fetus and associated with mortality in the perinatal period. In Rwanda there has been no accurate national estimate of malaria prevalence among pregnant women. In 2011, a cross-sectional study of 6 districts in 3 malaria transmission zones (low, medium and high) in Rwanda was conducted to estimate the prevalence of peripheral parasitemia in pregnant women. Data were collected from consenting women presenting to antenatal clinics (ANC) for the first time in their current pregnancy including age, parity, gestation, ITN availability and use. Blood was obtained for malaria testing using microscopy, rapid diagnosis tests and polymerase chain reaction (PCR). A total of 4,037 pregnant women were recruited with median age of 27 years, and 3,781 (93.7%) had usable PCR samples. The prevalence of MIP by PCR was 5.6%. Nearly 20% of women's families did not have a net, and 8.7% of these tested positive compared to 4.9% of women whose family owned an ITN. For those who did not sleep under an ITN the previous night, 8.1% tested positive compared with 4.8% who slept under an ITN. Malaria prevalence by parity ranged from 5.5% (parity 0-1), to 5.4% (parity 2-3), and 6.5% (parity 4 or more). The two districts that bordered highly endemic countries had MIP prevalence rates of 10% and above. Those testing positive were treated according to national guidelines. Despite a significant decline of 86% in malaria prevalence in the general population from 2005 to 2011, MIP prevalence remains high, especially in border districts. Our study also showed that ITN ownership and use among these pregnant women is below the national target. In order to address this gap, ITN distribution to achieve universal access, and educational campaign targeted at pregnant women on the use of ITN are recommended. Furthermore, early detection and treatment of MIP at ANC and regional collaboration to reduce cross-border malaria transmission should be prioritized.

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UNUSUAL HIGH GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* POPULATION IN BANGLADESH

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More than 90% of malaria cases of Bangladesh are caused by ***Plasmodium falciparum***. Despite the recommendation for the use of msp1, msp2, and glurp as markers in drug efficacy studies by WHO and their limited use in Bangladesh, still the circulating ***P. falciparum*** population genetic structure has not been assessed systematically in the country. The present study is the first comprehensive report of the circulating ***P. falciparum*** population structure based on msp1, msp2, and glurp in seven most malaria endemic districts out of thirteen malaria endemic districts of Bangladesh. Among the 130 pretreatment ***P. falciparum*** field isolates, 14, 20, and 13 distinct genotypes were observed for msp1, msp2, and glurp, respectively. We found 94.62% polyclonal infections, which is almost similar to some holoendmic areas of Sub-saharan Africa. The heterozygosity for msp1, msp2, and glurp was 0.89, 0.93, and 0.83, respectively. These MOI's fall within the range of MOI's reported in hypoendemic areas of Southeast Asia. Even though Bangladesh is a malaria hypoendemic country, the prevalence of polyclonal infection and the genetic diversity in ***P. falciparum*** do not represent hypoendemicity.

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POPULATION STRUCTURE: THE PHYLO-DYNAMICS OF *PLASMODIUM FALCIPARUM* IN PAPUA NEW GUINEA

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Papua New Guinea (PNG) has the highest burden of malaria outside of Africa with intense year round transmission ranging from hyper- to holo-endemic in the lowland and coastal areas to largely absent in the highlands. The country's extremely diverse biogeography contributes to variable parasite population dynamics and transmission, even across the highly endemic areas. Recently, we have shown that the population structure of ***Plasmodium falciparum*** on the north coast of Papua New Guinea (PNG) is fragmented. Should such population fragmentation and structure be observed throughout PNG then mapping of this geographical diversity will enable the monitoring of changes in populations, the identification of routes of migration, predict the spread of drug resistance, and pinpoint the source of outbreaks. Such knowledge will be valuable for the success of malaria elimination and control programmes as it will enable informed choices to be made. Using microsatellite markers, mitochondrial sequences, and genome wide SNP data, from three geographically distinct populations within PNG, we are investigating ***P. falciparum***'s genome dynamic nature, and demographic structure. Exploring fundamental biological questions regarding the genetic history. In addition, we are working towards defining a high-resolution map of parasite population networks and migration patterns throughout PNG. Using state-of-the-art Fluidigm Integrated Fluidic Circuit SNP genotyping, a panel of geographically informative single nucleotide polymorphisms (SNPs), and a national cross-sectional ***P. falciparum*** dataset of isolates covering all endemic areas of PNG. These results will have direct translational benefits by identifying isolated populations that may be targeted for elimination, and will provide a database of genotypes to map the origins of imported infections and outbreaks in areas where malaria is normally absent.

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A -1447 A>G POLYMORPHISM IN THE HUMAN CXCL10 GENE PROMOTER IS ASSOCIATED WITH CHILDHOOD MALARIA

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Background The risk factors for severity of malaria pathogenesis and the wide variation in clinical manifestations of malaria are poorly understood. Recent studies indicate that interferon gamma inducible chemokine, CXCL10, is a predictor of both human and experimental cerebral malaria severity. In addition, polymorphisms in the CXCL10 gene promoter has

been associated with increased CXCL10 production, which is linked to severity of malaria in Indian malaria patients. In the present study, we hypothesized that in a subset of Ghanaian malaria patients, susceptibility to malaria is associated with different variants of the CXCL10 gene. Method: We assessed basic demographics that may impact our assessment including age, gender, hemoglobin levels, sickle cell status CXCL10 plasma levels and CXCL10 polymorphism. We determined whether polymorphisms in the CXCL10 gene are associated with the clinical status of malaria patients. We tested several known polymorphisms and identified one reported single nucleotide polymorphism in the CXCL10 promoter (-1447A>G [rs4508917]) and compared 382 malaria and 115 non malaria cases using PCR-restriction fragment length polymorphism method. Results: The median age for malaria patients was 4 years and that for non-malaria patients was 13 years. There was significant difference with regards to hemoglobin, hematocrit, wbcs level, CXCL10 plasma levels between malaria patients and non-malaria patients, p=0.001. The -1447A>G genotype of the CXCL10 gene was significantly associated with malaria (adjusted odds ratio =2.55, 95% CI=1.13-5.74, p=0.024).). In addition, individuals with the 21447(A/G) genotype had significantly higher plasma CXCL10 levels than individuals with the 21447(A/A) genotype. Stratifying patients according to gender, the observed association of malaria with over expression of CXCL10 were more pronounced in females than in male patients (AOR = 5.47, 95% CI = 1.34-22.29, p = 0.018. Conclusion: These results suggest that the -1447A>G polymorphism in CXCL10 gene promoter could be partly responsible for malaria outcomes in Ghanaian malaria children.

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SURVEILLANCE OF MOLECULAR MARKER OF ANTIMALARIAL DRUG RESISTANCE IN SENEGAL BY USING MALARIA RAPID DIAGNOSTIC TEST (RDTS)

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In Senegal, strategies such as Intermittent Preventive Treatment in pregnancy (IPTp) (SP) and Seasonal Malaria Chemoprevention (SMC) using sulfadoxine-pyrimethamine (SP) and SP plus amodiaquine, respectively have been implemented while artemisinin-based combination therapies are used to treat uncomplicated malaria. These strategies have largely contributed to the decrease of malaria morbidity and mortality in the country. However, the successful control of malaria is highly dependent on continued effectiveness of these drugs which may be compromised by the spread of drug resistance. Therefore surveillance of drug resistance in the malaria parasites is essential. The objective of this study was to test the feasibility of routinely sampled malaria rapid diagnostic tests (RDTs) at a national scale to assess the temporal changes in the molecular profiles of antimalarial drug resistance markers of ***P. falciparum*** parasites. A low-cost sampling procedure of RDTs was established at 14 malaria sentinel sites across the country. Overall 4339 RDT positives were collected during 2014 out of which, a subset of 700 RDTs (50 RDTs per site) was randomly selected for initial SNPs analysis of the Pfcrt gene by PCR-SSOP ELISA methodology. Among the 700 selected and extracted RDTs, 598 (85.4%) was confirmed ***P. falciparum*** positive by Pfcrt PCR. The prevalence of the Pfcrt wild type CVMNK haplotype was above 75% in the North-eastern regions including Dakar while a lower prevalence at 65% and 56% was observed in the Central and South regions, respectively. In conclusion, this study showed that routine sampled positive RDTs can be successfully amplified by PCR and used for routine surveillance of antimalarial drug resistance. Further sampling of RDTs and analysis of other markers of drug resistance (e.g. Pfmdr, Pfdhfr, Pfdhps, K13) are ongoing which will provide temporal trends of these markers and potentially aid drug policy makers in timely decisions regarding choice of antimalarial drugs.

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TRANSCRIPTION PROFILING OF MALARIA-NAÏVE AND SEMI-IMMUNE COLOMBIAN VOLUNTEERS IN A *PLASMODIUM VIVAX* SPOROZOITE CHALLENGE

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In endemic regions the continued exposure to the malaria parasite induces significant levels of clinical immunity in individuals who develop lower morbidity and almost no mortality. This immunity is very complex and slow processes, but little is known about the immunological response to ***Plasmodium vivax*** in early infections and how the immune system may be boosted during vaccination. This study hypothesizes that the partial clinical immunity observed in semi-immune volunteers from Buenaventura (high prevalence) compared to naïve individuals from Cali (no transmission) is associated with altered peripheral blood gene expression. To explore the difference in gene expression between semi-immune and naïve individuals experimentally exposed to viable ***P. vivax*** sporozoites, we describe a transcript profile analysis in these volunteers using nanofluidic Fluidigm quantitative RT-PCR arrays and RNASeq. Previous malaria experience has a relatively minor variation effect, although it does separate the two clusters in the overall profiles of expression among the samples. There is little evidence for transcriptional changes prior to the appearance of blood stage parasite at diagnosis day (day 12 or 13). At the parasitemia onset, there is a strong interferon response reflected in up-regulation of co-regulated transcripts, while unexpectedly we also see down-regulation of transcripts related to TLR signaling and innate immunity. This differential expression was confirmed with the RNASeq, which also suggested differential expression of reticulocytes and a subset of T cell function. No obvious difference in the transcriptomes of naïve and semi-immune volunteers was seen, however several hundred genes were up-regulated in naïve individuals. Interaction analysis showed 175 genes with a significant Time-by-Population effect at $p<0.05$, most of these genes are more strongly up- or down-regulated in the naïve individuals. This study shows that gene expression is strong in naïve volunteers in comparison to semi-immune at the time of diagnosis. Gene expression of lymphocytes can thus be used to establish how semi-immune exposure modifies their activation.

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CHARACTERIZATION OF CIRCUMSPOROZOITE PROTEIN VARIANTS (VK210, VK247 AND *PLASMODIUM VIVAX*-LIKE) IN *P. VIVAX* ISOLATES WITH DIFFERENT PROFILES OF SENSITIVITY TO ANTIMALARIAL DRUGS

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The ***Plasmodium vivax*** Circumsporozoite Protein (CSP) is the most abundant polypeptide present in the sporozoite covering. Based on csp gene, two variants, VK247 and ***P. vivax***-like, have been described that differ from the classical form (VK210) by sequence variations in the central region of the gene. The distribution of these variants seems to be universal and an important issue is the possibility of differential response to treatment is depending on the genotype of the parasite. Here, we characterized the CSP variants circulating in Brazilian malaria-endemic area and associated the presence of these variants with different profiles of sensitivity to chloroquine and mefloquine. The ***P. vivax*** isolates were

collected in Manaus, Amazonas. The determination of CSP variants was performed by PCR-RFLP or PCR-sequencing and sensitivity to chloroquine and mefloquine was determined by the colorimetric DELI-test. VK210 was the most prevalent, detected in 92% of samples while VK247 variant was observed in 14.7% of samples. Single infection with VK210 was observed in 85.2% of samples, single infection with variant VK247 was observed in 7.9% of the samples and mixed infection (VK210 + VK247) was observed in 6.8% of samples. The mean IC_{50} in the presence of chloroquine were 61nM, 25nM and 21nM for VK210, VK247 and VK210+VK247, respectively. The mean IC50 in the presence of mefloquine were 24nM, 32nM and 10nM for VK210, VK247 and VK210 + VK247, respectively. No association was derived in the resistance or susceptibility profile or in the IC_{50} values in presence of chloroquine or mefloquine determined by DELI-test and the presence of the CS variants of ***P. vivax***. Conclusions: the classical form VK210 is more prevalent in the studied area and that the presence of VK210 appears not to be associated with susceptibility or resistance to chloroquine and mefloquine in the studied area.

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DECREASE OF HEMOGLOBIN LEVELS IN AFRICAN CHILDREN WITH SEVERE MALARIA ASSOCIATED WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY RATHER THAN HEMOGLOBINOPATHIES

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Red blood cell polymorphisms, including glucose-6-phosphate dehydrogenase (G6PD) deficiency and hemoglobinopathies confer relative protection from severe malaria. However, their true impact on clinical parameters during severe malaria remains poorly understood. In this study we determined the frequencies of accountable genetic variants and investigated their effects on baseline parasitemia and hemoglobin levels in African children with severe malaria. A total of 278 children aged 6 to 120 months with severe malaria were enrolled into this study. G6PD deficiency (G202A and A376G) and hemoglobin variants (HbC and HbS) were determined by direct sequencing of the genome regions harboring the variants. The overall prevalence of G6PD deficiency (G6PD*A-) was 12.9%; 14.4% of children were female heterozygous (G6PD*A), while 72.7% were G6PD normal (G6PD*B). 87.9%, 3.6% and 8.2% of children had the HbAA, HbAC and HbAS genotypes, respectively. Only one female child was homozygous for HbSS. HbCC and HbSC variants were absent. Using a multivariate regression analysis, G6PD variants were associated with decreased hemoglobin concentrations of 2.7 g/dL in G6PD heterozygous (P<0.0001) and 1 g/dL in G6PD deficient (P=0.009) children. However, there was no effect on adjusted log mean parasite densities (P=0.287). We found a significant association between the hemoglobin variants and the mean temperature (P=0.015). In fact, on admission, HbAS children had a higher temperature (37.8 ± 1.2°C), while lower temperatures (15.3 ± 4.4°C) were found in HbAC individuals. There was no effect of hemoglobin variants on parasitemia and hemoglobin levels. In addition, G6PD and hemoglobin variants did not have any association with severe malaria anemia. G6PD polymorphisms contribute to the reduction of hemoglobin levels in African children with severe malaria without leading to severe malarial anemia. This study confers further knowledge to help understand the effect of G6PD deficiency and hemoglobinopathies during malaria infection in endemic countries.

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PLASMODIUM FALCIPARUM ATOVAQUONE SUSCEPTIBILITY REMAINS INTACT IN NORTHERN CAMBODIA BASED ON *EX VIVO* THE MOLECULAR EVIDENCE

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Multidrug resistant ***P. falciparum*** (MDR Pf) malaria is a critical issue in Cambodia, with multiple artemisinin combination therapies losing their effectiveness in recent years. Atovaquone, in combination with proguanil (MalaroneTM) has been used in limited areas of Cambodia to treat MDR Pf. In a recent trial showing clinical failure of DHA-piperaquine, we evaluated ***in vitro*** atovaquone susceptibility and looked for point mutations in the Pf cytochrome bc1 gene, known to confer atovaquone resistance. Parasites from the last 62 of 101 adult patients treated with DHA-piperaquine in Northern Cambodia were genotyped for mutations at codon 268 in the cytochrome bc1 gene. A high resolution melting RT-PCR assay (HRM-RTPCR) offering faster turn-around times was compared to conventional DNA sequencing. ***In vitro*** drug sensitivity was assayed using an HRP-2 assay with W2 and C2B ***P. falciparum*** reference clones used as susceptible and resistant controls, respectively. The geometric mean atovaquone IC50 was 5.0nM for the sensitive W2 clone, 6.6 nM for 62 Cambodian isolates (no difference from W2) and 11,000 nM for the resistant C2B reference clone. None of the 62 isolates had cytb codon 268 mutations by either HRM-RTPCR or DNA sequencing. Despite recent ACT failures in the area, there does not appear to be significant atovaquone-proguanil resistance at this time. Although use as a first line agent is not recommended, atovaquone-proguanil remains a safe and effective alternative therapy for MDR Pf in northern Cambodia, and may serve as a stop-gap measure until more effective therapies are developed.

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ASSOCIATION BETWEEN GENETIC POLYMORPHIMS OF TCR RECEPTOR THE MALARIA VIVAX IN BRAZIL

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In Brazil, the ***Plasmodium vivax*** has been the most prevalent species, accounting for approximately 88% of malaria cases in the Brazilian Amazon region. Polymorphism in genes of molecules involved in immune response may influence the response and consequently the establishment of the parasite. The polymorphism TCRBV3S1 (C/T) has been widely investigated because it seems to significantly affect the repertoire of T cell receptors for modifying the development capacity of an imune response. The aim of this study was to identify the polymorphism, estimate the allele and genotype frequencies and associate these polymorphisms with parasitaemia of the individual.We analyzed 83 blood samples from patients from Goianesia, Para State, Brazil, with vivax malaria diagnosed by molecular biology and thick drop test. DNA samples were amplified by PCR and the resulting amplification fragments were 431bp, subsequently, were digested with the Pvu. Homozygous individuals were identified by the

presence of a single fragment of 431 bp. However, mutants homozygotes for the other allele were identified by the presence of a fragment of 352 bp and the heterozygotes by the presence of two fragments of 352 and 431 bp. All statistical analysis was performed using the R program v 2.11.1 (http://www.r-project.org). Differences in median parasitaemia in relation to genotypes were evaluated using the nonparametric Mann-Whitney test. P values < 0.05 were considered significant. The results for the polymorphism in TCRBV3S1 demonstrate that the most frequente genotype was the TC (45.8%) and the most frequent allele was C (82.5%). Polymorphism tested were in Hard-Weinberg Equilibrium. The parasitaemia ranged from 15 to 70,000 with a mediam of 1,500 parasites per microliter of blood. There was no difference in parasitaemia in relation to TCRBV3S1 genotypes (p = 0.19). No significant association was found between the polymorphisms tested and vivax malaria. The results suggest that genetic variant analyzed in gene segment of the TCR do not affect the functionality of the molecules so that it can interfere with parasitaemia of malaria caused by ***P. vivax***.

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GENOME SEQUENCING OF *PLASMODIUM OVALE WALLIKERI*

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Plasmodium ovale curtisi and ***P. ovale wallikeri*** are distinct malaria parasites both known to cause liver relapse as seen with ***P. vivax***. This ability to reside in the liver as hypnozoites provides a reservoir for continuous transmission of the parasite which is a threat to malaria control. Although ***P. ovale*** spp often occur in very low parasitaemias and as mixed infections with ***P. falciparum***, they contribute to the malaria burden. Recent evidence suggests that these two ovale parasites are very closely related yet genetically distinct. There is also a clear phenotypic difference between them related to the duration of pre-erythrocytic latency, and as such represents an ideal system in which to identify candidate genes linked to hypnozoisis. We hypothesized that if genome data were available for both both ovale parasite species, new insights into the species barrier between them could be gained using a comparative genomic approach. We attempted the first genome sequence of a ***P. ovale wallikeri*** isolate, derived from an imported case of ovale malaria in the UK, using an Illumina Miseq platform in-house at LSHTM. We generated the first ever partial genome sequence for ***P. ovale wallikeri***, which we will compare with partial published data for ***P. ovale curtisi***. The multicopy extra-chromosomal genomes of the apicoplast and mitochondrion were particularly well represented. These were compared against genome sequence data from other ***Plasmodium*** species, including ***P. falciparum***, ***P. knowlesi*** and ***P. vivax***.

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ASSOCIATIONS OF ANTIBODY RESPONSES THE PROTECTION FROM CLINICAL MALARIA IN A HIGHLAND KENYA AREA OF LOW TRANSMISSION

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Establishing markers of protection in low transmission settings is important in determining how to assess risk in these less-immune populations. It is inherently difficult to predict correlates of protection in low transmission settings due to the limited number of cases, but our longitudinal prospective study allowed for this testing of antibody responses to multiple antigens over a time period of 6 years. We performed a nested case-control study with 3 controls matched to every case on village and age. Plasma samples from a blood collection that targeted the entire cohort performed from April-June 2007 were used to test antibody responses to 11 antigens (10 using Multiplex assay, 1 using enzyme-linked immunosorbant assay). Cases were identified as having a measured fever or reported fever or headache in the presence of ***Plasmodium falciparum*** malaria detected by microscopy from the Ministry of Health dispensary-based surveillance performed from June 2007-June 2013. Controls were followed for the same time period as cases without detection of clinical malaria. Conditional logistic regression performed on laboratory results of 620 plasma samples (155 cases, 465 controls) identified 2 antigens where a positive antibody response, defined as >= 1 arbitrary unit, was statistically significantly associated with protection from clinical malaria over a 6 year period in a highland Kenya area with unstable malaria transmission. Specifically, subjects whom elicit a positive response to GLURP-R2 have a 42% decrease in odds of developing clinical malaria compared to subjects who do not elicit a positive response over this time period (OR = 0.58, 95% CI: 0.37, 0.90, p=0.016). Similarly, subjects whom elicit a positive response to LSA-NRC have a 42% decrease in odds of developing clinical malaria (OR= 0.58, 95% CI: 0.38, 0.89, p=0.013). Identifying markers of protection in less-immune individuals are important for future vaccine development as malaria control efforts continue to increase, transmission will continue to decrease, leaving many populations living in transmission settings similar to our highland cohort.

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MEMORY CD4 T CELL ACTIVATION IN CHRONIC MALARIA INFECTION

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Development of long-lived memory T cells is the cellular basis for vaccination. However, the potential for survival of protective effector memory T cells (Tem) in malaria is not well understood. It is well-documented that activated effector cells (Teff) increase glycolysis for proliferation while quiescent memory T cells predominantly rely on fatty acid oxidation for energy generation during homeostatic proliferation. However, Tem don't do homeostatic proliferation and the mechanisms allowing Tem to survive to the memory phase, and yet maintain their protective effector functions, is unknown. To understand the metabolic status regulating survival in Tem during chronic malaria infection, we compared gene expression profiles of ***Plasmodium chabaudi*** MSP-1-specific B5 TCR transgenic Teff and Tmem. Strikingly, we observed up-regulation of the genes involved in lipid biosynthesis, without concomitant upregulation of fatty acid oxidation, in Tem compared to Teff. These data were supported by high lipid content in Tem compared to Teff using Bodipy 409/502 staining by flow cytometry, and by electron microscopy, which nevertheless showed lipid droplets next to active mitochondria. However, the increase in fatty acid synthesis here was not coupled with an increase in mitochondria, suggesting a novel function of fatty acid in effector memory cells other than fatty acid oxidation. To assess whether fatty acid biosynthesis is required for Tem generation or maintenance in chronic malaria infection, we analyze activation and memory cell formation in malaria-specific T cells throughout infection in animals treated with drugs that inhibit fatty acid biosynthesis. Blocking fatty acid synthesis pathway reduces memory T cell survival. These findings will help to understand the metabolic pathways that control generation and maintenance of protective Tem during chronic malaria infection and may suggest new generation metabolic adjuvants for malaria vaccine development.

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IMPACT OF SEASONAL MALARIA CHEMOPREVENTION IN THE PRODUCTION OF MSP1 THE AMA1 ANTIBODIES IN SENEGAL

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Malaria remains a major disease in many African countries, caused an estimated 243 million cases of clinical malaria and 863 thousand deaths globally 2008. The acquisition of immunity to clinical malaria is usually acquired the first five years of life depending on the intensity of malaria transmission. Nowadays, many strategies such as IPTic /SP are used for prevention in children. Intermittent Preventive Treatment for children (IPTc) against ***Plasmodium falciparum*** malaria is administered at defined intervals curative doses independently of the presence of parasites or symptoms. IPTc could however delay the acquisition of the antibodies which are managed against the malaria to this group of children. In this optics we want to understand the impact of this strategy in kinetics of specific antibodies against malaria on the acquisition of antibody in children living in zone of unstable transmission. This study measure the kinetics of antibodies MSP-119 and AMA-1 by ELISA, which are recombinantes proteins specifically managed against the membrane of ***Plasmodium falciparum***. To evaluate the impact of IPTic /SP on antigenic variation in rural areas of three districts, all children aged 11mths-10years, in Senegal. Our results show that young children under 5 years are the ones who produce most antibodies and this production increases significantly with age (p=0, 0001). Production of AMA-1 antibody is more important (27, 09 %) than MSP-119 antibody (13, 69 %). Control zone produce more antibodies than intervention zone, the PSP is a factor which can modifies the production of antibody. Seroepidemiology can provide key information on malaria transmission for control programmes, when parasite rates are low.

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LONGITUDINAL CHANGES IN ΓΔ T CELL POPULATIONS FOLLOWING ACUTE MALARIA INFECTION AMONG CHILDREN IN A MALARIA ENDEMIC REGION

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Immune dysregulation caused by ***Plasmodium falciparum*** (Pf) infection may impair the control of malaria infection, but may on the other hand contribute to the clinical immunity that is observed to develop with increasing age. Although others have shown robust expansion of the malaria-responsive Vδ2 subset of γδ T cells during acute infection in previously naïve individuals, we have shown that heavy malaria exposure is associated with loss and dysfunction of these cells, and that this process is associated with tolerance to subsequent Pf infection. Our aim was to investigate the impact of age on changes in the absolute numbers of peripheral blood γδ T cells following an acute malaria episode in Tororo, Uganda, a setting of high malaria endemicity. Children in 3 age groups (1-3yrs, 4-6yrs, 7-10yrs) who presented with acute febrile malaria were enrolled from a larger cohort study, and a blood sample was drawn at enrollment. Participants were treated for malaria and follow-up blood draws were performed at 3, 6, and 9 weeks. Cells were stained with antibodies to CD3, CD4, Vδ2, and Vγ9, and acquired on a BD Accuri flow cytometer. To date, 41 children have been enrolled in this study (1-<4 yrs, n=12; 4-<7 yrs, n=15; 7-10 yrs, n=14). Overall, we observed a significant increase in the absolute count of Vδ2 (p=0.001), Vγ9 (p<0.001) and Vδ2+Vγ9+ T cells (p=0.004) between the acute malaria episode and at 3 weeks follow-up. However, we observed significantly greater induction of Vδ2+ T cells among children aged 1-<4 compared with children 7-10 years of age (P<0.001 utilizing repeated measures generalized estimating equations controlling for age and Day 0 parasite density.) This suggests that Vδ2 T cells significantly expand in the peripheral circulation following acute malaria infection, but there are age-associated differences in this expansion in heavily exposed children. These data are consistent with the hypothesis that alterations in Vδ2 T cell function may play a role in the development of clinical immunity to malaria.

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MECHANISMS UNDERLYING THE INDUCTION OF IL-27-PRODUCING CD4⁺ T CELLS DURING IMMUNE RESPONSES AGAINST INTRACELLULAR PATHOGENS

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CD4⁺ T cells play critical roles in protection against blood-stage malaria. During malaria infection, there is a coordinated upregulation of the integrins CD11a and CD49d. The upregulation these integrins can be used as surrogate markers to directly identify ***Plasmodium***-specific CD4⁺ T cells responding to blood stage infection. We have reported that during ***Plasmodium berghei*** ANKA (PbA) infection, a subpopulation of malaria-specific CD4⁺ T cells produce IL-27, a heterodimeric regulatory cytokine of the IL-12 family composed of p28 and EBI3. These IL-27-producing CD4⁺ T cells, which we have designated as Tr27 cells, inhibit IL-2 production as well as clonal expansion of effector CD4⁺ T cells, and express the T cell inhibitory receptors LAG-3 and PD-1. In this study, we investigated the conditions under which Tr27 cells are induced. First, we infected C57BL/6 mice with various malaria strains such as ***P. yoelii***, ***P. chabaudi***, ***P. vinckei***, as well as ***Listeria monocytogenes***. Specific CD4⁺ T cells responses during infection were investigated using $CD11a^{hi}CD49d^{hi}$ as markers for specific T cells by flow cytometry. Specific CD4⁺ T cells from all the ***Plasmodium***-infected mice expressed the inhibitory receptors LAG-3 and PD-1, whereas those from ***Listeria***-infected mice did not. ELISA results revealed that IL27p28 was produced by CD4⁺ T cells from ***Plasmodium***-infected mice but not by CD4⁺ T cells from ***Listeria***-infected mice, suggesting that Tr27 cells are induced in only ***Plasmodium*** species. MyD88 and TRIF are critical adaptor molecules in toll-like receptor (TLR) signalling. So we examined whether TLR signaling is critical for the induction of Tr27 cells. CD4⁺ T cells from both MyD88 KO and TRIF KO mice infected with PbA produced IL-27. Finally, we examined the involvement of LAG-3 and PD-1 signalling in induction of Tr27 cells. CD4⁺ T cells from mice treated with anti-PDL-1 and anti-LAG-3 mAbs produced IL-27p28 at levels lower than untreated mice during infection with PbA. Taken together, our results suggest that Tr27 cells develop during infection with ***Plasmodium*** species in a manner independent of TLR signalling but possibly dependent on LAG-3/PD-1 signalling.

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THE EFFECT OF *IN UTERO* EXPOSURE THE *PLASMODIUM FALCIPARUM* MALARIA ON CORD BLOOD T REGULATORY CELLS THE CYTOKINES IN KENYAN INFANTS

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It is well documented that infants born to mothers with placental malaria are more susceptible to ***Plasmodium falciparum*** malaria infections, other infections during infancy as well as impaired immune responses to vaccines. Immunologic differences are also evident in children born to mothers with placental malaria such as increased frequency of regulatory CD4+ T-cells (Tregs) in cord blood and alterations in cord blood cytokine levels. However, these previous studies evaluated malaria exposure based on placental malaria infection at parturition. In this study, we asked whether malaria exposure during pregnancy altered the cord blood cellular immune profiles. Clinical and parasitological data were collected from the expectant women during all their ANC visits and at delivery to determine their malaria histories during gestation. Immediately after delivery, cord blood was collected and processed immediately. CBMC were stained using a panel of monoclonal antibodies to identify ***ex vivo*** Treg cells by flow cytometry. The concentrations of circulating pro-inflammatory and anti-inflammatory cytokines and chemokines were evaluated in cord blood plasma by Luminex bead based assay. The frequency of CD4+ Treg cells was increased in cord blood of malaria exposed infants compared to unexposed infants. These Treg cells mainly expressed the naïve phenotype (CD45RA+). No difference in the frequency of CD4+ Treg cells in cord blood of infants who were exposed to malaria either early or late in gestation was observed. Cord blood from Kenyan infants had increased levels of pro-inflammatory cytokines TNF-α, IL-8, IL-2R and chemokines RANTES and MIP-1β compared to North American cord blood. Exposure to malaria ***in utero*** also results in the expansion of CD4+Treg cells in cord blood and may contribute to the increased susceptibility to infections as observed in children born to mothers with placental malaria.

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IMPACT OF *IN UTERO* EXPOSURE THE MALARIA ON VΔ2 T CELL RESPONSES

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Placental malaria remains a major cause of maternal and child morbidity in sub-Saharan Africa, and there is evidence that ***in utero*** exposure to malarial antigens leads to increased susceptibility to malaria in early childhood. The Vδ2 subset of $\gamma\delta$ T cells possess intrinsic reactivity to malaria antigens and may represent an important anti-malarial effector mechanism, but we have shown that repeated exposure to malaria in early childhood is associated with loss and dysfunction of this subset. Our aim was to investigate the impact of ***in utero*** exposure to malaria on Vδ2 T cells at birth, leveraging samples from a recently initiated double-blind placebo-controlled trial of antimalarial chemoprevention during pregnancy in Tororo, Uganda, a high endemicity setting. 300 pregnant women were enrolled at 12-20 weeks gestation and randomized to 1) 3-dose sulfadoxine-pyrimethamine (SP) (standard of care, given at 20, 28, and 36 gestational weeks); 2) 3-dose dihydroartemisinin-piperaquine (DP). 3) Monthly DP. Upon delivery, maternal peripheral blood, placental blood, and placental tissue were obtained to classify current or prior ***in utero*** exposure, and absolute counts of cord blood Vδ2 T cells were enumerated using flow cytometry. To date, 143 women have delivered; all women will have delivered by May 2015. Of 138 deliveries after 28 weeks gestation, 5.8% had either a positive placental (n=7) and/or maternal peripheral blood smear (n=6), with placental histopathologic diagnosis currently being performed. Compared to children born to mothers without a positive peripheral or placental blood smear at the time of delivery, malaria-exposed infants had significantly higher absolute counts of CD3+ Vδ2 cells in cord blood (21.69 vs 78.32 Vδ2 cells/μl, $p<0.001$) at birth. This suggests that ***in utero*** exposure to malaria may lead to expansion of Vδ2 T cells at birth. Results will be updated upon completion of the trial and will include chemoprevention assignments and histopathologic diagnoses.

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ANTIBODY RESPONSES THE *PLASMODIUM FALCIPARUM* AND *P. VIVAX* AND PROSPECTIVE RISK OF *PLASMODIUM* INFECTION POSTPARTUM

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Pregnant women are more susceptible to malaria due to alterations in the immune response and the appearance of pregnancy-specific parasites, but how these immunological changes influence malaria risk in the postpartum period is unknown. A cohort study at the Thai-Myanmar border, found that postpartum women experienced less ***Plasmodium falciparum*** infections, but more ***P. vivax*** infections, compared to non-pregnant controls. To investigate the immunological basis behind these observations we measured IgG levels to ***P. falciparum*** antigens (PfVAR2CSA-DBL5, PfAMA1, PfEBA-140, PfEBA-175, PfMSP2, PfRh2, PfCSP, PfDBL-alpha) and ***P. vivax*** antigens (PvAMA1, PvMSP1-19, PvDBP, PvCSP) at enrolment (delivery date for postpartum women) and every four weeks thereafter over 12 weeks in 201 postpartum and 201 non-pregnant individuals paired by residence, age and enrolment date. To investigate the association between postpartum status, antibody levels and the time to the first microscopically confirmed species-specific infection, a Cox-proportional hazards regression was performed, modelling antibodies as time varying exposures. Higher levels of antibodies against most ***P. falciparum*** targets were associated with increased risk of ***P. falciparum*** infection in postpartum women (Hazard Ratio (HR) range 1.55-2.83; p range <0.001-0.043) suggesting that antibodies are indicative of a history of exposure. In contrast antibodies against ***P. vivax*** targets were not associated with an increased risk of ***P. vivax*** infection in postpartum women (HR range 1.06-1.18; p range 0.03-0.63). Similar associations were seen in control women suggesting that antibodies do not play a role in the differential susceptibility to malaria in the postpartum period in this population. This study provides a comprehensive analysis of antibodies towards two ***Plasmodium*** spp. and contributes to our understanding of the association between antibodies and risk of infection postpartum. Further investigations examining antibody-independent mechanisms of the differential susceptibility of malaria in the postpartum period are warranted.

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NATURALLY ACQUIRED IMMUNE RESPONSE TO THE ICAM1 BINDING PF11_0521_DBL2B IS ASSOCIATED WITH REDUCED RISK OF HIGH DENSITY *PLASMODIUM FALCIPARUM* MALARIA IN YOUNG PAPUA NEW GUINEAN CHILDREN

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Cerebral malaria is characterized by adhesion of ***Plasmodium falciparum*** infected erythrocytes to the cerebral microvasculature. ICAM-1 has been proposed as the key host adhesion receptor in the brain for infected erythrocytes causing cerebral malaria. The PfEMP1 variant PF11_0521_DBL2β has been shown to bind host ICAM1 receptor. We examined the association of IgG responses to PF11_0521_DBL2β with subsequent risk of ***P. falciparum*** malaria at enrolment in 187 children, aged 1-3 years, compared to that for four domains from two distinct PfEMP1 variants (PF13_0003_NTS-DBL1; PF13_0003_CIDRγ; PFL1955w_NTS-DBL and PFL1955w_CIDR). Overall, there was a low prevalence of antibodies to all PfEMP1 proteins analysed in this study (9-32%) likely due to the young age of the participants. Seroprevalence for the NTSDBL and CIDR domains of the group B/C variant, PFL1955w was particularly low in these young children (10% and 9% respectively). However, seroprevalence of NTSDBLα1 and CIDRγ domains of the group A variant PF13_0003 was relatively higher (24% and 28% respectively), as was the seroprevalence of the ICAM1 ligand PF11_0521_DBL2β (32%). Antibodies to the three group A variant domains were positively associated with concurrent ***P. falciparum*** infection. Antibodies specific to PF11_0521_DBL2β were associated with protection against high-density (≥10000 parasites/μl) ***P. falciparum*** malaria (IRR = 0.63, $p = 0.007$) independent of age and exposure. These results indicate that PF11_0521_DBL2β antibodies provide functional immunity against high-density malaria in young PNG children. The results highlight the importance of parallel comparisons of multiple PfEMP1 domains in identifying serological markers of protection and support the further development of this antigen as a malaria vaccine candidate.

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IMPORTANCE OF VAR2CSA ANTIBODIES TO *PLASMODIUM FALCIPARUM* IN A LOW TRANSMISSION AREA

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In areas where ***Plasmodium falciparum*** is transmitted, infected erythrocytes (IE) accumulates in the placenta causing placental malaria (PM), thus increasing the risk of maternal anemia and low birth-weight (BW) babies. Maternal antibodies (Ab) to VAR2CSA have been shown to play a key role in reducing the severity of PM in high-transmission areas. However, their importance in reducing PM in low-transmission areas is less clear. In this study, plasma samples collected at delivery from 1, 377 women in Yaoundé, Cameroon (low transmission) with (PM+) and without PM (PM-) were measured by Luminex for Ab against full-length VAR2CSA (FV2) and its 6 DBL domains. Samples were collected prior to implementation of intermittent preventative treatment (IPT) and insecticide-treated bed nets (ITN), allowing us to study natural acquisition of Ab to VAR2CSA. Ab levels to FV2 and each of the domains were determined and correlated with presence of PM, baby weight, and anemia. Furthermore, repertoire of Ab to the 6 DBL domains was compared with gravidity and by PM status. In this low transmission area, results showed an association between PM+ and lower BW ($p<0.0001$) and increased anemia ($p<0.0001$), but presence of Ab to FV2 did not improve the outcome. PM+ women produced more Ab against FV2 than PM- women ($p<0.0001$). Ab levels increased approximately two-fold with gravidity (G1-G6+). Moreover, the number of DBL domains recognized by Ab was consistently higher in PM+ (G1=3.2 domains to ≥G6 = 4.2 domains) than PM- women (G1=1.6 domains to ≥G6 =2.9 domains). Therefore, a larger DBL repertoire was found in PM+ than PM- women. Thus, Ab levels to FV2 and the breadth of Ab response to DBL domains was associated with infection rather than protection from PM. These results differ significantly from those reported for high transmission areas and need be taken into consideration in vaccine development and creating prediction models based on Ab levels.

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PROTEOMIC PREDICTORS OF *PLASMODIUM FALCIPARUM* SPECIFIC IGG ANTIBODY RESPONSES IN AN AREA OF INTENSE SEASONAL MALARIA TRANSMISSION

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During an infection some antigens of the infecting pathogen elicit higher antibody responses than others, but the factors underlying this heterogeneity are unclear. In this study we sought to understand the factors underlying differential antibody reactivity to natural ***Plasmodium falciparum*** infections. Using a protein microarray containing 1087 ***P. falciparum*** proteins, we profiled ***P. falciparum***-specific IgG responses in plasma samples collected from 267 Malian subjects aged 3 months to 25 years exposed to intense seasonal malaria transmission every year. We examined the relationship between the level of ***P. falciparum*** antigen-specific IgG levels and a number of features of the antigens on the array including their subcellular location, presence of MHC class II epitopes, protein abundance, molecular weight, presence of human orthologs, and degree of polymorphism. We found that IgG reactivity was significantly higher to extracellular and plasma membrane proteins, proteins with MHC class II epitopes, highly abundant proteins and highly polymorphic proteins; whereas IgG reactivity was significantly lower to proteins with human orthologs. Multiple regression analysis revealed that extracellular location independently predicted higher IgG reactivity, whereas location in the plasma membrane predicted low IgG reactivity in highly conserved membranes, and conversely high reactivity in highly polymorphic membranes. We observed the same findings in our cohort the following malaria season. These results provide insights into the proteomic features of antigens that underlie the variation in antibody responses during a natural infection, information that could inform vaccine strategies.

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MEROZOITE SURFACE PROTEIN-1 FROM *PLASMODIUM FALCIPARUM* IS A MAJOR TARGET OF OPSONIZING ANTIBODIES IN INDIVIDUALS WITH ACQUIRED IMMUNITY AGAINST MALARIA

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Malaria, in particular when caused by ***Plasmodium falciparum***, represents a huge medical problem in many countries. Individuals living in malaria endemic regions can acquire immunity against this often deadly disease with increasing numbers of survived infections. This premunition is evidently mediated by serum antibodies controlling levels of blood stage parasites. Antibodies against various ***P. falciparum*** antigens were shown to interfere with parasite growth by inhibiting red blood cell invasion. In addition, increasing evidence points towards an important role of opsonizing antibodies, which bind to the cell-free merozoites and recruit immune effector cells such as macrophages or neutrophil granulocytes. The merozoite surface protein-1 (MSP-1) is a target of antibodies acquired during natural infection. Antibodies to MSP-1 can inhibit parasite growth and have been associated with protection against malaria in several epidemiological studies, making MSP-1 a promising vaccine candidate. Here we use the antibody-dependent respiratory burst (ADRB) assay to characterize serum antibodies from semi-immune individuals from Burkina Faso. While a few sera were identified, which directly inhibit growth of ***P. falciparum*** blood stage parasites ***in vitro,*** IgG from almost all individuals clearly mediated the activation of neutrophils. The level of neutrophil activation correlates with antibody levels to MSP-1 and affinity-purified MSP-1 antibodies mediate ADRB activity. Furthermore, immunization of non-human primates with recombinant full-length MSP-1 induces antibodies, which efficiently opsonize ***P. falciparum*** merozoites. Reversing the function by pre-incubation with recombinant antigens allows us to quantify the contribution of MSP-1 to the anti-parasitic effect of serum antibodies and to map MSP-1 subunits primarily recognized by opsonizing antibodies. Our data suggest that MSP-1 is an important target of opsonizing antibodies acquired during natural exposure to malaria. Induction of opsonizing antibodies might be a crucial effector mechanism for malaria vaccines based on full-length MSP-1.

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TACI IS NEEDED THE CONTROL *PLASMODIUM YOELII* PARASITEMIA THE ANTI-*P. YOELII* IGG3 ANTIBODY PRODUCTION

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The transmembrane activator and calcium-modulator and cyclophilin ligand receptor (TACI) is involved in B-cell survival, antibody switching and plasma cell generation. TACI expression is severely impaired in murine and human newborns as compared to adults. To assess the role of TACI in murine malaria infections, TACI-KO and the wild type (WT) C57BL/6 mice were infected (i.p) with 1 million ***Plasmodium yoelii*** (Py) parasites. After the infection, the parasitemia levels were significantly elevated in TACI-KO mice (61.8% at day 18) compared to WT mice (11.1% at day 11), and parasitemia clearance was substantially delayed in the TACI-KO (27 days) relative to WT control (18 days). In addition, to investigate the impact of TACI on anti-Py antibody production, sera from WT and TACI-KO mice were tested by ELISA and Western Blot (WB) assay. The ELISA results showed that the TACI-KO mice produce less anti-Py IgG-antibodies than the WT mice, and the WB results showed that TACI-KO mice were impaired in the anti-Py IgG3 production but not the WT mice. Several IgG3 specific bands (49, 70-190 kDa) detected in the sera of Py infected WT mice were absent in the sera of infected TACI-KO mice. (IgG3 levels following infection have been associated with clinical immunity to malaria). Importantly, measurement of the levels of the B cell activating factor (BAFF- a TACI ligand) revealed that serum BAFF concentrations were higher in TACI-KO mice than the WT mice after Py infection. Conclusion: The TACI receptor plays a key role in the control of Py NL infections, and it may mediate anti-Py antibody isotype production and BAFF secretion

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RESPONSES THE GAMETOCYTE-SPECIFIC *PLASMODIUM FALCIPARUM* ANTIGENS DETECTED BY PROTEIN MICROARRAY MAY IDENTIFY MARKERS OF GAMETOCYTE EXPOSURE

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Malaria elimination efforts would benefit from vaccines that block transmission of ***Plasmodium falciparum*** gametocytes from humans to mosquitos. A clear understanding of gametocyte-specific antibody responses in exposed populations could help determine if transmission-blocking vaccines (TBV) would be boosted by natural gametocyte exposure, and could also inform the development of serologic tools to monitor gametocyte exposure in populations targeted for malaria elimination. We reanalyzed previously published data from microarrays containing 1,204 ***P. falciparum*** proteins and probed with plasma from Malian children and adults collected before and after the 6-month malaria season. Using publicly available proteomic data we identified 91 proteins as gametocyte-specific and 69 proteins not expressed by gametocytes. The overall breadth and magnitude of gametocyte-specific IgG responses increased during the malaria season, although they were consistently lower than IgG responses to non-gametoctye antigens. Notably, IgG specific for the TBV candidates Pfs48/45 and Pfs230 increased during the malaria season. Additionally, IgG specific for the gametocyte proteins Pfmdv1, Pfs16, PF3D7_1346400 and PF3D7_1024800 were detected in nearly all subjects, suggesting that seroconversion to these proteins may be a sensitive marker of gametocyte exposure. These findings suggest that TBV-induced immunity would be boosted through natural gametocyte exposure, and that antibody responses to particular antigens may reliably indicate gametocyte exposure.

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POLYMORPHISMS IN CYTOKINE GENES CAN INFLUENCE ON ANTIBODY PRODUCTION AGAINST PVDBP IN BRAZILIAN PATIENTS WITH MALARIA VIVAX?

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In Brazil, the ***Plasmodium vivax*** has been the most prevalent species, accounting for approximately 88% of malaria cases in the Brazilian

Amazon region. A DBP (Duffy Binding Protein) appears to be a strong vaccine candidate. The aim of this study were evaluate SNPS of the TNF-α gene, in the IL-10 and IFN-γ can interfere with blood levels of antibodies to PvDBP. We analyzed 84 samples from patients from Goianésia of Pará city, Pará State, Brazil, with vivax malaria by microscopy and confirmed by molecular analysis. Three SNPs in the promoter region of the TNF-α gene (-238 G / A, -308 G / A and -1031 T / C) and two IL-10 (-819C/T and - 592C/A), one IFN-γ (+874A/T) gene were genotyped by PCR-RFLP e ASO-PCR. Standardized ELISA protocol was used to measure IgG total against PvDBP. The nonparametric Kruskal-Wallis test was used to determine the differences in the antibody levels in relation to the genotypes. Sixty two (73,8%) patients produced IgG antibodies against PvDBP. All polymorphisms tested were in Hardy-Weinberg equilibrium (p-value > 0.05). For the polymorphism at position - 1031 TT in the TNF-α gene, the TT genotype had the highest frequency (51%), at position -308GG, the genotype GG (80,6%) and position -238 was GG (88,7%) in the patients with antibody titers against PvDBP. For the same group, the most common genotypes of SNPs in the IL-10 were CT (56,5%) to the position-819, e and CA (56,5%) to - 592. The most common genotypes of SNPs in the IFN-γ +874 were AA (52,8%).No significant differences were observed in the frequencies of genotypes among individuals who were positive or negative for IgG antibodies against PvAMA-1.However, it was significant association (p = 0.03) for individuals with CGG haplotype of the TNF- α (-1031, -308, -208). This study indicated that individuals with a TNF-α CGG haplotype are more likely to possess antibodies to PvDBP and genetic polymorphisms may play a relevant role in the regulation of the antibody response in this population. Although no association observed in this study may not entirely exclude their possible link with malaria disease pathogenicity/severity.

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PERIPHERAL THE PLACENTAL BIOMARKERS IN WOMEN WITH PLACENTAL MALARIA: A SYSTEMATIC REVIEW

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Placental malaria (PM) causes significant morbidity in mothers and infants. Diagnosis of PM during pregnancy is problematic due to placental sequestration of parasites. To circumvent this problem, the immunological response in pregnant women to a malaria infection could potentially serve as an indicator of ongoing PM. We aimed to identify potential host biomarkers that can indicate PM infection caused by ***Plasmodium falciparum*** by performing a systematic review of the literature. The databases of PubMed, Embase and the Cochrane Library were searched using specific search terms. This search resulted in 2424 records, of which 47 were relevant. Both studies on peripheral and placental markers were included in our review. Most studies measured biomarkers at time of delivery and most focused on inflammatory markers. A trend was observed for increased peripheral levels of IL-10 and TNF-α in PM positive women at delivery, but due to heterogeneity a meta-analysis could not be performed. Although many other biomarkers were studied, of which several showed significant differences between PM positives and negatives, a few were considered to be of greater interest. These were inflammatory markers TNF-R2, CXCL-13, C5a, and suPAR, a lipid metabolism marker APO-B and a marker of angiogenesis sFlt-1; each of these had additional proof of an association with (placental) malaria or its detrimental effects. Although differences in design and test methods limited firm conclusions on potential biomarkers, it is unlikely that a single biomarker will result in a high enough sensitivity and specificity to detect PM. Therefore, it is proposed to study combinations of multiple biomarkers involved in different pathophysiological pathways of PM. Furthermore, as the majority of published studies tested biomarker levels only at delivery, more longitudinal cohort studies will be necessary to inform on the natural course of biomarker levels in pregnant women, as well as fluctuations during PM.

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ASSESSING SEROLOGICAL RESPONSES THE MOSQUITO SALIVARY GLAND GSG6 PROTEIN AS A MARKER OF EXPOSURE THE MALARIA VECTORS IN A REGION OF DECLINING TRANSMISSION IN SOUTHERN ZAMBIA

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Various methods for measuring exposure to malaria vectors are available. Human landing catches are the gold standard for quantifying vector exposure but are less accurate for estimating the entomological inoculation rate (EIR) in low transmission settings because of the large sample size required to obtain precise estimates. Serologic responses to mosquito salivary proteins may provide an alternative method to estimate vector exposure in low transmission settings. An enzyme immunoassay (EIA) to measure IgG antibodies to ***Anopheles gambiae*** salivary protein gSG6 was performed using both gSG6-1P peptide and recombinant gSG6 protein in a region of declining malaria transmission in Choma District, Southern Province Zambia. In this setting, a single rainy season, lasting from approximately December through March, is followed by a cool, dry season from April to July, and a hot, dry season from August to November. The malaria vector population, consisting of Anopheles arabiensis, peaks during the rainy season. The parasite prevalence as measured by active case detection using a rapid diagnostic test was <1% between 2009 and 2013. 152 blood samples were collected from 92 individuals participating in a longitudinal cohort study in June 2013, December 2013 and June 2014. The median age was 23 years (IQR: 4, 74) and 47% were female. The assay using recombinant protein performed better than the assay based on gSG6 peptides. When restricted to 16 participants with complete visits at three time points, the mean EIA optical density (OD) values were 0.193, 0.159 and 0.191 at June 2013, December 2013 and June 2014 respectively. The EIA OD in December 2013 was significantly lower than the EIA OD value in June 2013 (p=0.005). The higher OD values observed in June suggest increased exposure to anopheline mosquitoes during the prior rainy season, with a reduction in detectable antibodies by the end of the dry season. These results suggest that the EIA using recombinant gSG6 protein can be used to assess exposure to malaria vectors in a low transmission setting approaching malaria elimination.

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NEW ANSWERS THE HOW IMMUNITY IN MALARIA IS FORMED: DEVELOPMENT OF *PLASMODIUM FALCIPARUM* SPECIFIC B-CELLS DURING THE FIRST YEAR OF LIFE

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Plasmodium falciparum malaria is still a major health threat in endemic areas especially for young children. While it is recognized that antibody immunity plays an important role in controlling the disease, knowledge of the mechanisms of sustenance and natural boosting of immunity is very limited. Before, it has not been possible to investigate malaria specific B-cells in flow cytometry, making it difficult to know how much of a B-cell

response is due to malaria, or how much is due to other immunological stimulators. In this study, we have developed a new technique using quantum dots to be able to investigate ***P. falciparum*** specific B-cells directly in fresh/frozen PBMC samples, something that has not been done before. To start with, we compared immune and non-immune individuals and found around 20% of the CD19-positive cells to be specific for ***P. falciparum*** in immune samples, with the highest levels in ongoing infections. We then used this technique to study the development of malaria immunity during infancy from the time of delivery with repeated sampling up to 10 months of age, in combination with studies of the response in the mothers, in a study performed in 150 mother-baby pairs in Uganda. We see large differences in development of immunity correlating with changes in CD27-positive and FCRL4-positive ***P. falciparum*** specific cells. This gives new insights into how immunity against malaria is formed, something that is important to know for creation of a vaccine or in forming new medications.

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DIRECT COST OF SEVERE MALARIA MANAGEMENT IN PEDIATRIC HOSPITAL OF MBUJIMAYI, DEMOCRATIC REPUBLIC OF CONGO

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Leading cause of morbidity and mortality of the Congolese child, malaria especially severe is so much a source of the economic losses, both direct (related to curative treatment or prevention) and indirect (due to absenteeism or the decrease of productivity), non-negligible for Mbujimayi's population whose the majority lives below the poverty line. We conducted a prospective study of the cases admitted in observation and hospitalization from July 01, 2012 to June 30, 2013 in the Pediatric Department of the Provincial Hospital Dipumba in Mbujimayi, in order to determine the direct cost of the in-hospital hold in charge of severe malaria among children of 6 to 59 months as well as the factors influencing it. Epi info 2008 version 3.5.1 was used to analyze data. Severe malaria represented 70.9% of admissions (534 of 753) whose 45.5% and 36% of cases were respectively secondary to untreated or poorly treated malaria. The majority of the households concerned (81.5%) were very poor because earned less than $ 30/month. Each household contained an average 2.2 subjects of under five years. The mean direct cost of the hold in charge of a severe malaria episode rose to $ 38.6±11.2 (range $ 8.5-79.94 US) either 34740±10636 Fc (Congolese money) of which 78.3% were bound to medication, 10.7% to consultation, 8.3% to hospitalization and 7.1% to laboratory tests. The average direct cost was high in case of healing ($40.83±10.95), bad quality of treatment before admission, therapeutic failure notion, severe anemia and high gravity of the case at admission. Malaria is a costly disease in relation to the standard of living of our population. It is therefore necessary to reinforce the management capabilities of the cases correctly and early so much at home that in hospital and to streamline the prescriptions in order to reduce the costs led by malaria.

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REFERRAL PRACTICES AND MANAGEMENT OF SEVERE MALARIA IN CHILDREN: A RETROSPECTIVE STUDY OF PATIENTS MANAGED IN A CHILDREN EMERGENCY UNIT IN A TERTIARY HOSPITAL IN SOUTHWEST NIGERIA

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Severe malaria is a disease known to be fatal in children and a major cause of admission into emergency units in Nigeria. The Nigerian treatment guidelines for the management of severe malaria recommend laboratory diagnosis and treatment with injection artesunate. Majority of children with severe malaria are referred to secondary and tertiary centers for management. Pre referral treatment with rectal artesunate or injection arthemeter is always encouraged. The objective of this study was to assess the use of pre referral treatment, pattern of management and treatment outcome in children with severe malaria This study assessed the use of rectal artesunate and arthemeter injection as pre referral treatment and management practices of severe malaria a teaching hospital in south western Nigeria. Children managed in Otunba Tunwase Children emergency unit between April and September 2014 were recruited. A total number of 134 children with a diagnosis of severe malaria were seen during the period. Referral, demographic, laboratory, treatment and outcome data were collected using semi structured questionnaire. Analysis was done using SPSS version 17. 134 children were managed over a period of 6 months. Majority of children were >5years (52.2%) while 64(47.7%) were <5years. More males (50.7%) than females (49.2%) were seen. Some (21, 8%) were referred to the hospital from other health facilities while 78.2% were admitted directly into the emergency unit. None of those referred got the recommended pre referral treatment before referral. Laboratory diagnosis was made in 83.6% of cases while clinical diagnosis was made in 16.4%. The treatment regimen used was injection artesunate in 97.8% of patients while oral ACT was commenced in 80.6% of patients. Majority (91%) improved and were discharged while 11(8.2%) died and 1 (0.75%) absconded. In conclusion, despite largely complying with treatment guidelines, a high mortality rate was still recorded during the period under study. Referral system should be strengthened to ensure patients receive pre referral treatment.

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EXPANDING ACCESS TO MALARIA DIAGNOSIS AND TREATMENT IN LAO PDR WITH A PUBLIC-PRIVATE MIX PROGRAM

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As the Lao People's Democratic Republic transitions toward malaria elimination and attempts to limit the spread of artemisinin resistance, one of the country's primary objectives is to improve access to and quality of diagnosis and treatment services. To increase the coverage of case management services and ensure the availability of quality antimalarials, the Center for Malariology, Parasitology, and Entomology (CMPE) implemented a Public-Private Mix (PPM) program to integrate private sector providers into national malaria interventions. As part of the PPM program, private healthcare providers are trained to use rapid diagnostic tests kits (RDTs) for malaria diagnosis and ACTs treatment of uncomplicated malaria, while agreeing to adhere to national guidelines and surveillance protocols. The PPM program was initially piloted in 2008 in eight districts across four provinces with a total of 98 private pharmacies

and 10 physicians from private clinics. By 2015, the PPM program has been expanded to include 17 clinics and 242 private pharmacies across 22 districts in eight provinces in Lao PDR. Over a 66 month period of implementation, the PPM program accounted for 19.2% (22,060) of total patients testing positive for uncomplicated malaria in the areas where the program exists. While public health facilities still test and report the majority of positive uncomplicated malaria cases in those areas (80.8%; 93,232), it is clear that private providers have made a significant contribution in providing access to life-saving health interventions. Overall, the PPM partnership has improved compliance with national guidelines and the quality of malaria services available within the private sector as well as increased the robustness of malaria data received throughout the country, which will be necessary for achieving the goal of elimination.

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EFFECTS OF ARTEMETHER ON HEMATOLOGICAL PARAMETERS OF PATIENTS PRESENTING WITH UNCOMPLICATED MALARIA IN KISUMU COUNTY WESTERN KENYA

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Malaria is still the leaing cause of morbidity in sub sahah Africa.Artemether is an antimalarial used for the treatment of multiple drug resistant strains of ***Plasmodium falciparum*** malaria.Artemether has been shown to affect the hematological parameters of healthy animal models.Neutropenia has been reported following use of artemether in patients with uncomplicated malaria. In a clinical efficacy study conducted among patients presenting with uncomplicated malaria in Kisumu County, Western Kenya, 118 Subjects were enrolled and randomized to receive either artemether lumefantrine or artesunate mefloquine.These subjects were then admitted in the ward for thee days and thereafter discharged upon receipt of two consecutive negative malaria blood films.Data from AL arm were analyzed. Complete blood counts were done daily while subjects had positive malaria blood films and thereafter weekly till Day 42. ACT Diff 5 coulter machine was used to run the complete blood counts.All parameters were observed to decrease in the first 48-72hrs.Interestingly in the White blood cont differential count, only decreased in neutrophil count was observed while the other parameters were either not changed or actually increased. These findings confirm what others have found.It is therefore important to closely monitor hematological parameters especially haemoglobin levels in patients using artemether based artemisinin combination therapies.

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MALARIA PARASITEMIA, ANEMIA THE MALNUTRITION PREVALENCES THE INTERACTIONS AMONG PRESCHOOL-AGED CHILDREN IN RURAL RWANDA - A COMMUNITY-BASED SURVEY

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Malaria, anemia and malnutrition are 3 highly prevalent and frequently co-existing diseases that, individually, are associated with high morbidity and mortality. We measured the burden of, and assessed for interactions between, malaria, anemia and malnutrition among community based preschool-aged children. Analysis of data collected as part of a community-wide cross sectional survey was performed. Altogether, 1,882 children aged 6-59 months with complete data on outcomes of malaria slide positivity, anemia (Hb levels of lt 90g/L) and stunting, wasting and underweight were included. Multivariate logistic regression analysis was performed. The prevalences of malaria, anemia and stunting were 5.9%, 7.0% and 41.3% respectively. Malaria parasitemia risk was associated with age groups gt 24 months (odds ratio (OR) 3.3; P=0.014, OR 3.5; P=0.008 and OR 3.7; P=0.005 for age-groups 24-35, 36-47 and 48-60 months respectively), whilst a reduced risk was observed among children living in household of high socio-economic status (SES) (OR 0.37; P = 0.029). Risk of anemia was higher among children gt or equal to 12 months and those with malaria parasitemia (OR = 3.86; P = lt 0.0001). Underweight was associated with stunting (OR = 20.41; P = lt 0.0001) and wasting (OR 59.14; P = lt 0.0001), stunting was associated with underweight (OR = 20.26; P = lt 0.0001) while wasting was associated with underweight (OR = 60.71; P = lt0.0001). The risk of stunting was high among children with a fever history (OR = 1.33; P = 0.01) but low among children living in household of high SES (OR = 0.79; P = 008) and in household with gt or equal to 1 bednet (OR = 0.55; P = 0.017). Study findings showed high proportions of stunting and anemia but not malaria in the study group. A strong association between malaria and anemia with children aged gt or equal to 12 months at significantly high risk of anemia and malaria. Integrated rather than vertical programs providing nutritional rehabilitation, comprehensive malaria control, improvements in household SES and investments in better house structures are needed to optimize health outcomes among children lt 5 years.

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DEVELOPMENT OF MALARIA PARASITES PURIFICATION BY ANTIBODY IMMOBILIZED MAGNETIC NANOPARTICLES

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Malaria is the most important parasitic infectious disease that many researchers are interested. Due to its harmful, malaria researches are still ongoing to combat the disease in several fields including the progression on drug and vaccine. In this regard, intact malaria parasites are required material for the research experiment. Conventional method for malaria separation is gradient centrifugation which is complex, time consuming, and specific to only mature stage of the parasite. This study aimed to develop a novel technique for malaria purification with rapid and high specificity by using magnetic nanoparticles (MNPs). The MNPs with specific functionalized surface were modified by immobilization of anti-malarial antibodies, which were purified from acutely ***P. falciparum*** infected plasma. Afterwards, antibody immobilized MNPs (Ab-MNPs) was incubated with ***P. falciparum***-infected erythrocytes. Complexes of Ab-MNPs and infected erythrocytes were then selectively purified from normal red blood cells by magnetic force. The malaria parasites were smeared, stained, and examined under optical microscopic examination. The result of microscopic examination showed that all stages of the parasite were separated by Ab-MNPs with high purity. Moreover, we showed result of scanning electron microscopy that the high specificity between Ab-MNPs and malaria infected erythrocytes was observed. This developed technique would be potentially used in malaria research and also adapted in diagnostic field.

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ALTERED ANGIOGENESIS THE ADVERSE BIRTH OUTCOMES IN EXPERIMENTAL PLACENTAL MALARIA

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Each year ~125 million pregnant women are at risk of malaria infection. Placental malaria (PM) has a profound impact on maternal and child health, increasing the risk of stillbirth (SB) and low birth weight (LBW), and results in ~200 000 infant deaths per year. We have shown that

PM-mediated LBW is associated with dysregulated angiogenesis through the angiopoietin (Ang)-Tie2 axis; however, it is unknown whether these alterations are a cause or consequence of poor birth outcomes. We hypothesize that altered levels of Ang1/Ang2 during PM impairs placental vascular remodeling, directly resulting in poor birth outcomes. To test this, we used the ***Plasmodium berghei*** ANKA (PbA) mouse model of experimental PM (EPM) and found that levels of angiogenic (Ang1, Ang2, PlGF, VEGF) and inflammatory (sICAM-1, C5a) markers were altered with PbA infection. Further, an elevated ratio of Ang2/Ang1 in both maternal serum and placental mRNA was associated with PM-mediated LBW. To further interrogate the Ang-Tie2 pathway in PM, we compared Ang1+/- to WT mice and found that pups from infected Ang1-deficient dams were significantly smaller and had decreased viability compared to pups from PbA-infected WT mice, irrespective of pup genotype. This suggests that maternal Ang1 deficiency exacerbates SB and LBW associated with PM and that supplementing with recombinant Ang1 (rAng1) could potentially rescue these phenotypes. Combined with the genetic approach, the ability of rAng1 to prevent SB/LBW would establish a causal role for Ang1 in PM-associated adverse birth outcomes. To determine the impact of Ang1 deficiency on fetoplacental vasculature, we are employing microCT 3D imaging to compare placentas from PbA-infected Ang1+/- and WT mice to examine the number of vessels and degree of vascular remodeling. Overall, these studies will provide novel insights into mechanisms underlying malaria-complicated pregnancies and may identify novel interventions to prevent poor birth outcomes in PM. Further, our findings may have broad implications for other causes of adverse pregnancy outcomes associated with vasculopathy including preeclampsia and gestational diabetes.

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ASSESSMENT OF THE SUSCEPTIBILITY OF LABORATORY BRED *ANOPHELES GAMBIAE* M/S HYBRID MOSQUITOES TO *PLASMODIUM FALCIPARUM*

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Malaria is caused by the ***Plasmodium*** haemo-protozoan and transmitted by Anopheline mosquitoes***. The Anopheles complex*** comprises seven species out of which ***An. gambiae*** is the most effective vector in Sub Saharan Africa. This species is divided into two main forms based on chromosomal polymorphisms. These are: the Mopti (M) form and Savanna (S) form. Both forms exhibit high and equivalent levels of susceptibility to ***P. falciparum*** and malaria transmission potential. Currently, there are increasing reports of naturally occurring M/S hybrids in West Africa and further studies nullify assumptions that there cannot be hybridisation of M and S forms by demonstrating a significant level of gene flow between the two. Unfortunately, no profiling has been done on M/S hybrids with regards to malaria transmission despite the fact that both forms exist in sympatry in Central and West Africa and even in Ghana. The primary purpose of this study therefore, is to investigate the susceptibility of ***An. gambiae*** M/S hybrids to ***P. falciparum.*** This novel investigation would provide information on the malaria transmission potential of M/S hybrids which could be useful in vector control programmes and serve as a basis upon which further research may be performed. The study will be conducted at the Noguchi Memorial Institute for Medical Research, Accra. ***An. gambiae*** M and S forms would be bred under standard insectary conditions and mated to ensure fertilisation and production of viable eggs. 2- 5 day old naïve adult hybrid female mosquitoes would then be infected with mature stage 5 gametocytes (NF54 lab strain). Infection would be done with a membrane feeder using high and low gametocytaemia parasite cultures. Control cages would be fed with uninfected blood and 10% sugar solution. DNA extraction and PCR tools would be used to determine the presence of parasites in the salivary glands of mosquitoes 18–20 days post infection. It is expected that the susceptibility profile of the ***An. gambiae*** M/S hybrid would be known and from this, conclusive information can be drawn on the malaria transmission potential of ***An. gambiae*** M/S hybrids.

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ESTABLISHMENT OF A PBMC REPOSITORY FOR IDENTIFYING BIOMARKERS OF PROTECTION AND ASSOCIATED TARGET ANTIGENS FOR MALARIA

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Studies in the 1970s demonstrated that immunization with radiation-attenuated ***P. falciparum*** sporozoites (***Pf***RAS) via mosquito bite confers protection against malaria in human subjects. However, neither the mechanisms of protection nor the targeted antigens have been clearly identified. The BMGF is supporting a clinical trial (Immunization via Mosquito bite with Radiation-Attenuated Sporozoites, or IMRAS) in which up to 24 subjects will receive bites from ***Pf***RAS-infected mosquitoes (true-immunized) and 8 subjects will receive bites from irradiated, uninfected mosquitoes (mock-immunized). These subjects, plus infectivity controls, will undergo controlled human malaria infection (CHMI). To maximize statistical power, the trial is designed to generate approximately 50% protection in the true-immunized group by using two cohorts, where Cohort 1 will be conducted prior to Cohort 2. Samples collected throughout the trial using routine venipuncture for whole blood and leukapheresis for large numbers of PBMCs will be used to: discover biomarkers and correlates of protection using a range of approaches, including systems biology; and identify pre-erythrocytic ***Pf*** antigens associated with protection using agnostic and candidate approaches. Leukapheresis was performed on 14 subjects in Cohort 1 who completed a series of 5 true (n=11) or mock (n=3) immunizations and CHMI. Leukopaks were collected pre-immunization (n=14), day 14 post 3rd immunization (n=8), day 5 or 6 post-CHMI (n=12) and 4 months post-CHMI (n=9). PBMC were isolated by Ficoll-Hypaque density gradient separation, and cryopreserved at 20 million PBMCs/vial. The average yield per leukopak was 4.5 billion PBMCs (range 1.6-10 billion). Conduct of Cohort 2 is ongoing and will be completed in early 2016. The IMRAS Committee for Samples and Immunoassays has been established to prioritize the allocation of samples to maximize scientific benefit. This PBMC repository will be an invaluable resource for identifying biomarkers of protection and associated target antigens for malaria vaccine development.

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PRE-REFERRAL TREATMENT PROCESS OF SEVERE MALARIA CASES IN AREAS SOUTHEAST OF SENEGAL

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The new guidelines of the NMCP recommended by WHO includes the Pre-referral treatment of severe malaria cases with the use of rectal artesunate capsules .This strategy which mainly targets children under 5 years has been scaled up in all the health posts of the country especially in the South East districts where malaria mortality in this age group makes up about 40% of national mortality. So after drawing up the document for the implementation and management tools, we launched and held the

trainings in the targeted medical areas in February 2014. These trainings helped guide 63 agents on the strategy in the regions of Tambacounda, Kolda, Sedhiou and Kedougou between 14 and 26 March 2014;. This program enabled us to train 405 providers. The NMCP also includes a community component in Salemata and Saraya districts in the region of Kedougou to reduce the delay of managing severe cases in areas where access is difficult; So after 8 days of theoretical and practical sessions, 50 community health workers were trained. After that, the districts with the support of the NMCP proceeded to the establishment of the capsules Artesunate and the management tools in the health posts and community sites. The 1st national monitoring conducted from 22 to 23 October, 2014 showed 100% functionality of Home-based management (HBM) sites and health nuts. For the same period, the routine data showed that 24 severe malaria were referred to the health centers (including 6 cases from the community level) with 60% of cases who received pre-referral artesunate-based treatment; which have contributed to the reporting of severe malaria cases in children under 5 years and their early treatment to prevent a fatal outcome; However, the impact of both seasonal malaria chemoprevention (SMC) and HBM along with a more active screening of cases have certainly reduced the incidence of severe cases in these highly endemic malaria areas. Looking ahead, the NMCP contemplates doing an overall assessment of the strategy in May 2015 in order to share this experience and expand the strategy to other districts with high malaria mortality and thus further reduce malaria death risk in this vulnerable age group.

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MATERNAL HOST FACTORS INFLUENCE NEONATAL MALARIA IN SOUTHEASTERN NIGERIA

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This study aims at documenting the maternal host factors influencing the prevalence of malaria in neonates in South Eastern Nigeria. Fifty-seven neonates who had a positive blood smear for the malaria parasite were included in the study and their socio-demographic and clinical correlates reviewed. The prevalence of neonatal malaria in this study is 35.67% and 77.3% of children with neonatal malaria presented fever. 99 (42.9%) and 132 (57.6%) of the mothers had parasitaemia in maternal peripheral blood and placental blood respectively. Obstetrics factors like parity was found to have a significant association ($\chi 2=7.30$, p=0.026) with neonatal malaria. Neonatal malaria was more likely to occur in babies of primigavid mothers and those mothers who attended ANC outside the tertiary health facilities ($\chi 2=6.75$, p=0.009). Neonatal malaria is not as rare as was previously thought and its morbidity and mortality in Sub-Saharan Africa is increasing and thus, there is need for educating of pregnant mothers on the necessity of early care-seeking for newborns.

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DHFR MUTATIONS IN *PLASMODIUM KNOWLESI* DO NOT APPEAR LINKED TO SELECTIVE DRUG PRESSURE FROM PUTATIVE HUMAN-TO-HUMAN TRANSMISSION IN SABAH, MALAYSIA

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Malaria due to zoonotic ***Plasmodium knowlesi*** is increasing in Eastern Malaysia. Despite demonstrated vector competency, it is unknown whether human-to-human transmission is occurring naturally. We sought evidence of drug selection pressure from the antimalarial sulfadoxine-pyrimethamine (SP) as a potential marker of human-to-human transmission. The ***P. knowlesi*** dihydrofolate-reductase (pkdhfr) gene was sequenced from 449 ***P. knowlesi*** malaria cases from Sabah (Malaysian Borneo) and genotypes evaluated for association with clinical and epidemiological factors. Homology modelling using the pvdhfr template was used to assess the effect of pkdhfr mutations on the pyrimethamine binding pocket. Fourteen non-synonymous pkdhfr mutations were detected, with the most common being at codon T91P (10.2%) and R34L (10.0%), resulting in 21 different genotypes, including the wild-type, 14 single mutants, and six double mutants. Whereas 145 (32%) of patients had pkdhfr single mutants, 14 patients harboured double-mutants. In contrast, among the 47 ***P. falciparum*** isolates sequenced, three pfdhfr genotypes were found, with the double mutant 108N+59R being fixed and the triple mutants 108N+59R+51I and 108N+59R+164L occurring with frequencies of 4% and 8%, respectively. Two non-random spatio-temporal clusters were identified with pkdhfr genotypes. There was no association between pkdhfr mutations and hyperparasitaemia or severe disease, both hypothesized to be indicators of H-H transmission. The orthologous loci associated with resistance in ***P. falciparum*** were not mutated in pkdhfr. Subsequent homology modelling of pkdhfr revealed gene loci 13, 53, 120, and 173 as being critical for pyrimethamine binding, however, there were no mutations at these sites among the 449 knowlesi isolates. Although common, the pkdhfr mutations in Sabah do not appear due to selective drug pressure. While not providing evidence for naturally-occurring human-to-human transmission, this mode of transmission is not excluded.

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STRATEGIC PROCUREMENT MANAGES THE CHALLENGE POSED BY THE ACCUMULATING OF LONG-LASTING INSECTICIDE-TREATED BED NETS (LLIN) PACKAGING WASTE

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Long-lasting insecticide-treated bed nets (LLINs) can successfully prevent the transmission of malaria worldwide, but for some recipient communities, one unwanted result of this success has been the accumulation of used LLIN packaging. If managed incorrectly, this used packaging can expose local populations to toxic substances. Three procurement options are available to help donors, programs, and the

malaria prevention community respond to this challenge: 1. not specifying any particular type of LLIN packaging; 2. procuring LLINs in individual bags, but stating that a specific packaging be used; 3. procuring LLINs that are packaged in bulk instead of individual bags. With each option, programs and stakeholders should review the potential ramifications and contextual issues before deciding on the best solution. Ultimately, any decision that will contribute to a well-managed LLIN packaging waste plan will contribute to an improved malaria prevention program and a reduced risk of contaminating the environment.

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LESSONS FOR INTEGRATING INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN SCHOOL SYSTEMS IN LOW INCOME SETTINGS: EXPERIENCES FROM UGANDA

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Evaluations of mass drug administration suggest that these programmes are not always well received by communities. Concerns regarding side-effects and the time and effort required to implement programmes, and doubts over the intentions of such programmes, have been raised. Discourses of foreigners harming local residents are often called into play in making sense of such initiatives that involve standardized treatment of asymptomatic people with drugs that are viewed as a precious commodity. The START-IPT trial was conducted in primary schools in Jinja district to assess if treating schoolchildren with dihydroartemisinin-piperaquine monthly to prevent malaria could improve the health of children and reduce the burden of malaria in the community. A qualitative study was conducted alongside the main trial to investigate the potential feasibility for integrating this intervention into routine health services and school systems. Ethnographic observations were conducted of the sensitization and consenting process at selected schools for one day each. During the roll-out of the intervention, the same schools that had been observed during the sensitization and consenting process were observed for the first three days of treatment distribution. A total of 19 in-depth interviews were held with district and national stakeholders, teachers, health workers and Village Health Team members. Three focus group discussions were held with study staff. We will present the findings of the qualitative study highlighting: 1) considerations for integration of health programmes into schools; and 2) potential supporting intervention methods and content of messages for IPT through schools. Key themes emerging in this context related the IPT programme to post-colonial concerns, economies of opportunity and operational issues. We situate these findings with those of others identified through a systematic literature review to provide considerations relevant to those planning to conduct mass treatment in schools in Africa.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE OR ARTESUNATE-AMODIAQUINE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA-MALAWI, 2014

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Malaria remains a major public health problem in Malawi, with an estimated 4 million cases in 2013. The first- and second-line treatments for uncomplicated malaria are artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ), respectively. Globally, emerging antimalarial drug resistance threatens treatment efficacy. We evaluated the efficacy of AL and ASAQ for the treatment of uncomplicated malaria in Malawi. During March-July 2014, febrile children aged 6-59 months with microscopy-confirmed uncomplicated ***Plasmodium falciparum*** malaria (1,000-200,000 parasites/μL) were enrolled in an ***in vivo*** efficacy trial at 3 sites, 1 each in northern, central and southern Malawi. Children were randomized 3:1 to AL or ASAQ arms. Sample size was sufficient to estimate site-specific efficacy for AL and overall efficacy for ASAQ. Blood was collected for malaria diagnosis by microscopy and malaria parasite molecular testing on days 0-3, 7, 14, 21, and 28. If treatment failure occurred, polymerase chain reaction (PCR) was used to differentiate recrudescence from reinfection; in PCR corrected analyses, reinfections were censored on the day they occurred. The primary outcome was the proportion of children with PCR-corrected adequate clinical and parasitological response (ACPR) on day 28. We enrolled 453 children; 307/338 (90.8%) and 102/115 (88.7%) reached a study endpoint in the AL and ASAQ arms, respectively, with no treatment failures on or before day 3. PCR uncorrected ACPR was 97% (95% confidence interval [CI]: 92-99%) for ASAQ and 77% (95% CI: 72-82%) for AL (84% [95% CI: 76-91%], 69% [95% CI: 59-78%], and 78% [95% CI: 68-86%] in the northern, central, and southern regions, respectively). PCR-corrected ACPR was 99% (95% CI: 95-99.9%) in the ASAQ arm and 99% (95% CI: 98-99.9%) in the AL arm, with 99-100% efficacy in each of the sites. Both AL and ASAQ remain efficacious treatments for uncomplicated malaria in Malawi. Recurrent parasitemia, primarily a result of reinfection, was significantly lower with ASAQ than with AL. This is expected given the shorter half-life of lumefantrine (3-6 days) compared to amodiaquine (9-18 days).

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HOW TO ACCOUNT FOR SEASONALITY WHEN PLANNING NATIONAL-LEVEL MALARIA ORDERS FOR COUNTRIES

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Learning objectives: Participants will understand how to account for seasonality when planning orders for malaria products. Quantification is the process of estimating the quantities and costs of the products required for a specific health program, and determining when the products should be delivered to ensure an uninterrupted supply for the program. The process includes both a forecasting and a supply planning step. A supply plan is based on forecast consumption, quantities on order, stocks on hand, and program minimum and maximum stock levels. The supply plan is the final output of the quantification; it lists in detail the quantities, costs, and arrival dates of the shipments. The traditional approach to supply planning is to calculate the annual forecast consumption and divide by 12, which yields an average monthly consumption (AMC). Shipments are scheduled to arrive when national stocks are at a minimum; stocks are then increased to the maximum level. Using a standard AMC for shipment scheduling for seasonal products can cause overstocks in the dry season and stockouts in the rainy season. An alternative is to develop a seasonality index to apply to forecast consumption_this will produce a monthly consumption that accounts for seasonal variation. Shipments can be planned accordingly. To develop a seasonality index, a random month is first selected as a reference month. Then, historical consumption for each month is divided into the consumption for the reference month. This yields monthly ratios, which capture the general shape of the annual consumption pattern, and show the relationship of consumption in a particular month to that of the reference month. For example, a peak malaria month may show consumption that is 2.5 times higher than a non-peak month; or consumption during a month of the dry season may be less than half the consumption in a rainy season month. After developing the seasonality index, these ratios are applied to the total forecast consumption for one year, which will yield an estimate of monthly consumption. When supply plans are developed, shipments should be scheduled to arrive prior to the peaks in consumption.

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FOOD INSECURITY AND THE COST OF MALARIA ILLNESS IN RURAL TANZANIA

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Understanding the impact of malaria on endemic rural African communities can assist governments and donors in developing appropriate and sustainable malaria control strategies. In Muheza, Tanzania, a cluster randomized trial is assessing the cost and effectiveness of non-pyrethroid insecticide-treated durable wall lining (DL). To project savings from DL, we estimated the current expenditures on malaria treatment in conjunction with a cross-sectional epidemiological household survey. We mapped and enumerated households and invited 4,200 randomly selected residents aged>6 months from 60 village clusters to participate in 3 rounds of epidemiological surveys. We asked the subset of respondents randomly selected for a parasitemia test (mRDT) and who reported a malaria episode within prior 30 days to respond to socio-economic surveys which focused on care seeking behavior, household expenditures on malaria care, and catastrophic expenditures and food insecurity. We complemented the surveys with macro-costing analysis implemented at district hospital to project the economic cost of care. Of the 89 respondents in the latest round of the socio-economic survey (Jan-Feb 2015), 55% were children, 53% reported some impact of the illness episode on food security in their household (7% to a great extent), 8% were hospitalized, 88% sought care in an ambulatory setting, and 4% were treated at home. On average, household direct medical expenditures on a malaria episode, including private sector, averaged $3.95 ($3.77 child, $4.17 adult) for ambulatory cases, $75.81 ($29.11 child, $94.42 adult) for hospitalized cases, and zero for home cases. The corresponding economic costs, including government financing, were $5.38 ($5.17 child, $5.63 adult) for ambulatory, $89.17 ($78.13 child, $93.58 adult) for hospitalized, and zero for home cases. The direct non-medical cost (transportation, food and lodging) averaged $1.93 for ambulatory and $23.51 for hospitalized cases. Malaria's impacts on household food security and finances are substantial. Additional preventive programs would generate important cost offsets.

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IMMUNE RESPONSES AGAINST TRANSMISSION BLOCKING TARGET ANTIGENS IN CHILDREN TREATED FOR SCHISTOSOMIASIS FROM A MALARIA MODERATE TRANSMISSION REGION IN ZIMBABWE

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Malaria remains a leading killer in sub-Saharan Africa with the most vulnerable group being children under 5 years. Although there has been successful decline in the malaria cases, the focus so far has mainly been on control. Eradication of malaria is still a long term goal and the development of effective vaccines remains to be achieved. Transmission blocking vaccines (TBV) represent a valuable approach to malaria elimination. Several antigens (Pfs230, Pfs49/45 and Pfs25) expressed in the sexual stages have been identified as target antigens and pre-clinical studies on TBVs have shown marked efficacy, 96-100%. The study was carried out in school-going children (7-16 years age, N=150) in Makoni, District, Zimbabwe, undergoing MDA for schistosomiasis. The prevalence of malaria diagnosed by microscopy and rapid diagnostic kit (Paracheck) TM was 1.2 % and 30.3 %, respectively and infected children received CoartemTM.treatment. We wished to assess presence of naturally occurring transmission-blocking immune responses. Serum samples were used for ELISA and in membrane feeding assays (MFA) to assess functional malaria transmission blocking immunity, and whole blood samples were used for PCR detection of ***Plasmodium falciparum*** malaria. Analysis of serum samples by ELISA revealed >90% sero-positivity using crude asexual lysates. Most significantly, 66 % and 63% samples showed ELISA reactivity to the recombinant Pfs48/45 and Pfs47 proteins, respectively. A few randomly selected sera samples were also tested in MFA. The MFA demonstrated measurable transmission blocking ability- 4 out of 20 sera revealed 56 to 84% transmission reducing activity. The results provide evidence for the presence of transmission blocking antibodies in these children co-infected with malaria and schistosomiasis. A TBV vaccine induced immunity further boosted by natural immunity may play significant role in the elimination of malaria transmission even in people co-infected with other helminthes.

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PRE-CLINICAL EVALUATION OF GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) AS A POTENTIAL MULTI-STAGE, PAN SPECIES MALARIA VACCINE

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Malaria is a major global health burden, causing approximately 584, 000 deaths annually, especially in children under the age of five. There are five ***Plasmodium*** species that infects humans, of which ***Plasmodium falciparum*** (P.f.) and ***P. vivax*** (P.v.) are the two most prevalent. Currently, no effective vaccine against malaria exists, hence the global effort aim to develop a vaccine to prevent infection, limit disease and interrupt mosquito transmission for all ***Plasmodium*** species. Glycosylphosphatidylinositol (GPI) is a potential target as it is a conserved glycolipid anchor of many essential parasite proteins found across most differentiated stages and ***Plasmodium*** species. Additionally, GPI is also a toxin that causes immuno-pathological symptoms of malaria. Previously, we proved that vaccination against GPI protects mice against severe malarial disease. This study further investigates the potential of the synthetic anti-GPI vaccine in preventing infection and blocking parasite transmission into the mosquito vector in a pre-clinical rodent malaria model, ***P. berghei*** (P.b.). The vaccine showed significant efficacy in reducing liver burden following sporozoite challenge, blood stage infection and parasite transmission to the mosquito. This was further validated by passively immunizing mice with anti-GPI antibodies prior to mosquito infection. When assessed over a complete life-cycle, i.e. sporozoite challenge followed by blood stage infection and mosquito feeding, sustained reduction in oocyst numbers were observed. Thus the anti-GPI vaccine shows pre-clinical efficacy against sporozoite, blood stage and sexual stages of malaria.

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HETEROLOGOUS PRIME-BOOST VACCINATION WITH CANDIDATE MALARIA VACCINES CHAD63-MVA ME-TRAP IS SAFE AND HIGHLY IMMUNOGENIC FOR EFFECTOR T-CELL INDUCTION WHEN CO-ADMINISTERED WITH EPI VACCINES IN HEALTHY GAMBIAN INFANTS AND NEONATES

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Despite the decline in the global burden of malaria cases and deaths, an effective malaria vaccine is still crucial to complement existing control strategies against the devastating effect of malaria: approximately 584,000 deaths occur annually, mostly in young children. We report here the interim findings of evaluation of co-administration of malaria vectored vaccines with EPI vaccines in Gambian infants and neonates. Sixty-five healthy infants and neonates aged 16 weeks, 8 weeks and 1 week were sequentially enrolled and randomized to vaccine or control (EPI vaccines only) arm. Participants in the vaccine arm received high dose ChAd63 ME-TRAP prime vaccination, followed by administration of MVA ME-TRAP boost vaccination eight weeks later. Each vaccination was accompanied by administration of EPI vaccines appropriate for the participants' ages. Safety of the vaccines was assessed by the description of adverse events related to vaccinations ascertained through clinical assessment, biochemical and haematological tests. Immunogenicity was evaluated by interferon-gamma ELISPOT, and intra-cellular cytokine staining and flow cytometry. Antibody testing was performed to assess possible interference of the candidate vaccines with the EPI vaccines. The median haemoglobin, white cell counts, alanine transaminases and creatinine at pre and post-vaccination visits in the vaccine and control arms were within the acceptable ranges. Frequently observed adverse events that appeared related to the vaccinations included fever and induration at injection sites. Overall, the vaccination regimes were very well tolerated. High level antigen-specific T cell responses were generated and sustained beyond Day 168 among infants in the malaria vaccine arm. Prime-boost effects were also observed with significant increase in geometric mean T-cell responses at Days 21 and 63. In conclusion, our findings suggest that administration of ChAd63-MVA ME-TRAP together with EPI vaccines continue to exhibit satisfactory safety and potent T-cell immunogenicity in very young infants living in a malaria-endemic area.

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FACING THE CHALLENGE OF VOLUNTEER RECRUITMENT IN PHASE I MALARIA CLINICAL TRIALS: EXPERIENCE FROM BAGAMOYO CLINICAL TRIAL UNIT, TANZANIA

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As a way to speed up clinical development program of investigational products such as vaccines to the community in need. The need to establish phase I clinical trial facilities in Africa has been realized, now that several research sites in Africa are capable to conduct early phase one clinical trial, Ifakara Health Institute is one among those sites. In the past three years, Ifakara Health Institute was able to conduct different three Phase I clinical trials to assess for the safety and immunogenicity of two promising malaria vaccine candidates. Volunteers for these trials were recruited from higher learning institutions in Dar es Salaam based on the pre-defined inclusion and exclusion criteria. Considering the fact that, such trials were for the first time conducted, there were challenges associated with the execution of these trials, which over time, a research team have learnt how to overcome them. One of those challenges associated with process of volunteer recruitment and encountered by establishment of volunteer database to assure easy and timely recruitment. Volunteers were invited in the series of sanitization meetings and those who met initial inclusion criteria attended screening at BCTU. Out of 702 volunteers who attended first sensitization meeting, 524 (74.6%) attended the second sensitization meeting. Almost three quarters 384 (73.3%) of the volunteers who attended second sensitization meeting met initial inclusion criteria and invited to the screening at BCTU. Out of 384 underwent both clinical and laboratory screenings at BTCU, 242(63%) were declared eligible and their contact address entered in the volunteer database ready for PfSPZ and P27A malaria clinical trials. So far, the screening of PfSPZ and P27A malaria phase I trials screening has utilized 153 (63.2%) volunteers from the existing volunteer database. Consequently, volunteer's data base has ensured the timely recruitment and enrollment, although there was a delay in getting approval from ethical committees and regulatory authority.

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CONSTRUCTION OF TRANSGENIC *PLASMODIUM BERGHEI* TO EVALUATE THE TRANSMISSION BLOCKING VACCINES BASED ON *P. VIVAX* TARGET ANTIGEN PVS48/45

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Plasmodium vivax is geographically widely distributed species of human malaria parasite, with nearly 40% of the world population at risk. Because of the lack of a continuous ***in vitro*** culture system, research on ***P. vivax*** parasites has relied on access to blood from infected patients or primates, resulting in relatively fewer vaccine studies in comparison to ***P. falciparum***. Transmission blocking vaccine (TBV) specifically targeting the sexual development of the malaria parasite in the mosquito vector offers an effective way to reduce or stop malaria transmission. Known TBV target antigens in ***P. falciparum*** include Pfs230 and Pfs48/45 expressed prior to fertilization of gametes and Pfs25 expressed after fertilization. Targeted gene disruption studies with P48/45 have also shown that it plays a critical role in male gamete fertility. The long-term goal of our research is to evaluate Pvs48/45 (***P. vivax*** homologue of Pfs48/45) as a TBV candidate. We focused our studies to generate transgenic ***P. berghei*** parasites by replacing the endogenous Pbs48/45 gene with Pvs48/45. In one transgenic parasite line we replaced the full length Pbs48/45 with Pvs48/45 gene sequence. In the second line we replaced Pbs48/45 sequence with chimeric sequence that consist of the signal and anchor sequences from Pbs48/45 and the middle section from Pvs48/45. Both transgenic parasites showed similar asexual blood-stage growth kinetics to the wide type ***P. berghei*** parasite in mice. More importantly, these transgenic parasites were found to be transmission competent and resulted in complete transmission success through ***Anopheles*** vector. These studies demonstrated the functional conservation of P48/45 proteins in evolutionarily distant species of ***Plasmodium*** with about 60% identity at the protein level between ***P. vivax*** and ***P. berghei***. We are now characterizing stage specificity of transgene expression by RT-PCR, Western blotting and IFA. In the next step, these transgenic parasites will be employed to evaluate transmission blocking activity based on the Pvs48/45 recombinant antigen, monoclonal antibodies and vaccine formulations being developed in our lab.

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A NOVEL SIMIAN ADENOVIRUS VECTOR IS ABLE TO ELICIT HUMORAL AND CELLULAR RESPONSES PROTECTIVE AGAINST AN EXPERIMENTAL *PLASMODIUM* CHALLENGE

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Malaria remains a public health burden despite efforts to improve infection control. In 2013 there were an estimated 198 million cases and 584,000 deaths, 78% of which occurred in children under 5 years, making malaria a leading cause of death in children of this age. Although malaria is treatable with ACT, reports indicate that less than 26% of children with malaria received ACT in 2013. A malaria vaccine is needed to reduce the burden of this disease in the areas where access to medication is logistically demanding. The development of an effective malaria vaccine has proven challenging. Although inoculation of volunteers with radiation attenuated sporozoites is capable of producing sterilizing immunity through induction of targeted antibody and CD4 and CD8 T cell responses, this method remains impractical for widespread use. Furthermore, clinical trials with the leading vaccine candidate RTS,S have failed to produce long lived efficacy, likely due to its inability to induce strong CD8 T cell responses. We have reported the design of a fusion protein based on chimeric ***Plasmodium yoelii*** CSP and MSP1 antigens designated PyLPC-RMC. This construct is able to induce robust antibody and CD4 T cell responses. Based on the evidence that viral vectors increase CD8 T cell mediated immunity we tested heterologous prime-boost immunization regimens that include human adenovirus serotype 5 vectors (Ad5). While Ad5 remains a popular vector for vaccine studies, the high prevalence of pre-existing immunity to Ad5 severely compromises its utility. The use of non-human Ad species is an alternative to Ad5-based vaccination. Here we use simian adenovirus 36 (SAd36) as candidate for a vectored malaria vaccine since there is little to no pre-existing immunity to this virus in human populations. Our studies show the induction of specific CD8 T cell response and similar antibody titers when compared to a prime-boost immunization regimen that includes Ad5PyLPC-RMC. This robust immune responses induced by SAd36PyLPC-RMC are translated into a lower parasite load and higher hemoglobin levels after a ***P. yoelii*** challenge when compared to naïve and mice immunized with Ad5PyLPC-RMC.

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ENHANCING THE PROTECTIVE EFFICACY OF A CHIMERIC MULTI-STAGE RECOMBINANT MALARIA VACCINE WITH THE USE OF ADENOVIRAL VECTORS IN HETEROLOGOUS PRIME-BOOST IMMUNIZATION REGIMENS

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Although significant improvements in malaria control have occurred in the past few years, an effective vaccine is needed to control the disease. Given the complexity of the parasite, an ideal malaria vaccine should target several stages of the parasite life cycle. A major challenge for the development of an effective multi-stage vaccine is to induce balanced and robust cellular and humoral immune responses. However, a regimen able to induce such responses is not available. We have reported ***Plasmodium yoelii*** chimeric recombinant proteins derived from CSP and MSP-1 that express cognate promiscuous T cell epitopes. These vaccines have superior efficacy compared to non-chimeric vaccine constructs. Based on the reported chimeric proteins, we developed a single fusion protein called PyLPC-RMC. This protein was able to induce multi-stage immune responses mediated by CD4 T cells and neutralizing antibodies. With the aim of eliciting effective CD8 T cell responses, we produced recombinant adenovirus (Ad) vectors expressing PyLPC-RMC as a transgene and tested several prime-boost immunization regimens with the reported fusion protein in an effort to improve protective efficacy. A major concern in the use of the Ad5 vector is the high prevalence of anti-vector neutralizing antibodies against capsid proteins following natural infection in humans which limits the immunogenic potential of the vector. To overcome this limitation, we developed a chimeric Ad5/3 vector where the Ad5 knob region was replaced with the orthologous region from the rare Ad3 human serotype, allowing the vector to circumvent preexisting anti-Ad5 immunity. Comparative experiments demonstrated that the breadth of the immune responses elicited by immunization with Ad5 or recombinant Ad5/3 were comparable. Our data highlights that immunization with the recombinant Ad5/3 vector induces a protective immune response against ***P. yoelii*** infection that depends on antibodies, CD4 and CD8 T cells. To our knowledge this is the first time that the chimeric Ad5/3 vector has been used for malaria vaccine development. The proposed immunization regimen and correlates of protection will be discussed.

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PHASE 2A/B PROTECTIVE EFFICACY OF *PLASMODIUM VIVAX* CS DERIVED PROTEIN FORMULATED WITH MONTANIDE ISA 51

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Plasmodium vivax is one of the most widely distributed malaria parasites, generating a global burden estimated in 75-85 million cases every year, which represents a great public health problem, particularly in the Asian and American continents. Malaria vaccine is considered the most economical and best strategy that may contribute to accelerate the reduction or elimination of malaria in high to low areas of transmission. Despite the traditional shortage of funding for ***P. vivax*** malaria research, significant efforts are being invested in the development of ***P. vivax*** vaccines and several phase I clinical trials have been conducted in recent years involving two different parasite antigens, the circumsporozoite (CS) protein and the oocyst/ookinete Pvs25 protein. The MVDC has invested efforts in the development of a ***P. vivax*** vaccine program and successfully assessed the safety, tolerability, and immunogenicity of a mixture of three long synthetic peptides (LSP) derived from the ***P. vivax*** CS protein in two phase 1a/b clinical trials, in addition, to the establishment of the ***P. vivax*** sporozoite infectious challenge. The protective efficacy of the ***P. vivax*** CS LSP formulated in Montanide ISA 51 adjuvant is being assessed in a phase 2a/b randomized, double-blind, controlled trial. Sixteen malaria naïve volunteers and sixteen previously exposed volunteers (pre-immune) were included. Ten and six volunteers from each group were randomly assigned to the experimental (E) and control group (C), respectively. E volunteers will receive three doses of the mixture of LSP (150 µg) and C volunteers a placebo at months 0, 2 and 6. Thirty days after the last immunization all volunteers will be challenged with viable ***P. vivax*** sporozoites. Currently the first two immunizations have shown to be safe and well tolerated and no serious adverse events have occurred, however a local self-limited adverse event and a few self-limited systemic adverse events such as fever, headache, chills, malaise and diarrhea have developed in some volunteers within the first three days. Results of the immune response as well as the protective efficacy will be presented.

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FROM LAB TO FIELD: PRACTICAL CHALLENGES TO PERFORMING SMFA WITH LOW MEAN OOCYSTS IN THE CONTROL

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The standard membrane-feeding assay (SMFA) is one of few functional assays in which the transmission blocking activity (TBA) of test antibodies is evaluated in pre-clinical and clinical studies. There are two different readouts to express SMFA results: % inhibition in oocyst intensity (transmission reducing activity, TRA) and % inhibition in prevalence (transmission-blocking activity, TBA). Our lab and others have shown that TBA results from different assays depend on the mean number of oocysts in the controls. Therefore, if one wants to use TBA data directly from SMFA to estimate efficacy in the field, the SMFA should be performed with a similar mean number of oocysts in the control, i.e, means of more than 0 but less than 4-5, as is usually seen in mosquitoes in the field. However, at present it is very challenging to target the mean number of oocysts in the control within a certain restricted range (e.g., the mean number of oocysts is within 1-4, which we call "restricted SMFA"). Based on our SMFA data, we have compared TBA estimates from "restricted SMFA" and model-based TBA estimates. The "restricted SMFA" should have a pre-specified target mean number of oocysts in the control (e.g., 2.5, the midpoint of the restricted range), while the model-based TBA was calculated with observed TRA using any (i.e., non-restricted) controls. Our simulations show that the "restricted SMFA" required more feeding experiments compared with the model-based approach to achieve the same level of accuracy in TBA estimates. These results suggest that it might be more practical to estimate TBA based on the model rather than performing "restricted SMFA".

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PROFILING OF ANTIBODY RESPONSES AGAINST *PLASMODIUM FALCIPARUM* PROTEIN ARRAY IN UGANDAN CHILDREN FOR IDENTIFICATION OF NOVEL BLOOD-STAGE VACCINE CANDIDATES

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Immunity to ***Plasmodium falciparum*** malaria can be acquired albeit slowly and after repeated infections. Parasite specific antibodies are considered to play a critical role in this immunity. Despite malaria being an enormous global health burden, the key targets of protective antibodies remain largely unknown. Identification of these targets could accelerate development of much-needed malaria vaccine. In this study, we attempted to profile antibody responses to 1848 recombinant proteins representing ~35% of the ***P. falciparum*** entire proteome, to identify novel blood-stage malaria vaccine candidates. The recombinant proteins were expressed by wheat germ cell-free system; a robust eukaryotic protein expression system that allows synthesis of natively folded plasmodial proteins as compared to prokaryotic expression system. Serum samples were obtained from children and young adults (n=66) who are indigenous residents of Lira, a malaria holoendemic region in Northern Uganda. They were enrolled at the start of the rainy season and prospectively monitored for clinical malaria episodes for a year. Antibody (IgG) responses against the 1848 ***P. falciparum*** protein array were measured by modified AlphaScreen® system, a homogeneous assay in solution which has the advantage of retaining antigens in natural conformations. As an outcome, levels of 195 antigen-specific antibodies were significantly associated with protection from clinical malaria (Hazard ratio <1, P<0.05). Of these, 78 antigens were predicted to have signal peptide and/or transmembrane domain(s) suggesting expression on parasite surface hence viable targets of protective immunity. Overall, 32% (25/78) of selected antigens were previously uncharacterized hence considered novel blood-stage vaccine candidates. Our data offers new and wide options for malaria blood-stage vaccine candidate discovery.

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PARASITE GENETIC DIVERSITY AND PROTECTIVE EFFICACY IN A PHASE 3 TRIAL OF THE RTS,S/AS01 MALARIA VACCINE

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The RTS,S/AS01 vaccine targets the circumsporozoite (CS) protein of ***Plasmodium falciparum*** and confers partial protective efficacy against clinical and severe malaria disease in infants and children for 12 months post vaccination (NCT00866619). We investigated whether vaccine efficacy was specific to parasite genotypes at CS. We employed PCR-based next-generation sequencing of DNA extracted from 4985 participant samples to survey polymorphisms in CS. We evaluated the impact of polymorphic CS positions and several haplotypic regions on vaccine efficacy (VE) against first or only episodes of clinical malaria within a year of vaccination. VE was significantly greater against clinical malaria with infections matching the vaccine strain in several haplotype regions and individual amino acid positions of the CS C terminus in the 5-17 month old per-protocol category of 4557 RTS,S/AS01 vaccinated and 2328 control vaccinated participants. For matched versus mismatched malaria based on the entire CS C-terminus, VE based on a hazard ratio was 62.7% (95% CI, 51.6 to 71.3) versus 54.2% (95% CI, 49.9 to 58.1), P = 0.06; and 1-year cumulative VE after vaccination was 50.3% (95% confidence interval [CI], 34.6 to 62.3) versus 33.4% (95% CI, 29.3 to 37.2), P = 0.04 for differential VE. In the 6-12 week old category, VE against matched and mismatched malaria was similar. Given the low frequency of parasites matching the vaccine strain at many of the study sites, these results suggest that parasite genotype contributes to the partial nature of protection conferred by RTS,S/AS01 vaccination in 5-17 month old children.

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NOROVIRUS-VLPS EXPRESSING MALARIA ANTIGENS INDUCE FUNCTIONAL IMMUNITY AGAINST *PLASMODIUM* PARASITES

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Despite the development of novel prophylactic anti-malarial drugs and practices to prevent infection, malaria remains a major health concern in tropical regions. Preclinical testing of novel malaria vaccine strategies achieved through rational antigen selection and novel particle-based delivery platforms is yielding encouraging results in preclinical models. Self-assembling virus-like particles (VLP) and capsid-like particles (CLP) are safer than attenuated live viruses, and have been approved as vaccination tools by the FDA. Moreover, a theoretical potential for a dual-application vaccine, against both the vector particle and the heterologous insert, could greatly impact public health. Here we explore the use of Norovirus sub-viral particles that lack the natural shell (S) domain forming the interior shell but retain the protruding (P) structures of the natural virus as vaccine vector. Epitope selection and their combination into multiple epitope presentations may focus antigen specific immune responses to crucial epitopes. Several recombinant P-particles displaying epitopes from two protective malaria antigens, namely CelTOS and CSP, were evaluated for immunogenicity and their ability to confer protection in murine challenge models. Immune responses induced in mice resulted either in sterile protection (particles displaying PfCelTOS epitopes) or in antibodies with functional activity against sporozoites (particles displaying PfCSP epitopes) as measured by an ***in vitro*** liver-stage development assay (ILSDA). These results are encouraging and support further evaluation of this platform as a vaccine delivery system for malaria antigens.

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HOW TO ADDRESS THE METHODOLOGICAL AND LOGISTICAL CHALLENGES OF PERFORMING MALARIA VACCINE EFFECTIVENESS STUDIES IN THE SUB-SAHARAN COUNTRIES

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RTS,S/AS01 is the most advanced candidate vaccine and might be the first to be introduced for children living in sub-Saharan Africa (SSA). A Phase III pivotal trial (NCT00866619) demonstrated a partial vaccine efficacy against uncomplicated and severe malaria due to ***Plasmodium falciparum***. Pre- (NCT02374450) and post-licensure safety studies are planned in five SSA countries with a Health and Demographic Surveillance System in place. The effectiveness and impact of the vaccine, when used in addition to other malaria control interventions, needs to be assessed in a larger population setting. We have explored the optimal study design for SSA settings to estimate effectiveness and impact of RTS,S/AS01 against malaria disease. A methodological assessment of possible study designs was conducted, taking into account post-licensure uncertainties regarding recommended age groups and booster dose requirement. To account for effect modifiers and potential confounding factors, other determinants (such as malaria endemicity, vaccine coverage and field feasibility) were also considered. A stepped wedge cluster trial was considered unsuitable as simulations demonstrated that this design requires long study duration to account for malaria seasonality and a high number of clusters, which might be logistically complex to achieve in the field. A self-controlled case series design was considered inappropriate because of possible natural immunity against malaria existing before vaccination and the difficulty of identifying a control period during the same malaria season. Case-control design increases the risk confounding and does not allow for estimation of vaccine impact effects. The optimal design was considered to be a cohort study, this would allow calculation of vaccine effectiveness and vaccine attributable rate reductions, and could assess these measures for a variety of outcomes. Therefore, the effectiveness and impact of the RTS,S/AS01 vaccine will be first assessed through a cohort study embedded in and taking advantage of the infrastructure developed for the safety study in 5 SSA countries.

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EXPLORATORY ENDPOINTS IN THE BK-SE36 CLINICAL TRIAL AND FOLLOW-UP STUDY IN UGANDA

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One of the constraints in the development of malaria vaccines is the limited availability of experimental models that can be correlates of protection. Randomized, controlled trials remain as the gold standard in providing scientific evidence regarding the efficacy of a malaria vaccine candidate. In clinical trials, several factors interplay with regards to interactions of the pathogen and host immune response. An evidence-based approach would expedite the development of a specific candidate. Here, we present the experiences we have encountered during our phase 1b clinical trial and follow-up study using the SE36 antigen from the blood stage ***Plasmodium falciparum*** malaria parasite. SE36 antigen was formulated with aluminum hydroxyl gel (BK-SE36). An age de-escalation (age cohorts: Stage 1: 21-40y; Stage 2: 16-20y, 11-15y, 6-10y) trial, tested BK-SE36 in participants after 2 vaccinations, 21 days apart. The vaccine was found to be safe and well tolerated. In our attempts to obtain additional data on the possible promise of BK-SE36, stage2 subjects were age-matched to 50 control individuals to compare malaria episodes 130–365 days post-second vaccination. In our assessment, we used a wide range of clinical outcomes from infection: using comparisons of risks, rates or hazards, depending on various endpoints. Each endpoint has its own advantages and disadvantages but gives important information for the developmental pathway of BK-SE36. Our longitudinal data constitute a first description of a blood-stage vaccine candidate that gave statistically significant level of protection up to one-year post-vaccination.

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INITIAL EVALUATION OF PFS25-EPA AND PFS230-EPA CONJUGATES ADJUVANTED WITH ALHYDROGEL®

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Both Pfs25 and Pfs230 proteins of ***Plasmodium falciparum*** are leading malaria transmission-blocking vaccine candidates. Pfs25 is a surface protein expressed on the zygotes and ookinete stages in the infected mosquito and Pfs230 is expressed in gametocytes in the human host and on the surface of gametes in the mosquito host. To enhance immunogenicity, recombinant Pfs25 and Pfs230 domain 1 (identified as Pfs25M and Pfs230D1M, respectively) were chemically conjugated to recombinant nontoxic Pseudomonas aeruginosa ExoProtein A (rEPA) in conformance with current good manufacturing practices (cGMP). The clinical grade conjugates were formulated following cGMP on Alhydrogel at 78 µg/mL of Pfs25M and 50 µg/mL of Pfs230D1M, respectively. In order to meet the regulatory requirements for a phase 1 human

clinical trial, these drug products were extensively evaluated. The initial characterization performed on the clinical lots included appearance, endotoxin content, sterility, general safety, strength (protein content tested by o-Phthaldialdehyde (OPA) assay and aluminum content determined by atomic absorption), identity (SDS-PAGE and Western blot after extraction of antigen from Alhydrogel), integrity (pH, percent protein bound to Alhydrogel, SDS-PAGE, Intrinsic Fluorescence CD, Direct Alhydrogel Formulation Immunoassay), and efficacy (mouse potency assay). Our results showed that the Drug Products Pfs25M-EPA and Pfs230D1M-EPA formulated on Alhydrogel are in conformance with the regulatory specifications and are considered suitabe for human clinical trials.

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DEVELOPMENT OF SELF-ADJUVANTED SELF ASSEMBLING NANOPARTICLES FOR USE AS MALARIA VACCINE CANDIDATES

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The need for a safe, effective, and affordable malaria vaccine is currently one of the most critical global public health concerns. Currently, no malaria vaccine candidate meets all of these criteria. RTS,S, the most successful candidate to date, induces protection, but these levels quickly fall; particularly in the most vulnerable population young children. In light of this situation there is a pressing need for new vaccine technologies to induce higher and longer lasting levels of protection. Our lab has developed the Self Assembling Protein Nanoparticle (SAPN) technology. Our SAPNs are rationally designed from coiled coil folding motifs to be similar in size and shape to small icosahedral viruses. Each SAPN is decorated on its surface with 60 copies of the antigen of choice. Currently, we have multiple malaria SAPNs in all stages of development from preclinical to clinical trials. One important aspect of vaccine development is adjuvant formulation. We have begun to expand upon our SAPN design to generate self-adjuvanted particles. These self-adjuvanted SAPNs contain a fragment of ***Salmonella enterica*** flagellin, a known TLR5 agonist, as well as a malaria antigen. Previous studies have indicated that TLR5 activation by flagellin has the potential to function as a successful adjuvant by activating cells in both the innate and adaptive immune system. This activation leads to a strong adaptive immune response and ultimately memory. ***In vitro*** studies in a model cell culture system indicate that SAPNs containing flagellin stimulate TLR5 in a concentration dependent manner. Initial animal studies are ongoing to determine the effect on protection against sporozoite challenge. Our self-adjuvanted SAPNs are viable new malaria vaccine candidates that may potentially eliminate the need to contain a separate adjuvant in the vaccine formulation.

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HIGH-THROUGHPUT AUTOMATED ASSAYS FOR ANTIBODY BINDING AND INHIBITION OF SPOROZOITE INVASION

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In recent trials, Sanaria® ***Plasmodium falciparum*** (Pf) sporozoites(PfSPZ)-based vaccines, composed of aseptic, purified, cryopreserved, radiation attenuated PfSPZ Vaccine, or non-attenuated PfSPZ with chloroquine (PfSPZ-CVac) were safe and highly protective. PfSPZ Vaccine protected 100% and 92% of non-immune individuals after 5 doses and 87% after 3 doses in 2 independent trials and provided durable and heterologous (75%) strain protection. PfSPZ-CVac was 100% efficacious in non immunes. Under natural exposure settings in Africa, PfSPZ Vaccine provided ~50% protection of semi-immune individuals over 6 months. Analysis of the humoral immune responses induced in these volunteers has uncovered a striking correlation between anti-PfCSP antibodies measured by ELISA and their protective status. Because the activity of antibodies in response to PfSPZ is believed to target SPZ before they enter hepatocytes, we assessed 2 additional measures of antibody function: the capacity to bind PfSPZ (anti-whole PfSPZ Immunofluorescence Assay or IFA) and the ability to prevent invasion into HC-04 cells (inhibition of Sporozoite Invasion or ISI). Initially IFA was performed by manual inspection of antibody-stained dried PfSPZ preparations. We have enhanced the throughput of this assay coating sporozoites in 96-well format and using an Acumen eX3 laser cytometer for fluorescence detection and quantification. The IFA readouts are highly correlated (75%-90%) with anti-PfCSP responses.The ISI assay is similarly performed with HC 04 cells grown in 96-well plates followed by a 3-hour invasion of PfSPZ. Both assays are reproducible over multiple iterations with specificity, sensitivity and statistical significance to predict clinical outcome in non-immune volunteers. Analysis of semi-immune individuals is ongoing. The miniaturized IFA and ISI assays are also useful screening tools in human monoclonal antibody development. They are expected to provide the malaria community with antigen-unbiased assays to interrogate antibody responses across diverse pre-erythrocytic vaccine platforms in an automated high-throughput format.

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DIFFERENTIAL ANTIBODY RESPONSE TO THE *ANOPHELES STEPHENSI* AAPP AND *PLASMODIUM* ANTIGENS IN INDIVIDUALS NATURALLY EXPOSED TO BITES OF AFROTROPICAL MALARIA VECTORS

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To identify the efficacy of control efforts of intervention trials against malaria transmission, the development of sensitive tools for evaluation of malaria risk and frequency of the vector exposure is required. During the exposure to the anopheles mosquitoes, antibodies against some of the salivary gland proteins are raised in the hosts. Recently, we have identified that anopheles mosquitoes expressed anopheline anti-platelet protein (AAPP), a one of the major component of the salivary protein, and the anti-AAPP antibody was well induced in mice frequently bitten by the mosquitoes. To examine the possibility of anti-AAPP antibody as a new tool for the evaluation system of malaria risks, recombinant AAPP as well as a series of malaria antigen were purified, and ELISA was conducted for the sera of residents of Sumba Island, Indonesia. Throughout the rainy season we examined, anti-malaria antibodies such as anti-PfCSP IgG and anti-PfMSP1 IgG were significantly increased in the individuals infected with ***Plasmodium*** as compared with normal hosts. In contrast, IgG responses to AAPP were increased after the exposure to the mosquitoes during the rainy season, and those with high titer of anti-AAPP antibody was significantly correlated with infection with ***Plasmodium*** spp. Geographical variations of mosquitoes number were associated with the anti-AAPP IgG responses, further supporting that anti-AAPP antibody response was depended on the variation of vector exposure. Taken together, anti-AAPP IgG can be a suitable marker for the evaluation of the vector exposure and malaria risks.

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MALARIA CONTROL WITH SOLAR-POWERED MOSQUITO TRAPPING SYSTEMS: SOCIO-ECONOMIC AND PERCEIVED HEALTH OUTCOMES OF HOUSE LIGHTING IN RUSINGA ISLAND, WESTERN KENYA

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In 2012, a proof of principle study was launched to eradicate malaria from Rusinga Island using solar-powered mosquito trapping systems (SMoTS). In addition to the mosquito trap, two light bulbs and a mobile telephone charging port were provided for use in each homestead in which a SMoTS was installed. Prior to receiving SMoTS, residents mainly used kerosene tin lamps for lighting. The effectiveness of new preventive health interventions is enhanced if, in addition to clinical efficacy, they are socially and culturally acceptable, and are widely adhered to in the longer-term. Social science studies on the project aim to understand socio-cultural and behavioural aspects of adherence to use and maintenance of SMoTS. We assessed socio-economic and perceived health outcomes of house lighting using in-depth interviews and focus group discussions with selected early recipients of SMoTS. The main economic benefit of solar lighting was reduced or eliminated expenditure on kerosene. Additionally, some residents charged mobile telephones for neighbours without SMoTS for pay. Kerosene traders, however, attracted fewer customers which led some to abandon the trade. Electricity reportedly reduced risks of respiratory infections, fire outbreaks from tin lamps, and physical accidents prone to poor house lighting. However, bright lights reportedly attracted mosquitoes into houses. Social outcomes included improvements in spousal relations due to reduced squabbles over expenditure on kerosene, while extended lighting periods facilitated unhurried social networking in the evenings, and night-time studying. Respondents also perceived improved social status as a result of owning a SMoTS. Negative social outcomes were strained relationships among women in polygamous households and envy from households that did not receive SMoTS. Although data on malaria prevention is not yet complete, there is evidence of enhanced socio-economic and emotional well-being of study participants which may increase the desire of community members to sustain the intervention beyond the research period.

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CONTROLLING MALARIA VECTORS IN EASTERN RWANDA THROUGH A COMMUNITY-BASED LARVAL SOURCE CONTROL APPLICATION OF BTI

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Larval source management (LSM) with existing vector control strategies such as insecticide treated nets (LLINs) and indoor residual spraying (IRS) are expected to contribute to further malaria reduction. We evaluate the process of a community-led LSM using bacillus thuringiensis israelensis (Bti). The research is being conducted in Ruhuha sector, a malaria endemic zone of the eastern province of Rwanda, geographically surrounded by marshlands and mainly occupied by rice farming. Since January 2015, four main steps were achieved; baseline studies involving entomological and socio economic aspects, training of larval control and mosquito surveillance teams; larval control implementation and lastly self-assessment using designed toolkits. Overall, 90% of rice farmers highlighted stagnant water/irrigation ditches as common mosquito breeding sites and 92% cited rice fields as significantly contributing to larval habitats. Only 12% had ever heard about LSM. However, 90% and 88% of the rice farmers were confident of the safety of the intervention to rice consumers and farmers, respectively. Nearly all were confident that the intervention would reduce the number of mosquitos and thereby reduce malaria transmission. Weekly application of Bti in marshlands resulted in a decrease of late stage mosquito larvae as compared to the baseline data. A self-assessment done using checklists coupled with weekly meetings highlighted contextual aspects and challenges to the implementation of the intervention and resulted in adjustment of self-assessment tools. The community-based LSM was found highly acceptable in the area. Newly identified open water sources have had implications on the amount of product to be used as well as the general timeframe allocated to the intervention. In conclusion, this novel approach is deemed to contribute to significant malaria reduction in the area and is expected to strengthen community know-how and promote local (1) ownership, (2) sustainability and (3) long term application.

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NO DIFFERENCE IN THE INCIDENCE OF MALARIA IN HUMAN-LANDING MOSQUITO CATCH COLLECTORS AND NON-COLLECTORS IN A SENEGALESE VILLAGE WITH ENDEMIC MALARIA

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To study the various vectors that transmit malaria and estimate their aggressiveness, most entomological studies opt to capture mosquitoes using human landing catches (HLC), which remains the gold standard method. However, this method has raised safety concerns due to a possible increased risk of malaria or other mosquito-borne diseases among the mosquito collectors. The aim of this study was to evaluate the incidence of malaria attacks among mosquito collectors and to compare these results with those of non-collectors living in a Senegalese village. From July 1990 to December 2011, a longitudinal malaria study involving adult mosquito collectors and non-collectors was performed in Dielmo village, Senegal. During the study period, 4 drugs were successively used to treat clinical malaria, and long-lasting insecticide-treated nets were offered to all villagers in July 2008. No malaria chemoprophylaxis was given to mosquito collectors. Incidence of uncomplicated clinical malaria and asymptomatic malaria infection were analyzed among these two groups while controlling for confounding factors associated with malaria risk in random effects negative binomial and logistic regression models, respectively. A total of 3,812 person-trimester observations of 199 adults at least 15 years of age were analyzed. Clinical malaria attacks accounted for 6.3% both in collectors and non-collectors, and asymptomatic malaria infections accounted for 21% and 20% in collectors and non-collectors. A non-significant lower risk of malaria was observed in the collector group in comparison with the non-collector group after adjusting for other risk factors of malaria and endemicity level (clinical malaria: adjusted incidence rate ratio = 0.89; 95% confidence interval [95%CI] = 0.65-1.22; p= 0.47). Being a mosquito collector in Dielmo was not significantly associated with an increased risk of malaria both under holoendemic, mesoendemic and hypoendemic conditions of malaria epidemiology. This result supports the view that HLC, the most accurate method for evaluating malaria transmission, can be used in areas with endemic malaria without ethical concern.

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USING MACHINE-LEARNING FEATURE SELECTION APPLIED TO NEAR INFRARED SPECTRA TO CHARACTERIZE AGES OF MALARIA VECTORS

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One way to evaluate the degree of risk of a particular mosquito population on malaria transmission is by determining its age and species composition. Currently, mosquito age-grading is best done by hand-dissection of ovaries to identify mosquitoes that have previously laid eggs (i.e., likely to be old and potentially infectious), and those that have not previously laid eggs (likely to be young and non-infectious). Studies show that the minimum age of mosquitoes to lay eggs is three days. Such a mosquito cannot be infectious, as the malaria parasite needs 10-14 days in mosquitoes to complete its development. A mosquito has to be at least 10 days old to be infectious. This means knowing a mosquito's egg laying status is not enough information to tell whether it is infectious. This implies a need for a new method to age grade a particular mosquito population to determine its infectiousness. Studies have shown that Near-Infrared Spectroscopy (NIRS), a low-cost high-throughput method, is 95% accurate on age-grading laboratory-reared mosquitoes. Based on calibration from lab-reared mosquitoes, NIRS classified 95% of 1740 wild samples to be <10 days old and 5% to be at least 10 days old. Despite of these promising results on age grading wild mosquitoes, its application still needs validation of its accuracy. Once hand dissection of ovaries were thought to be a means of validating accuracy of NIRS, but dissection does not provide chronological age needed to validate NIRS inferred ages. Identifying specific spectral feature associated with age is one way of validating these results. Using supervised machine learning (samples used have labels), we applied feature extraction techniques to characterize patterns associated with age from spectra collected from lab-reared mosquitoes. Machine learning feature extraction uses computer tools to develop features from a huge data set. 700 spectra collected from lab-reared mosquitoes with different known ages were used in this analytical study. It was found that there are features in spectra that support classification of lab-reared mosquitoes by age, a step toward validation of NIRS for age-grading of wild mosquitoes.

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WHAT IS THREATENING THE EFFECTIVENESS OF ITNS? A COMPARISON OF PHYSICAL, BEHAVIORAL, ENTOMOLOGICAL AND ENVIRONMENTAL FACTORS

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Insecticide-treated nets are the cornerstone of global malaria control and have been shown to reduce malaria morbidity by 50-60%. However, some areas are experiencing a resurgence in malaria following successful control. We enrolled 442 children hospitalized with malaria in an endemic area of western Kenya where coverage with ITNs is high. We paired them with age, time, village and gender-matched controls. We completed comprehensive household and neighborhood assessments including entomological surveillance. The variables were grouped into five domains - ITN ownership, compliance, physical integrity, vector susceptibility and facilitating factors. After variable selection, case-control data were analyzed using conditional logistic regression models and mosquito data were analyzed using negative binomial regression. Measures of ITN coverage and physical integrity were not correlated with infection in our study . However, ITN compliance(AOR=0.23, 95%CI:0.12-0.43) presence of nearby larval sites (AOR=1.43, 95%CI:1.05-1.95), and specific types of crops were significantly correlated with infection amongst children who owned an ITN. The odds of infection increased nearly three-fold when one other household member had symptomatic malaria infection (AOR=2.76, 95%CI:1.83-4.18). Overall, perfect household adherence could reduce the probability of infection to less than 30% and reducing environmental risk factors could reduce the probability of infection to less than 20%. We conclude that availability of ITNs is not the bottleneck for malaria prevention in this community. Behavior change interventions to improve compliance and environmental management of mosquito breeding habitats may greatly enhance ITN efficacy. A better understanding of the relationship between agriculture and mosquito survival and feeding success is needed.

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EFFECTIVENESS OF CONTINUOUS DISTRIBUTION IN SUSTAINING ACCESS TO LONG-LASTING ITNS: RESULTS FROM FIVE COUNTRIES

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Sustained universal coverage with ITNs is essential to malaria control. While mass campaigns which deliver nets in a single time-limited operation, continuous distribution systems (CD) use existing infrastructure such as clinics, schools, religious leaders and community-based workers to deliver nets continuously over time. To assess the effectiveness of CD, the NetWorks project piloted different models in five settings: Cross River State, Nigeria (schools and ANC), Nasarawa State, Nigeria (community and ANC), Ghana (multiple: schools, ANC and child clinics), Madagascar (community) and South Sudan (community). The number of nets distributed ranged from 26,686 to 150,000 per pilot. Pre-post representative cross-sectional household surveys were carried out in Nigeria, Ghana and South Sudan; in Madagascar, the study was a post-only design. Results showed improvements or, for Madagascar, continuation of high rates across several key indicators such as ownership of at least 1 ITN (Cross River 51% to 77%, Nasarawa 17% to 37%, Ghana 81% to 88%, Madagascar 97% and South Sudan 66% to 82%) and population access to an ITN (Cross River 34% to 55%, Nasarawa 16% to 34%, Ghana 57% to 67%, Madagascar 84% and South Sudan 38% to 66%). For the proportion of households with at least 1 ITN per 2 people, improvements were mixed with positive outcomes in Cross River (17% to 30%) Madagascar (62%) and South Sudan (31% to 63%) and slight declines in Nasarawa (25% to 17%) and Ghana (50% to 40%). Few households were oversupplied with nets and there was little overlap in source of nets. However, in Nasarawa, which had the lowest coverage outcomes at endline, gains were constrained by stock-outs, insufficient awareness of the program (only 32% of households knew about the program), and misconceptions among providers. Regardless, overall results suggest that CD contributes to sustained ownership levels and household access to ITNs over time. The quality of implementation contributed measurably to redemption rates for community distribution. Pilots with strong partnerships, sensitization, and supervision systems were successful.

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HEALTH SYSTEMS STRENGTHENING: IMPROVING QUALITY OF SERVICES FOR PREVENTION OF MALARIA IN PREGNANCY THROUGH THE STANDARDS-BASED MANAGEMENT AND REWARD APPROACH IN KENYA

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Performance quality improvement (PQI) is one of Jhpiego's 9 health systems strengthening components in provision of health services towards improvement of maternal health including better pregnancy outcomes. The Standards-Based Management and Reward (SBM-R) approach has been used in improving as well as assessing the quality of services provided at health facilities. Kenya developed 15 malaria in pregnancy (MIP) SBM-R standards for use by service providers in provision of MIP services and is also used by supervisors to assess the quality of services provided at service delivery points. Facility incharges were trained on the 15 MIP SBM-R performance standards and they oriented service providers in their facilities on use of the performance standards. A baseline on SBM-R practices was done in all facilities before orientation in Kakamega east and Kakamega central subcounties and 1st assessment on practices done after three months of practice. A total of 30 health facility incharges from the two malaria endemic subcounties ***(Kakamega east 16 Kakamega central 14)*** were trained on the 15 MIP SBM-R performance standards. The facility incharges oriented 291 service providers ***(127 Kakamega east, 164 Kakamega central)*** on use of SBM-R performance standards in provision of MIP services in health facilities. Baseline assessment had an average score of 57% for Kakamega east and 58% for Kakamega central. 1st assessments were conducted after three months of practice and showed an average score of 76% for Kakamega east and 64% for Kakamega central giving an overall increase in score of 19% and 13% between baseline and 1st assessment for Kakamega east in Kakamega central respectively. Use of MIP SBM-R performance standards ensures services provided at health facility level are in line with WHO recommendations and national guidelines. Establishment of PQI as a health systems strengthening component is feasible and is an approach that would make available quality MIP services at facility level. Provision of quality MIP services ensures protection of pregnant women against the effects of malaria in pregnancy.

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DESIGN, MONITORING AND IMPLEMENTATION OF THE SECOND ROUND OF SCHOOL NET DISTRIBUTION TO MAINTAIN ACCESS TO LONG-LASTING INSECTICIDAL NETS IN SOUTHERN TANZANIA

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Tanzania has been successful in implementing mass distribution of nets through a number of campaigns conducted to scale up the coverage of insecticide-treated nets (ITNs) in the country. In order to sustain these gains, the government has adopted a school-based approach as a Keep-up Strategy for the continuous distribution of long-lasting insecticidal nets (LLINs). In 2013, it piloted a school net project (SNP1) for distribution of LLINs in the Southern Zone. A second round of school net project (SNP2) was undertaken to maintain high access to LLINs. This study reports on the design, implementation and outcomes of the SNP2 project to sustain high LLIN coverage in Southern Tanzania. The SNP was conducted in Lindi, Mtwara and Ruvuma regions of Tanzania to distribute nets to students and teachers of primary and secondary schools (Standard 1, 3, 5 and 7 and Forms 2 and 4). In Lindi region, two additional classes (class 2 and 4) were targeted for net distribution. Each student from the selected classes received one LLIN for his/her household. In addition, a net was issued to each of the primary and secondary school teachers in the three regions. Training and sensitization activities, planning of logistics, data quantifications and revision of tools and manuals from SNP1, took place prior to net distribution. We designed a database that used an android software application for collection, entry and management of SNP2 data. A total of 487 trainers and 4,583 implementers were trained for distribution of the nets to schools. A total of 2,337 schools, 473,700 students and 25,269 school teachers participated in SNP2. A total of 507,775 LLIN were distributed to schools. A total of 464,893 (98.0% of registered) students and 24,206 (95.8% of registered) school teachers received LLIN. After net distribution was over, 18,676 (3.8% of those distributed) LLIN remained. The SNP2 reached 98% of registered eligible students. LLIN ownership and use in the community can be expected to increase and therefore reduce the burden of malaria in the three SNP regions.

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COVERAGE, USE AND MAINTENANCE OF BED NETS AND RELATED INFLUENCE FACTORS IN KACHIN SPECIAL REGION II, NORTHEASTERN MYANMAR

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Myanmar is one of the 31 highest burden malaria countries worldwide. The study combined a quantitative household questionnaire survey and qualitative direct observation of households to investiagte the extent to which bed nets were used and which factors influence bed net use among the population in Kachin Special Region II, Northeastern Myanmar. The results of study showed that the bed net to person ratio was 1:1.96 (i.e., more than one net for every two people). The long lasting insecticidal net (LLIN) to person ratio was 1: 2.52. Also, the percentage of households that owned at least one bed net was 99.7% (666/688). 3262 (97.3%) residents slept under bed nets the prior night, 2551(76.1%) of which slept under ITNs/LLINs the prior night (SUITNPN). The poorest families (OR: 4.67, 95% CI: 3.59 to 9.12; P<0.0001), those with thatched roofing (OR: 1.57, 95% CI: 1.33, 2.24; P<0.0001), those who use agriculture as their main source of family income (OR: 1.66, 95% CI: 1.45, 2.70; P<0.0001), household heads who knew that mosquitoes transmit malaria (OR: 1.88, 95% CI:1.45-3.47; P<0.0001) and those who used bed nets to prevent malaria (OR: 1.56, 95% CI: 22, 2.67; P=0.0003) were significantly more likely to be in the SUITNPN group. However, residents in lowlands and foothills were significantly less likely to be SUITNPNs (OR: 0.63, 95% CI: 0.44, 0.71; P<0.0001). Finally, head of household attitude towards fixing bed nets influenced MCHI (F=8.09, P=0.0046). The coverage and usage rates of bed nets were high, especially among children and pregnant women. Family wealth index, geographical zones, household roofing, source of family income, household head's knowledge of malaria transmission and of using bed nets as tools for malaria prevention are independent factors which influence use of ITNs/LLINs in KR2. The attitudes of household heads toward mending bed nets influenced the intactness of bed nets. Maintaining high coverage and use rate of bed nets should be a priority for the war-torn population of KR2 to ensure equity and human rights.

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A LONGITUDINAL STUDY OF THE DURABILITY OF LONG-LASTING INSECTICIDAL NETS IN ZAMBIA

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Universal access to long-lasting insecticidal nets (LLINs) is key to malaria control. Quantifying how many nets are needed to achieve and maintain universal coverage requires knowing when to replace worn nets. Net lifespan is often thought to be 3 years. We aimed to describe attrition, physical integrity, and insecticide persistence of LLINs over time to better estimate net lifespan. In 2 highly endemic provinces in Zambia, LLINs randomly selected from distribution records were followed every 6 months from 12-30 months of age. Net owners were surveyed on net care and reasons for attrition. Holes were counted and sizes estimated (< thumb =1.23 cm^2, ≥thumb but <fist =28.28 cm^2, ≥fist but < head=240.56 cm^2, and ≥head=706.95 cm^2). Proportional hole index (pHI) was calculated by dividing hole area by 1.23 cm^2 (smallest hole area). Functional survival (FS) was defined as nets present at follow up with a pHI <643 (WHO working group definition). Generalized estimating equation models of log transformed pHI and survival analysis on nets with endpoints of attrition and pHI≥643 were done. At 12 and 24 months old, a subset of nets was studied for insecticidal activity and concentration using bioassay and chemical analysis. We enrolled 999 LLINs; 505 PermaNet and 494 Olyset nets. Of these, 74 were removed for insecticide studies, and 925 had full follow-up. At 30 months old 325 (33%) nets remained. Attrition at 12-30 months old was primarily due to disposal (29%). Olyset nets, repairs and use over a reed mat were associated with larger log pHIs. Only 56% of remaining nets met FS criteria. FS was shorter in nets with repair ($p<0.05$), but longer in nets used the night before ($p<0.05$) and never washed ($p<0.05$). At 30 months, nets had a 34% chance of functionally surviving. Median survival was longer in PermaNets than Olysets ($p<0.05$). Insecticide activity and content was lower at 12 months old. Bioassay and chemical results were poorly correlated, likely due to small sample size. Replacing nets every 3 years may not be enough to maintain high level coverage with LLINs in this setting. A better measure of net survival incorporating insecticidal effectiveness is needed.

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TYPHOID TRANSMISSION: A HISTORICAL PERSPECTIVE ON MATHEMATICAL MODEL DEVELOPMENT

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Mathematical models of typhoid transmission have been developed for nearly half a century. To facilitate a better understanding of the historical development of this field, we reviewed mathematical models of typhoid and summarized their structures and limitations. Eleven models, published in 1971 to 2014, were reviewed. While models of typhoid vaccination are well developed, we highlight the need to better incorporate water, sanitation and hygiene interventions into models of typhoid and other foodborne and waterborne diseases. Mathematical modeling is a powerful tool to test and compare different intervention strategies which is important in the world of limited resources. By working collaboratively, epidemiologists and mathematicians can build better mathematical models of typhoid transmission that will be useful in epidemiological practice.

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A SYSTEMATIC REVIEW OF DIARRHEAL DISEASE: ITS DIFFERENTIAL BURDEN BETWEEN GENDERS AND THE ROLE OF WOMEN IN THE ABATEMENT OF THIS EPIDEMIC?

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Even though research on diarrheal diseases has been done in the past, some aspects have remained unexplored. One of these aspects is the differential disease burden and vulnerability to disease between males and females. We try to shed light on this important issue by performing a systematic review of relevant articles chosen from the literature. We searched PubMed for peer-reviewed articles, and included grey literature from the World Health Organization, Water and Sanitation for the Urban Poor and Water Supply and Sanitation Collaborative Council. All articles that dealt with the public health relevance of diarrheal disease, focused on access to clean water and care taker role in access to clean water, role of gender in sanitation, helminth infections and differential gender burden were included in this study. Articles which do not address diarrheal diseases and the role of sanitation and WASH interventions in the amelioration of diarrheal diseases and helminth infections, articles that are not epidemiologically linked or articles that deal with rare pathogens or diseases, therapeutic regimens or drug resistance were excluded from the study. All articles that dealt with respondents 6 years of age and older were included in the study. From our systematic review, we concluded that the burden of diarrheal disease falls more on females qualitatively than males. Women empowerment in making household and community level decisions with regard to sanitation may be of greater benefit to the well-being of society in developing countries. Some limitations of our study are: The study participants in most of the studies belonged to the 6-18 year age group, which could have resulted in age bias. Secondly, because of the large quantity of articles that were retrieved, there is a small but very unlikely chance that some relevant articles might have been missed. Lastly, we provide qualitative evidence of differential burden of diarrheal disease between genders. A meta-analysis will be performed to consolidate our current findings.

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AUTOMATED VS. NON-AUTOMATED ANTIBIOTIC SUSCEPTIBILITY TESTING: A COMPARATIVE ANALYSIS OF METHODS FOR ASSESSING RESISTANCE IN *SHIGELLA* AND *ESCHERICHIA COLI*

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Substantial milestones have been achieved in automation of microbiology assays for identification and antimicrobial susceptibility testing of bacterial pathogens. However, non-automated methods are still widely utilized across the globe. Both automated and non-automated ***in vitro*** methods are available in Kenya to determine the minimum inhibitory concentrations (MIC) for multiple antibiotics against bacterial pathogens. A comparison study was performed using 60 common diarrheal clinical isolates of ***Shigella*** spp (30) and E. coli (30) on two representative methods: the Etest® (Biomérieux), a non-automated gradient diffusion test, and the Negative Breakpoint Combo 34 panel (NBPC 34) for the MicroScan (Siemens), a micro broth dilution-based automated platform. The resistance patterns for these isolates against five commonly used antibiotics in Kenya were interpreted according to the Clinical and

Laboratories Standards International (CLSI M07-09-2012) guideline. The resistance patterns measured by both MicroScan and Etest® were highest to Tetracycline (90%) and Trimethoprim/Sulphamethoxazole (> 80%) for both pathogenic E. coli and ***Shigella*** spp. Resistance patterns were much higher for E.coli for the following anibiotics by MicroScan and Etest® respectively: Ampicillin/Sulbactam: 60% and 50% and Ampicillin: 90% and 80% as compared to ***Shigella*** spp: Ampicillin/Sulbactam: 3% and 33% and Ampicillin: 53% and 50%. Resistant patterns among E. coli pathotypes were similar while among ***Shigella*** spp, S. flexneri exhibited higher resistance than S. sonnei to three out of the five antibiotics tested. Ciprofloxacin, whose use in treatment for acute diarrhea is increasing in Kenya, was more than 100% effective against E. coli and 90% against ***Shigella*** spp. There was significant correlation of the results obtained between the two methods ($p < 0.01$). Therefore, either of these methods can be used for reliable ***in vitro*** antimicrobial susceptibility testing for clinical or research purposes.

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IDENTIFICATION OF *SALMONELLA ENTERICA* SEROVAR PARATYPHI A ANTIGENS EXPRESSED DURING CHRONIC BILIARY PARATYPHOID CARRIAGE

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Enteric fever, caused by ***Salmonella enterica*** serovar Typhi (***S.*** Typhi) and serovar Paratyphi (***S.*** Paratyphi) A, B, or C, is a life-threatening systemic disease, responsible for significant morbidity and mortality worldwide. A subset of individuals infected with enteric fever become asymptomatic chronic carriers, with the gallbladder and biliary ducts being primary sites of chronic colonization. Because ***S.*** Typhi and ***S.*** Paratyphi are human-restricted pathogens, asymptomatic carriers can act as critical reservoirs for further spread of enteric fever. We have previously used ***in vivo***-induced antigen technology (IVIAT) to identify potential bacterial biomarkers unique to ***S.*** Typhi chronic carriers. Here, we report a similar approach to identify bacterial antigens expressed in humans with ***S***. Paratyphi A isolated from cholecystectomy specimens in Kathmandu, Nepal. In brief, we pooled sera from ***S.*** Paratyphi A carriers and adsorbed it against ***in vitro***-grown ***Escherichia coli.*** We then used this sera to screen a genomic inducible expression library of ***S.*** Paratyphi A (500-1500 bp fragments) in ***E. coli*** BL21DE3. We identified 70 clones (representing 133 genes of interest) that were reactive with the sera from paratyphoid A carriers but not against sera from Bangladeshi healthy controls or patients convalescing from acute paratyphoid A infection. Thus far, we have subcloned 98 of the 133 genes of interest, and identified 48 proteins with higher immunoreactivity in chronic paratyphoid A carriers compared to healthy individuals from a typhoid endemic area (Dhaka, Bangladesh). Many of the genes encode proteins involved in carbohydrate transport/metabolism and antimicrobial peptide resistance, whereas others encode uncharacterized proteins which may play an important role in surviving in the nutrient-limited biliary environment or in the formation of biofilms. Further assessment of these proteins may lead to the discovery of diagnostic biomarkers of ***S.*** Paratyphi A carriers, and may lead to improved understanding of the survival adaptations of ***Salmonella*** in biliary tissue.

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CHARACTERIZATION BY PCR-RFLP OF STRAINS OF *CAMPYLOBACTER JEJUNI* IN CHILDREN FROM 0- 5 YEARS WITH DIARRHEA IN THE PERUVIAN AMAZON

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Campylobacter is one of the most common causes of bacterial gastroenteritis in children living in developing countries. To date, 18 species of ***Campylobacter*** have been described, with C. jejuni being the most common cause of gastrointestinal infections in most settings. Molecular biology provides a wide variety of techniques for genotypic sub–typing of ***Campylobacter*** spp. The use of the polymerase chain reaction (PCR)–restriction fragment length polymorphisms (RFLP) technique allows for the higher resolution of pathogen identification to assist in source tracing and outbreak investigation. Application of the PCR–RFLP assay of Fla A gen was performed to determine the genetic diversity of ***Campylobacter*** spp. found circulating in the Amazonian jungle community of Santa Clara, Peru between 2002 to 2006 in stool samples of children from 0–5 years of age.***Campylobacter***–specific restriction fragment length polymorphisms (RFLP) were described in asymptomatic, dysenteric and non–dysenteric diarrhea stools. Specific RFLP patterns showed a high level of diversity among isolates and specific RFLP patterns were highly associated with the having clinical dysentery, as opposed to watery diarrhea or no diarrhea. The utility and feasibility of the realization of molecular techniques in this remote region at low cost demonstrates that this test to be incorporated in epidemiological studies to improve the understanding of disease transmission of ***Campylobacter*** in children under five in endemic areas.

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ANTIBIOTIC RESISTANCE IN *CAMPYLOBACTER* AND *SHIGELLA* SPECIES ISOLATED FROM AMAZONIAN CHILDREN UNDER FIVE YEARS BETWEEN 2010-2014

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Campylobacter and ***Shigella*** species are common cause of diarrhea in children from developing countries. Due to the emergence of antimicrobial resistance in these species, therapeutic options have dramatically reduced causing considerable morbidity and mortality in children from developing countries. In this study, we cultured 12,304 stool samples and analyzed the antibiotic resistance profiles of 875 ***Campylobacter*** and 218 ***Shigella*** species isolated between 2010-14 from children under five years old near the Peruvian Amazon city of Iquitos. Fecal samples were cultured on standard growth media plates for bacterial identification and antibiotic susceptibility determined on ***Campylobacter*** and ***Shigella*** isolates by disk diffusion. Of the ***Campylobacter*** isolates, C. jejuni was most prevalent (64%) followed by C. coli (34%), whereas S. flexneri was most prevalent (69%) ***Shigella*** spp. isolate followed by S. sonnei (20%), S. boydii (10%) and S. dysenteriae (1%). Of the ***Campylobacter*** isolates, 84% were resistant to trimethoprim/sulfamethoxazole, followed by ciprofloxacin (77%), nalidixic acid (67%), ampicillin (53%), tetracycline (50%) and 12% to azithromycin and erythromycin each. Of all tested ***Shigella*** species, the highest resistance rate was found against tetracycline (89.5%), followed by trimethoprim/sulfamethoxazole (86.2%), ampicillin (76%), erythromycin

(72.5%). Only 5% of ***Shigella*** species were resistant to azithromycin, while 16.5% exhibited intermediate resistance profiles. Similarly, nalidixic acid resistance in ***Shigella*** was detected in 3% of the isolates and 11% showed intermediate resistance, with none of the isolates resistant to ciprofloxacin (0%) and only 0.9% demonstrating intermediate resistance. These high antibiotic resistance rates in ***Campylobacter*** and ***Shigella*** represent a serious public health concern for children living in the Amazon, especially in remote regions where trimethoprim/sulfamethoxazole remains the first line of therapy against dysentery. Based on these results, strategies for treatment of diarrheal diseases in this region should be adjusted to reflect emergence of resistance.

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RECOMBINASE POLYMERASE AMPLIFICATION AS A DIAGNOSTIC FOR TOXIN PRODUCING *CLOSTRIDIUM DIFFICILE* IN POINT-OF-CARE SETTINGS

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Clostridium difficile Infection (CDI) has become a significant global health concern over recent years. With the emergence of highly virulent strains and continuing spread throughout the world, innovations in CDI detection and treatment are severely needed. The most accurate current methods for diagnosis are costly and use equipment not currently available in clinical labs worldwide. Recombinase Polymerase Amplification (RPA) is an isothermal method for DNA amplification. Unlike PCR, the reagents for RPA are thermostable and do not require expensive thermal cyclers for amplification; which allows the entire RPA reaction to be ran on the bench top at point-of-care facilities. The detection of the resulting amplicon can be made using the novel process of lateral flow analyses (LF). This detection method requires a very short amount of time and is graded using the naked eye, advancing the value of this method. The goal of this project is to utilize our RPA-Lateral Flow (RPA-LF) protocol to detect ***C. difficile*** in DNA samples and to further identify if the sample contained DNA coding for toxin A or B, a critical diagnostic determination. We accomplished this by designing specific primers for the toxin A and B genomes which were then paired with a specific genetic probe that allows for detection via the lateral flow strips. By the RPA-LF method, we have detected 10^3 toxin A producing bacteria and 10^4 toxin B producing bacteria. Further exploration has demonstrated the specificity of RPA when testing ***C. difficile*** samples alongside other enteric pathogens; ***C. difficile*** was positively identified while non-***C. difficile*** samples were continuously negative. The RPA-LF protocol was successfully utilized to identify the presence of ***C. difficile*** in the stools of infected mice. RPA-LF is currently being tested against PCR results from human stool samples. If successful, this data will further strengthen the potential of RPA-LF as a future, vital point-of-care diagnostic. Induction of this protocol as an acceptable method for CDI detection would also aid in the advancement of a global monitoring system for the spread of ***C. difficile***.

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REDUCTION IN DIARRHEAL RATES THROUGH INTERVENTIONS THAT PREVENT UNNECESSARY ANTIBIOTIC EXPOSURE EARLY IN LIFE

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Antibiotic treatment of common childhood illnesses, such as acute gastroenteritis and upper respiratory infections, is generally not indicated. Antibiotic exposure before 6 months of age has also recently been associated with increased rates of subsequent diarrhea. However, because the treatment of some illnesses with antibiotics is necessary, we cannot prevent all antibiotic exposures early in life. Here, we estimated the impact of realistic interventions that would prevent only unnecessary antibiotic exposures on childhood diarrheal rates. In data from a prospective observational cohort study conducted in Vellore, India, we used the parametric g-formula to model diarrheal incidence rate differences contrasting the observed incidence of diarrhea to the incidence expected under hypothetical interventions. The interventions prevented antibiotic treatments for non-bloody diarrhea, vomiting, and upper respiratory infections before 6 months of age. More than half of all antibiotic exposures before 6 months (58.9%) were likely unnecessary. The incidence rate difference associated with removing unnecessary antibiotic use before 6 months of age was -0.28 (95% confidence interval: -0.47, -0.11) episodes per 30 child-months. This implies that preventing unnecessary antibiotic exposures in just 4 children would reduce the incidence of diarrhea by one from 6 months to 3 years of age. When targeted only to children who had stopped exclusive breastfeeding, the impact of the interventions was smaller because many antibiotic exposures occurred during exclusive breastfeeding. These results suggest that a general intervention applied to all children before 6 months of age would be most effective. This work provides an example application of statistical methods which can further the aim of presenting epidemiologic findings that are relevant to public health practice. Interventions to reduce unnecessary antibiotic use among young children could result in an important reduction in diarrheal rates.

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A CONJUGATE VACCINE FOR CHOLERA CONTAINING THE O-SPECIFIC POLYSACCHARIDE (OSP) OF *VIBRIO CHOLERAE* O1 INABA AND A RECOMBINANT FRAGMENT OF TETANUS TOXIN HEAVY CHAIN (OSP:RTTHC) INDUCES SERUM, MEMORY AND LAMINA PROPRIAL RESPONSES AGAINST OSP, AND PROTECTION AGAINST WILD TYPE CHOLERA CHALLENGE IN MICE

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Vibrio cholerae is the cause of cholera, a severe watery diarrhea. Protection against cholera is serogroup specific. Serogroup specificity

is defined by the O-specific polysaccharide (OSP) component of lipopolysaccharide (LPS). Here we describe a conjugate vaccine for cholera prepared via squaric acid chemistry from the OSP of V. cholerae O1 Inaba strain PIC018 and a recombinant heavy chain fragment of tetanus toxin (OSP:rTTHc). Immunized mice developed prominent anti-OSP and anti-TT serum IgG responses, as well as vibriocidal antibody and memory B cell responses following intramuscular or intradermal vaccination. Mice did not develop anti-squarate responses. Intestinal lamina proprial IgA responses targeting OSP occurred following intradermal vaccination. We assessed a range of vaccine doses based on the OSP content of the vaccine (10-50 µg), and vaccine compositions varying by molar loading ratio of OSP to rTTHc (3:1, 5:1, 10:1). In general, we found comparable immune responses in mice immunized with these variations, although memory B cell and vibriocidal responses were blunted in mice receiving the highest dose of vaccine (50 µg). We found no appreciable change in immune responses when the conjugate vaccine was administered in the presence or absence of immunoadjuvant alum. Administration of OSP:rTTHc resulted in 55% protective efficacy in a mouse survival cholera challenge model. Development of an effective cholera conjugate vaccine that induces high level and long-term immune responses against OSP would be beneficial, especially in young children who respond poorly to polysaccharide antigens.

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HIGHLIGHTING THE ESSENTIAL SECONDARY METABOLITES OF THREE OYSTER MUSHROOMS RESPONSIBLE FOR ANTIMICROBIAL ACTIVITIES AGAINST MULTIDRUG RESISTANT *SHIGELLA* SPP.

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Growing resistance to available antibiotics is becoming a serious issue now and thus exploring all natural resources to find an effective antimicrobial agent is of paramount importance. Shigellosis, a highly prevalent diarrheal disease in developing countries is presently associated with multidrug resistance (85%) ***Shigella*** isolates having higher MIC values against commonly used antimicrobials, isolated from fresh stools and rectal swab samples of infected persons. Although antimicrobial activities of edible mushrooms are well known against common pathogens like ***Staphylococcus***, ***E. coli*** etc. the antimicrobial activities of edible mushrooms against ***Shigella*** spp. is largely unknown. Thus the present study was aimed to evaluate the ***in-vitro*** antibacterial activities of ethanol and aqueous extracts (hot) of three oyster edible mushrooms namely ***Pleurotus ostreatus, Pleurotus eous*** and ***Pleurotus florida*** against ***Shigella flexneri*** type 4a, ***Shigella sonnei***, ***Shigella boydii*** and multidrug resistant ***Shigella flexneri*** type 2a. Methodologies adopted were agar disc diffusion assay and minimum inhibitory concentration (MIC) assay along with phytochemical screening assay (PSA) was performed for the detection and estimation of specific antimicrobial components. All the ethanol based crude mushroom extracts showed inhibitory activities against all the ***Shigella*** spp. especially upon the multidrug resistant strain. Presence of essential secondary metabolites such as phenol, flavonoid, terpenoid, steroid and saponin were confirmed in the tested mushrooms. Significant difference (ANOVA, P value < 0.0001) in the phenol and flavonoid content of the mushrooms were correlated for their differences in antimicrobial activities. Therefore, this study revealed anti-shigellosis potency of edible mushrooms highlighting specific essential secondary metabolites of them which may be responsible for such activities.

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AGGREGATIVE ADHERENCE AND INTESTINAL COLONIZATION BY ENTEROAGGREGATIVE *ESCHERICHIA COLI* ARE PRODUCED BY INTERACTIONS AMONG MULTIPLE SURFACE FACTORS

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Enteroaggregative ***Escherichia coli*** (EAEC) are important diarrheal pathogens worldwide, and particularly in developing countries. EAEC are exceptional colonizers that are defined by the characteristic stacked-brick pattern they produce on epithelial cells. This defining phenotype is convergent. Strains exhibiting it typically express one of several varieties of Aggregative Adherence Fimbriae (AAF). Research in our laboratory has revealed that a non-structural adhesin, the integral outer membrane Heat-Resistant Agglutinin 1 (Hra1), is sufficient to produce aggregative adherence and is encoded on many EAEC chromosomes. Hra1, like AAF, confers autoaggregation and biofilm formation ***in vitro***. An hra1 mutant of EAEC strain 042 adheres but is deficient in true stacked-brick formation and ***in vivo*** colonization but shows no defects in the ***in vitro*** phenotypes. We hypothesized that Hra1 is sterically masked by one or more other surface factors and unveiled only when required for host colonization. Physically or genetically removing fimbriae from EAEC strain 042 reveals that the AAF/II fimbriae do not mask Hra1. By contrast, deletion of the gene encoding a secreted antiaggregation protein (Aap) resulted in enhanced ***in vitro*** colonization-associated phenotypes and ***in vivo*** clumping in a Caenorhabditis elegans colonization model. Hyper-autoaggregation by aap mutants was previously attributed to loss of Aap-AAF interactions. We demonstrate that enhanced autoaggregation and biofilm formation by aap mutants is unrelated to the presence of AAF/II but is Hra1-dependent. The data suggest that Aap masks Hra1 ***in vitro*** and that the aggregative adherence phenotype is a complex one mediated by multiple surface factors.

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CARRIAGE OF NASAL *STAPHYLOCOCCUS AUREUS* AND RHINOVIRUSES AMONG HEALTHY INDIVIDUALS IN THREE RURAL AREAS OF GHANA

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Colonisation of the nares with ***Staphylococcus aureus*** is known to be associated with skin and soft tissue infections. Data on its occurrence is however limited in many developing countries including Ghana. This study therefore sought to describe the burden of Staphylococcus aureus, risk factors for infection and co-colonisation with rhinoviruses. We conducted a cross-sectional study among healthy individuals in three rural areas of Ghana. Nasal swabs were collected from study participants and tested for the presence of methicillin resistant Staphylococcus aureus (MRSA), methicillin susceptible ***Staphylococcus aureus*** (MSSA) and coagulase negative staphylococci (CoNS) bacteria using conventional bacteriological techniques. Nasopharyngeal swabs were also collected and tested for the presence of rhinoviruses using Reverse Transcriptase Real-Time Polymerase Chain Reaction. Staphylococci bacteria were identified in 91 (25.4%; 95% CI = 21% - 30.3%) out of the 358 study subjects enrolled. Of all bacteria isolated, 51 (56.0%) were MSSA, 32 (35.2%) were CoNS, 6 (6.6%) were MRSA and 2 (2.2%) were methicillin resistant coagulase negative staphylococci (MR-CoNS). The overall prevalence of MSSA was 14.2% (95% CI; 10.8% - 18.3%) and that of MRSA was 1.7% (95% CI; 0.6% - 3.6%). Of the total 205 samples, 78 (38.0%; 95% CI = 31.4% - 45.1%) tested positive for rhinoviruses. Nine (4%) were positive for both MSSA and rhinoviruses while one (1) was positive each for MRSA and MR-

CoNS. There was no association between human rhinovirus detection and MSSA (p = 0.52) or MRSA colonisation. In conclusion, the present study has further corroborated other findings that MSSA and MRSA are still significant reservoirs of human nasopharynx in rural areas of Ghana. There is a need to look at the disease transmission dynamics and a further strong public health education on practices that reduce the transmission of these pathogens within communities.

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IDENTIFICATION OF NEW ANTIGEN CANDIDATES OF *BARTONELLA BACILLIFORMIS*

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Bartonella bacilliformis is a fastidious Gram-negative bacterium associated with Carrion disease, a neglected illness endemic in Peru. In the acute phase of the disease, ***B. bacilliformis*** invades erythrocytes and mortality rates are 44-88% in absence of adequate treatment. A relevant problem is the lack of an effective diagnostic to overcome misdiagnosis and treat asymptomatic carriers (about 45% of people living in endemic areas). The objective of this study was to identify new ***B. bacilliformis*** antigenic candidates that could lead to a new diagnostic tool able to be implemented in rural areas. Serum samples from 177 people were collected in 5 different localities of northern Peru (in 4 of them an outbreak occurred few months earlier and the another one is an endemic region). Clinical data were recorded and ELISA for IgM / IgG with whole cell as antigen was done. After sonication, total bacteria were separated via gel electrophoresis and electrotransferred onto a PVDF membrane. Seroreactive antigens were detected by Western blot analysis with each serum both for IgG and IgM. The candidate proteins detected were cut out and N-terminal amino acid sequencing was performed. The presence of at least one symptom compatible with Carrion disease was reported by 34.5%. After Western blot analysis and taking into account the ELISA levels obtained, four proteins were considered potential antigenic candidates, two detected by IgM and two by IgG. The amino acid sequencing identified Pap31 and GroEl, already described in the literature but with no optimal results, and two new antigenic candidates (both subunits of the same protein). One has 30.1 kDa and was detected with IgM while another has 42.75 kDa and was detected with IgG. These new antigenic candidates are involved in the tricarboxylic acid cycle and one was recently described as being able to play a role in the invasion process and in the pathogenesis of other Bartonella spp. infection. The fact that these new antigens were identified with these sera highlights their possible usefulness in the development of a rapid diagnostic tool.

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PRECLINICAL VALIDATION OF ANTI-BURULI ULCER PLANTS USED IN TRADITIONAL MEDICINE

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Buruli ulcer (BU) is the third most prevalent mycobacteriosis, after tuberculosis and leprosy. West Africa bears more than 90% of the disease burden and mainly in remote rural areas. The recommended drug combination Rifampicin-Streptomycin is not affordable for the majority of affected populations who thereby rely mainly on herbal remedies. The systematic investigation of ethnobotanical facts is a reasonable first step to unveiling more efficacious remedies as affordable and more readily accessible treatment for BU locally. In the aim of validating the use of 15 traditional plant remedies used by the rural population for the treatment of BU, their biological activity and safety were assessed. The identification of the 15 plants used in traditional medicine to manage BU was achieved through a short survey based on ethnobotanical reports. Maceration of plants samples resulted in 18 hydroethanolic extracts. The activity of extracts against ***Mycobacterium ulcerans*** NM2O9 was tested using the Resazurin Microtiter Assay. The safety of promising extracts' fractions was assayed on WRL68 human hepatocyte cell line by Resazurin reduction assay. Generally, the selected plants are locally used as decoction, infusion and maceration and are taken orally or applied on the ulcerated lesions. Six extracts from 5 plants demonstrated activity with Minimum inhibitory concentrations (MIC) between 16.12-31.25µg/mL. ***Mangifera indica*** root and leaf, ***Azadirachta indica*** stembark, ***Vernonia amygdalina*** leaf, ***Alchornea cordifolia*** leaf and ***Spathodea campanulata*** root showed the lowest MIC value of 16.12µg/mL, while that from ***Zanthoxylum zanthoxyloides*** root showed the highest MIC value of 31.25µg/mL. Apart from ***V. amygdalina*** extract with CC_{50} value of 10.27µg/mL, promising extracts were not cytotoxic (CC_{50}≥40.9µg/mL) according to the American National Institute for Cancer criterion (CC_{50}<30µg/mL). These results support the traditional use of the 5 promising plants in the treatment of BU. However, detailed studies are required to unveil the active ingredients in the lead extracts and elucidate their mechanisms of action for further anti-BU drug development.

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'A SOCIAL KILLER': LEPROSY, STIGMA, AND THE HISTORY OF THE CONTROL OF A NEGLECTED DISEASE IN CAMEROON, 1916-1974

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Just as disease eradication has social benefits, a highly stigmatized disease such as leprosy can lead to the social death of its sufferers as well as affect control measures. I assume that leprosy is both a 'social epidemic' and a 'social killer,' as the illness of an individual affects their immediate and whole community, and defines their personhood and social relations within their given social ecumenes. Studies have zoomed in on the degree of stigma that characterizes neglected tropical diseases (NTDs) such as leprosy. However, equal measure of attention has not been accorded the association of stigma and the control of NTDs from a historical perspective. This research aims to highlight the dynamic interrelationship between disease, medicine and society through history, building on the conjoined phenomena of tropical disease and tropical medicine. I argue that disease outbreaks and interventions can illuminate divisions within a society as they affect different groups of people differently. Using essentially primary data (archival and oral sources), it maps out the institutionalization and contours of stigma, marginality, and social change, and how those can be understood within the broader social, economic, and political forces that animated developments in Cameroon. The study also focuses on how the disease undermined the integrity of the body of leprosy patients, and how colonialism and political change transformed leprosy into a 'stigmatized phenomenon' in spite efforts to 'destigmatized' it in the various leprosy institutions in colonial and postcolonial Cameroon. Results from this study will inform us on the checkered history of global health and the control of NTDs, and how the problem of stigma has animated the cultural and social issues in the epicenters of leprosy in Cameroon violating racial, social, economic, and political boundaries.

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ANTIBACTERIAL PROPERTIES OF EXTRACTS FROM *PSIDIUM GUAJAVA* (L) AGAINST MULTIDRUG RESISTANT *STAPHYLOCOCCUS AUREUS*

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Pathogenic ***Staphylococcus aureus*** causes infections such as septicemia, pneumonia and renal abscess. It produces extracellular enzymes and heat stable enterotoxins that can cause food poisoning. Multi-drug resistance (MDR) in ***S. aureus*** is a major public health concern and methicillin resistant ***S. aureus*** (MRSA) remains a global health threat. It is difficult to successfully treat infections caused by such resistant strains since treatment options are limited. Medicinal plants have played important roles in drug discovery resulting in successful treatment of many diseases. ***Psidium guajava*** (apple guava) has been traditionally used to treat various diseases including malaria, gastroenteritis, diarrhea, coughs and sore throat. The study investigated the antibacterial activities of ***P. guajava*** leaves extracts against MDR-SA isolates identified and characterized from 5 major health facilities in Ghana. To ensure the viability of the 30 MDR-SA isolates, Mueller Hinton agar plates were spread with 50 µl of each isolate suspended in biological peptone followed by incubation at 37°C for 24 h. The antibacterial activities of the aqueous, 70% and absolute ethanolic crude extracts against the MDR-SA were investigated by the agar-well diffusion in duplicates and mean zones of inhibition recorded. The extracts showed effective antibacterial activities and in some cases inhibitions zone of 16, 14.5, 13.5, 11.5 and 10.5 mm were recorded for 200, 100, 50, 25, 12.5 mg/ml of the extract respectively. The 70% ethanolic extract appeared much more effective against the MDR-SA screened. The MIC value for each isolate was found to be considerably strong ranging from 1.56 to 6.25 mg/ml while the ATCC-25923 isolate had 3.13 mg/ml for both the extract and ciprofloxacin. The ***P. guajava*** extracts obtained may have broader antibacterial activities than the isolates tested. It is expected that the active phytochemical will the identified to develop more effective anti-MDR-SA agent that could be helpful in better management of infections associated with multi-drug resistance ***S. aureus***.

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MOLECULAR DETECTION OF GENES CONFERRING ANTIBIOTIC RESISTANCE IN UROPATHOGENIC *ESCHERICHIA COLI* STRAINS (UPECS) ISOLATED IN MEXICO

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The emergence of UPEC strains multiresistant to antibiotics is considered as a serious health concern. The aim of this work was to determine the frequency of genes conferring resistance to antibiotics commonly used in Mexico, and to other genes coding for extended spectrum betalactamases (ESBLs) in a total of 194 UPEC strains isolated from community-acquired urinary tract infection patients from Unidad Médica Familiar No. 64 (Mexican Institute for Social Security of Tlalnepantla, Edo. de México, México). The ***Escherichia coli*** strains were identified by biochemical tests and by PCR amplification of 16S rRNA gene. Genes coding antibiotic resistance and betalactamases were identified by single and multiplex PCR. Percentages of antibiotic-resistance-conferring genes among UPEC strains were as follows: 30.9% (n=60) carried the ***sul1*** gene (sulfonamide resistance); 33.5% (n=65) ***tetA*** (tetracycline resistance); 17% (n=33) ***tetB*** (tetracycline resistance); 12.3% (n=24) ***dfrA1***(trimethoprim resistance); 11.8% (n=23) ***cat1*** (chloramphenicol); 4.1% (n=8) ***cmlA*** (chloramphenicol); 2% (n=4) ***aadA1*** (streptomycin) and 0% carried ***aac(3)-IV*** (gentamycin) or ***qnr*** (quinolone). Frequencies ESBLs genes among UPEC strains were: bla_{TEM} 26.3% (n=51); 13.9% (n=27) bla_{SHV}; 23.1% (n=45) $bla_{OXA\text{-}1\ Like}$, 22.6% (n=44) $bla_{CTX\text{-}M}$ phylogenetic group 1; 0% $bla_{CTX\text{-}M}$ phylogenetic group 2; and 3% (n=6) $bla_{CTX\text{-}M}$ phylogenetic group 9. Finally, 8.7% (n=17) of the strains carried the $bla_{OXA\text{-}48}$ gene coding for carbapenem-resistant betalactamase. These results shows that antibiotic resistance is common among UPEC strains, and notably high to sulfonamide, tetracycline and to cephalosporins. These data may be useful to document that patterns of antibiotic resistance in UPECs vary with patient population and geographic region.

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RODENT RESERVOIRS AND ENVIRONMENTAL SOURCES OF *LEPTOSPIRA* ALONG THE TRANS-OCEANIC HIGHWAY IN THE SOUTHERN AMAZON BASIN OF PERU

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Rodents are the reservoir for numerous zoonotic pathogens, including ***Leptospira***, which are shed in the urine of infected animals. Transmission occurs by direct animal contact or through exposure to contaminated environmental sources such as stagnant water, exacerbated by flooding during rainy seasons. Deforestation, agricultural expansion, and human settlements can perturb rodent habitats, potentially increasing contact with humans and transmission of ***Leptospira***. Such perturbations have resulted from the construction of the trans-oceanic highway though the Madre de Dios Region in the southern Peruvian Amazon, increasing the potential for zoonotic infections among residents in newly established communities along the highway. We set out to determine the prevalence of ***Leptospira*** in rodent populations and in the environment along the highway in Madre de Dios. Urine and tissue samples from captured wild rodents, surface water samples, and soil were collected from 6 different locations in 4 communities along the highway, each with varying levels of habitat perturbation, during both dry and rainy seasons. Pathogenic ***Leptospira*** were detected by amplification of the ***lipL32*** gene by PCR. During the dry season, 21 environmental samples were collected from non-disturbed areas (3), border areas (7), disturbed areas (4), and from locations within the communities (7). Thirty-eight samples were collected during the rainy season, including non-disturbed areas (8), border areas (12), disturbed areas (6), and from locations within the communities (12). To date, 2/21 (9.5%) environmental samples from the dry season and 8/38 (21%) samples from the rainy season were PCR positive. Testing is underway on 136 rodent kidney and urine samples, as well as phylogenetic analysis of the 16S rRNA gene sequence to characterize the ***Leptospira*** species diversity in both rodent and environment samples. Our data are consistent with a higher prevalence of ***Leptospira*** in the environment during the rainy season and provides valuable data on the species circulating in the southern Amazon Basin.

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INJECTIONAL ANTHRAX: AN EMERGING GLOBAL PUBLIC HEALTH THREAT

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Anthrax (caused by ***Bacillus anthracis***) traditionally has been identified through three routes of exposure: cutaneous, gastrointestinal or inhalation. With the numbers of individuals using illicit drugs steadily rising since the turn of the 21st century, particularly in Europe where intravenous drug use is 4.6 times the global average, a new form of infection has cropped up. Coined injectional anthrax, contaminated heroin is administered into the individual whom proceeds to suffer from severe soft tissue infection, symptoms similar to cutaneous exposure, septic shock,

and death. The goal of this presentation is to examine existing data on injectional anthrax and to provide a detailed synopsis over the last fifteen years for physicians and government officials. Clearly, injectional anthrax represents an emerging infectious disease of public health importance.

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RECOMBINANT TETANUS TOXIN FRAGMENT C DIPSTICK: A RAPID, COST-EFFECTIVE ASSAY FOR MEASURING VACCINE EFFICACY

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Tetanus vaccination efforts in developing countries require population-level monitoring to assess post-vaccination protective immunity. Tetanus toxin-based ELISAs are commonly used, but require venous whole blood, time, laboratory equipment, and training. Expression and purification of native tetanus toxin is a complex, inconsistent process, leading to added cost and variable performance with clinical specimens. We describe development of a single-use immunochromatographic test ("dipstick") for the rapid evaluation of protective immunity to tetanus toxin. The assay uses purified recombinant tetanus toxin Fragment C (rFragC) as a surrogate marker for native tetanus toxin. rFragC production yields >50mg/L of culture with >99% purity by Coomassie staining of SDS-PAGE gel fraction. The dipstick was compared to a commercial ELISA ("TBS," The Binding Site) using human 1) plasma from a vaccine-defined cohort in Dhaka, Bangladesh (ICDDR,B), 2) plasma from freshly collected US panels of random volunteers (Bioreclamation IVT), and 3) whole blood from freshly collected US panels of random volunteers (Bioreclamation IVT). Dipsticks were read visually using a scoring card and quantitatively using the ESE Quant lateral flow reader (Qiagen). TBS ELISA values ≥0.1 IU/mL were considered positive for a protective anti-tetanus toxin titer, while TBS ELISA values <0.1 IU/mL were considered negative. Plasma dipsticks from the Bengali panel (n=40, 38 positive, 2 negative on TBS ELISA) had a clinical correlation of 100%, and quantitative comparison yielded a bivariate fit line with R^2=0.51, p<0.001. Plasma dipsticks from the US panel (n=158, all positive on TBS ELISA) had a clinical correlation of 99%; bivariate fit line with R^2=0.64, p<0.001. Whole blood dipsticks from the US panel (n=18, all positive on TBS ELISA) had a clinical correlation of 100%; bivariate fit line with R^2=0.23, p=0.04. A future goal is to include more tetanus antibody-negative samples. The rFragC dipstick offers a simple, sensitive, inexpensive alternative to ELISAs for detecting tetanus antibody in plasma and whole blood at the point-of-care during vaccination programs.

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USEFULNESS OF MODIFIED SIMPLIFIED CHINESE INK TECHNIQUE FOR DETECTION (VISUALIZATION) OF THE CAPSULE OF BACTERIA, FUNGI AND PARASITES

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There are few reports on a technique to visualize the capsule of bacteria, fungi and parasites. Here we present a technique to visualize the capsule of bacteria: ***Bacillus anthracis***, pneumococcus; fungi: ***Cryptococcus***; and parasites: ***Blastocystis*** in clinical specimens and cultures. We worked with clinical specimens and cultures of the collection of the microbiology department of the National Institute of Child Health, strains of ***Bacillus anthracis*** collection as well as ***Cryptococcus neoformans***; fecal culture and ***Blastocystis spp.*** samples. To display the capsule, we used the Modified simplified Chinese ink technique, and recorded images in photomicrographs. Direct examination with this technique shows the capsule of those microorganisms, some with intracellular colors and details ***Cryptococcvus neoformans*** and ***Bacillus anthracis***, also in ***Blastocystis spp.*** Consequently, the modified simplified Chinese ink technique allows visualizing the capsule of bacteria, fungi and parasites, which is useful for laboratory diagnosis, teaching and research.

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PREVALENCE OF NEONATAL TETANUS IN NORTHEASTERN NIGERIA

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Although efforts have been made towards improving the health of children across the globe with notable results, neonatal tetanus (NNT) remains a major contributor to the neonatal death rates in Nigeria. This problem calls for a concerted effort by the government to achieve the revised global NNT elimination deadline of 2015. The purpose of this cross-sectional quantitative study using secondary data was to establish the prevalence of NNT in Nigeria's northeast region and to ascertain if there was any significant difference in frequency of antenatal care (ANC), trained traditional birth attendants (TBAs), and umbilical cord treatments, using single sample proportions test and chi-squared tests of independence. The framework for this research was the theory of planned behavior. The participants (N = 312) were mothers of NNT babies. In spite a continual decline in the NNT cases between 2010 (26%) and 2013 (9%), the prevalence rate of NNT was unacceptably high at 28.815%. Also, significant differences existed as mothers who gave birth to NNT babies received significantly fewer or no ANC ($p < 0.001$), received significantly fewer or no attention from TBAs ($p < 0.001$), and reported significantly fewer incidences of proper umbilical cord treatments ($p < 0.001$). The chi-squared tests of independence resulted in significant differences in the frequencies of mothers who received ANC between Nigerian provinces ($p < 0.001$) and mothers who had their baby's umbilical cord treated ($p = 0.005$). This study will contribute to social change by guiding health care policy makers and immunization program managers on maternal and newborn health care services and indicate ways to build capacity of the TBAs for safe home delivery/hygienic handling of umbilical cord of newborns.

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GROUP B STREPTOCOCCUS IN THE GAMBIA - TWENTY YEARS ON

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A report from 20 years ago indicated that GBS genital colonization in Gambian mothers was reportedly predominantly due to serotype-V. However, tThe trivalent capsular polysaccharide conjugate vaccine currently in phase III trials includes only serotypes Ia, Ib and III. Here We therefore aimed towe define the current epidemiology of GBS in Gambian mothers and babies. Rectovaginal swabs from Gambian mothers and nasopharyngeal and rectal swabs from their infants were collected in a prospective cohort study. Swabs were pre-cultured in Todd Hewitt Broth (THB), followed by culture on selective agar. Culture nNegative samples were analysed for the presence of DNA via real-time PCR. Positive isolates were serotyped using Conventional multiplex PCR and gel-agarose electrophoresis. 750 women/infant pairs were recruited to the study. 270 women (36%) were found to be GBS-colonized (260 by culture alone, 10 by culture and PCR). 134 infants were colonized (25%) at birth and all but one remained colonized at six days. By three months, 44 infants remained colonized (6%) and 12 infants were newly colonized (2%). The predominant serotypes were: serotypes V (40%), II (28%), Ib (20%), Ia (10%) and III (2%). 12 colonized infants were treated for presumed neonatal sepsis and 4 for presumed meningitis. Blood cultures were positive for GBS (serotype-V) in one case, equivalent to 1.4/1000 live-

births. In conclusion, the serotype distribution among colonizing GBS strains in the Gambia remains unchanged over the last twenty years with serotype V predominating. Knowledge of the current serotype prevalence in regions such as the Gambia is vital to ensure vaccine development matches regional requirements to maximize its impact in these settings.

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PHYLOGENETIC VARIANTS OF *RICKETTSIA AFRICAE*, AND INCIDENTAL IDENTIFICATION OF "*CANDIDATUS RICKETTSIA MOYALENSIS*"

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Rickettsia africae, the etiological agent of African tick bite fever is widely distributed in sub-Saharan Africa. Contrary to reports of its homogeneity, a localized study in Kenya reported high genetic diversity. In the present study, gene concatenation phylogeny of gltA, ompA, ompB, 17kDa and sca4 genes was used to re-analyse ***R. africae*** samples from diverse regions of Kenya that had been collected in a previously reported study. The bona fide ***R. africae*** isolates formed two distinct clades. Clade I isolates (98%) branched with the validated ***R. africae*** str ESF-5, while clade II (two isolates) formed a distinct sublineage of clade 1. Some isolates were determined to be ***R. aeschlimanii*** and not ***R. africae***. One isolate turned out to be a novel rickettsiae and an interim name of "Candidatus Rickettsia Moyalensis" is proposed. In conclusion, this data supports the use of multilocus gene concatenation as opposed to individual gene trees for phylogenetic inferences. It is determined that, though only recently emerged, ***R. africae*** lineage is diverse.

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ANALYSIS OF NEONATAL SEPSIS IN KUMASI, GHANA THROUGH PAPER-BASED MEDICAL RECORDS

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Reducing infant mortality is Millennium Development Goal 4 in Ghana, though it has remained high and unchanged. Improving care and reducing health complications related to infections and respiratory distress has shown to decrease the prevalence of neonatal deaths and preterm births. The objective of this study is to present data originating from an electronic medical records (EMR) pilot project and communicate the results of an analysis of neonatal sepsis using health information from paper-based medical records to identify characteristics of the neonatal patients at the Komfo Anokye Teaching Hospital in Kumasi, Ghana. Medical records from the Mother Baby Ward (n=198) from 2009-2014 were processed using scanners connected to laptop computers. Health information was manually extracted and verified by two researchers for quality assurance purposes and included sepsis, respiratory distress, cough, difficulty feeding, lethargy, seizures, jaundice, birth history, birth maturity and birth location. Regression analysis revealed a significant association between sepsis and birth location (p=0.0180, 95% CI) as well as sepsis and jaundice (p=0.0446, 95% CI). A descriptive profile of the population revealed that 63.6% of infants comprised of 97 males and 101 females were differentially diagnosed as having sepsis. There were 21 (10%) twins observed. Of the 198 cases included in the analysis, there were 127 (64%) full-term births, 105 (53%) cases reporting respiratory distress and 93 (46%) reporting jaundice. The process of manually scanning and converting paper-based medical records to later use for manual data extraction provides a safe and secure way to evaluate health information related to infant and neonate health.

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GROUP B STREPTOCOCCUS COLONIZATION AMONG PREGNANT WOMEN IN LUBUMBASHI, DRC, 2015

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Globally, invasive bacterial infections account for nearly a quarter of neonatal mortality. In the US, group B streptococcus (GBS) is the leading cause of early-onset neonatal sepsis, even after the introduction of routine prenatal screening and intrapartum antibiotic prophylaxis to prevent transmission from colonized women to their infants. GBS colonization is also associated with risk of preterm or stillbirth, and the risk and severity of invasive GBS disease are higher in infants born before term. In sub-Saharan Africa, there are few estimates of perinatal GBS colonization or disease, and none at all from the Democratic Republic of the Congo (DRC). As a first step to assessing the contribution of GBS to DRC's high neonatal mortality rate, we will conduct a cross-sectional study of GBS colonization in pregnant women attending antenatal care in Lubumbashi, Upper Katanga Province, DRC. We will report the prevalence of GBS colonization in these women, the antimicrobial susceptibility of isolates, and the feasibility of intrapartum antibiotic prophylaxis administration in the setting of a referral hospital. We believe these results will be important to estimate the role of GBS in perinatal mortality in the DRC, the potential impact of a maternal vaccine, and the feasibility of studies to evaluate non-antibiotic prophylaxis measures that might be introduced while awaiting vaccine development, approval, and introduction.

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THE EMERGENCE OF ESBL *SALMONELLA TYPHIMURIUM* EXPRESSING BLA CTX-M-15 IN MOZAMBIQUE

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We observed several cases of bacteremia due to Extended Spectrum Beta-Lactamase producing (ESBL) ***Salmonella typhimurium*** at an Urban Mozambican hospital. We sought to identify antibiotic resistance genes in these strains, and phylogenetically characterize them in the context of other African and non-African strains, including ST313 ***S. typhimurium***, the dominant lineage causing epidemic invasive disease in sub-Saharan Africa. ***S. typhimurium*** strains were isolated from the blood of adults on the wards of Maputo Central Hospital in Maputo, Mozambique between 2011 and 2013. Older isolates were obtained from the blood of subjects in a cohort of HIV+ adults in Entebbe, Uganda from 1995-97. All subjects were HIV+ with a mean CD4 count of 150 cells/ml. Isolates typed as ***S. typhimurium*** using the Kaufman-White scheme, and confirmed with PCR. Antibiotic susceptibility was tested with the Kirby-Bauer disc diffusion method and confirmed with the automated Vitek 2 system. ESBL phenotype was confirmed with the double-disc diffusion method. Whole genome sequencing was done with the Ion Torrent PGM system. Genomes were assembled using D23580 ***S. typhimurium*** and virulence plasmid pSLT-BT as references, both of which are representative of epidemic invasive ST313 strains from sub-Saharan Africa. Chromosome and plasmid phylogenies were created based on variable sites. ESBL genes were identified with PCR and then sequenced. The phylogeny indicates that the Mozambique strains collected in 2011-2013 are descendants of Ugandan strains from 1995-1997. Non ESBL Mozambican strains are genetically similar to invasive epidemic strain D23580 (all separated by <0.02 substitutions/variable site; 100% bootstrap support), whereas the Mozambican ESBL isolates represent a distinct and a comparatively long branch of the tree (0.8 substitutions/variable site, 100% bootstrap support). All strains contain highly similar virulence plasmids with identical

Tn21- like elements containing antibiotic resistance genes, characteristic of epidemic lineage ST313. All ESBL strains contained identical ***bla***CTX-M-15 genes found on nearly identical 300 kb plasmids.

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EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE OF INVASIVE SALMONELLOSIS, RURAL THAILAND, 2006-2014

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Invasive salmonellosis commonly causes bloodstream infection in Southeast Asia. Limited epidemiologic and antimicrobial resistance data are available. We captured blood cultures performed in all 20 hospitals in Nakhon Phanom (NP) and Sa Kaeo (SK) provinces in a surveillance system. Cultures were performed as clinically indicated in hospitalized patients; patients with multiple cultures had only the first included. Salmonella isolates were identified at the serogroup level using serological testing. Antimicrobial resistance was assessed by disk diffusion and interpreted using 2015 CLSI guidelines. 522 invasive salmonellosis cases were identified (214 in NP and 308 in SK); 12% were ≤5 years old and 18% were ≥65 years old. Salmonella was the 5th most common pathogen (522/144,271 cultures). Overall incidence increased from 3.9/100,000 person-years in 2006 to 7.7 in 2008; from 2009-2014 the annual incidence ranged from 3.5-6.4. From 2006-2010, mean incidence was higher in SK than NP (4.3/100,000 vs. 7.7 p = 0.01). Overall, the most common serogroups were Group C (42%), Group D (35%), and Group B (9.8%). Group D was the most common serogroup in NP (44%), followed by C (18%). In SK, Group C was the most common (59%), followed by D (28%) and B (7%). Groups E and A were uncommon in both provinces. Serogroups were not identified for 21% of isolates in NP and 5% in SK. Antibiotic resistance was 67% (326/490) for ampicillin, 18% (89/499) for trimethoprim-sulfamethoxazole (TMP-SMX), 16% (79/504) for cefotaxime, and 2% (9/468) for ciprofloxacin. 56% had intermediate ciprofloxacin resistance. Group C had the highest proportion of isolates resistant to ampicillin (92%, n = 194), cefotaxime (37%, n = 79), and TMP-SMX (37%, n = 78). Group D had the highest proportion of isolates with intermediate resistance to ciprofloxacin (65%, n = 109). There were no temporal trends in antibiotic resistance. Bloodstream Salmonella infection in rural Thailand is commonly resistant to ampicillin, cefotaxime, and TMP-SMX. Intermediate resistance to ciprofloxacin is common. Serogroup distribution and antibiotic resistance may differ throughout Thailand and the region.

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SEROLOGIC EVIDENCE FOR THE GEOGRAPHIC DISTRIBUTION OF BACTERIAL ZOONOTIC AGENTS IN KENYA

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Diseases of zoonotic origin substantially contribute to the high burden of febrile illnesses in developing countries. We evaluated serologic evidence of previous exposure to Bacillus anthracis, Brucella spp., spotted fever group rickettsioses (SFGR) and typhus group rickettsioses (TGR) from HIV-negative samples of persons aged 15-64 years collected during a nationwide HIV serosurvey conducted in 2007 in Kenya. The national adjusted seroprevalence by pathogen was: Bacillus anthracis, 11.3% (141/1091); Brucella spp, 3.0% (27/968); SFGR, 23.3% (191/770); and TGR, 0.6 % (12/770). On bivariate analysis, positive titers to B. anthracis were only significantly associated with province of residence while sex, education level and wealth were significantly associated with positive titers to Brucella spp. Significant associations for SFGR seroprevalence included age, education level, wealth and province of residence while TGR was only significantly associated with province of residence. Wealth and province remained significantly associated with positive titers to B. anthracis on multivariate analysis while sex and age remained significant for Brucella spp. Significant associations for SFGR seroprevalence were sex, education level and province of residence on multivariate analysis while TGR had no significance. High IgG sero-prevalence to some of these zoonotic pathogens suggests that a large proportion of individuals have previous exposure, symptomatic or inapparent. Given that a substantial proportion of exposures to these pathogens result in illness, these pathogens should be considered in the differential diagnosis of febrile illness in Kenya.

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

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

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MAPPING BACTERIAL BLOODSTREAM INFECTIONS: A METAGENOMICS APPROACH

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Deep sequencing of the 16S rRNA gene has been widely used to profile environmental bacterial communities. Albeit extremely powerful, the

applicability of this technology has not been demonstrated yet for the characterization and diagnosis of bacterial sepsis. In this study we have developed and evaluated a 16S metagenomics approach to profile the bacterial diversity in the blood of febrile patients. Seventy-five children (median age 15 months) with severe febrile illness were recruited at St. Camille District Hospital in Nanoro, Burkina Faso, between January and April 2013. A laboratory diagnosis could be obtained on site for 12 bacterial sepsis cases with positive blood culture, and 41 malaria cases with positive thick blood films or positive malaria rapid diagnostic tests. A variable volume of whole blood was available for all patients (200 - 1000 µl) and was used for DNA extraction and subsequent amplification of the V3-V4 regions of the bacterial 16S rRNA genes. The resulting PCR products were deep sequenced on the Illumina MiSeq platform. Reads were curated using the mothur pipeline and taxonomy assigned using both homology and phylogenetic placement approaches. Bio-informatic pipeline validation and data analysis is currently ongoing. We will present the design of the metagenomics assay and the bacterial diversity identified in the bloodstream of the study participants.

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BLOODSTREAM BACTERIAL INFECTION AMONG OUTPATIENT CHILDREN WITH ACUTE FEBRILE ILLNESS IN NORTHEASTERN TANZANIA

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Fever is a common clinical symptom in children attending hospital outpatient clinics in rural Tanzania, yet there is still a paucity of data on the burden of bloodstream bacterial infection among these patients. The present study was conducted at Korogwe District Hospital in north-eastern Tanzania. Patients aged between 2 and 59 months with a history of fever or measured axillary temperature ≥ 37.5°C attending the outpatient clinic were screened for enrolment into the study. Blood culturing was performed using the BACTEC 9050® system. A biochemical analytical profile index and serological tests were used for identification and confirmation of bacterial isolates. In-vitro antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. The identification of ***Plasmodium falciparum*** malaria was performed by microscopy with Giemsa stained blood films. A total of 808 blood cultures were collected between January and October 2013. Bacterial growth was observed in 62/808 (7.7%) of the cultured samples. Pathogenic bacteria were identified in 26/808 (3.2%) cultures and the remaining 36/62 (58.1%) were classified as contaminants. Salmonella typhi was the predominant bacterial isolate detected in 17/26 (65.4%) patients of which 16/17 (94.1%) were from patients above 12 months of age. Streptococcus pneumoniae was the second leading bacterial isolate detected in 4/26 (15.4%) patients. A high proportion of Salmonella typhi 11/17 (64.7%) was isolated during the rainy season. Salmonella typhi isolates were susceptible to ciprofloxacin (n = 17/17, 100%) and ceftriaxone (n = 13/17, 76.5%) but resistant to chloramphenicol (n = 15/17, 88.2%). ***Plasmodium falciparum*** malaria was identified in 69/808 (8.5%) patients, none of whom had bacterial infection. Bloodstream bacterial infection was not found to be a common cause of fever in outpatient children; and Salmonella typhi was the predominant isolate. This study highlights the need for rational use of antimicrobial prescription in febrile paediatric outpatients presenting at healthcare facilities in rural Tanzania.

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COXIELLA BURNETII ANTIBODIES ARE PREDOMINANT AMONG PATIENTS WITH UNDIFFERENTIATED FEVER IN AFGHANISTAN

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Diagnosis of infectious diseases in Afghanistan remains a challenge with limited ability for pathogen isolation and identification. Baseline data on the prevalence of etiologies causing undifferentiated fever is lacking in Afghanistan. Herein we screened serum of Afghan patients suffering from undifferentiated fever for antibodies against number of pathogens, including ***Coxiella burnetii***, ***Leptospira*** spp.and typhoid fever. Patients > 5 years old with undifferentiated fever who meet the WHO case definition and presented at Kandahar provincial hospital (KDH), Helmand provincial hospital (LG) and a tertiary hospital in Kabul (KID) were enrolled and consented into a surveillance study between 2007 and 2012. A single serum sample was collected and tested by ELISA for the detection of IgM and IgG against Q fever ***(C.burnetii***, Panbio®), ***Leptospia*** spp. IgM (Panbio) and total immunoglobulins of ***Salmonella enterica*** serovar Typhi. A total of 566 patients were screened. Cases from KDH showed the highest frequency of ***C. burnetii*** antibodies (n=178, 36% IgG and 7.9 % IgM), followed by those from LG (n= 82, 23.2% IgG and 4.9% IgM) and KID (n= 303, 16.5% IgG and 1.3% IgM). ***Leptospia*** IgM was evident in 11.2% of patients, 12.9% in KID, 10.7 in KDH and 6.1 in LG. Typhoid fever titers >320 were found in11.2% of all patients, being higher in LG (15.9%) and KDH (12.9%) than KID (8.9%). Almost half of the ***C.burnetii*** IgM-positive cases (12/22) did not mount immune responses to other pathogens. The data suggest that both acute and past Q fever infections were evident within patients tested. The increased seropositivity rates in cases from KDH and LG provincial hospitals compared to those of KID in Kabul city may be attributed to limited sanitary measures in these areas. While typhoid fever is transmitted by ingestion of sewer polluted food and water, both Q fever and ***Leptospira*** are spread by contact with animals and their contaminated products or excreta. The obtained results provide initial disease burden information for Afghanistan and will be useful to health authorities in guiding hygiene improvement plans and disease prevention strategies.

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REVERSE-TRANSCRIPTASE PCR DETECTION OF *LEPTOSPIRA* IN RIO DE JANEIRO: POOR AGREEMENT WITH SINGLE-SPECIMEN MICROSCOPIC AGGLUTINATION TESTING

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Leptospirosis is a potentially fatal zoonotic disease caused by bacteria of the genus ***Leptospira***. In the Americas, Brazil reports the majority of cases, though incidence remains underestimated due to limitations in available diagnostics. The reference standard for the diagnosis of leptospirosis remains microscopic agglutination testing (MAT) on acute and convalescent serum. However, paired specimens are rarely sent for testing, and positive MAT results from a single specimen (titer ≥1:800) are often used to provide a presumptive diagnosis. The purpose of this study was to test serum samples from patients in Rio de Janeiro with a real-time reverse-transcriptase PCR (rRT-PCR) targeting the Leptospira 16S rrs gene and compare these results to detection with MAT. Improved analytical sensitivity of ***Leptospira*** detection was shown using rRT-PCR compared to optimized real-time PCRs with the same primers and probe. We then tested up to 55 archived serum samples per month from 2008, for a total

of 478 samples. Thirty-five (7.3%) samples tested positive by rRT-PCR with no clear seasonality in the percent of cases detected. Clinician-reported day of disease information was available for 282 samples (18 rRT-PCR positive). ***Leptospira*** RNA was detected in samples collected as late as day 30, and cycle thresholds did not vary based on the day of disease of sample collection. The percentage of positive samples also did not differ when samples were categorized as acute [≤ 7 days; 8/127 (6.3%)]; late acute [8 to ≤14 days; 6/69 (8.7%)]; or convalescent [>14 days; 4/86 (4.7%)]. Thirty-three (6.9%) samples tested positive by MAT using a regional panel of 19 Leptospira strains. Only three samples tested positive by both rRT-PCR and MAT. Of the 282 samples with day of disease information, 19 (6.7%) were positive by MAT, and one sample tested positive by both methods. In conclusion, rRT-PCR and single-specimen MAT demonstrate poor agreement for the diagnosis of leptospirosis and identify distinct patient populations. The accuracy of using a single MAT result, even at a titer of ≥1:800, for the diagnosis of acute leptospirosis should be re-evaluated.

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PATHOGEN-SPECIFIC FEATURES OF THE HUMAN PERIPHERAL BLOOD TRANSCRIPTIONAL PROFILE IN PATIENTS WITH SCRUB TYPHUS AND MURINE TYPHUS

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Scrub and murine typhus are important causes of fevers in Southeast Asia. The nonspecific clinical presentation and lack of sensitive acute-phase diagnostic tests hinder their early recognition. To identify pathogen-specific features of the host response to infection, we used oligonucleotide arrays to examine genome-wide patterns of whole blood gene expression in patients with scrub typhus (n=8) and murine typhus (n=6) admitted to Mahosot Hospital, Vientiane, Laos. We compared these patterns with those of healthy controls (n=12), as well as patients with dengue (n=13) and ***E. coli*** bacteremia (n=6). The average change in abundance of 15,643 transcripts following infection with ***Orientia tsutsugamushi*** and ***R. typhi***, causative agents of scrub and murine typhus, respectively, was similar to those seen in patients with dengue (Pearson's r=0.81 and 0.66, respectively). All three groups had elevated abundance levels of transcripts associated with the mitotic cell cycle and mitochondrial activity; these levels were highest with dengue and lowest with murine typhus infection. The transcriptional profile of patients with ***E. coli*** was distinct (r=0.23 and r=0.49 compared to scrub and murine typhus, respectively), and characterized by elevated abundance levels of transcripts associated with myeloid gene expression. Principal components analysis indicated that there were differences in gene expression that distinguished scrub and murine typhus patients from those with dengue and ***E. coli*** infection, and we found that gene sets associated with T and NK cells were expressed at higher levels in scrub and murine typhus. We also identified a set of 10 transcripts that correctly predicted 13 of 14 ***O. tsutsugamushi*** and ***R. typhi*** infections and 18 of 19 dengue and ***E. coli*** infections (10-fold cross-validation). Eight of 9 scrub typhus and murine typhus patient samples from Kathmandu, Nepal were also correctly predicted as rickettsial infections using the same gene set. Further validation will establish the potential of these gene expression patterns to improve diagnostic capabilities and our understanding of the early host responses to rickettsial infections.

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MICROGEOGRAPHIC DIFFERENCES IN TRANSMISSION OF LEPTOSPIROSIS IN THE PERUVIAN AMAZON

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Leptospirosis is hyperendemic in the Peruvian Amazon, and different transmission contexts seem to coexist within a small region. In this study we looked at the prevalence of leptospirosis in three areas around the city of Iquitos: the Belen neighborhood (part of which gets flooded seasonally), the riverine semi-rural community of Mazan, and the roadside community of Los Delfines. We enrolled adults and children over 5 years old in a household survey of the three communities. GIS and demographic data were collected and participants provided a blood samples, a urine sample, or both. Serology using the microagglutination test (MAT) was performed on all serum samples. Seroprevalence in Belen was 9.3 % (46/493), and it was significantly different between the flooded area (16%) and the non-flooded area (5.2%) ($p<0.01$). Seroprevalence was 5.5 % (12/238) in Mazan and 7.4% (17/229) in Los Delfines. We did not find any specific risk factors associated to seropositivity, besides living in a flooded area. Seroprevalence to the intermediate ***Leptospira licerasiae*** was 92.5% and 91.5% in the flooded and dry areas of Belen, and 88.2% and 85.6% in Mazan and Los Delfines respectively. A qPCR assay directed at ***Leptospira*** 16S rDNA , was done on 27 urine samples from MAT positives from Belen and 55% were positive. A sub group of urine samples from MAT negative was also assayed, 18% (11/61) were positive. The most prevalent serovar in Belen and Los Delfines was serovar Bratislava, which has been described to be mostly associated with pigs. We find that the seroprevalence to pathogenic ***Leptospira*** varies in different contexts, and that even within the area on Belen, exposure seems to be higher in the flooded area. The prevalence in the non-flooded area is similar to that in semi-rural areas not considered hot spots. Although MAT does not definitely define infective serovar, because of cross-reactivity within strains, our findings suggest an important role for pigs in the transmission of leptospirosis in this area.

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THE EFFECT OF CIVIL WAR ON CUTANEOUS LEISHMANIASIS "ALEPPO BUTTON" IN ALEPPO CITY

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In the ancient northern Syrian city of Aleppo, CL has been present for hundreds of years (if not longer), where it is known as the "Aleppo evil", "Aleppo ulcer", "Aleppo boil", or "Aleppo button" which is Cutaneous Leishmaniasis. Aleppo ulcer is a disfiguring condition that disproportionately occurs on the face, especially of young people. It typically lasts one or 2 years before the lesion heals spontaneously, and is often known locally as "one-year sore". However, in many cases specific anti-parasitic chemotherapy can hasten the healing process and improve clinical and cosmetic outcomes. A major problem with one-year sore is that the scar can produce permanent disfigurement of the face. It is well known about the rise and fall and then a rise again in the incidence of the disease in the city of Aleppo. During the 1950s the number of cases of CL fell after an insecticide campaign aimed at controlling malaria, but it then rose again during the 1960s. However, CL was mostly controlled during the 1980s.There is no doubt that the areas of Syria affected by the civil war are experiencing an increase in cutaneous leishmaniasis, and this will also be seen in the refugee camps in Jordan and Turkey. This is due to garbage collection, open sewage, and poverty which promote the habitats of ***Phlebotomus*** sandflies that transmit CL. Interestingly, a clinical trial conducted prior to the current civil conflict found that use of insecticide-treated bednets (ITNs) could prevent CL in Aleppo. Recently, WHO reports out of Syria indicate the emergence of epidemic cutaneous leishmaniasis

in the besieged city of Aleppo, adding further to the misery there, perhaps the international community needs to focus on refugees and refugee encampments to ensure local control and patient access to treatments.

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DISTRIBUTION OF ESCHAR IN CHILDREN WITH SCRUB TYPHUS

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Scrub typhus, caused by ***Orientia tsutsugamushi***, and transmitted by the bite of a trombiculid mite chigger is widely prevalent in the 'tsutsugamushi triangle' of the world. Prompt and appropriate antibiotic therapy is important in decreasing morbidity and mortality. Presence of eschar at the site of chigger bite is an important finding in the early diagnosis of scrub typhus. The chigger is microscopic and the eschar is painless. A careful examination is required to identify an eschar. Describing the distribution of these eschars is beneficial to clinicians. In our study, we describe the distribution of eschars in all children < 15 years of age admitted with a confirmed diagnosis of scrub typhus based on a positive scrub typhus IgM serology or a Weil Felix test OX K > 80 over a 3 year period. There were 286 children admitted with confirmed scrub typhus during this period. An eschar was present in 155(54.5%) children with 94(60.6%) males and 61(39.4%) females. The eschars were distributed in the following areas: scalp 2(1.3%), ears 5(3.2%), eyelids 4(2.6%), neck 15(9.7%), axillae 35(22.6%), chest and abdomen 21(13.5%), buttocks 2(1.3%), genitalia 25(16.1%)(scrotum 24 and labia 1), leg 3(1.9%), arm 3(1.9%), groin 24(15.5%), shoulder 9(5.8%) and back 7(4.5%). The commonest sites of eschars were scrotum 24/94(25.5%) and axillae 14/94(14.9%) in males and axillae 21/61(34.4%) and groin 14/61(23%) in females. Eschars were seen within skin folds in 84/155(54.2%) children. The distribution is children is predominantly in the axillae and genitalia whereas in adults, as described in literature, the distribution is predominantly over the chest, abdomen and the groin. Recognizing an eschar is the most useful clue to diagnose scrub typhus in children presenting with acute febrile illness. In endemic regions, children should be carefully examined for the presence of eschar especially in the skin folds of the genitalia, axillae and groin to make an early diagnosis of scrub typhus.

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SPECTRUM OF WINTER DERMATOSES IN RURAL YEMEN

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Surveys have been carried out to determine the prevalence of skin diseases in rural Yemen are scarce or even not available. This study was undertaken to investigate spectrum of winter dermatoses in a rural Yemeni community. At the dermatology outpatient clinic of Al-Helal Specialized Hospital at Radaa' district of Al Bayda' governorate, this retrospective study was conducted, by data analysis of 700 selected records of patients managed during 4 months of 2013-14 winter season. Results 700 patients with 730 diseases were reported in this study, the major bulk of patients (46.57%) were in >18-40 years age group, and females outnumbered males. By far, Dermatitis, eczematous and allergic disorders (38.49%) topped the list of the most frequent skin disorders groups, followed by skin infections and infestations (20%) and pigmentary disorders group (13.70%). Contact dermatitis (10.68%) was the most prevalent skin disorder, followed by hyperpigmentations (8.77%), acne (8.08%), viral infections (5.75%), atopic dermatitis (5.62%), and parasitic infestations (5.34%). In conclusion, this survey has documented spectrum of winter dermatoses in a rural Yemeni community, but also reflects pattern of common dermatoses in the whole country. Dermatitis, eczematous and allergic disorders, skin infections and pigmentary disorders are the commonest groups. Contact dermatitis is the most prevalent disorder, and leishmaniasis is the most prevalent skin infectious disease. Climate, occupational, social, and environmental factors are of main contributors. Such statistics can form an important basis for community-based health policies.

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IRON DEFICIENCY DURING PREGNANCY AND NEURODEVELOPMENT OF ONE-YEAR-OLD CHILDREN

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Iron deficiency (ID) in infancy is a known risk factor of short and long term neurocognitive deficits. Although evidence exists on the impossibility of completely reversing impaired cognition caused by early iron deficiency, little is known about the impact of prenatal ID on the neurocognitive function of children. The objectives of this study were to assess the impact of prenatal ID on the cognitive and motor functions of one-year-old children in Benin, and to determine the epidemiologic pathway underlying this relationship. Our prospective cohort study included one-year-old children born to women recruited at their first antenatal care (ANC) visit, before 29 weeks of pregnancy, within the MiPPAD trial comparing sulfadoxine-pyrimethamine and mefloquine. Pregnant women were enrolled if they had not taken any anthelminthics, iron or folic acid and were HIV negative prior to first ANC visit. Serum ferritin and C-reactive protein concentrations of pregnant women were determined from venous blood samples collected at first and second ANC visits of at least, one-month interval and at delivery. Women were given oral iron, folic acid and anthelminthics as part of the ANC package in Benin. A total of 636 children (76.8% of eligible children) were assessed for cognitive and motor functions, using the Mullen Scales of Early Learning (MSEL), at twelve months of age by trained research nurses. Prevalence of ID was 33.3%, 35.2% and 30.4% at first ANC visit, second ANC visit and delivery, respectively. There was no significant difference in the cognitive and motor functions between children whose mothers were iron deficient and those whose mothers were not iron deficient during pregnancy. Although we observed an increased risk of ID, RR = 2.3 (95% CI 1.9-2.8) and RR = 1.7 (95% CI 1.4-2.1) at second ANC and delivery, respectively, if pregnant women had ID at first ANC visit, persistent ID throughout pregnancy was not related to infant neurocognitive function. Preliminary analyses show no association between prenatal ID and early neurocognitive development of children.

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INFLAMMATORY AND ANGIOGENIC FACTORS AT MID-PREGNANCY ARE ASSOCIATED WITH SPONTANEOUS PRETERM BIRTH IN A COHORT OF TANZANIAN WOMEN

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Preterm birth (PTB) is the leading cause of perinatal mortality worldwide, with the greatest burden occurring in resource-constrained settings. Based on the hypothesis that altered placental angiogenesis and inflammation early in pregnancy lead to PTB, we examined whether levels of inflammatory and angiogenic mediators, measured early in pregnancy, were predictive of spontaneous PTB (sPTB). Plasma samples were collected from a prospective cohort of primigravid Tanzanian women between 12-

27 weeks gestation. A panel of 18 markers was screened on a training cohort of 426 women. Markers associated with sPTB in the training cohort were repeated in a test cohort of 628 women. All markers were measured by ELISA. In both the training and test cohorts plasma levels of IL-18BP, sICAM-1, sEndoglin and CHI3L1 were elevated and Leptin was lower at enrollment in women who subsequently experienced sPTB. In multivariate analysis women with plasma levels of CHI3L1, C5a, sICAM-1, AngptL3, sEndgolin, sFlt-1 and IL-18BP in the highest quartile had an increased risk of sPTB compared with those in the lowest quartile. Women with Leptin and Ang2 in the highest quartile had a reduced risk of sPTB compared with women in the lowest quartile. Levels of angiogenic and inflammatory mediators measured at mid pregnancy were associated with subsequent sPTB. These findings provide insight into mechanisms underlying sPTB and suggest biomarkers that may have clinical utility in risk-stratifying pregnancies.

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THE IMPACT OF THE AMAZON HOPE MEDICAL PROGRAM ON THE PREVALENCE OF ANAEMIA IN CHILDREN FROM THE PERUVIAN AMAZON

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The Amazon Hope medical boat health program offers care to 167 impoverished communities of the Peruvian Amazon. In 2010, we performed a study into childhood anaemia in these communities, which identified a high prevalence (89%) of anaemia in children under 5 years old attending the boat clinics. Subsequently, we started an iron supplementation and anti-parasite program and studied its impact on anaemia prevalence post-intervention. Eligibility criteria included being a child from 6 to 59 months attending the boat clinic, diagnosed with anaemia in the initial study and whose guardian gave informed consent to participate. Participants with a haematocrit of <33% were offered operationally-defined "anaemia treatment" with 2-3mg/kg/day of oral iron for three months. Participants with a haematocrit of 33-35% received operationally-defined "iron supplementation treatment" with 0.5-1mg/kg/day of oral iron for two months repeated every three months for a total of one year. In addition, 400mg albendazole was given every three months to all children aged over 2 years and 200mg to children between 1 and 2 years. In 2011, haematocrit levels were repeated on visits to the study site communities. Data from 869 of the children originally tested for anaemia were available for analysis. Mean anaemia prevalence in participants decreased from 89% in 2010 to 20% in 2011. Older children had the greatest reduction in anaemia prevalence following the intervention: 68% (97-29%) in those aged 6-12 months, 62% (91-29%) in those aged 12-23 months, 65% (86-21%) in those aged 24-35 month, 73% (87-14%) in those aged 36-47 months, and 77% (84-7%) in those aged 48-59 months. Iron supplementation and anti-parasite therapy were associated with a marked reduction in anaemia prevalence in attending children. The largest reduction was seen in children over two years old, which perhaps relates to increased intake and adherence to iron supplementation. Further research is required to inform regional policy makers to consider the scope for potential implementation and scale-up of similar interventions to correct or prevent anaemia in these communities.

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CONGENITAL TOXOPLASMOSIS AND PREGNANCY MALARIA DETECTION POST-PARTUM; EFFECTIVE DIAGNOSIS AND EFFECTIVENESS OF APPROVED CHEMOTHERAPEUTIC REGIMENS IN GHANA

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Congenital toxoplasmosis (CT) and Pregnancy malaria (PM) have been individually reported to cause severe negative outcomes in pregnancies but the diagnostic method is still debatable. This study sought to estimate the prevalence of PM and CT single and co-infections in pregnant women by using various specimens including serum and placental tissues. Genomic DNA extracted from the placenta, cord blood or blood of mothers was tested by PCR. Conventional method of immunodiagnosis was done for CT. We tested 79 pregnant women and the mean age was 28±1.06. There was a big difference in the prevalence of ***Plasmodium falciparum*** infection determined by PCR between two specimens tested: PCR positive for mother's peripheral blood was 6.3% while 57.3% for placental tissues ($p < 0.001$). PCR testing for placental tissues showed 29.2% positive for ***Toxoplasma gondii***, while 76.0% of mothers had serum IgG against T. gondii. It should be noted that 6.32% of the placental tissues showed positive for SAG 3 in PCR, a marker of active infection in T. gondii. Although there were no enhanced fetal disorders at birth in our study, there is a possibility of active transmission of ***T. gondii*** from mothers to foetus even in immune mothers. Our study suggests that foetus were exposed to ***P. falciparum*** and ***T. gondii in utero***, and placenta PCR is a sensitive method for detecting such episodes. In cases of PCR-positive, clinical follow-up after birth may be important.

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CHANGES IN THE PROFILE OF CIRCULATING B CELLS AND IGG AND COMPLEMENT RECEPTORS CAN BE ASSOCIATED WITH THE DEVELOPMENT OF *ERYTHEMA NODOSUM LEPROSUM*

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Reversal reaction (RR) and erythema nodosum leprosum (ENL) are severe complications of leprosy. We previously observed differences in the profile of circulating B cells and in the frequency of CD32 and CD21 between paucibacillary and multibacillary. We hypothesized that those differences may be associated with the pathogenesis of reactions. Peripheral blood mononuclear cells were obtained from 14 household contacts (HC), 43 patients without reaction, 7 tuberculoid, 20 borderline and 7 lepromatous, and 12 patients with reactions (9 RR and 3 ENL). Total lymphocytes and subpopulations of B cells, frequency of CD32 and CD21 were evaluated ***ex vivo*** by flow cytometry. A decrease in the frequency of lymphocytes was seen in borderline (61.0%; p= 0.0165) and lepromatous (61.2%; p= 0.0428) when compared to HC (71.2%). The frequency of B cells were higher in lepromatous (13.1%) when compared to HC (7.1%, p= 0.0300), tuberculoid (8.6%, p= 0.0021) and ENL (5.3%, p= 0.0121). There were no differences in the frequency of transitory, naïve or memory B cells between leprosy clinical forms. However, ENL had a higher frequency of memory B cells when compared to borderline (35.9% vs. 24.1%, p= 0.0344), lepromatous (22.3%, p= 0.0485) or RR (23.49%, p= 0.0182). Plasmoblasts were more frequent in lepromatous (8.9%) when

compared to tuberculoid (5.5%, p= 0.0108). Interestingly, there was a higher frequency of CD21+ B cells in lepromatous (92.2%) compared to tuberculoid (88.1%, p= 0.0090), borderline (89.1%, p= 0.0500), RR (81.84%, p= 0.0111) or ENL (82.3%, p= 0.0242). Furthermore, lepromatous (84%) presented a lower frequency of CD32+ B cells when compared to HC (93.5%, p= 0.0021), tuberculoid (91.5%, p= 0.0106) or ENL (95.6%, p= 0.0121). Lepromatous (70.3%) also presented a lower frequency of CD32+ plasmoblasts when compared to ENL (90.1%, p= 0.0303). Despite the differences in the profile of circulating B cells in clinical forms of leprosy, the increased frequency of CD21+ B cells and decreased frequency CD32+ B cells in lepromatous patients could contribute to exacerbation of the humoral immune response and increase the risk of developing ENL.

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THROMBOCYTOPATHY CONTRIBUTES TO THE DEVELOPMENT OF BLEEDING COMPLICATIONS IN HUMAN LEPTOSPIROSIS

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Leptospirosis is a widespread zoonotic disease in the tropics caused by the pathogen Leptospira interrogans. Bleeding is one of the most important complications of which the pathogenesis is poorly understood. We hypothesized that impaired platelet function (thrombocytopathy) contributes to the bleeding complications of leptospirosis. We conducted a prospective study on 23 hospitalized patients with leptospirosis in Semarang, Indonesia, of whom 9 developed clinical bleeding. Platelet function and the binding of von Willebrand factor (vWF) to platelets was investigated using flow cytometry. The latter was included as vWF-platelet binding was recently suggested to cause thrombocytopathy. Leptospirosis was associated with ***in vivo*** platelet activation with increased expression of the platelet granule marker P-selectin and increased fibrinogen binding to αIIbβ3. However, upon ***ex vivo*** stimulation with the platelet agonists adenosine-diphosphate (ADP) and thrombin-receptor activating peptide (TRAP), P-selectin expression and fibrinogen binding were significantly lower compared to controls, suggesting thrombocytopathy. Patients with clinical bleeding had the most pronounced thrombocytopathy and these patients also had increased vWF binding to platelets. In conclusion, our study identifies thrombocytopathy as a contributing factor to bleeding in leptospirosis. Excessive platelet activation and platelet-vWF binding may be an underlying mechanism behind this thrombocytopathy.

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ASSESSMENT OF THE EFFECTIVENESS AND UTILITY OF THE NEW WHO GUIDELINES FOR THE MANAGEMENT OF HEPATITIS B IN NORTHERN UGANDA

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Hepatitis B virus (HBV) kills 750,000 people a year, overwhelmingly in low and middle income countries (LMICs), despite the widespread use of HBV vaccine. However there is little research conducted into hepatitis B in sub-Saharan Africa. WHO published its first guideline for the investigation and management of hepatitis B in 2015. It highlights the lack of evidence to guide management in LMICs, particularly where HBV DNA testing is unaffordable. The emergence of tenofovir as a safe, effective and increasingly inexpensive treatment for hepatitis B has only served to emphasise the need for evidence-based criteria applicable to this setting. Previous guidelines for the management of hepatitis B (eg. from the American Association for the Study of Liver Diseases) have placed HBV DNA testing at the centre of patient assessment. The new WHO guidelines include guidance for management without this where it is not feasible. However little data is available to validate this approach. We aim to demonstrate that assessment of patients with hepatitis B without the use of HBV DNA testing is feasible and results in acceptable allocation to treatment. St Mary's Hospital Lacor is a regional referral hospital just outside Gulu in Northern Uganda. The local prevalence of hepatitis B is 17%, making this a significant cause of morbidity and mortality. HBV DNA tests in this setting must travel 335 miles to Kampala and cost $120, putting them far beyond the means of most patients. We performed a pragmatic prospective study to compare the therapeutic allocation of hepatitis B patients by the WHO guidelines without HBV DNA testing, taking a parallel assessment with HBV DNA as our gold standard. 100 patients with hepatitis B diagnosed at St Mary's Hospital were recruited. Liver ultrasound, liver and renal function tests, full blood count and HIV serology were performed. Investigators made a treatment decision while blinded to the HBV DNA result. HBV DNA results were then unblinded and patients were re-allocated to treatment or observation and the appropriate management commenced. We present the sensitivity and specificity of the without-HBV DNA WHO guideline in this setting.

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USE OF ANTIBIOTICS AND ANTIMALARIALS IN THE MANAGEMENT OF FEBRILE ILLNESSES IN CHILDREN IN PUBLIC HEALTH FACILITIES IN WESTERN KENYA

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Malaria is a global tropical disease associated with high morbidity and mortality, especially among the children. Many diseases present clinically with fever and Integrated Management of Childhood Illnesses (IMCI) advocates for the use of antibiotics for other infections and antimalarials for malaria. With the signs and symptoms of malaria resembling those of other diseases, many febrile conditions are treated clinically as malaria. Without laboratory confirmation of malaria, continued use of artemisinin-based combination therapy on non-malaria cases may soon lead to resistance due to their unnecessary use. A randomized control study was put in place with the primary objective of testing whether financial incentives offered (to intervention group) at the facility level improve targeting of antimalarials to patients with parasitologically diagnosed malaria. This is a sub-study of the above describing the role of antibiotics and antimalarials in the management of febrile illnesses among children. The main objective was to describe the prescription habits of both antimalarials and antibiotics. This was a comparative, records-based cross-sectional study carried out in 17 public health facilities of high and low malaria endemicity in the western region of Kenya. Health facility records were reviewed by use of a checklist and data was analysed using STATA analysis package. 6086 children under the age of 5 years were included in the study with a mean age of 2 years and 51.2% being female. Among the 2124 study subjects who received antibiotics and other treatment regimens, (37.5% of intervention and 31.8% of control) most of them received cotrimoxazole dispensed at 46%. Positive blood smear results for Western and Rift Valley Provinces were 26.5% and 11.6% respectively. Among those who received medication, 70% of those with a negative blood smear result were given antibiotics and 68% of those with a positive blood smear result got AL.

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SIMPLE, ECONOMICAL RABIES VACCINATION: WHY INTRADERMAL ADMINISTRATION SHOULD BECOME ROUTINE WORLDWIDE

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Rabies prophylaxis is expensive and unsatisfactory globally. Thousands of people die an agonising death unnecessarily because rabies vaccine is either unavailable or unaffordable. Shortages may occur anywhere. Dog rabies virus encephalitis is always fatal in unvaccinated patients, and post-exposure prophylaxis (PEP) may fail if it is incomplete. Doctors are understandably afraid of trying the low dose intradermal (ID) regimens. Confusingly, the WHO currently recommends 9 different vaccine regimens. It is now urgent to agree and then strongly recommend highly immunogenic, economical, safe and simplified methods suitable for all. This is now possible using the ID route for all three essential types of rabies vaccine regimens: Pre-exposure, Post-Exposure Booster for those previously immunised and Primary Post-exposure. The WHO already approves ID Pre-exposure: (0.1 ml, Days 0, 7, 28) and the PEP Booster: (Single Day ID 0.1 ml at 4-sites). For Primary PEP, the mandatory rapid immune response can be achieved by inoculating small doses of vaccine at multiple ID sites. Two 4-site ID candidate regimens have been proposed: a 'One Week' version suffers several disadvantages compared with the '4-site ID One Month' regimen, which uses the same total dose of vaccine and timing as the original 8-site ID regimen that was regarded as highly immunogenic by WHO. The new 4-site method involves 3 clinic visits, minimises vaccine wastage, accommodates inexperienced ID technique, is economical compared with all other regimens, and gives the same dose of vaccine antigen dispensed in either 1ml or 0.5 ml vials. The '4-site ID One Month' schedule is: Day 0, a whole vial ÷ ID 4-sites; Day 7, half a vial ÷ ID 2-sites; Day 28, 0.1/0.2 ml (one fifth of a vial) ID at 1-site. The result is three essential ID regimens: a Pre-exposure 3 dose (0,7,28); a Booster PEP Single Day 4-site; and a Primary PEP 4-site 3 dose (0,7,28). Should these ID regimens become routine globally? This strategy could pave the way to a new ID pre-exposure regimen and a Primary PEP regimen of only 2 visits.

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UPDATING THE CDC YELLOW BOOK DENGUE MAP FOR CLINICIANS TO IMPROVE UNDERSTANDING OF DENGUE RISK AREAS

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The *CDC Health Information for International Travel* (Yellow Book) publication is used by clinicians both during a pre-travel consultation to prepare travelers for international travel and when evaluating ill patients after travel. For the 2016 Yellow Book, CDC Dengue and Travelers' Health Branches sought to provide clinicians clearer, more rigorous guidance regarding dengue risk by improving the map that shows where travelers should employ mosquito bite prevention measures and where dengue should be considered in the differential diagnoses of ill travelers. Instead of one "dengue risk" category which included only areas with known dengue cases and potentially omitted areas with sporadic risk, CDC moved to describe two categories of risk: "frequent or continuous (FC)" or "sporadic or uncertain (SU)." FC areas are those with strong evidence of recent dengue cases over multiple years or reports of more than 10 cases in at least 3 of the previous 10 years. SU areas are those with weak evidence of transmission or locations with at least one locally acquired dengue case reported in the last 10 years that do not fit the FC definition. Evidence used to categorize geographic areas included dengue outbreak and surveillance data from official reports, ProMED reports, and published scientific research compiled by Oxford University and CDC. Because not all dengue outbreaks are reported worldwide, expert opinion was used when evidence for a given locale was missing, since the absence of cases does not indicate an absence of risk. The inclusion of the SU category, which augmented the geographic expanse of potential dengue risk areas, helps provide clinicians with a more risk-based map to inform both recommendations for avoiding dengue and differential diagnoses for returning ill travelers. An international traveler's risk for dengue virus infection depends on the local prevalence of dengue and exposure to vector mosquitoes. Although risk areas change over time, the revised dengue map incorporates a larger body of direct evidence and a more detailed assessment of risk to improve the information available to clinicians consulting the 2016 Yellow Book.

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FACTORS CONTRIBUTING TO ADHERENCE TO THE BURULI ULCER ANTIBIOTIC TREATMENT REGIME: A CASE-CONTROL STUDY AT THE AMASAMAN SUB-DISTRICT IN GHANA

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Buruli Ulcer is a debilitating disease caused by the mycobacterium ulcerans. In 2005 antibiotic regime was introduced in the treatment of the disease in addition to surgical treatment for advanced stages. The study aimed at finding the factors that contributed to adherence to the antibiotic treatment for Buruli ulcer, in Amasaman sub-district. A structured questionnaire which focused on patients access to treatment both geographically and financially, counseling patients prior to start of treatment and outcomes of the having the disease such as stigmatization and cumbersomeness of daily wound care was used to answer the identified study objectives. A case-control study was carried out with 100 each of cases and controls. The study found that patients knowledge about the disease, support from family, and effect of patient seeking treatment on daily activity were associated with patients adherence to treatment. Showing an OR 0.38(CI 95% 0.18-0.74) for patients knowledge about the disease, an OR 2.27(CI 95% 1.17-4.45)) for support from family and OR 0.30(CI 95% 1.54-7.34)) for effect of patient seeking treatment on daily activity. Other factors such as patients access to health with OR 11.29(CI 95% 5.56-23.11), experience with medication side effect with OR 2.53(CI 95% 1.03-6.65), patient being counseled before treatment having an OR 4.51(CI 95% 2.31-8.93) and quality of care given to patients by health care providers with OR 0.15(CI 95% 0.02-0.71) were also found to be associated with adherence to treatment. It is recommended that, staffs providing care for Buruli Ulcer patients should be well trained to deliver good counselling to patients prior to the start of treatment so as to enhance patients understanding the treatment regimen and the benefits of completing appropriately.

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DENGUE AND COMORBIDITIES IN THE PERUVIAN AMAZON: 2010-2014

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Dengue illness is endemic throughout urban centers in the Peruvian Amazon, especially in Iquitos city, causing high morbidity and mortality

every year. Arterial hypertension, diabetes, rheumatoid arthritis, and other chronic diseases have been shown to be risk factors for severe dengue elsewhere, but little is known about the impact of these comorbidities on hospitalization and mortality rates in this region. To assess this impact, we analyzed the epidemiology and clinical data from patients, with acute undifferentiated febrile illness (AUFI), who were enrolled through clinic-based passive surveillance in Health facilities (3 hospitals, 9 health centers) located in Iquitos, from December 2010 to December 2014. We obtained acute and convalescent blood samples as well as clinical history of any comorbidity (hypertension, diabetes, asthma, rheumatoid arthritis and others). Dengue virus infection (DENV) was confirmed by real time PCR or virus isolation in the acute sample or by seroconversion (4-fold increase in IgM antibodies) between acute and convalescent samples. All probable cases (presence of IgM without a rise in titer) were excluded. We confirmed DENV in 1,941 of 4,435 AUFI cases screened. Of these, 1,493 were treated as outpatients compared to 448 who were hospitalized. Of the 60 DENV+ patients with comorbidity, 18 required hospitalization and 42 did not, a similar ratio to those without comorbidity (OR=1.42, CI95%: 0.81 to 2.5). Hypertension was the most common comorbidity. Shock and death occurred in four patients who did not have any comorbidity. Although the 2009 World Health Organization Dengue Guidelines list certain co-morbidities in their criteria for hospital admission, none of the comorbidities we evaluated were associated with hospitalization. Longitudinal research is necessary to assess the true impact of comorbidities on DENV outcomes in this region.

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CORRECTING IRON DEFICIENCY PREVALENCE FOR PLASMA INFLAMMATION IN BOLIVIAN INFANTS

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Iron deficiency (ID) in infancy can cause cognitive and developmental deficits that may be irreversible after the age of 2. Plasma ferritin (Fer), reflective of iron stores, is often used as a sensitive measure of ID, yet levels of Fer are affected by inflammation. The goals of this study were to quantify the prevalence of ID among 6 - 8 month-old infants in a high-altitude population of Bolivia and compare different methods of correcting ID for the effect of inflammation. Healthy infants were recruited from 2 hospitals in El Alto, Bolivia, and followed from 1 to 8 months of age. Blood taken at 2 and 6 - 8 months was analyzed for ferritin (Fer), C-Reactive Protein (CRP), and alpha(1)-acid-glycoprotein (AGP). ID was defined as Fer < 12 µg/L. Inflammation was defined as CRP ≥ 5 µg/L or AGP ≥ 1 mg/L. Six methods were tested for correcting Fer for inflammation: exclusion, internal correction factors, meta-analysis-derived correction factors, and 3 linear regression models of Fer on CRP and AGP (each used different CRP and AGP reference values). The ID prevalence values generated from these 6 methods were compared to the crude (uncorrected) prevalence. At 2 months of age, only 1 of 160 infants (< 1%) demonstrated evidence of ID, and 6 (4%) were inflamed. At 6 - 8 months of age, the prevalence of inflammation in this cohort was 20.2% (33/163). Uncorrected ID prevalence was 41.1% (67/163 infants measured). Exclusion yielded a prevalence of 44.6% (58/130), while different correction methods showed ID prevalence between 41.7% and 49.7% at 6 - 8 months of age. This analysis suggests that adjusting Fer for inflammation may lead to a more sensitive and valid measure of ID, depending on the correction method chosen. While infants in this population were born with adequate iron stores, by 6 - 8 months these stores were depleted to the point of ID in nearly half of the infants, a strikingly high figure. This analysis confirms that inflammation can significantly affect ferritin, and even in a low-inflammation setting can cause ID prevalence using Fer to be underestimated by nearly 10 percentage points. Interventions to prevent ID and inflammation in early infancy should be further explored.

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TRENDS IN CARE SEEKING FOR CHILDREN'S RECENT FEVER IN LIBERIA: EVIDENCE OF IMPACT OF THE MALARIA COMMUNITIES PROGRAM (MCP)

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Through the Malaria Communities Program (MCP), the President's Malaria Initiative (PMI) awarded small grants to local organizations in 12 countries to implement projects around malaria prevention and treatment. One such organization, EQUIP Liberia, was awarded an MCP grant to work in two counties in Liberia: Nimba and Sinoe. In these counties, the EQUIP project piloted integrated community case management (iCCM), trained general Community Health Volunteers (gCHVs) and used behavior change communication to generate demand and increase care seeking. This study analyzed the 2007 Liberia Demographic and Health Survey (LDHS) and 2011 Liberia Malaria Indicator Survey (LMIS) data to evaluate how trends in care seeking for children's fever in counties covered by the EQUIP project compared with care seeking trends in non-project counties. Two logit regression models were developed to assess the odds that care was sought from any public facility and from any private facility. Results show that the increase in care seeking from public facilities was significantly greater in EQUIP project areas compared with the rate of increase in areas with no EQUIP presence, after adjusting for socio-demographic characteristics (adjusted OR for the additional increase associated with project counties: OR=3.6, p<0.05). Similarly, the rate of decline in care seeking from private facilities in EQUIP project areas was significantly more rapid than the rate of decline in areas with no EQUIP presence (adjusted OR for additional decline associated with project counties: OR=0.14, p<0.001). While the observed patterns could be explained by external factors such as urbanization, health system expansion, or other programs implemented during the same period, results provide some observational evidence of impact of EQUIP's malaria community-based malaria control program on care seeking in two counties in Liberia.

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ANEMIA AND MICRONUTRIENT DEFICIENCIES IN ECUADOR: RESULTS FROM THE ECUADORIAN NATIONAL HEALTH AND NUTRITION SURVEY (ENSANUT-ECU)

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The burden of malnutrition in Latin America is high, although there is limited data from Ecuador. The recent Ecuadorian National Health and Nutrition Survey is the first national level nutrition survey since 1987, and provides a unique opportunity to examine the burden of anemia and micronutrient deficiencies in Ecuador. A total of 21,479 individuals were surveyed, including 11,325 adults (>19y), 3,646 adolescents (12-18y), 4,459 school-aged children (5-11y), and 2,049 children under five. Socio-demographic data and venous blood samples were collected and hemoglobin and micronutrient concentrations were analyzed. The prevalence of anemia and micronutrient deficiencies (zinc, vitamin A, iron, vitamin B12, folate) were calculated and mapped using ArcGIS. Binomial and linear regression models were used to examine the associations of micronutrient status and socio-demographic characteristics. Micronutrient deficiencies were common, including zinc deficiency (Zn<65.0 µg/dL; 41%), anemia (Hb<11.0 g/dL; 8%), and vitamin B12 insufficiency (vitamin B12<221.0 pmol/L; 30%). Coastal Ecuador had the highest prevalence of anemia (13%; RR: 2.46, 95% CI: 2.22-2.72, p<0.01) and zinc deficiency

(46%; RR: 1.32, 95% CI: 1.24-1.42, p<0.01), compared to other regions. Anemia was more prevalent in urban areas compared to rural settings (RR: 1.15, CI: 1.05-1.26, p<0.01). Anemia (16%; RR: 2.32, 95% CI: 2.08-2.59, p<0.01), vitamin A deficiency (serum retinol<20.0 µg/dL; 25%; RR: 3.50, 95% CI: 3.06-4.00, p<0.01) and iron deficiency (serum ferritin<12.0 µg/L; 9%; RR: 1.75, 95% CI: 1.51-2.03, p<0.01) were more prevalent among children, compared to other age groups. The highest prevalence of anemia (16%; RR: 2.08, 95% CI: 1.76-2.46, p<0.01), vitamin A deficiency (15%; RR: 2.39, 95% CI: 1.89-3.00, p<0.01), and zinc deficiency (50%; RR: 1.24, 95% CI: 1.14-1.34, p<0.01) was reported among Afro-Ecuadorians; the indigenous population had the highest prevalence of vitamin B12 insufficiency (35%; RR: 1.35, 95% CI: 1.13-1.61, p<0.01). Findings suggest the burden of micronutrient deficiencies is high in Ecuador, particularly in urban and coastal settings.

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SECRETED FILARIAL SMALL RNAS - LOCALIZATION IN BIOFLUIDS AND BIOMARKER POTENTIAL FOR ONCHOCERCIASIS

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Extracellular small RNAs, in particular miRNAs, are found in a wide variety of bodily fluids and have been proposed as biomarkers for diseases. More recently, reports have suggested that parasitic nematodes secrete specific miRNAs in exosomes and these can be found in serum of infected patients, with major implications for diagnosis and evaluation for treatment efficacy. We have identified microRNAs derived from three different filarial nematodes (Clade III): ***Litomosoides sigmodontis*** (murine filariasis), ***Onchocerca volvulus*** (human filariasis) and ***O. ochengi*** (bovine filariasis), in serum or nodule fluids obtained from their definitive hosts. Specifically, miRDeep revealed a total of 62 mature miRNAs from 52 distinct pre-miRNA candidates in nodule fluids from cattle infected with ***O. ochengi*** of which 59 are identical in the genome of the human parasite ***O. volvulus***. Six of the extracellular miRNAs were also identified in sequencing analyses of serum and plasma from humans infected with ***O. volvulus*** from endemic regions in Cameroon and Ghana. Also, fourteen parasite-derived miRNAs were found in mouse serum during the patent stage of the infection, all of which were detected in either human serum or bovine nodule fluid samples from endemic geographical regions in Cameroon. These results suggest that common miRNAs are secreted by filarial parasites and we have carried out an initial assessment of the ability of these miRNAs to detect infection in the serum of mice infected with ***L. sigmodontis***, suggesting high sensitivity and specificity (80/100). Interestingly, among all of the secreted miRNAs described to date, whether in secretory-excretory products or detected in host body fluids, there are common secreted miRNA such as miR-71 and miRNA families including miR-100 and bantam, as well as specific differences across the clades. These results confirm the conserved nature of RNA secretion by nematodes and also suggest that there might be specific secreted signatures depending on each parasite, their life cycle, developmental stage and the niche that they occupy within the final host.

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IN VIVO EFFECTS OF DRUGS USED IN LYMPHATIC FILARIASIS MDA PROGRAMS ON *BRUGIA MALAYI* IN GERBILS

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Lymphatic filariasis threatens nearly 20% of the world's population and has already handicapped one-third of the 120 million people currently infected, making continued control and research efforts a global health priority. Control is managed through mass drug administration (MDA) programs with three drugs: ivermectin (IVM), albendazole (ALB), and diethylcarbamazine (DEC), of which only ALB is well-understood. The lack of clarity regarding the mechanism of action of IVM and DEC is compounded by the disparity in results obtained ***in vitro*** versus ***in vivo***. The alteration of motility is the most simple and rapid way to gauge drug response ***in vitro***, making it the current standard for anthelmintic efficacy. In order to impair parasite motility, IVM requires drug concentrations orders of magnitude higher ***in vitro*** to even approach ***in vivo*** effects. In ***Brugia malayi***, the IC50 for microfilariae (Mf) was 43 µM, 10,000 times the amount of drug that clears Mf in human patients. DEC and ALB yielded less profound differences, with IC50 values 5.8 and 1.8 times the peak plasma concentration. These findings, and others suggest that the rapid clearance of Mf observed after MDAs with IVM or DEC is aided by the host's immune system. Given that ***in vitro*** experiments have proven to be inauthentic substitutes for studying antifilarial drug action, we have used an ***in vivo*** model with ***B. malayi*** in gerbils. Gerbils were intraperitoneally administered the infectious L3 and the worms allowed to develop to adulthood and begin producing Mf. They were then treated with 6 mg/kg DEC, 1 mg/kg ALB, or 0.15 mg/kg IVM mirroring human MDA dosages. Adults and Mf were collected 1 and 7 days post-treatment and RNA was isolated for transcriptomic analysis. Preliminary data analyzing the effects of IVM on adult females and Mf revealed changes in transcripts related to muscle regulation and locomotion as well as those encoding muscle protein and collagen expression, suggesting that IVM alters filarial neuromusculature and protein secretion. Further analysis of the effects of IVM, ALB, and DEC ***in vivo*** will provide a better understanding of how these drugs clear filarial parasites.

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MOLECULAR STUDIES OF THE PHYSIOLOGICAL ROLE OF THE ECDYSONE RECEPTOR IN FILARIAL PARASITES

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A homologue of the ecdysone receptor (EcR), a master regulator of development in insects has previously been identified and shown to be responsive to 20- hydroxyecdysone (20HE) in transfected ***Brugia malayi***. As the EcR is not found in vertebrate animals, it and the regulatory pathways it controls represent a attractive potential chemotherapeutic targets. In order to deduce the role the EcR plays in filarial parasites, adult female ***B. malayi*** were treated with 20HE in culture and microfilarial output and embryograms were monitored in treated and control parasites. RNAseq of the transcripts of adult females treated with 20HE was conducted to observe changes in gene expression. Proteomic analysis of the total protein extract of adult female worms treated with 20HE was also conducted to observe changes in gene expression at a biochemical level. Females treated with 20HE ecdysone produced significantly more microfilaria than control worms, implicating the EcR in regulation of microfilarial development. RNAseq identified 30 genes whose expression was genes significantly upregulated in the treated parasites compared to untreated controls. Of these, 18% were involved in regulating transcription. The proteomic analysis revealed 932 proteins to be significantly upregulated. Of these, 384 exhibited a greater than 2 fold difference in between the induced and uninduced parasites. A total of

15% of the upregulated proteins were involved in transcription regulation. Structural Activity Relationship (SAR) modeling was used to predict molecules capable of actively binding to the BmEcR. This has identified the diacylhydrazine family of molecules as potential agonists or antagonists of the receptor. These studies should assist in the development of inhibitors for the BmEcR that may be evaluated as potential lead compounds for development of a new class of drugs against the filaria.

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WORMBASE-PARASITE: A COMPREHENSIVE, OPEN-ACCESS RESOURCE FOR HELMINTH GENOMIC DATA

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WormBase-Parasite (parasite.wormbase.org) is a major new resource for storing, analyzing and exploring the genomes of helminth parasites. The public database, developed jointly by EMBL-EBI and the Wellcome Trust Sanger Institute, contains 97 annotated genomes from a total of 89 helminth species. A large number of the genomes were sequenced as part of the International Helminth Genomes Initiative - the largest collection of helminth genomic data ever assembled. The majority of the genome assemblies in WormBase-ParaSite are as yet unpublished so this resource provides unprecedented access to this high quality genomic data. WormBase-ParaSite is based on the well-established Ensembl infrastructure and provides several tools for exploring and analyzing the helminth data: a BLAST tool for aligning sequence to multiple genomes; a BioMart data-mining tool and Compara gene trees for comparative genomics analysis. We plan to add tools for the exploration of transcriptomic data for the next release of WormBase-ParaSite. This resource will allow researchers to perform critical investigations for example to identify of orthologs for existing drug targets, to discover new 'druggable' candidate genes, or to study the evolution of parasitic traits such as the ability to infect through skin. WormBase-ParaSite is closely integrated with WormBase, the genomic database for C. elegans and related species. Key reference parasitic genomes are incorporated in WormBase, as they become established and stable, where they are more richly curated. We welcome submissions from the helminth research community to gradually improve the phylogenetic coverage and build a robust resource for future research.

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LOCALIZATION OF ACETYLCHOLINE RECEPTOR (ACHR) SUBUNIT PROTEINS IN *BRUGIA MALAYI* GAMETES AND EARLY EMBRYOS

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AChRs are required for body movement in nematodes, and they are targets of several "classical" drugs that have been used to treat nematode parasite infections. We have recently reported that AChR subunit genes are highly expressed in reproductive tissues and muscle in ***Brugia malayi*** male and female adult worms and suggested that this may partially explain effects of drugs such as levamisole on worm reproduction. We now report results of parallel protein localization studies for AChRs. Anti-peptide antibodies to AChR subunit proteins Bm-unc-29 and Bm-acr-26 were used to localize the proteins in histological sections of ***B. malayi*** adult worms. Both of these proteins were detected in oocytes in the ovary and in early embryos (morulae through early pretzel stages) in females. The proteins were also present in spermatogonia and spermatocytes in the male testis. The uterus wall adjacent to stretched microfilariae (Mf) and the vas deferens adjacent to mature sperm were also strongly labelled by the anti-peptide antibodies. However, the uterus wall adjacent to developing embryos was not labeled. These results support the hypothesis that AChRs are involved in gameteogenesis and early embryo development and that they are also involved in the release of Mf and mature sperm. While the latter findings are consistent with neuromuscular signaling for Mf and sperm release, there are no nerves in oocytes or spermatogonia, and early embryos are not motile. Since acetylcholine has been shown to be a paracrine signaling molecule in several systems, the presence of AChR subunit proteins in germ line cells and in early embryos suggests that ACh may have an autocrine or paracrine function in filarial worms that promotes gametogenesis and growth of early embryos.

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BIOINFORMATIC IDENTIFICATION OF NEW DNA BIOMARKERS FOR *LOA LOA* INFECTION SUITABLE FOR LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Loa loa infections have emerged as a serious public health problem because of severe adverse neurological reactions in patients after treatment with ivermectin for onchocericasis or lymphatic filariasis. This necessitates the need for careful mapping of ***L. loa***, ***O. volvulus*** and ***Wuchereria bancrofti*** infections in regions were these parasites are co-endemic. Loop-mediated isothermal amplification (LAMP) has become a widely adopted screening method because of its operational simplicity, rapidity and versatility of visual detection readout options. Previous publications have described LAMP assays for ***L. loa*** using targets developed for PCR. In this study, we present a LAMP and the development of a species-specific LAMP assay for ***L. loa***. This pipeline identified ~140 new ***L. loa*** specific DNA repeat families as putative biomarkers of infection. The consensus sequence of one of these, repeat family 4 (RF4), was compiled from ~ 350 sequences dispersed throughout the ***L. loa*** genome. PCR and LAMP primers sets targeting RF4 only amplified ***L. loa*** and not ***W. bancrofti***, ***O. volvulus***, ***B. malayi***, human or mosquito DNA. Using turbidity as the readout, RF4 LAMP detects as little as 0.060 pg of ***L. loa*** DNA (~1/1600th of mf) purified from spiked blood samples in ~ 50 minutes, well within the 60 minute cut off time for the assay. The equivalent of one mf worth of DNA (100 pg) was consistently detected by RF4 LAMP in 25-30 minutes. In summary, we have successfully employed a bioinformatics approach to mine the ***L. loa*** genome for species-specific repeat families that could serve as biomarkers for LAMP. The species-specificity and sensitivity of the RF4 LAMP assay suggests that it shows promise as a field tool for the implementation and management of MDA programs and warrants further testing on clinical samples as the next stage in development towards this goal.

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POPULATION GENOMICS ESTIMATES HISTORICAL PREVALENCE OF *WUCHERERIA BANCROFTI* POPULATIONS

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To assess the progress of elimination we must first have a reference point for comparison. For ***Wuchereria bancrofti*** (Wb), elimination programs are recent. Therefore, there is an absence of past prevalence data for many newly mapped endemic areas. Without a reference measure it is difficult to evaluate elimination progress in light of stochastic fluctuations. We present a solution whereas we utilize population genomic models to infer the past demography of Wb populations in regions currently undergoing elimination. We apply the Pairwise Sequential Markovian Coalescent (PSMC) model to reconstruct infection history from a single Wb genome. We then use a more recent, multi-genome, version of PSMC to resolve more recent demography, closer to the start of elimination programs. Our methods demonstrate that information critical to Wb elimination can be obtained from a very small investment. Our results determine that Wb populations in Papua New Guinea were in decline before human

intervention, possibly as early as 500 AD. With population genomics we now have a metric for which to compare the progress of any future elimination program.

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IMPACT OF COMMUNITY DIRECTED TREATMENT WITH IVERMECTIN ON FOREST TYPE OF ONCHOCERCIASIS IN TANZANIA, EAST AFRICA: FROM CONTROL TO ELIMINATION

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Onchocerciasis is a public health problem in Tanzania and ivermectin mass drug administration has been used to control the disease since 1994. After evidence from Mali and Senegal showed that onchocerciasis elimination can be achieved in Africa by ivermectin mass treatment, Tanzania shifted its target from control to elimination by 2020. However, more empirical evidence is required to understand where and when onchocerciasis elimination can be achieved by ivermectin mass treatment and how that depends on the pre-control endemicity level, achieved coverage in mass drug administration programmes and the duration of mass treatment. Here, we present empirical data from epidemiological evaluation carried out in seven onchocerciasis ***foci*** in Tanzania, located four regions. These ***foci*** had received 9 to 12 years of ivermectin treatment with more than an average of 75% reported coverage. Per focus we selected 10-20 communities, based on their high pre-control endemicity level and proximity to vector breeding sites of the rivers. Mf prevalence and intensity were measured in through skin snips in all individuals aged 5 years and older who consented to the procedure. In total 23,638 individuals from 132 communities were examined. Based on the extensive epidemiological evaluation conducted and the statistical significance testing made during analysis, focal onchocerciasis infection elimination might already have been achieved in three of the seven ***foci*** evaluated namely Tanga, Tukuyu and Tunduru, although interruption of transmission remains to be confirmed by additional epidemiological surveys and/or entomological evaluations. Infection levels were still high in Mahenge and Kilosa ***foci***. However, due to poor implementation of mass drug administration in some the evaluated ***foci*** onchocerciasis is still highly prevalent which might need an alternative strategy to accelerate elimination in Tanzania. This will guide the policy decision and next steps to achieve elimination of onchocerciasis by 2020 in Tanzania.

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ONCHOCERCIASIS: SHIFT FROM CONTROL TO ELIMINATION-VECTOR CONTROL SHALL NOT REMAIN UNDER A BUSHEL!

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A little more than two decades ago, the approach to human onchocerciasis control was to attack the Simulium sp. vector through large scale and expensive aerial spraying, executed in the well-known Onchocerciasis Control Program (OCP) of West Africa. Larviciding was aimed at reducing vector densities to levels where transmission of the disease was eliminated and in some East Africa ***foci*** where it achieved local vector elimination. With the discovery of the drug ivermectin (Mectizan®) in 1987, there began a shift from vector control to annual mass treatment with ivermectin for onchocerciasis control that began with the launching of APOC in 1996, and culminated in the closure of OCP in 2002. APOC, with some small exceptions in Equatorial Guinea, Tanzania and Uganda, was focused on the Community Directed Treatment with Ivermectin (CDTI), where community volunteers are trained and utilized to treat their respective community members. It seems that since the establishment of APOC "vector control" become a "forbidden" term among polite onchocerciasis circles. Recently however vector control has been resurrected with the stated goal of attaining elimination in most of Africa by 2025. Vector control directed against Simulium larval stages through ground larviciding using WHO approved and environmentally safe insecticides was successful in eliminating S. yahense in Bioko Island, Guinea Bissau, S.neavei in 2003 and 2008 in Itwara and Mt. Elgon ***foci*** of Uganda respectively. It is also an important way forward in areas of onchocerciasis transmission that have concomitant ***Loa loa*** hyper-endemicity. Vector control encourages best practices in mapping breeding sites and defining clearly transmission zones and transmission seasons while focusing on elimination efforts in geographic areas where the resources are needed. Ground larviciding can be done at an affordable level by endemic countries and their partners in many instances, thus accelerating transmission interruption. The era of vector control taboo is gratefully over; it must be considered as a complementary tool for accelerating onchocerciasis elimination.

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EVALUATION OF ONCHOCERCIASIS STATUS IN SIXTEEN SELECTED CDTI ENDEMIC VILLAGES IN EDO, ENUGU AND DELTA STATES NIGERIA 2014

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We conducted an evaluation of the status of Onchocerciasis control in 16 villages (10 random and 6 Sentinel)April 2012 and April, 2014 after 19 years of annual mass drug administration - MDA, During the survey,1,203 persons aged 2 - 82 years were interviewed and examined for clinical Onchocerciasis, microfilaria - mf and 741 persons for ocular disease. We interviewed 16 village heads, 170 heads of households, 32 community directed distributors - CDDs and 10 health workers on CDTI. Entomological study in 6 sentinel villages, 6,695, S. damnosum s.l were examined by Polymerase chain reaction (PCR). We found mean skin mf (40.2% mf) in 10 random villages($p<0.001$) and 12.9% in 6 sentinel villages, all age groups affected, increasing with age and highest for age 50+ (49.3%) $p<0.05$. Community microfilaria load was above 0 mf/mg skin and highest in Utesse (1.8mf/mg skin). Notably, 60% of random villages were excluded from CDTI as hypo-endemic but showed evidence of onchocerciasis transmission of skin mf (33%), (29%)nodules with viable embryonic mf ***in utero*** and live male worms (9.5%). PCR showed infective O. volvulus mf L3 in the head in two villages (Oke, 5.8/10,000 and Idumogo, 30.7/10,000). Children <10 were found to have mf in skin (1.4%) $p<0.01$. Low therapeutic (49.5%) and low geographic (74%) coverage rates were observed indicative of failed CDTI. Few CDDs per village in random villages(1: 5,207), community involvement (54%) and willingness (60%) in contrast to the 6 sentinel villages (100%). Government funding was zero resulting in poor supervision. However, ocular and clinical skin lesions significantly reduced or not found. We concluded that Onchocerciasis prevalence following ivermectin has significantly reduced but the disease transmission is still ongoing. Villages considered hypo-endemic were found to be transmitting onchocerciasis. This is indicative of failed CDTI based on ONCHOSIM model. There is need to urgently modify current APOC/CDTI implementation strategy, conduct entomological assessments and adopt twice annual MDA in high transmission zones

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CHALLENGES IN THE DETECTION OF *WUCHERERIA BANCROFTI* MICROFILARIA IN AREAS WITH MULTIPLE FILARIAL INFECTIONS IN THE DEMOCRATIC REPUBLIC OF CONGO

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The Democratic Republic of Congo (DRC) is endemic for a variety of filarial infections. In 2014, surveys were undertaken in 13 sites (villages) to provide baseline data for the national lymphatic filariasis (LF) program. During the daytime 300 to 500 people in each site were tested using immuno-chromatographic tests (ICT) and filarial test strips (FTS). Nighttime capillary blood samples were then drawn only from participants who tested positive for LF antigenemia (by ICT and/or FTS). The smears were stained, fixed and examined microscopically. Antigenemia was detected in 319 individuals from 8 sites, with microfilarial (mf) parasites being visualized in 129 of the 319 (40.4%) - 86 with ***Wuchereria bancrofti*** (Wb), 72 with ***Mansonella perstans***, 7 with ***M. sterptocerca*** and 3 with ***Loa loa***. The only co-infections identified microscopically were between Wb and ***M. perstans*** (30 individuals or 34.9% of those with Wb mf), but since the 43 other individuals with non-Wb mf were also ICT and/or FTS-positive, it is likely that they too were co-infected since many individuals with Wb infection are amicrofilaremic. The median intensity of infection for Wb was 2004 mf/µl (range 33.4- 12558.4), 517.7 mf/µl (range 33.4-25818.2) for M. perstans, 116.9 mf/µl (range 50.1-183.7) for M. streptocerca and 2054 mf/µl (range 492 - 4108.2) for ***L. loa***. In the context of these multiple filarial infections, the identification of Wb infections is programmatically challenging. Heavy M. perstans infections can obscure Wb mf in night-blood exams; and even the generally diurnally periodicic ***L. loa*** and the 'subcutaneous' mf of M. streptocerca could be found in nighttime blood smears. While diagnosis of Wb is most effectively made by ICT or FTS antigen detection, determining exactly how mass drug administration for LF should be implemented in the complex environments of DRC may require not only antigen detection but also the assistance of specific antifilarial antibodies as well.

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MAPPING THE DISTRIBUTION AND ENVIRONMENTAL DRIVERS OF *LOA LOA* IN NIGERIA: PREPARING FOR THE SCALE UP OF INTERVENTIONS AND ELIMINATION OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS

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The distribution of ***Loa loa*** filariasis (also loiasis or tropical eye worm) in Nigeria is potentially a major obstacle to the elimination of lymphatic filariasis (elephantiasis) and onchocerciasis (river blindness) due to the risk of severe adverse events associated with the standard drug regime including ivermectin. Understanding the distribution and environmental drivers of the ***L. loa*** parasitic disease transmitted by the forest Chrysops spp. may help to predict high risk co-endemic areas, and where alternative treatment strategies are required to interrupt transmission of lymphatic filariasis (caused by ***Wuchereria bancrofti***, transmitted by Anopheles spp. mosquitoes), and onchocerciasis (caused by ***Onchocerca volvulus***, transmitted by riverine Simulium spp). This study aimed to develop a historical ***L. loa*** database for Nigeria from all available public scientific sources, including information on the location, time period, vector, diagnostic methods and prevalence of infection of microfilaria or history of eyeworm. All data were geo-referenced and mapped to identify high risk areas, and data on climate, vegetation, land cover and river systems examined. The results to date include more than 40 publications since the year 1910, and encompass more than 200 data points, in over 130 study sites across 19 states and five zones of the country. The majority of data were found in the southern region of the country, with ***Chrysops silacea*** and ***C. dimidiata*** as main vectors. Parasitological examination of blood films for microfilaria were the main diagnostic method until the last decade where the new Rapid Assessment Procedure for loiasis (RAPLOA) has been used. Prevalence varied across geographical areas, and was more predominant in the tropical forested areas. Detailed environmental analysis is currently underway and will be presented and discussed. This extensive ***L. loa*** database will be a critical resource to the lymphatic filariasis and onchocerciasis elimination programmes. It will help to predict problems areas and where alternative strategies such as different drug regimes and vector control can be implemented safely and effectively.

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BEYOND MASS DRUG ADMINISTRATION: IDENTIFYING OPTIMAL DIAGNOSTIC TOOLS FOR LYMPHATIC FILARIASIS POST-TREATMENT SURVEILLANCE

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Significant progress has been made toward the global goal to eliminate lymphatic filariasis (LF). Many LF endemic countries have implemented successful mass drug administration (MDA) programs and have stopped MDA in some areas based on reduced infection in children, including Ghana and the Philippines. Post-treatment surveillance (PTS) guidelines include a recommendation to detect new ***foci*** of transmission using available diagnostic tools, including antigen and antibody tests. While antigen tests (ICT) are widely used, the utility of antibody tests for monitoring LF transmission has not been well-defined. In order to define optimal strategies for the use of antibody tests for PTS, it is necessary to have a better understanding community-wide clearance of antibody responses following MDA. To characterize antigen and antibody responses post-MDA, samples from Ghanaian adults (15-45 years) and individuals from the Philippines (4-85 years) were tested by ICT and two LF ELISAs (Wb123, Bm14). For both countries, ICT prevalence was below 2%, the threshold that warrants MDA (Ghana – 10/988 (1.0%); Philippines – 10/1153 (0.9%)). Overall Wb123 antibody prevalence was low (Ghana – 5/749 (0.7%); Philippines – 34/1131 (3.0%)) but increased with age in the Philippines where > 90% of positive individuals were ≥20 years of age. Of the samples that were also tested for Bm14 antibodies, the prevalence of Bm14 was higher than Wb123 (Ghana – 39/96 (40.6%); Philippines – 38/118 (32.2%)). There was no correlation between ICT and antibody positivity to either Wb123 or Bm14. Additional samples from both countries are currently being analyzed by Bm14 ELISA. The observed difference between Wb123 and Bm14 results suggests a lower sensitivity for Wb123 compared to Bm14 antibodies post-MDA, perhaps indicative of a more rapid clearance of Wb123 antibodies. These results may have implications for selecting the optimal tool to monitor incident infections in young children, but strategies to monitor the decline in antibody prevalence in adult populations may be appropriate for PTS.

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FAMILIAL AGGREGATION AND HERITABILITY OF *WUCHERERIA BANCROFTI* LYMPHATIC FILARIASIS

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Lymphatic filariasis (LF) is responsible for severe disabilities across the world, especially because of lymphedema. Although immune factors were identified to explain the development of lymphedema in infected individuals, few studies have investigated the genetic susceptibility to infection. To assess familial aggregation and heritability of ***Wuchereria bancrofti*** infection, we conducted a study in a village of the Republic of Congo. Pedigree was built for 829 individuals (broken down in 267 households and 36 families) from which 143 were positive at the immunochromatographic card test (prevalence: 17.3%) and 44 (5.3%) had ***W. bancrofti*** microfilariae (mf). Analyses were adjusted on individual risk factors for LF (age, sex, outdoor activities, usage of bednet) and for environmental factors (eg: distance between house and nearest river), and accounted for possible household effect. Patterns of familial aggregation, assessed using S.A.G.E. software, showed that the presence of antigenemia was very slightly but significantly correlated within families ($0.06 < r < 0.10$; with $0.001 <$ P-value < 0.013). Regarding microfilaremia, correlation values were much higher: $r = 0.45$ between fathers and sons (P-value = 0.013), $r = 0.78$ between mothers and sons (P-value = 0.034), and $r = 0.94$ between fathers and daughters (P-value < 0.001). Heritability was estimated using SOLAR software. Genetic factors explained 13% (P-value = 0.226), 61% (P-value = 0.166) and 51% (P-value = 0.048) of variation in the presence of antigenemia, presence of mf, and in mf density, respectively. Household effect was never found significant. Our results show that the acquisition of ***W. bancrofti*** infection (as assessed by antigenemia) barely depends on genetic factors and is thus mainly due to exposure factors. However, both the presence of mf and variation in ***W. bancrofti*** mf density seem significantly influenced by genetic factors. Additional genetic studies are needed to confirm this finding.

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HUMAN ONCHOCERCIASIS: MODELLING THE POTENTIAL LONG-TERM CONSEQUENCES OF A VACCINATION PROGRAM

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Currently, the predominant onchocerciasis control strategy in Africa is annual mass drug administration (MDA) with ivermectin. However, there is a consensus among the global health community, supported by mathematical modelling, that onchocerciasis in Africa will not be eliminated within proposed time frameworks in all endemic ***foci*** with only annual MDA, and that novel and alternative strategies are urgently needed. Furthermore, use of MDA with ivermectin is already compromised in large areas of central Africa co-endemic with loiasis and there are areas where suboptimal or atypical responses to ivermectin have been documented. An onchocerciasis vaccine would be highly advantageous in these areas. We used a previously developed onchocerciasis transmission model (EPIONCHO) to investigate the impact of vaccination in areas where loiasis and onchocerciasis are co-endemic and ivermectin is contraindicated. We also explore the potential influence of a vaccination programme on infection resurgence in areas where local elimination has been successfully achieved. Based on the age range included in the Expanded Programme on Immunization (EPI), the vaccine was assumed to target 1 to 5 year olds. Our modelling results indicate that the deployment of an onchocerciasis vaccine would have a beneficial impact in onchocerciasis-loiasis co-endemic areas, markedly reducing microfilarial load in the young (under 20 yr) age groups. An onchocerciasis vaccine would reduce the onchocerciasis disease burden in populations where ivermectin cannot be administered safely. Moreover, a vaccine could substantially decrease the chance of re-emergence of ***Onchocerca volvulus*** infection in areas where it is deemed that MDA with ivermectin can be stopped. Therefore, a vaccine would protect the substantial investments made by present and past onchocerciasis control programmes, decreasing the chance of disease recrudescence and offering an important additional tool to mitigate the potentially devastating impact of emerging ivermectin resistance.

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IMMUNO-EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTH INFECTIONS AFTER REPEATED SCHOOL-BASED DEWORMING: A COMMUNITY-WIDE CROSS SECTIONAL STUDY IN WESTERN KENYA

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The development of human immunity to soil-transmitted helminths remains poorly understood despite their widespread endemicity in tropical and subtropical countries. Infected individuals in endemic areas do not appear to develop fully protective immune responses, and the factors driving possible partial immunity acquired are unclear. With the increasing number of endemic countries introducing school-based deworming programs, there is a need to understand the effect of anthelminthic treatment on immune responses to helminths, not only in school-age children but also in younger and older members of the treated community. This study investigates both the development of humoral immunity against ***Ascaris lumbricoides*** and hookworm in an endemic community, and the effect of school-based and community-based anthelminthic treatment on antibody responses. The study took place in 2014 in four villages of Bungoma County, Western Kenya, where annual school-based deworming has been taking place since 2012. Stool and finger-prick blood samples were collected from over 1300 individuals aged 2 to 88 years, before and three months following community-wide treatment with 400mg albendazole. Parasite egg counts were obtained using Kato-Katz thick smears and antibody seroprevalence was measured by enzyme-linked immunosorbent assay (ELISA). Prevalence of ***A. lumbricoides*** and hookworm infections was 7.3% and 6.2%, respectively, at study baseline, and 2.6% and 2.0% at follow-up. Individual antibody profiles against ***A. suum*** haemoglobin (AsHb) and ***Necator americanus*** larval (Na-ASP2) and adult (Na-SSA-2) antigens were obtained and analysed by age-group, village and population level. Correlations between antibody seroprevalence and intensity of soil-transmitted helminth infection were investigated at both sampling time-points, taking into consideration a series of confounding factors including malaria co-infection and socio-economic and hygiene and sanitation ranks. Changes in antibody seroprevalence levels post community treatment with albendazole were also investigated, with particular emphasis on differences between age-groups.

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COMPARISON OF KATO KATZ AND MINI-FLOTAC FOR ESTIMATION OF PREVALENCE AND INTENSITY OF INFECTION WITH SOIL-TRANSMITTED HELMINTHS IN THE PERUVIAN AMAZON

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Women of reproductive age are considered a high-risk group for soil-transmitted helminth (STH) infections. A randomized placebo-controlled trial on maternal postpartum deworming with single-dose 400 mg albendazole is currently underway in Iquitos, Peru. In order to evaluate re-infection rates, stool specimens were collected six months after treatment allocation. In a substudy of participants, stool specimens were analyzed by both the Kato Katz (KK) and mini-FLOTAC (MF) methods. The aim of this substudy was to compare the diagnostic accuracy of the World Health Organization recommended quantitative diagnostic method, KK, to the MF method. All laboratory personnel were blinded to the results of the other test, using a code switching technique. Eggs per gram of stool were calculated for each species using a multiplication factor of 24 for KK and 10 for MF. The total number of positive specimens detected by either method was taken as the diagnostic reference standard for each parasite species. Of the 306 women screened for STH infections, 41% were found to be positive for at least one of the helminth species, using either diagnostic method (17% ***Ascaris***, 34% ***Trichuris***, and 7% hookworm). The KK method had a higher sensitivity compared to MF for ***Ascaris*** (98% vs. 81%), and hookworm (90% vs. 60%), but not ***Trichuris*** (84% vs. 91%), though these differences were not statistically significant. The strength of the agreement (k Cohen coefficient) between the two methods was high, ranging from 0.86 for ***Ascaris***, 0.80 for ***Trichuris***, and 0.65 for hookworm. The KK method diagnosed a statistically significant higher number of eggs for all three helminth species compared with MF. These results contribute to the on-going discussion of which diagnostic method is optimal for assessment of STH prevalence and intensity in field conditions.

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IMMUNOLOGICAL CHARACTERIZATION OF HUMAN HOSTS TO *ASCARIS LUMBRICOIDES* AND *TRICHURIS TRICHIURA* INFECTION IN A POPULATION LIVING IN THE RURAL MUNICIPALITY OF COLOMONCAGUA, HONDURAS

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Soil-transmitted helminth (STH) infections, specifically ***Ascaris***, ***Trichuris***, and Hookworm, compose three of the most prevalent Neglected Tropical Diseases, infecting over a billion people worldwide. Recurrent childhood STH infections have been shown to cause stunted physical growth, reduced physical fitness, and decreased school performance. ***Ascaris*** and ***Trichuris*** are of notable interest, given their abrupt decline from a peak prevalence and intensity in pre-adolescence to adulthood, with little published works evaluating the possible underlying immunological mechanism in human. We used modern molecular techniques to (a) determine the burden of disease and (b) evaluate the immune profile of 30 adolescents and 51 adults between the ages 13-45, in rural Honduras, endemic for ***Ascaris*** and ***Trichuris***. Using quantitative PCR of DNA extracted from stool we quantified the burden of disease of both ***Ascaris*** and ***Trichuris***. Positive samples were stratified into groups based on the degree of infection, and controlled for with samples positive for 6 other common gastrointestinal parasites: ***Necator americanus, Ancylostoma duodenale, Entamoeba histolytica, Strongyloides stercoralis, Giardia lamblia***, and ***Cryptosporidium parvum***. Each group was immunologically characterized for serum Th1, Th2, and Th17 cytokines using LUMINEX analysis. Further immunological work-up was done using ELISA analysis to identify ***Trichuris*** and ***Ascaris*** specific IgG, IgM, and IgE antibodies. Select putative serum to ***Trichuris*** and ***Ascaris*** may be used to screen vaccine antigen candidates. Results from this pilot study identify the basic serum immune profile associated with protection against ***Ascaris*** and ***Trichuris*** and serve as the foundation for a second, more comprehensive study.

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A CONTEXTUAL FRAMEWORK TO ASSESS HETEROGENEITY IN IMPACT OF NATIONAL NEGLECTED TROPICAL DISEASE CONTROL PROGRAMS: EVIDENCE FROM SCHOOL-BASED DEWORMING IN KENYA

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The majority of countries endemic for soil-transmitted helminths are now implementing mass drug administration programmes, either as part of school-based deworming (SBD) efforts or lymphatic filariasis control programmes. MDA implementation happens however in a heterogeneous social and economic environment, so that the success of STH treatment programmes will vary according to the context. The impact of STH treatment programmes on reducing infection and reinfection is theoretically influenced by a variety of factors, including: drug efficacy, treatment coverage, and intensity of transmission, which itself is influenced by climate and levels of water, sanitation and hygiene (WASH). Based on data from the national SBD programme in Kenya, we describe the heterogeneity in programme impact and investigate the influence of contextual factors. Indicators of the domains STH epidemiology, capacity to deliver treatment, operational feasibility and financial capacity were developed based on open access data and basic school WASH questionnaires. Associations of these variables with relative prevalence and intensity reductions were investigated using mixed effects linear regression analysis at the school-level. Our findings demonstrate that relative prevalence and intensity reductions for ***Ascaris lumbricoides*** and hookworms varied significantly by county (equivalent to district) and within counties by school. Multivariable analysis of factors associated with programme impact showed evidence for a higher reduction of ***A. lumbricoides*** among schools with ventilation improved pit (VIP) latrines compared to pit latrines and among schools located in areas with a higher land surface temperature. Whereas, higher hookworm reductions were found among schools in locations with higher community-level access to improved sanitation. In conclusion, this study demonstrated the influence of contextual factors on the implementation of deworming programmes and highlights the importance of improved sanitation in support of deworming efforts.

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THE USE OF *ASCARIS SUUM* HAEMOGLOBIN AS DIAGNOSTIC ANTIGEN FOR HUMAN ASCARIASIS

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The human roundworm ***Ascaris lumbricoides*** infects some 800 million people in the developing world. Standard diagnostic tests for ascariasis depend on detection of parasite eggs in stool samples. This technique has some important limitations in terms of both application and interpretation. A new antibody test has recently been developed for detecting ***Ascaris*** infections in domestic pigs using ***A. suum*** hemoglobin antigen (AsHb). Since ***Ascaris*** worms in humans and pigs are very similar, the objective of this study was to evaluate the diagnostic value of native and recombinant AsHb for community diagnosis of human ascariasis. Initial results showed that humans living in an endemic area in Indonesia had high rates of IgG4 antibodies to AsHb. Antibody rates and titers significantly decreased in the community following two annual rounds of mass treatment with albendazole. Unfortunately, further studies showed that sera from patients with hookworm infections contain cross-reactive antibodies to AsHb. Interestingly, antibodies in ***Ascaris*** and hookworm infection sera do not bind to recombinant AsHb produced in ***E. coli*** or to AsHb after treatment with PNGaseB. This suggests that the antibodies bind to carbohydrate epitopes. This study has provided a proof of principle that antibody testing may be useful for monitoring the effects of deworming programs in communities. While antibodies to shared carbohydrate antigens may have some value, we are now searching for antigens that can provide species-specific diagnoses.

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PERSISTENCE OF HIGH PREVALENCE OF SOIL TRANSMITTED HELMINTHS IN CHILDREN IN SUBURBAN AREA OF DAKAR SENEGAL, DESPITE MASS DE-WORMING STRATEGIES

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Soil-transmitted helminths are the most widespread Neglected Tropical Diseases (NTDs). Senegalese ministry of health has implemented mass drug administration with mebendazole and albendazole since 2006 as per recommended by WHO. This study aimed to describe the burden of these diseases among children several years after the scale up of these strategies. A retrospective study was conducted in a pediatric clinic in a hospital located in suburban area of Dakar, Senegal between March and December 2013. Stool samples from children under 15 years old attending hospital, were collected and examined by microscope using the modified Ritchie concentration techniques. For each child, hematologic parameters and nutritional status were also assessed. Out of 402 children surveyed, 207 (53.9%) were infected with one or more species of intestinal parasites. The prevalence rate was 120 (55.3%) for male and 97 (44.7%) for female. The prevalence was high (43.7%) in age group of 1-5 years compared to other age group. Infections with soil-transmitted helminths were more important (65.9%) compared to protozoan (18.4%). ***Ascaris lumbricoides*** was the predominant isolate (34.1%), followed by Trichirus trichiura (22.5%) and Giardia intestinalis (9.6%). Among the 140 (64.5%) infected children with anemia, 23 (16.4%) presented severe anemia with hemoglobin level below 8g/l. Severe stunting was also noted in 35 (15.6%) infected patients. Prevalence of soil-transmitted helminths remains high among children in suburban area despite the mass de-worming strategies. So, it's urgent to conduct more epidemiological survey in these areas to assess the impact of the mass drug administration.

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PERFORMANCE OF REAL-TIME PCRS FOR THE DETECTION AND THE QUANTIFICATION OF GASTROINTESTINAL PARASITES IN CLINICAL SAMPLES FROM SENEGAL

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Gastrointestinal parasites infections represent one of the major public health problems in the world. Therefore, appropriate innovative tools for clinical diagnosis and epidemiological investigations are needed for assessing interventions to control these infections. This study aimed to compare the performance of real time PCR (qPCR) systems to microscopic examination in the detection of intestinal parasites.One hundred fecal samples were collected from patients and control groups attending Senegalese hospitals. Microscopic examination was made on fresh stool samples, after modified Ritchie and modified Ziehl Neelsen concentration techniques. Species-specific primers/probes were used for 21 common gastrointestinal protozoans and helminths. Positive frequency, sensitivity and specificity of each qPCR system were compared to conventional microscopic examination. Real-time PCR was positive in 37 of 103 samples (35.9%) for 18 parasite species tested while microscopic examination was positive in 19 (18.4%) samples ($p < 0.05$). QPCR enabled to identify 30 single infections and 7 multiples infections. Among the most detected protozoa, comparative results between qPCR and microscopy showed respectively 12 positive cases (11.6%) vs 7 (6.8%) for ***Giardia intestinalis***, 3 (2.9%) vs 1 (0.9%) for ***Entamoeba histolytica*** and 4 (3.8%) vs 0 (0.0%) for ***Dientamoeba fragilis***. In helminths group, the number of positive cases by qPCR vs microscopy for the most detected parasites was respectively at 8 (7.7%) vs 5 (4.8%) for ***Trichuris trichiura***, 4 (3.8%) vs 1 (0.9%) for ***Taenia saginata*** and 3 (2.9%) vs 0 (0.0%) for ***Strongyloides stercoralis***. Considering results obtained by both methods as gold standard, overall sensitivity and specificity of qPCR were at 90.2% and 100% respectively.QPCR seems to be superior to microscopic examination for the detection of protozoan and helminths in stool samples. However, these are preliminary results which should be confirmed during next steps.

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IMPROVEMENT OF REAL-TIME PCR DIAGNOSIS OF *TRICHURIS TRICHIURA* USING ETHANOL PRESERVED STOOL SAMPLES AND A BEAD-BEATING PROCEDURE

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Real-time PCR has proven to be a highly specific and comparatively sensitive tool in the detection of virtually all clinically relevant stool parasites, with the exception of ***Trichuris trichiura***. The robustness of the eggs seems the most likely cause of diminished DNA yields for this helminth. Here we evaluated different sample preparation procedures in order to optimize PCR-based detection of ***T. trichiura***. Stool samples (n=60) from a ***T. trichiura*** endemic community were used to compare four different sample preparation procedures. All samples were microscopically examined for intestinal helminths, while two aliquots were taken for DNA detection. One aliquot was frozen directly without preservation; the other was mixed with 96% ethanol. After transportation to a centralized laboratory, the present ethanol was washed away. Thereafter a bead-beating procedure was performed and compared to non-beating controls. DNA isolation was done using a spin column-based method followed by multiplex real-time PCRs for the detection of six helminth species and four protozoa. ***T. trichiura*** DNA could be detected in 40% of the directly frozen samples using the standard procedure. Higher detection levels

were found by microscopy (45%), ethanol preservation (45%), bead-beating (52%) and the combination of the latter two (55%). A significant correlation was seen in all used procedures between microscopy egg counts and the PCR cycle threshold (Ct) value, representing the detected parasite DNA load. At the same time Ct-values decreased significantly with the combination of ethanol preservation and bead-beating, reflecting increased efficiency of the DNA isolation. The various procedures hardly influenced the detection rate of the other parasites present, being ***Ascaris lumbricoides*** (≈60%), ***Necator americanus*** (≈60%), ***Dientamoeba fragilis*** (≈50%) and ***Giardia lamblia*** (≈12%). In this study we showed that preservation of stool samples using 96% ethanol in combination with a bead-beating step before DNA extraction is performed, improves the DNA yield of ***T. trichiura*** without hampering the real-time PCR detection levels of other intestinal parasites.

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FACTORS ASSOCIATED WITH NATIONAL DEWORMING COVERAGE OF SCHOOL-AGE CHILDREN FOR SOIL-TRANSMITTED HELMINTHIASIS

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The World Health Organization (WHO) has established a target of providing preventive chemotherapy (PC) for soil-transmitted helminthiasis (STH) to at least 75% of at-risk school-age children (SAC) in each endemic country by 2020. According to World Health Organization, 29 of 106 endemic countries reached or surpassed this threshold in 2013. To identify factors associated with higher PC coverage in SAC, we compared national treatment data from the WHO Preventive Chemotherapy Databank with indicators for economic development, health systems, infrastructure, school enrollment, and related programmatic metrics (e.g., coverage of preschool children). We categorized countries into two groups by reported coverage, with 28 countries reaching at least 75% of at-risk SAC and 27 reaching fewer than 75%. Coverage data were not available for 51 endemic countries. Considering endemic countries by WHO region, 26% (11/42) of Africa, 25% (6/24) of the Americas, 50% (4/8) of South-East Asia, 13% (1/8) of the Eastern Mediterranean, 25% (2/8) of Europe, and 27% (4/15) of the Western Pacific achieved at least 75% coverage. Compared to countries reporting <75% coverage, countries with ≥75% coverage had a 11% higher average per capita gross domestic product ($4848 vs. $4384) and 53% higher physician density (0.65 doctors per thousand people vs. 0.42). Countries with higher coverage for SAC also reported much higher coverage for preschool-age children as well (57% vs. 8%), suggesting underlying factors may be linking performance for both metrics. Other metrics, such as net school enrollment and access to improved sanitation, differed by <10% for both groups. While only limited causal insights can be drawn from this descriptive exploration, our findings highlight the need for a more rigorous longitudinal analysis that considers a wide range of indicators and programmatic variables, some of which may not be readily available. These results also underscore the potential value of improving the detail of treatment data, which could help identify predictors of success as the global community strives to cover 75% of children by 2020.

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DELINEATING THE REGULATION AND FUNCTION OF HUMAN RESISTIN USING TRANSGENIC MICE AND CLINICAL SAMPLES FROM SOIL-TRANSMITTED HELMINTH INFECTIONS

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Resistin-like molecules (RELM) belong to a family of secreted proteins that are expressed in multiple helminth infections with important effects on the host immune response. However the importance of human RELM proteins in helminth infections is less well understood. To investigate this, we utilize transgenic mice in which the human resistin gene along with its transcriptional regulatory elements was inserted. We recently showed that infection with the hookworm ***Nippostrongylus brasiliensis*** caused significantly increased human resistin expression in the infected lung and intestine. Human resistin expression was detrimental to the host and provoked a monocyte-rich inflammatory response in the lung, increased expression of inflammatory cytokines such as TNFα and impaired parasite expulsion. Additionally, ***Ascaris***-infected children from Ecuador had elevated serum resistin levels, which were positively correlated with parasite egg counts in the stool and with serum inflammatory cytokines. Together, these studies identify a detrimental role for human resistin in instigating a non-protective inflammatory response following helminth infection. In ongoing studies, we are investigating the genetic determinants that regulate resistin expression including single nucleotide polymorphisms in the gene and putative transcription binding sites in the promoter. Preliminary analysis has identified two STAT6 binding sites in the human resistin promoter implicating the Th2 cytokine pathway in promoting human resistin expression, and we have validated STAT6 induced resistin expression ***in vitro*** and ***in vivo***. Identifying the cellular and molecular signals that regulate resistin expression may have important diagnostic and therapeutic implications for helminth infection.

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DEVELOPMENT OF A CONTROLLED HUMAN INFECTION MODEL FOR TESTING THE EFFICACY OF EXPERIMENTAL HOOKWORM VACCINES

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A controlled human hookworm infection model is being developed to provide early proof-of-concept that experimental hookworm vaccine candidates are feasible and efficacious. The proposed model consists of vaccinating healthy, hookworm-naïve adults with a candidate hookworm vaccine, followed by challenging them with infectious ***Necator americanus*** larvae (L3) to assess the effect of vaccination on infection. Currently, a feasibility study is underway in Washington, DC, in which different doses of L3 are administered to healthy adult volunteers to determine the optimal dose that is safe, well-tolerated and results in consistent levels of infection. 3 cohorts of 10 healthy, hookworm-naive adult volunteers are receiving 25, 50, or 75 L3 in a dose-escalating design. L3 are obtained from the feces of an infected donor who is regularly screened for blood borne pathogens. Batches of L3 are tested for identity, motility/viability, and bacterial/fungal growth prior to release for use. Individual doses are prepared by counting motile L3 by microscopy; these are then applied to a gauze pad that is placed on the subject's forearm for 1 hour. Subjects are seen weekly until 12 weeks post-infection, when they are treated with albendazole. Fecal and blood samples are collected at regular time points. Preliminary results from the 25 L3 dose cohort indicate that it is well tolerated by volunteers (n=10). Early manifestations of infection included pruritus, erythema, pain, and papulovesicular rash (duration: 4-48 days) at the application site. Gastrointestinal complaints (abdominal bloating, flatulence, nausea and abdominal pain) are frequent starting between weeks 4-5 post-infection. Eosinophilia developed in 8 of 10 (range: 0.5-4.9 x 10^3/mm^3). As of 9 weeks post-infection, 3/10 subjects have eggs detectable by microscopy in their feces. Additional investigations being performed include video capsule endoscopy to visualize adult worms in the intestine, serology for crude and defined hookworm antigens, fecal PCR for hookworm egg antigen, and fecal worm counts post-treatment. Full results, including those from the remaining cohorts, will be presented.

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KATO KATZ VERSUS LUMBRERAS RAPID SEDIMENTATION TEST TO EVALUATE HELMINTH PREVALENCE IN THE SETTING OF A SCHOOL BASED DEWORMING PROGRAM

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The sensitivity of the Kato Katz (KK) test is suboptimal for the evaluation of intestinal helminths prevalence. Moreover, during mass deworming with albendazole, when helminths egg burden decreases, the KK sensitivity is likely to be even lower. The Lumbreras rapid sedimentation is a low cost non quantitative test, but may provide useful information in low burden areas. A descriptive study comparing the prevalence of intestinal helminth infections assessed by the KK and the Lumbreras rapid sedimentation test was performed. A database of an ongoing study in the Anta province of Cusco where school based mass albendazole treatment is provided twice a year was used. Lumbreras rapid sedimentation tests were read in a Petri dish at 100x (dish) and in a slide at 100x and 400x (slide). Kato Katz, slide, and dish tests were performed following standard procedures, in different days, by 3 different observes blinded to other test results. The sensitivities were compared using the McNemar test with a significant $p<0.05$. A total of 774 children were included in the study and each provided 3 stool specimens for testing. The prevalence of Ascaris infection was 6.6% by KK, 6.6% by slide, and 7.4% by dish. Similarly, the prevalence of Trichuris was 0.5% by KK, 1.2% by slide, and 1.3% by dish and the prevalence of hookworm was 0% by KK, 0.5% by slide, and 0.8% by dish. The prevalence of other helminths like Strongyloides (0% KK, 0.6% slide, 1.8% dish) and Hymenolepis nana (15.4% KK, 16% slide, 19.6% dish) also varied with the diagnostic method used. Using the combined outcome of the 3 different stool tests as the standard, the sensitivities of the KK and dish sedimentation tests were 83.6% and 93.4% ($p=0.070$) for Ascaris, 77.3% and 98.7% ($p<0.001$) for H. nana, 36.4% and 90.9% ($p=0.031$) for Trichuris, 0% and 100% ($p=0.031$) for hookworm, and 0% and 77.8% ($p<0.001$) for Strongyloides respectively. The rapid-sedimentation and Petri dish reading was able to detect more infections with intestinal helminthes than the other methods. When compared with this method, Kato Katz demonstrated significantly lower sensitivity, missing most Trichuris, hookworm, and Strongyloides infections

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AURANOFIN REPURPOSING: A NEW CURE FOR SOIL-TRANSMITTED HELMINTHES INFECTION

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Soil-transmitted helminth (STH) are nematode parasites (hookworms, ***Ascaris*** and ***Trichuris***) and are key contributors to morbidity and poverty worldwide. Few anthelmintics are available for treatment, and only one anthelmintic, albendazole, are considered adequate for mass drug administrations, even though there are more than 1 billion people are infected. New anthelmintics and treatment strategies are greatly needed, in particular as albendazole resistance is inevitable given its current method of usage and given the widespread resistance to benzimidazoles (like albendazole) in veterinary use. Due to the cost and time of developing new drugs, researchers have been working on "repurposing" the FDA approved drugs for the new usages. A gold complex named as auranofin received more attention for its great potential to be repurposed for multiple therapeutic applications. Auranofin is approved for the treatment of rheumatoid arthritis by WHO in 1985. More recently, it was found auranofin has great potential as a treatment for a number of parasitic infections including lymphatic filariasis, onchocerciasis, African trypanosomiasis, malaria, and schistosomiasis. We hypothesize that auranofin has efficacy against STHs and other nematodes as well. We exposed Auranofin to four different nematodes, including a free-living nematode ***Caenorhabditis elegans*** and the various intestinal parasitic nematodes such as ***Ancylostoma ceylanicum, Trichrus muris***, and ***Heligomasmidoes polygyrus in vitro.*** The ***in vivo*** efficacy of auranofin against all these three parasitic worms also was evaluated in rodents. Here we present the results of these studies and the potential efficacy of auranofin against STH infections.

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IMMUNODIAGNOSIS OF *STRONGYLOIDES STERCORALIS* INFECTION IN CANDIDATE PATIENTS FOR TRANSPLANTATION

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Strongyloidiasis is an intestinal infection caused by the nematode ***Strongyloides stercoralis***. Most cases progress to a benign chronic condition, however hyperinfection and dissemination may occur, especially in immunocompromised patients. The aim of this study was to evaluate RIFI, ELISA and WB techniques for the diagnosis of ***S. stercoralis*** in candidate patients for transplantation. In order to validate the tests were used serum samples from immunocompetent patients. Feces and serum samples from candidate patients for transplantation were used as follows: 50 for renal transplant (RT), 50 for liver transplant (LT), 50 for bone marrow transplant (BMT). Fecal samples from all patients were analyzed by spontaneous sedimentation, Rugai and Agar plate culture techniques. Filariform larvae of S. venezuelensis were used as source of antigen. For RIFI, sera at 1:40 and human anti-IgG conjugate fluorescein at 1:500 were diluted in PBS. For ELISA, 10µg of antigen (saline and alkaline soluble fractions), sera diluted at 1:200 and conjugate (human anti-IgG peroxidase) at 30.000 in PBS 0.05% Tween 3% of milk were used. For WB, sera at 1:100 and conjugate (human anti-IgG peroxidase) at 1:1000 in were diluted in Tris-HCl 5% of milk. Among the patients candidates for transplantation, 9.3% (14/150) were positive by parasitological techniques; agar plate culture detected 6.6% (10/150). With RIFI positivity was 16.6% (25/150), among which 22% RT, 18% LT and 10% for BMT. By ELISA technique positivity was 11.3% (17/150), among which 14% in patients candidates for RT, 14% LT and 3% BMT, using alkaline antigen, and 24.6% (37/150), among which 18% in patients candidates for RT, 50% LT and 6% BMT, using saline antigen. By WB technique positivity was 20.6% (31/150), among which 18% in patients candidates for RT, 32% LT and 12% BMT, using alkaline antigen, and 18.6% (28/150) among which 10% in patients candidates for RT, 14% LT and 32% BMT, using saline antigen. Application of immunodiagnostic techniques may be indicated in screening of candidate patients in transplant, however limitations of serological reactions in immunosuppressed patients should be considered.

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PEDIATRIC STRONGYLOIDIASIS AND POVERTY IN TUMBES, PERU

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Strongyloides stercoralis is a helminth that causes chronic infections in humans worldwide. Although zoonotic transmission has been described for many helminthes, studies about the zoonotic potential of ***S. stercoralis*** and possible role of domestic animals are scarce, especially in resource-limited areas. This precludes the identification of risk factors and the development of potentially effective interventions. We conducted a population-based cross-sectional study in 14,833 children aged 2-15 years old who resided in 115 rural villages of Tumbes, a low-resource, low-endemicity setting in Peru. We used a generalized linear model to estimate the prevalence ratio (PR) of strongyloidiasis according to socioeconomic factors and zoonotic potential such as dog ownership, after controlling for potential confounders. The overall prevalence of ***S. stercoralis*** was 0.7% and the 50% of the positive children had dogs at home. We didn´t find a significant association between strongyloidiasis and dog ownership (adjusted PR [aPR] = 0.97; 95% CI = 0.66-1.44; p = 0.883). Compared with houses built with brick and cement, the prevalence of strongyloidiasis was higher in adobe and thatch houses (aPR = 1.79; 95% CI = 1.05-3.05; p = 0.032), and houses built with mats and other local material (aPR = 3.03; 95% CI = 1.31- 6.99; p = 0.009). Also, households that disposed stools in the open field had a higher prevalence of strongyloidiasis than those with sewage facilities (aPR = 1.89; 95% CI = 1.04 - 3.44; p = 0.037). Children four years or older had higher prevalence than children of three years or less. Infections by ***S. stercoralis*** in low-resource, low-endemicity setting in Peru, are strongly associated to poor living conditions without evidence of canine-human transmission. The results highlight the role of poverty-alleviation and sanitary interventions for controlling strongyloidiasis.

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RECOMBINASE POLYMERASE AMPLIFICATION-BASED ASSAY TO DIAGNOSE SOIL-TRANSMITTED HELMINTHS IN STOOLS

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Soil-transmitted helminths (STH) are parasitic nematodes that populate the human intestine and affect more than 1 billion people worldwide, causing impairment to physical, nutritional, and cognitive development in children. The global strategy to control STH infection involves periodic mass drug administration (MDA) with mebendazole and albendazole. The standard microscopy method used to measure disease prevalence has diminished sensitivity as intensity of infection decreases. As the prevalence and intensity of infections are reduced due to continued MDA, improved diagnostic tools to support control program decisions are needed. To identify available diagnostic technologies and potential biomarkers, a landscape analysis was conducted. Based on the landscape analysis, a nucleic acid amplification test based on recombinant polymerase amplification (RPA) technology is being developed to detect STH in stool. Primers and probes specific to ***Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale*** and ***Necator americanus*** were designed and the assay was optimized. Comparison with an established polymerase chain reaction (PCR) assay showed that each species-specific RPA assay is as sensitive as real-time PCR, detecting 5 to 20 copies of the cloned target sequences after incubation at 39°C for < 20 minutes. Also, the assay was able to amplify the target region in DNA extracted from human stool samples that were positive for STH based on Kato-Katz, with no cross-reactivity of the non-target genomic DNA. This suggests that RPA is highly specific for rapidly detecting A***. lumbricoides, T. trichiura, A. duodenale*** and ***N. americanus***. Studies using stool from patients with light, moderate, and heavy intensity STH infections will be performed to further evaluate its performance.

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PRELIMINARY STUDY ON THE PREVALENCE OF INTESTINAL PARASITES IN AN AREA FROM THE ECOLOGICAL REGION OF THE GRAN CHACO, ARGENTINA

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A previously published systematic review of the literature has shown that there is an incomplete prevalence map of soil-transmitted helminths (STHs) in Argentina. Until now, the highest prevalences were observed in the northeast and northwest regions of the country. In this study we centered on the Argentinian area of an ecological region denominated Gran Chaco that is located between both regions and comprises the provinces of Chaco, Santiago del Estero, Formosa and parts of Santa Fe, Cordoba, San Luis, Salta, Tucumán, La Rioja Catamarca and Corrientes. The Argentinian Gran Chaco is divided into a sub-humid, arid and serrano region each with its own climate and characteristic vegetation. This study was conducted in the surrounding areas of the city of Añatuya, Department of General Taboada, Province of Santiago del Estero located within the arid region of the Gran Chaco. Since there is no record of prevalence for either STHs or other intestinal parasites in this area, the aim of this study was to determine the prevalence of intestinal parasites in different rural settlements surrounding the city of Añatuya. Even though the communities included had similar characteristics with respect to water, sanitation and hygiene (WASH), the prevalences found varied and were unexpectedly low with regards to STHs.

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IN VIVO EVALUATION OF PLANT NATURAL PRODUCTS FOR ACTIVITY AGAINST THE HOOKWORM *ANCYLOSTOMA CEYLANICUM*

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Hookworms are blood feeding intestinal parasites causing iron-deficiency anemia, weight loss, stunted growth and malnutrition to more than 700 million people worldwide. Major control strategies rely on mass treatment with albendazole or mebendazole. However, there is increasing evidence that hookworms and other soil-transmitted nematodes are developing resistance to these drugs. In an attempt to find alternative control tools and considering that in several endemic areas, local populations use plant products to treat several ailments including parasitic diseases, compounds from plants were tested ***in vitro*** for their anthelminthic activity against the adult stage of the hookworm, ***Ancylostoma ceylanicum***. Extracts from five plant species and chromatographically-enriched fractions of the most active one were screened. These plants were collected from the western United States. Extracts from two of the plants namely Dalea ornata and Oemlaria cerasiformis showed anthelminthic activity (mortality and/or reduced motility) of their crude extracts and enriched fractions against A. ceylanicum. Associated worm mortality rates ranged from 25% at

24 hours to 100% at 120 hours, after incubating worms with the test compounds. Three concentrations of the compounds were tested (100, 50, 10 mg/mL). Our ***in vitro*** data showed a dose-dependent activity where the lowest concentration (10 mg/mL) achieved 100% mortality 120 hours post exposure while the same activity level was obtained at 48 hours with 100 mg/mL. We are currently assessing the anthelminthic potentials of these candidates using our hamster model of hookworm infection. Their toxicity to mammalian cells is also being evaluated. Studies aiming at purifying and testing active components of the extracts ***in vitro*** and ***in vivo*** are underway. The anthelminthic activity of these compounds in the animal model of the disease is being evaluated using clinical, parasitological and immunological criteria such as weight gain, anemia, egg output, worm burden, immune cell proliferation potentials, and immune cell population types and sizes by flow cytometry.

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STRONGYLOIDES STERCORALIS INFECTION AND HYPERINFECTION SYNDROME DURING MEDICAL INTERNISHIP (2014): EXPERIENCE FROM A MEDICAL STUDENT PERSPECTIVE IN PERU

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Strongyloides stercoralis is an intestinal parasite with a worldwide distribution and potential life threatening capacity. However, it is still a neglected disease by the health care in endemic areas. The aim of this study was to describe ***S. stercoralis*** cases during the Internal Medicine internship in a public hospital in Peru. A total of 5 cases were identified during the period June- December 2014. Medical information was collected from chart review. 60% (N= 3/5) of the patients were male and 60% (N= 3/5) were ≥ 50 years. 60% (N= 3/5) resided at Lima city at the moment of admission and 60% (N= 3/5) had a travel history to an endemic area for ***S. stercoralis*** infection. All the patients were farmers either from the coast, rainforest or the highlands. Previous history of diarrhea was present in 20% (N= 1/5) of the patients, whereas 80% (N= 4/5) reported constipation and abdominal pain as chronic disturbances. Eosinophilia was present in 60% (N= 3/5) of the patients. 60% (N= 3/5) of the cases developed Hyperinfection syndrome (HS) with progressive recovery in 66.66% (N= 2/3) of them. HS risk factors identified in this subgroup consisted of: (1) High dose corticosteroid use and (2) Cancer along with HTLV-1 infection. Interestingly, 33.33% (N= 1/3) did not present a known risk factor for HS. Diagnosis of HS was made by means of microscopic examination of the sputum sample collected from Bronchoalveolar Lavage (BAL) in 66.66% (N= 2/3) of the patient whereas in 33.33% (N=1/3), positive fecal samples along to a compatible clinical picture led to the diagnosis. Overall, survival rate was 80% (N= 4/5) and HTLV-1 infection was present only in the patient who died. All the patients received ivermectin and were followed 1, 6 and 12 months after discharge with 100% (N= 4/4) clearance of the larvae (except for the one who died). Conclusion: Awareness of ***S. stercoralis*** as an important parasitic infection in endemic areas should be encouraged in health care professionals and medical students in training in an attempt to achieve an early diagnosis and appropriate treatment.

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HIV/MALARIA CO-INFECTION AMONG PREGNANT WOMEN IN ADAMA AND 'AWASH SEBAT KILO' ETHIOPIA: A CROSS-SECTIONAL STUDY

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Due to the high prevalence of HIV and malaria in Sub-Saharan Africa, co-infections are very common. This study was undertaken to determine the prevalence and severity of malaria in HIV positive pregnant and non-pregnant women who receive ART. Demographic information was collected through questionnaire. Blood samples were taken from the study participants and thick and thin blood smears prepared. Malaria parasite detection and parasite density was done microscopically. CD4+ T cell count was determined by BD FACS Count (Becton, Dickinson and Company (BD), USA machine and Hb value determined by the CELL DYN 1800 machine (Abbott Company, USA). 500 HIV positive women from Adama hospital and 'Awash Sebat Kilo' health center participated in the study. Out of these, 22.2% were malaria infected. Among the pregnant HIV positive women, 44.6% were malaria infected. Pregnant HIV/malaria co-infected women, on the average, had a significantly higher (P<0.001) malaria parasite density (26,595 15,309 versus 15,400 12,278), a significantly lower (P=0.05) Hb values (7.49 3.34 versus 8.37 3.13) and lower mean CD4+ T cell count (195 123 versus 220 140) compared to non- pregnant HIV positive women. Compared to pregnant women infected with only HIV, malaria/HIV co-infected pregnant women had significantly lower (P=0.005) CD4+ T cell count (195 123 versus 279 151) and significantly lower (P<0.001) mean Hb level (7.49 3.34 versus 10.53 2.96). Lower CD4+ T cell count and Hb level and higher parasite density were recorded in primigravid HIV/malaria co-infected pregnant women than in the multigravid ones. The study revealed high malaria parasite density, reduced Hb level and CD4+ T cell count in HIV positive pregnant women, indicating that pregnancy has an adverse effect leading to severe malaria in HIV positive women.

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PREVALENCE AND COMPARATIVE DIAGNOSIS OF CRYPTOSPORIDIOSIS IN HIV INDIVIDUALS IN OSOGBO, NIGERIA

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Cryptosporidiosis is an important opportunistic infection responsible for significant morbidity and mortality in HIV/AIDS patients. The conventional diagnosis of ***Cryptosporidium*** with conventional modified Ziehl-Neelsen (ZN) staining techniques requires observation of the infective oocysts that fail to detect cases of cryptosporidiosis in many immunocompromised patients. This study compare the diagnostic efficacy of modified ZN and Enzyme Linked Immounosorbent Assay (ELISA) for detection of ***Cryptosporidium*** in HIV and AIDS individuals attend HIV clinic in LAUTECH Stool samples from 172 (67(39.0%) males; 105(61.0%) females) HIV-seropositive cases were examined for ***Cryptosporidium*** spp using the modified ZN technique and ELISA. Cyflow machine was used to measure their CD4+ count. The overall prevalence of ***Cryptosporidium*** spp. detected with ZN technique and ELISA was 59 (34.3%) and 98 (57%) respectively (p<0.05). Using a composite reference method generated from the two diagnostic methods, 49 (28.5%) patients were found to be truly infected and 61 (35.5%) truly uninfected. ELISA had a sensitivity of 79.0%, specificity of 56.5%, positive predictive value (PPV) of 51.0%, and negative predictive value (NPV) of 82.4% while ZN had sensitivity of 51.0%, specificity of 82.4%, PPV of 79.0%, and NPV of 56.5%. There was a significant association between ***Cryptosporidium*** infection and

CD4+ count (P=0.0001), with the highest parasite prevalence observed among patients who had the lowest CD4+ count (<200 cells/mm3). There was no statistical significant difference (P=0.979) among the age groups, with the age group 30-39 having the highest prevalence 70(40.7%) of infection. The ZN staining technique was less sensitive for the detection of ***Cryptosporidium*** in comparison to ELISA in this study. ELISA method can therefore have considerable advantages in the treatment of immunosuppressed individuals allowing early diagnosis thereby decreasing morbidity and the mortality.

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PROCYANIDIN TRIMER C1 DERIVED FROM *THEOBROMA CACAO* REACTIVATES LATENT HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 PROVIRUS

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Despite remarkable advances in combination antiretroviral therapy (cART), human immunodeficiency virus type 1 (HIV-1) infection remains incurable due to the incomplete elimination of the replication-competent virus, which persists in latent reservoirs. HIV-1 latency can be defined as a reversibly nonproductive infection of a cell which is usually interpreted to refer to an integrated provirus that is replication-competent but transcriptionally silent. In light of recent evidence, this definition might be expanded to include proviruses that express some but not all gene products in the absence of virion production.Multiple approaches to reactivation and depletion of the latent reservoir have been attempted clinically. These efforts aim to reactivate latently infected cells so as to render them susceptible to viral cytopathic effects, an antiviral immune response, or other means of targeted cell killing while protecting uninfected cells by cART. However, complete depletion of the latent reservoir remains a long-term goal. We screened medicinal plant extracts for compounds that could reactivate the latent HIV-1 provirus and identified a procyanidin trimer C1 derived from Theobroma cacao as a potent activator of the provirus in human T cells latently infected with HIV-1 . This reactivation largely depends on the NF-κB and MAPK signaling pathways because either overexpression of a super-repressor form of IκBα or pretreatment with a MEK inhibitor U0126 diminished provirus reactivation by C1. A pan-PKC inhibitor significantly blocked the phorbol ester-induced but not the C1-induced HIV-1 reactivation. Although C1-induced viral gene expression persisted for as long as 48 h post-stimulation, NF-κB-dependent transcription peaked at 12 h post-stimulation and then quickly declined, suggesting Tat-mediated self-sustainment of HIV-1 expression. These results suggest that procyanidin C1 trimer is a potential compound for reactivation of latent HIV-1 reservoirs.

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INVESTIGATING CLIENT PERCEPTION AND ATTITUDE TO DECENTRALIZATION OF HIV/AIDS TREATMENT SERVICES TO PRIMARY HEALTH CENTERS IN THREE NIGERIAN STATES

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The views and opinions of end-users in decentralization provide insights into level of uptake of services and improvement in access. We examined clients' perception and attitude towards decentralization of antiretroviral treatment services to primary health centers (PHCs). A cross-sectional survey was undertaken in three Nigerian states. Study sites were purposively selected and respondents were equally sampled from each site. A total of 1265 interviews were conducted with HIV/AIDS clients exiting health facilities. High level of perception of decentralization of antiretroviral services to PHCs as beneficial to HIV/AIDS control (70%) of the respondents, as well as high stated community support for decentralization to PHCs were found. The difference in willingness to accept decentralization between the three states was found to be statistically significant (<0.05). However, over 90% of respondents in all three states felt decentralization of ART services to PHCs would be beneficial in controlling HIV/AIDS in Nigeria; the difference in respondents' perception across the three state was found to be statistically significant (p<0.001). These imply that scaling up of treatment services to PHCs would be widely accepted; and probably result in increased uptake. However, this must be accompanied by targeted behavior change interventions for clients who for the fear of disclosure and stigma would still not access care from proximate facilities.

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GEOGRAPHIC INFORMATION SYSTEM-BASED MODELING OF THE HIV/AIDS EPIDEMIC IN ECUADOR USING NATIONALLY COLLECTED DATA

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Ecuador holds a disproportionate burden of HIV in Latin America and reports an increase in HIV/AIDS cases after 2008. It is extremely important to understand HIV/AIDS spatiotemporal dynamics. This study uses Geographic Information System (GIS)-based disease modeling to describe, predict and identify areas of higher HIV activity in Ecuador. Nationally collected data on HIV screening, number of HIV cases and AIDS incidence rates during 2009 and 2010 were used for GIS-based disease modeling. Descriptive cartographic representation of the geospatial distribution of each of these variables was conducted. Different interpolation algorithms were tested on their performance to predict the values of HIV cases and AIDS incidence rates for unsampled geographical locations. Finally, spatial autocorrelation using Moran's I statistics indexes was conducted as part of a Hot Spot analysis to identify areas of significantly higher clustering of HIV cases (i.e. HIV activity). Overall, AIDS incidence rates were highest in the Coast, mid-level in the Andes and lowest in the Amazon basin. HIV testing and screening rates were highest in different provinces located across the different regions (i.e. Coast, Andes and Amazon basin). Ordinary Kriging was the interpolation algorithm that best fit the data being analyzed. Autocorrelation models suggested that the province of Santa Elena (near the port of Guayaquil) represents a Hot Spot for AIDS incidence rate in Ecuador. It has been suggested that increased screening efforts have led to higher reported number of AIDS cases in Ecuador. However, nationally collected data evidences a mismatch between screening and AIDS incidence rates. Further analysis showed one Hot Spot in the Province of Santa Elena, near the main port and largest city of Ecuador, Guayaquil. This study helps in the understanding of the geospatial distribution and statistically significant association, aggregation and autocorrelation of HIV/AIDS cases in Ecuador. Further research is needed to identify geospatial locations where HIV socio-structural determinants collude to increase HIV/AIDS transmission in the local population.

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TUBERCULOSIS DISEASE AMONG HIV POSITIVE ADULTS ON ANTIRETROVIRAL THERAPY IN MALAWI

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Tuberculosis (TB) and HIV co-infection is common and associated with a high mortality rate. The incidence of TB among people who are stable on antiretroviral therapy (ART) is not well described. We used data from the screened and the enrolled participants in a clinical trial of adults on ART to determine the prevalence and incidence of TB infection in patients who have symptoms that are consistent with TB and assessed the impact of TB infection in this population. We screened all participants in our clinical trial for typical symptoms of TB. Participants with TB symptoms submitted samples for GeneXpert testing. We only enrolled adults with CD4 count>250 and undetectable viral load into our prospective study. Pulmonary TB (PTB) was diagnosed if in patients with typical TB symptoms, there was a positive TB test (GeneXpert) or positive chest x-ray finding. Extrapulmonary TB (XTB) was diagnosed based on clinical and radiological findings. We screened 1416 participants for enrollment in our clinical trial and 41 participants had symptoms suggestive of TB. One participant with a positive test and one with a negative test but with a typical chest X-ray finding were diagnosed with PTB. The prevalence of PTB in the adult ART population was 0.1% and among those with symptoms of PTB, prevalence was 4.9%. We enrolled 900 participants and accumulated 1,117 years of follow up. The incidence rates were 8.4, 0.8, 0.6 and 0.9 per 100 person years for PTB symptoms, PTB diagnosis, PTB laboratory confirmed diagnosis and XTB, respectively. There were no deaths among the 9 cases of PTB but 5/11 participants with XTB died (case fatality rate 45%). PTB was not associated with changes in CD4 cell count or viral load. Further analysis of the effect of PTB and XTB on clinical, immunological and virological outcomes is being conducted. PTB and XTB are rare in adults who are stable on ART. When PTB is actively diagnosed and treated, it was not associated with adverse outcomes in our small sample of cases. Extrapulmonary TB has a poor prognosis even when patients are on appropriate ART.

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HIV-1 EXPOSED UNINFECTED AND UNEXPOSED INFANTS HAVE SIMILAR ANTIBODY RESPONSES TO CHILDHOOD VACCINES, BUT THE RESPONSES AGAINST HEPATITIS B VACCINE DECAY BY TWENTY ONE MONTHS OF AGE

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HIV-1 exposed uninfected infants (HEU) residing in regions with high burden of infectious diseases, have high mortality and morbidity. The underlying mechanisms causing the increased vulnerability of HEU compared to unexposed infants (HUU) are not clear. While childhood vaccination against most infections protects majority of the vaccinees, it is not known whether it provides equal protection in all children including those with prenatal exposure to infectious diseases that may alter infants' immune responses. Vaccine-specific IgG responses were compared among three groups of children: HEU N=13, HIV-1 unexposed/malaria exposed (HUME) N=25 and HIV-1/malaria unexposed (HMU) N=18 infants to address the hypothesis that in-utero exposure to HIV-1 alters infants capacity to develop long lasting protective humoral immune responses to childhood vaccines. Antibody levels against hepatitis B virus (HBV), measles, tetanus toxoid (TT) and diphtheria toxoid (DT) were measured by ELISA at multiple time-points beginning from birth up to 21months of age in a longitudinal cohort study conducted in malaria holoendemic region of western Kenya. Pre-vaccine antibody levels were lower for HBV and DT compared to measles and TT, but were similar between infant and mother. These antibodies tended to be lower in HEU compared to HMU or HUME. Peak IgG responses were also lower for HBsAg and DT compared to measles or TT in all the groups. Overall, the three groups responded similarly to vaccination (Kruskal Willis test: p=0.85 for measles, p=0.845 for TT, p=0.434 for DT and p= 0.365 for HBV) with varying peak responses across the vaccines. Measles, TT and DT antibody responses were maintained at high levels even by 21 months of age. However, responses against HBV decayed to pre-vaccine levels by 21 months of age, suggesting that even though HBV vaccine elicits robust antibody responses in all infants, these responses are not long lasting. These data suggest that HEU respond equally well as HUU to vaccines test here, but long-term efficacy of HBV vaccine needs to be evaluated particularly in infants residing in malaria holoendemic regions.

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BASELINE CHARACTERISTICS OF PATIENTS RECEIVING CARE FOR HIV/AIDS AT THE ANTI-RETROVIRAL CLINIC OF PANTANG HOSPITAL: A LONGITUDINAL COHORT STUDY

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The recommended medical care for HIV/AIDS patients consist of providing antiretroviral therapy (ART) for viral suppression, management of comorbidities, and interventions to reduce HIV transmission. In Ghana, 123,245 HIV/AIDS patients are on ART. Characterization of the infected population under treatment creates a baseline for monitoring the HIV care continuum, identification of those most likely to fall out of care, and possible interventions to improve retention in care and ongoing viral suppression. In this study we describe socio-demographic characteristics, self-reported adherence to medication, behavioral risk factors, and access to prevention services among patients receiving care at Pantang hospital as part of a longitudinal cohort study. A cross-sectional study design was used to recruit participants 18 years or older, HIV positive, receiving care with the ability to provide informed consent for interview using a structured in-person closed-ended questionnaire. 211 HIV/AIDS patients (mean=44) participated in the study. There were 44(20.85%), and 167 (79.15%) heterosexual males and females respectively. 17(8.06%) were not yet on ART, whereas 173 (81.99%) are on ART medication. 122 (62.24%) have never experienced any side effect of their medication while 11(5.61%) rarely did. 143 (67.77%) reported they followed the specific instruction for taking their medication, while 17(9.0%) don't follow those instructions. 151 (71.56%) strongly disagreed while 22 (10.43%) strongly agreed that they will have unprotected sex if their partners are also HIV positive. 152(72.04%) reported they never took alcohol before sex, while 10(4.74%) had ever had alcohol before sex. Majority (186, 88.15%) have never used drugs in their lifetime. 164 (83.67%) never received any free condoms during the past 12 months while only 18 (9.18%) said they had. 155 (79.08%) have never been diagnosed with active TB, whereas only 5(2.55%) have ever had TB. Only 2 (1.02%) of the participants have ever had pneumocystic pneumonia infection. Targeted HIV/AIDS prevention programs at this cohort will be beneficial in reducing HIV transmission.

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LEVERAGING ON COMMUNITY-BASED PEPFAR PROGRAMS ACHIEVEMENTS IN STRENGTHENING SURVEILLANCE, PREVENTION AND CONTROL TOWARD HIV/AIDS EPIDEMIC FREE GENERATION IN SUB-SAHARA AFRICA

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About 3.2 million children under the age of 15 are living with HIV/AIDS globally and 91 percent burden of whom is in Africa. The US President's Emergency Plan For AIDS Relief (PEPFAR) partnership scaling up for impact and efficiency on HIV/AIDS programs has be laudable in alleviating morbidity and mortality and sustainably accelerate core prevention and care interventions quality services estimated at $19.1 to $22-24 billion made available in LMIC including in reshaping health policy reforms and programs across African resources limited countries in 2013-2015. Strategic and geographical allocations in Community evidence-based programs promote accessible and cost-effective service delivery to populations at greatest risk communities across Sub-Saharan Africa. A strategic systematic search on Medline and acknowledged PEPFAR programs partners was used to assess publications from 2005- 2015 in Sub-Saharan Africa, scrutinized and categorised upon type and nature in improving strategic planning, quality of care and outcomes. Our findings showed that PEPFAR programs support community-based capacity building and social service systems delivery through sensitization and participatory activities on HIV risk factors, voluntary screening and care seeking for PLWHIV and caregivers, treatment adherence, ABCs practice attitudes, and empowering health systems paramount in strengthening HIV/AIDS free generation. Community's partnership impact highlights the differential benefits and issues related to transparency and accountability as useful indicators of communities' programs performance impacts and outcomes. The urgent need to maximize on PEPFAR and related programs in fostering sustainable country-driven and ownership of integrated community-based HIV/AIDS surveillance, prevention and control must be prioritized. Implementing effective core capabilities and better coordination in proven PEPFAR and national Ebola immunization programs (NEIP) has potential asset to inform Africa's policy decisions and health systems investments toward deadly viral disease free generation.

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REDUCED PLACENTAL TRANSFER OF IGG TO *PLASMODIUM FALCIPARUM* MALARIA IN HIV-EXPOSED UNINFECTED CAMEROONIAN INFANTS

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Although mother-to-child transmission of HIV has dramatically declined, the number of ***in utero*** HIV-exposed uninfected (HIV-EU) infants is on the rise. HIV-EU infants are at a greater risk of mortality and morbidity compared to their non-HIV exposed counterparts. Poor health outcomes in this pediatric population are, in part, explained by increased susceptibility to and severity of infections, including pneumonia and malaria. Passively acquired immunity through placental transfer of IgG from the mother is fundamental for protection of infants against infectious agents during the first year of life. Transplacental transfer of IgG to tetanus, measles, pneumococcus, VZV and haemophilus influenza type b is reduced in HIV-EU newborns. However, conflicting results are reported for the influence of maternal HIV on placental transfer of IgG to malarial antigens. We conducted a case-controlled study, in which HIV-positive (cases) and HIV-negative (controls) pregnant Cameroonian women were recruited. Maternal peripheral and cord plasma was used to measure IgG to malarial pre-erythrocytic (CSP, LSA-1) and erythrocytic (AMA-1, MSP-1, MSP-2, MSP-3, EBA-175, RESA, PfEMP1) antigens, that are important for protection and tetanus toxoid. Cord IgG levels to malarial sporozoite and merozoite proteins, as well as tetanus toxoid, were reduced in full-term HIV-EU newborns compared to non-HIV exposed newborns (all p values <0.05). Since significant differences in IgG levels to the above antigens were not found between HIV-positive and HIV-negative women, reduced IgG levels in HIV-EU infants were not due to reduced levels in their mothers. The results suggest that an alternative mechanism is responsible for low IgG levels in HIV-EU cord blood. Additional studies are in progress to determine possible mechanisms responsible for HIV-induced decrease in transplacental transfer of IgG.

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PERINATAL HIV-INFECTION AND LONG-TERM DEFICITS IN COGNITIVE EXECUTIVE FUNCTION AMONG SCHOOL-AGED UGANDAN CHILDREN

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The study was undertaken to evaluate the hypothesis that perinatal HIV infection predicts long-term deficits in cognitive executive function (CEF) among Ugandan children 6 -18 years old. Perinatal HIV-infection was diagnosed by end of breast-feeding via DNA polymerase chain reaction test. Current HIV status was confirmed with HIV-rapid diagnostic test. Proxy report of Child CEF was measured with behavior rating inventory of executive function (BRIEF). Descriptive analyses estimated means, standard deviations (SD), numbers and percentages by perinatal HIV status. Multivariable linear regression estimated HIV-related differences (β) in CEF scores and 95% confidence intervals (CI). HIV-infected (n=56), exposed negative (n=56) and unexposed children (n=54) were enrolled. Dysregulation (i.e. higher scores) in CEF domains - including significant elevations for emotional control, inhibition, initiation and working memory sub-scales, were noted for perinatally HIV-infected compared to HIV-unexposed children. CEF scores was highest (mean=54.2,SD=12.8), for HIV-infected intermediate (mean=48.4,SD=11.2) for HIV-exposed negative and lowest (mean=46.8, SD=8.8) for HIV-unexposed children. Overall, clinically relevant CEF elevations (BRIEF t-score ≥65 vs. <65) were noted in 12(20.7%), 5(9.1%) and 2(3.8%) HIV-infected, HIV-exposed and HIV-unexposed children respectively (p-value=0.016). Normal t-scores across all BRIEF sub-scales was least prevalent in HIV-infected (34.5%) vs. 50.9% in HIV-exposed negative and 60.4% HIV-unexposed children. Conversely, dysregulation in ≥2 subscales was more prevalent in HIV+(44.8%), vs. 30.9% in HIV-exposed negative, and 20.7% in HIV-unexposed children (P =0.0598). CEF scores were significantly elevated for HIV+ (β=5.4, 95%CI:1.4,9.4) but not HIV-exposed negative (β=-0.81,95% CI: -5.0,3.4) relative to HIV-unexposed. In conclusion, perinatal HIV-infection is a significant predictor of low CEF. Specific interventions to improve executive function may improve long-term functional status in HIV-infected children.

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HEPATITIS E VIRUS IMMUNOLOGICAL MARKERS IN HIV-INFECTED INDIVIDUALS, DOMINICAN REPUBLIC

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Hepatitis E virus (HEV) is an RNA (+) virus, primarily transmitted through foecal-contaminated water. HEV is endemic to various countries and currently considered the most common cause of viral hepatitis worldwide. In immunocompetent hosts causes a mild viral acute hepatitis, although in pregnant women it causes acute fulminant hepatitis. Recent studies have shown persistent infection by HEV in immunocompromised hosts, especially those infected by the HIV. The purpose of this investigation was to determine the presence of HEV specific antibodies in the Dominican Republic. Two cohorts were obtained from an outpatient clinic located in Santo Domingo, Dominican Republic; one consisted of 36 HIV (+) patients while the other was composed of 54 HIV (-) patients, both with persistent elevated liver enzymes and negative laboratory tests for hepatitis B or C. Informed consent was obtained from each participant. An epidemiological form was completed with relevant data of each participant; and their HIV status was confirmed by either rapid testing or record files. Blood samples where drawn from each participant to whom a rapid IgG test for HEV was performed, posteriorly a rapid IgM test for HEV was done to those who were positive to confirm chronic markers of HEV infection. Ten of the fourty-three (n=43) patients were positive for IgG against HEV. Of those 10, two were positive for HEV-IgM, both of which were on antiretroviral therapy and had a CD4 count over 200 cells/ml. Sixty percent of the patients who had a positive IgG against HEV had an ALT over 80 U/L, and an AST over 70 U/L. HEV should be considered as a differential diagnosis in HIV + patients with elevated liver enzymes in the Dominican Republic. Chronic infection with HEV should also be considered as an opportunistic infection in these patients in warm countries with poor water and sanitation quality.

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VIROLOGICAL RESPONSE OF FIRST LINE COMBINATION ANTIRETROVIRAL THERAPY (CART) AMONG PEDIATRIC PATIENTS IN LONGITUDINAL CAMBODIAN COHORT

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Increase access to cART for HIV+ children in resource-limited settings is expanding, although documented experiences remain limited. In most pediatric studies virological response rates are highly variable and inferior to adults, with reasons being not well understood. We describe experiences with virological responses to cART among perinatally infected children in Cambodia, followed since 2003 with a mean age at enrollment of 6.9 (SD±3.1). HIV viral load measurements became available from 2008, but remain not fully integrated in the routine monitoring due to prohibitive cost. NNRTI-based triple regimen (WHO-prequalified generic fixed-dose combinations) and boosted protease inhibitor (PI) with 2 NRTIs are standard first and second line therapies. Virological failure was defined as sustained HIV RNA ≥1000 copies/mL while on cART or persistent virological rebound above ≥1000 copies/mL after initial virological response. Of 113 children, 107 (95%) started cART based on WHO criteria and 29 (27%) had virological failure to the first line cART, although all initially restored immunologically. Time to switch to the second line therapy from the first documented viremia was an average of 16 months (95% CI: 11-21) with median HIV RNA 14734 copies/mL (474-294451). 10 (34%) children with virological failure had a period when adherence was not observed and 5 (17%) had cART interruption due to side effects. Despite excellent medication adherence the remaining 14 (48%) developed virological failure. Clinico-immunological monitoring and documented medication adherence were not sensitive enough to predict poor virological response resulting in late diagnosis of treatment failure. Prolonged treatment failure was associated with accumulated NRTI and NNRTI cross-resistance with the most common mutations M184V (21), K103N (16), V75M (4), and Q151M (3). Our experience showed that children tend to be maintained longer on failing regimens, mainly because of challenges associated with high cost of viral load, genotype testing, and limited treatment options. Access to virological monitoring should be expanded to enable early failure detection.

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LARGE PROPORTION OF HIV POSITIVE CHILDREN WITHOUT RECEIPT OF ANTIRETROVIRAL TREATMENT (SLOW PROGRESSOR, SPG) FOR 10 AND MORE YEARS IN PHNOM PENH, CAMBODIA

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More than 90% of children infected with HIV live in Africa and Asia. 2,5 million children live with HIV infection worldwide, and 370 000 children are newly infected every year. The aim of this study is to present a cohort of children who were not received highly active antiretroviral therapy (HAART) (slow progressor, SPG) for 10-16 years because of not decreased CD4 or increased viral load neither presented with opportunistic infections and AIDS-related comorbidities. HIV positive children on active antiretroviral therapy (HAART) were observed within 2002 - 2015 (12 years) in Phnom Penh. Subgroup of SPG children (n=32) did not need therapy at least 10 years from the year they have been diagnosed to be HIV positive. Because they were fully asymptomatic, and because of the guidelines valid in that time, they did not received HAART. Therapy has been started (due to the change of the guidelines) in all children of them in 2013. Group slow progressor was compared with non-slow progressor (NPG). 32 children of 140 (23%) did not present any sign of AIDS (clinical, immunological and virological) for 10 years after diagnosis of HIV. Average age, when they received 1st line HAART was 12 years in Group SPG and 9,2 years in the NPG Group. Average number of opportunistic infections was significantly higher in NPG (1,3% vs. 0,7%; P= 0,04), as well as the incidence of tuberculosis (52,3% vs. 29%; P=0,04). First line therapy was not significantly more common among SPG, as well as mortality (SPG vs. non- SPS) which was similar in both groups (2,9% vs. 0% P=NS). In conclusion, children infected with HIV have more opportunistic infections and therefore receive more prophylactic or therapeutic antibiotics and may be more colonized or infected with resistant organisms.

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TUBERCULOSIS DIAGNOSIS AND TREATMENT MONITORING AT PEDIATRIC HIV CLINICS IN SUB-SAHARAN AFRICA

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Among people living with HIV, Tuberculosis (TB) accounts for almost a quarter of all deaths. Given the overlapping burden of disease between HIV and TB worldwide, integration of TB and HIV care and preventive services is essential , especially among children, where is there is a paucity of data related to TB-HIV co-infection. Given the complexity of diagnosing childhood TB coupled with the poor sensitivity of confirmatory tests in this population, it is important to evaluate the feasibility of implementing WHO guidelines and recommendations related to TB care in the context of resource poor settings. The Global TB Program of Texas Children's Hospital

and Baylor College of Medicine has developed a comprehensive approach to integrating, monitoring and evaluating TB best practices within the Baylor International Pediatric AIDS Initiative (BIPAI) network of pediatric HIV clinics that spans six sub-Saharan African countries. We describe the process of monitoring and evaluation of TB programming that has been implemented throughout the BIPAI clinical network. Fourteen TB indicators were identified and incorporated into the standardized medical record. The indicators cover a broad range of aspects dealing with TB care including preventive services, quantification of TB disease, diagnostic services, drug resistance, and treatment in relation to HIV. Indicators are stratified by age and past treatment history when available. On a quarterly bases each clinical site is able to generate summary statistics with accompanying visual aids so that each site is able to independently evaluate their performance and make appropriate quality improvement decisions. Providing practical interpretations of WHO TB guidelines for program monitoring and evaluations to clinics in resource poor environments is possible after addressing clinic sustainability, training, and standardization of metrics.

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QUALITATIVE PROCESS ASSESSMENT OF GENE-ENVIRONMENT HEALTH EDUCATION INTERVENTION IN SOUTHERN ETHIOPIA: ADDRESSING GENOMIC LITERACY GAPS IN THE CONTEXT OF PODOCONIOSIS DISEASE

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Though scientific knowledge on the etiology and prevention of podoconiosis rapidly advanced the last few decades, little effort has been made so far to curb the lay people's misconceptions and enhance their motivations for preventive behavior. In this study, we qualitatively assessed the impact of skills training trial on lay peoples' understanding of gene-environment-contributions to podoconiosis etiology and their motivations for behavioral change. Sixty-five affected and unaffected adult participants in the trial were purposively selected and involved in semi-structured individual interviews (IDIs) or Focus Group Discussions (FGDs). Most of the participants indicated great enthusiasm for the household training and had retained messages related to environmental and behavioral risk factors and pinpointed barefoot exposure to mineral particles in the soil as an important cause. Using various metaphors, participants also discussed the joint role of heredity and environment: distinguishing 'inherited susceptibility' from that of 'inherited disease', specifying the pathways barefoot exposure to irritant mineral particles operate with inherited susceptibility, stating the contribution of heredity in population-risk variation, identifying strategies for behavioral control of genetic expression, and indicating favorable attitude towards interpersonal interactions. However, some still faced difficulties in understanding the mode of inheritance of podoconiosis. These participants either persisted with beliefs in either genetic or environmental essentialism in podoconiosis etiology. Younger participants seemed to have better understanding of hereditary risk than older participants. Economic hardship was the major barrier perceived to impede translation of the skills training into action. Community-wide dissemination of linguistically and culturally adapted gene-environment messages through younger populations may encourage accuracy of understanding, while integrating this with development packages may contribute to sustained preventive behavior.

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MULTISECTORAL APPROACHES TO HELMINTH CONTROL WITH DEWORMING AND WASH IN AN HIV-INFECTED POPULATION

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The London Declaration calls for a reduction in helminth-associated morbidity by 2020 and as a result it is important to understand how infection can be prevented through mass treatment as well as how transmission may be disrupted by removing reservoirs of infection from the environment with water, sanitation, and hygiene (WASH) strategies. We aimed to estimate the association of different chemotherapeutic and WASH "packages" with helminth infection status and infection intensity. This study is a retrospective cohort study nested within the Helminth Eradication to delay ART Trial (HEAT). Participants are HIV-infected patients recruited from clinics at three sites in Kenya (Kisii Provincial Hospital, Kisumu District Hospital, and Kilifi District Hospital). The exposures of interest are four WASH and helminth protection categories, including (1) access to deworming and WASH, (2) access to deworming only, (3) access to WASH only, and (4) access to neither. Because WASH access is actually comprised of a multitude of protective factors, the analysis is performed considering combinations of four definitions of WASH access, including: individuals who purchase purified water or treat water independently (filtration, chlorination, etc), individuals who have access to a flush toilet or pit latrine in their house or on their compound, individuals who always report hand washing post-toilet, and individuals who live in houses in which the floors of the house are made of cement, iron, stone, or timber (non-earthen). Although microscopy was previously performed, the 740 stool specimens were also analyzed for helminth infections using multiplex real time PCR analysis. We performed logistic and multiple linear regression to estimate the association between different helminth protection categories and presence of infection and infection intensity, by helminth species. Thus this study uniquely identifies the WASH factors that alone and in combination with deworming can reduce helminth infections in this immunocompromised patient group.

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INTEGRATED HEALTHCARE DELIVERY: IDENTIFYING AND HARMONIZING MULTILEVEL STAKEHOLDER PERSPECTIVES REGARDING INTEGRATED NEGLECTED TROPICAL DISEASE PROGRAMS

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One of the major challenges facing Ministries of Health in less developed countries is the ad-hoc manner in which health systems have been built to vertically address single diseases at a time. To strengthen health systems sustainably, many global health leaders promote integration of vertical programs into shared delivery infrastructures. Due to their significant degree of co-endemicity, the neglected tropical diseases (NTDs) are an example of a group of diseases in which synergistic integration is possible. The goal of this research is to harmonize different stakeholder approaches to integrated program delivery and, as a result, identify strategies for increasing the effectiveness of integrated programs and reducing NTD prevalence. Thus this study's primary research question is: how do perceptions regarding the role, effectiveness, and implementation of integrated NTD programs differ among NTD stakeholders? We conducted key informant interviews with stakeholders at each level of the integrated delivery implementation spectrum including: the World Health Organization, Ministry of Health workers in endemic countries,

implementation partner organizations, donor partners, local health providers, and community members. The study design is mixed methods; baseline quantitative surveys informed development of the qualitative study and guided the purposeful sampling of stakeholders. Based in an inductive grounded theory approach, this research utilizes a mix of respondent and informant questions during semi-structured interviews to identify how stakeholders define, prioritize, and execute integrated NTD programming. We present key findings from 43 interviews and propose a framework for integrated NTD delivery to harmonize stakeholder perspectives. Thus this study offers important insights for health systems more broadly by serving as an opportunity for understanding how to promote inclusive frameworks for cross-program coordination.

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HOW CAN INTEGRATING SANITATION AND HYGIENE INTO NTD CONTROL PROGRAM ACCELERATE REDUCTION IN NTDS? BURKINA FASO CASE STUDY

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Burkina Faso, one of the poorest countries in the world, has low sanitation coverage, inadequate hygiene practices, and high levels of morbidity associated with poor water, sanitation and hygiene (WASH), all factors that contribute to high rates of neglected tropical diseases. For example, WHO recommends the use of the Surgery, Antibiotics, Face Cleanliness and Environmental Improvement (SAFE) strategy for trachoma, and without proper WASH, zones of high trachoma prevalence will continue to exist and jeopardize any progress achieved as a result of addressing other SAFE strategy elements. United States Agency for International Development's WASHplus project identified WASH interventions to assist in eliminating and/or controlling trachoma, soil transmitted helminths, and schistosomiasis. The project conducted a desk review and chose to develop an integrated pilot program to integrate sanitation and hygiene into NTD programming in Burkina Faso, which has had a national NTD control program since 2007. Integration is gaining traction in the public health community but the means to monitor progress and identify the most effective indicators is still under discussion by the NTD and WASH communities. This activity aims to develop a model for an integrated WASH-NTD program, working with multiple stakeholders (predominantly Government and UNICEF), that can be scaled up in Burkina Faso by other implementers and be adapted and replicated in other countries. To achieve this objective, the intervention will be a comprehensive, community-focused WASH-NTD program implemented using multiple behavior change approaches and channels to support the adoption of crucial practices for disease prevention, including programming for caregivers, through schools and through local health workers, and radio. The comparison area in this area will use only one channel: local radio. Currently implementing the baseline study in Gnagna province, WASHplus will describe the intervention, present the combined WASH/NTD indicators developed to measure this integrated WASH-NTD program and highlight the preliminary findings gleaned through the baseline survey.

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PATENT EXTENSION VOUCHER: A POTENTIAL INCENTIVE FOR NEGLECTED TROPICAL DISEASES RESEARCH

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A Neglected Tropical Disease (NTD) is a condition that, despite its frequency is not necessarily low, has been for different reasons, especially for affecting the poorest people of the world, submitted to the ostracism of low investment to find better therapeutic options. The burden of disease coming from the NTDs is really high and is very close to other very prevalent conditions in terms of disability-adjusted life years (DALYs). But, none less important is the burden of annual losses on productivity within the low-income countries affected by NTDs. NTDs are not only a health problem, they are also an economic and social problem that is delaying the economies of these countries and why not, the whole world. Therefore, it is important to find the best way to stimulate the funding in the NTDs research arena. Unfortunately, it seems to be that not only good intentions are enough in order to obtain the funds to shorten the pipeline to find new compounds for these diseases. In 2006 a group of academics proposed what today we know as the FDA's NTDs Voucher. The idea is quite simple; if a pharmaceutical company succeeds getting the approval from FDA of a compound for one of the NTDs, this company will obtain a Priority Review Voucher (PRV). This means that the time it takes FDA, within the Fast Track Program (FTP), to review a new drug application is reduced. The goal for completing a Priority Review is six months. This is supposed to be a tool that can be very useful to put new compounds for NTDs into the market but the outcomes so far are not like they were expected at the beginning. An improved version of the voucher to stimulate the development of drugs for NTDs is proposed. The idea is based on granting a Patent Extension Voucher (PEV) for the companies that achieve in marketing an NTD compound, but taking into account the possibility of second use compounds for NTDs, a demonstrated effectiveness, impact on the targeted NTD and the current advantages of the FDA's PRV. Finally, a way to calculate the value of the proposed PEV is explained.

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BURDEN OF NEGLECTED TROPICAL DISEASES IN MACHALA, ECUADOR

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Neglected tropical diseases (NTDs) are a major threat to public health, particularly in Latin America where the disease burden remains high. We examined the burden of neglected tropical diseases in Machala, Ecuador, a coastal city in southern Ecuador near the Peruvian border. A total of 398,919 records were analyzed from a citywide database of clinic visits in Machala, Ecuador in 2014, to report the prevalence of NTDs, including helminthic, viral, bacterial, and protozoan infections. Binomial regression was used to examine the associations of NTD occurrence with socio-demographic factors. There were 1,919 NTD-related clinic visits in Machala in 2014; viral infections were the most commonly reported (85.0%), followed by helminthic (13.9%), bacterial (<1.0%), and protozoan (<1.0%) infections. Dengue fever was the most common NTD, with 1,631 cases throughout the year, accounting for over 95% of viral NTD cases. Ascariasis was the most common helminthic infection (73.8%), followed by schistosomiasis (9.7%). Children under five had a 2-fold greater risk of presenting with ascariasis (RR: 1.95, 95% CI: 1.44-2.64, $p<0.0001$), and school-age children (5-11y) had a 7-fold higher risk of presenting with strongyloidiasis (RR: 6.84, 95% CI: 1.53-30.55, $p<0.05$), compared to all other age groups. The burden of dengue fever was the highest in adolescents (12-18y), with a 3-fold higher risk (RR: 3.05, 95% CI: 2.73-3.40, $p<0.0001$), compared to all other age groups. Bacterial infections were most common in the elderly (≥65y), with a 16-fold higher risk of bacterial NTDs (RR: 16.19, 95% CI: 5.44-48.18, $p<0.0001$), and a seven times greater risk of yaws (Treponema pertenue) (RR: 6.94, 95% CI: 1.74-27.75, $p<0.01$), compared to other age groups. Men had a nine times greater risk of cysticercosis (OR: 8.79, 95% CI: 1.90-40.68, $p<0.01$), compared to women. The burden of NTDs, including dengue fever and helminthic infections, is high in coastal Ecuador, and varies by age and sex. Findings highlight the need for continuous active surveillance to identify risk groups and target preventive interventions to reduce the burden of neglected tropical diseases in Ecuador.

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IMPACT OF ONCE AND TWICE YEARLY MASS DRUG ADMINISTRATION ON BANCROFTIAN FILARIASIS AND SOIL TRANSMITTED HELMINTH INFECTION IN CENTRAL JAVA, INDONESIA

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Lymphatic filariasis is still prevalent in many areas of Indonesia. The Global Program to Eliminate Lymphatic Filariasis is based on annual mass drug administration (MDA) of the at-risk population. Although previous studies have shown a significant decrease of microfilaremia (Mf) after MDA in some areas, elimination from all endemic areas may need many years. Therefore, intensified MDA would be more useful in terms of duration, cost and compliance. The aim of the study was to compare the impact of annual and semi-annual MDA on W. bancrofti infections and on co-endemic soil transmitted helminth (STH). Two sub-urban villages Pekalongan District, Central Java with Mf prevalence rates ranging from 2%-7% received either once or twice yearly MDA with single dose of DEC/albendazole. Blood and stool samples were collected for detection of filarial antigen in blood by the ICT card test, Mf by three-line blood smear, and STH eggs in stool by duplicate Kato Katz smear. There was no significant difference in Mf prevalence before and after treatment in both groups; however, the decrease of antigen rates was significant on both regimens: once-yearly treatment (p0.1). Trichuris prevalence decreased significantly either with once a year treatment (p=0.004) or twice yearly MDA (p<0.0001). In conclusion, short-term evaluation showed that in our study area an additional round of DEC/albendazole did not lead to stronger reduction of Mf rates, but the beneficial effect on STH was larger after twice yearly MDA.

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DEVELOPMENT OF A SIMPLE DIPSTICK ASSAY FOR OPERATIONAL MONITORING OF DDT FOR VISCERAL LEISHMANIASIS AND OTHER VECTOR CONTROL PROGRAMS

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Indoor residual spraying (IRS) of insecticides can be extremely effective but only if correct quantities are applied. Unfortunately, disease control programmes are being hampered by a lack of affordable, user-friendly tools to monitor insecticide concentrations as part of quality assurance activities. The application of DDT insecticide continues to be a frontline option for the control and ultimately elimination of visceral leishmaniasis in India. We have developed a simple dipstick assay for DDT quantification. The assay is specific for active DDT (p,p`-DDT). It has been tested against ~2000 field samples from residential houses in Bihar state in India and validated against high performance liquid chromatography (HPLC). The dipstick assay matched HPLC results very closely and appeared to indicate a large variation in the actual levels of DDT being sprayed. Further field trials to confirm performance are planned. With the continuing use of DDT spraying as a strategic intervention for VL control, the simple dipstick assay provides a much needed quality assurance tool for monitoring DDT levels to promote the effective use of the insecticide.

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CO-IMPLEMENTATION OF NTD MASS DRUG ADMINISTRATION AND IMMUNIZATION: THE TANZANIA EXPERIENCE

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The Tanzania NTD control program aims to eliminate LF by 2020 through five years of annual MDA treatment. Immunization services are provided free of charge as part of Primary Health Care (PHC) in all public and private Reproductive and Child Health (RCH) clinics. IVD's recent expansion of activities has resulted in coverage of >85% of health facilities in the country and is projected to reach 95% in 2015. At the same time, Tanzania had 166,743 children unvaccinated for measles from 2011 to 2013. This huge number of unvaccinated children puts the country at risk of measles outbreaks and thus a need to catch up in 2 -3 years interval. In 2014, the Ministry of Health (MOH) planned to conduct two separate campaigns in roughly the same time period: annual mass drug administration (MDA) for children and adults aged 5 and older with ivermectin and albendazole to treat LF, STH, and onchocerciasis where endemic in 101 districts, and a national immunization campaign to vaccinate children 5-15 for measles and rubella and distribute mebendazole and vitamin A to children < 5 in 166 districts. Given the short interval between the two campaigns and overlap in target populations, the MOH decided to coordinate the campaigns to increase coverage and decrease cost and effort. A total of 20,529,629 (97%) children targeted were vaccinated with Measles With respect to Vitamin A, a total 8,136,451 (109%) children targeted were supplemented. Moreover, Mebendazole coverage was also 109%. And a total of 20,864,594 (76%) people were reached with Ivermectin and Albendazole. Furthermore, the NTD program conducted mop-up visits to districts with low program coverage. The joint campaign demonstrated the importance of adequate micro planning and mapping of service areas to ensure all communities, including the hard-to-reach, receive interventions. This experience demonstrated that integrated vaccination campaigns can be used as a vehicle to increase the uptake other important health interventions including NTD medicines with relatively low implementation costs and time when compared if the two interventions were implemented vertically.

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ARE WE ACHIEVING TREATMENT COVERAGE TARGETS FOR NTD ELIMINATION AND CONTROL PROGRAMS?

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Treatment coverage is a key performance indicator to measure whether NTD programs are on track to achieve their control and elimination goals. Identifying persistent pockets of disease transmission due to low coverage is important in order to prevent derailing a country's progress towards achieving elimination. The question explored here is whether countries supported by United States Agency for International Development are meeting the minimum coverage targets set for mass drug administration (MDA) for NTDs. NTD MDA treatment datasets created by national programs in 19 countries supported by United States Agency for International Development between 2007 and 2015 were analyzed. Program coverage was calculated as the number treated as a percentage of the number persons targeted and eligible for treatment. Results were measured against the global program coverage target of 80%. In 2013, mean program coverage was 80% or higher for all of the diseases in 12 out of the 17 countries supported, and between 2007-2015 districts achieved sufficient coverage 79% of the time. This means that about one

fifth of MDAs have fallen short of the targets set, with 15% of all districts found to be "repeat offenders" - achieving <80% coverage in at least two consecutive years. The percentage of MDAs meeting coverage targets varied across diseases, ranging from an average of 86% for districts treated for onchocerciasis to 39% for districts treated for schistosomiasis. Reasons for low reported coverage include data quality concerns such as accurately estimating the target population, and program implementation issues including MDA timing, staff and volunteer retention, and social mobilization. These results highlight the need for national programs to analyze their data after every MDA in order to identify districts with persistently low coverage, diagnose the cause, and take action. Actions may include checking coverage estimates by implementing post-MDA coverage surveys or conducting formal data quality assessments, collecting information on reasons for not participating in the MDAs, and adjusting program implementation strategies such as tailoring social mobilization approaches among populations with poor compliance.

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ASSESSING DATA QUALITY OF NEGLECTED TROPICAL DISEASE INTEGRATED CONTROL PROGRAMS: THE TANZANIA EXPERIENCE

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Neglected tropical diseases (NTDs) are endemic in all parts of Tanzania. This puts 47 million Tanzanians at risk of infection of two or more NTDs. In 2014 alone, over 57 million treatments were distributed reaching 21 million people countrywide. The program is expanding its activities and the geographical coverage is expected to rise from 64% in 2014 to 100% by 2016. As the program expands, maintaining good quality data becomes even more important. A data quality assessment (DQA) was implemented in May 2014 using pilot tools developed by the World Health Organization (WHO) and partners to assess the quality of the reported data from lower levels and the ability of the program to collect and report quality data. A brief desk review of programmatic reporting challenges was completed and four indicators for DQA were identified. Three regions that reported good to moderate coverage were purposefully selected to take part in the assessment. One district per region was randomly selected. Two health facilities per district and four communities (service delivery points (SDPs)) per health facility were selected using population proportionate to size. A recount of data was carried out on paper at all levels and a systems assessment was completed using a Microsoft excel-based system. DQA results indicate that the Tanzania program has a sound data management and reporting system (scores of 2.1-3) and produces quality data (verification factor of 0.8 to 1). However, there was sub-optimal performance across all service delivery points and health facilities, calling for more support and training to be provided at these lower levels of data collection to ensure quality data is generated and reported properly. Despite the strong reporting system, quality data across all intermediate levels and the SDPs needs greater attention. It was concluded that these types of assessments should be done routinely and frequently at district level to help provide more immediate feedback, which can facilitate timely corrective actions.

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A NATIONWIDE INTEGRATED EVALUATION OF PROGRESS WITH ONCHOCERCIASIS, SCHISTOSOMIASIS, SOIL-TRANSMITTED HELMINTH AND LYMPHATIC FILARIASIS CONTROL IN TOGO

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Togo has numerous national programs aimed at the control of neglected tropical diseases. Togo's Onchocerciasis Control Program has implemented mass drug administration (MDA) for onchocerciasis for over 15 years and has drafted a national plan for onchocerciasis elimination, the Lymphatic Filariasis (LF) Elimination Program just successfully completed its second post-MDA Transmission Assessment Survey (TAS), and the Integrated NTD Control Program has implemented integrated MDA for schistosomiasis (SCH) and soil-transmitted helminths (STH) for six years. In February 2015, the Togo Ministry of Health launched a nationwide, integrated evaluation for these three programs to 1) measure the impact of multiple years of MDA for SCH and STH, 2) gather preliminary data on Ov16 seroprevalence, 3) validate the results of the final post-MDA LF TAS using the Wb123 ELISA, and 4) examine the feasibility, challenges and cost of this complicated integrated activity. The survey used the same sampling scheme as that used for the 2009 baseline mapping of SCH and STH prevalence. At the same two schools in each of the 560 peripheral health units sampled in 2009, a convenience sample of 15 school-going children aged 6 to 9 years submitted stool and urine samples. Stool was examined for SCH and STH using the Kato-Katz method. Urine reagent strips were used to detect hematuria as an indirect measure of ***S. haematobium***. Eight of the 15 children in each school were tested for onchocerciasis using the Ov16 rapid test. In 8 districts previously endemic for LF and 1 district containing villages with a persistence of onchocerciasis, all children tested by Ov16 rapid test provided blood spots on filter paper for later testing by Ov16 ELISA and Wb123 ELISA for LF. More than 16,000 children at 1,128 schools were tested for SCH, STH and onchocerciasis and more than 2,700 children were tested by ELISA for onchocerciasis and LF. Preliminary results show low prevalence of onchocerciasis and LF, and significantly reduced prevalence of SCH and STH. Though the survey posed logistical challenges, it proved to be an effective and cost-saving means of assessing multiple programs simultaneously.

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THE GEOGRAPHIC DISTRIBUTION OF LEPROSY, SCHISTOSOMIASIS AND LEISHMANIASIS IN FOUR MUNICIPALITIES IN MINAS GERAIS, BRAZIL: IMPLICATIONS FOR CONTROL OF NTDS IN AN ENDEMIC AREA

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While it is known that leprosy is associated with poverty, the exact mechanisms remain to be elucidated. Limited data suggest that helminth infections could shift its presentation towards the lepromatous end of the spectrum; however, there is little published on the geographic overlap of leprosy and parasitic infections in Brazil. This study aims to investigate the geospatial distribution of leprosy, ***Schistosoma mansoni*** infection, and visceral leishmaniasis reported in 4 municipalities of Minas Gerais, Brazil over the last 8-10 years. Using the Brazilian national notifiable disease surveillance system (SINAN), data were collected on cases of leprosy, S. mansoni infection and visceral leishmaniasis between January 1, 2007

(January 1, 2005 for leprosy only) and February 28, 2015 reported in the following municipalities: Vespasiano, Pedro Leopoldo, Confins, and Santano do Riacho. Demographic characteristics such as age, gender, occupation, neighborhood and municipality of residence were tabulated. Analyses including those using geospatial information system (GIS) are underway. During the study period, there were 183 cases of leprosy, 200 cases of ***S. mansoni*** infection, and 318 cases of visceral leishmaniasis. For leprosy, the median age at notification of disease was 48 years (range 6-97) and 46% were female. Multibacillary disease predominated at 76% of cases. For ***Schistosoma mansoni*** infection, the median age was 30.5 years (range <1 to 84) and 33% were female. The median age for leishmaniasis was 32.5 years (range <1 to 89) and 38.7% were female. The highest proportions of cases of leprosy (42.1%) and of schistosomiasis (34.0%) were in the same municipality, which also had the second highest number of leishmaniasis cases (33.5%). Geospatial mapping will inform the epidemiology of these infections to lay the foundation for more in-depth studies of potential interactions between them. With the immunologic consequences of chronic parasitic diseases, investigating co-infections and their role in the presentation of leprosy has the potential for significant impact on the control of leprosy and other neglected tropical diseases.

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IMPACT OF FOUR TO FIVE YEARS OF INTEGRATED MASS DRUG ADMINISTRATION ON SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS IN TOGO

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Schistosomiasis and soil-transmitted helminths (STH) are prevalent throughout Togo. In 2009, the Ministry of Health of Togo (MOH) mapped the prevalence of schistosomiasis and STH throughout Togo in preparation for treatment through mass drug administration (MDA). All 560 peripheral health units (PHU) outside the capital of Lomé were mapped, except for 11 PHU mapped in 2006 during a pilot phase. Since 2010, the MOH has implemented integrated MDA for the control of schistosomiasis, STH and onchocerciasis based on the prevalence of disease and according to WHO guidelines. Reported coverage for the MDAs has been high, averaging 97.0% for schistosomiasis and 99.2% for STH during the past three years. In February 2015, Togo's MOH conducted a nationwide assessment (integrated with onchocerciasis and lymphatic filariasis surveillance) to measure the impact of MDA and amend MDA targets based on the new prevalence data. The sampling scheme was identical to that used during the baseline mapping. The two schools sampled at baseline in each of the 560 PHU were revisited and a convenience sample of 15 school-going children aged 6 to 9 years submitted stool and urine samples. Stool was examined for schistosoma and STH eggs using the Kato-Katz method. Urine reagent strips were used to detect hematuria as an indirect measure of ***S. haematobium***. A subset of urine samples were filtered and examined for eggs. More than 16,000 children at 1,128 schools were tested for schistosomiasis and STH. Preliminary results show a significant reduction in the prevalence of schistosomiasis and STH compared to 2009. Implementation of future MDAs will be based on the new prevalence data for schistosomiasis and STH, however, new population targets must be developed with consideration of the risk of disease recrudescence if MDA ceases. The findings from this study provide cause for optimism in the long-term control of schistosomiasis and soil-transmitted helminths. Efforts will be made to engage partners to support additional interventions (snail control, improvements in water, sanitation and hygiene) to achieve greater and sustained reductions in these diseases.

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HEALTHY HOUSING FOR HEALTHY LIVING: HOME IMPROVEMENT TO CONTROL VECTORIAL TRANSMISSION OF CHAGAS DISEASE IN LOJA PROVINCE, ECUADOR

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Healthy Homes for Healthy Living (HHHL) is a health promotion strategy aimed at interrupting Chagas disease transmission by creating living environments designed to prevent the presence of triatomines in homes of three rural communities of southern Ecuador. These living environments consider the conditions of domestic and peridomestic areas, as well as practices associated with hygiene and organization of the space according to social, cultural and economic practices of this region. Based on a participatory decision-making processes that stimulated knowledge sharing between local communities and researchers, HHHL built the first prototype of an entirely reconstructed anti-triatomine house in 2013. Subsequently, HHHL partnered with two new families in 2014 to determine infrastructural and health promotion processes required to improve homes that do not require full reconstruction but minimal improvement. Anti-triatomine measures in this case included screen in doors and windows, installation of false ceilings to improve internal temperature, roof marquee to facilitate entrance of natural light, and walls plastering and painting that facilitate localization of triatomines. Local materials were used to create fences, storage units, and animal shelters in the peridomestic areas. Particular attention was given to health promotion activities emphasizing the relationship between health, living environments, and Chagas disease. Health promotion efforts included regular reinforcement of practices previously identified as protective against triatomines presence such as sweeping with natural insecticides, prevention of cohabitation with domestic animals, and reduction of materials piled around the home. A Healthy Home Guardian was selected amongst the children of the family. Most Significant Change evaluative framework was used to assess appropriation of the space after a year of use of the anti-triatomine measures.

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COMMUNITY HEALTH WORKERS SPECIALIZED IN CHAGAS DISEASE: A NEW APPROACH TO OVERCOME UNDERDIAGNOSIS

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Chagas disease has overcome borders and become global. Spain is the most affected country of Chagas disease in Europe, and the second globally in terms of infection among migrants (after US). More than 60% of the estimated people with Chagas disease (more than 45,000 adults) are women of child-bearing age. Mother to child transmission of ***Trypanosoma cruzi*** is feasible in non-endemic countries. Europe still faces an underdiagnosis of 90%. Among population from endemic areas, lack of knowledge, stigma and fear are still linked to the disease. From February to September 2013, we recruited and trained in Madrid four mothers ***T. cruzi*** as community health workers (CHW) specialized in Chagas disease ("Mothers Committed To Chagas' Disease: Taking Action Here And There"® program). They came from Bolivia (Cochabamba and Santa Cruz), and average age was 34.7 (28-47y). Qualitative research

was performed concurrently in order to evaluate the program and the evolution of their experiences related to Chagas disease. After the training, all mothers shown improved knowledge about Chagas disease and maternal and child health, and their evaluations of the program were excellent. Their way to face the disease also changed. They started to perform activities among their communities, showing their commitment to the program. Since then (14 months), in Spain, population at-risk (par) have been informed: 424 through 30 chats given to groups, 460 par individually (in person/by telephone), around 300 have been tested thanks to the program (35 people were accompanied to the consultation by the CHW), and more than 7,000 par have received informative material). Last July (2014) in Cochabamba (Bolivia): 185 par were informed through 25 chats performed in hospitals, open spaces and churches. These CHW specialized in Chagas disease represent a global, pioneer and very useful tool in our settings (here, non-endemic countries) and in their countries of origin (there). The program is going to be replicated in Madrid and Barcelona in 2015.

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THE CRITICAL ROLE OF SCIENTIFIC RESEARCH IN THE FIGHT AGAINST THE NEGLECTED TROPICAL DISEASES: EXPERIENCE FROM THE AFRICAN PROGRAM FOR ONCHOCERCIASIS CONTROL

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The African Programme for Onchocerciasis Control (APOC, 1995-2015) is one of the most successful Public-Private health partnerships in Africa. The programme was set up following the donation of ivermectin by Merck and Co. Inc, to implement a sustainable distribution treatment system with ivermectin in all onchocerciasis-endemic countries in Africa. Currently onchocerciasis is controlled in most of the 31 endemic African countries with Malawi, Niger, Senegal, Mali and Chad performing surveys to see whether transmission is interrupted and interventions can be stopped. A key factor underlying this phenomenal public health achievement has been the quick uptake of research results for interventions and strategy adjustments. We review the interplay between science and policy, that has shaped APOC operations from mapping the extent of the disease endemicity, adoption of the CDTI strategy for implementation, resolving implementation challenges such as ***Loa loa*** endemicity, change in objective from control to elimination and development of new tools and methodologies including modelling in the assessment of the impact of interventions and progress towards elimination. For example, recent research findings from studies on vector species distribution have led to the development of maps delineating transmission zones which has been used to modify operational procedures to assess interruption of transmission. Another example is a quick research uptake of the Esperanza window trap and its introduction in the operations of APOC for transmission assessment. The most recent finding of some critical research such as modelling the spread of possible non-response to ivermectin and its impact on elimination will also be presented. APOC will cease by the end of 2015 paving the way for the establishment of a new entity for the control and elimination of neglected tropical diseases amenable to preventive chemotherapy (PC-NTDs). As illustrated by the APOC experience, for the fight against the PC-NTDs to be successful, it is critical that strong structures remain in place to steer critical research and ensure the rapid uptake of results.

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INVESTIGATING CHANGES IN MONOCYTE PHENOTYPES AND FUNCTIONS IN ACTIVE VISCERAL LEISHMANIASIS PATIENTS

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Immune mechanisms underlying Visceral Leishmaniasis (VL) pathology are not well characterised. We hypothesised that M2 macrophages play an important role in disease pathogenesis during VL, and aimed to establish whether macrophages became polarised towards an M2 phenotype. We examined the dynamics of M1 and M2 macrophage frequencies and phenotypes in the whole blood of active VL patients, at intervals of 7 days for four weeks until their drug treatment was complete.We found only minor changes in the frequency of M1 and M2 macrophages, but observed significant changes in the expression of cell surface markers. In particular, we found that M2 macrophages increased in frequency 14 days after drug treatment commenced.We also observed significantly reduced expression of CD14 on monocytes from active VL patients, but exacerbated TNF-α production in response to LPS, compared with the same cells from drug cured and endemic control samples. Although we found a strong correlation between CD14 expression and TNF-α production in response to LPS in cells from endemic controls, no such correlation was found in VL patients, suggesting a hyperactive phenotype in VL patient monocytes. Together, our findings indicate dynamic changes to macrophage and monocytes populations in VL patients over the course of drug treatment, and suggest that the functions of these cells may change at different stages of disease.

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TRYPANOSOMA CRUZI CAUSES POLYARTERITIS NODOSA-LIKE LESIONS IN MICE DRIVEN BY PATHOGEN-SPECIFIC TYPE I IMMUNITY

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Infectious agents are often considered potential triggers for chronic inflammatory disease, including autoimmunity; however, direct evidence is usually lacking. Recently we have established an experimental murine model for studying chronic chagasic heart disease, the most important clinical manifestation of Chagas' disease. In mice experimentally infected with the myotropic Colombiana strain of ***Trypanosoma cruzi***, parasitemia was controlled following acute infection. However, low levels of parasites persisted in tissue associated with development of a severe chronic systemic vasculopathy having the pathologic hallmarks of the human disease polyarteritis nodosa (PAN). Lesions occurred in many but not all organs and tissues, with hind limb skeletal muscle arteries most severely affected, resulting in associated myositis, atrophy, paresis/paralysis and death. Histopathology showed fibrinoid necrosis, rare perilesional amastigote nests within skeletal myocytes, and massive leukocyte infiltrates composed mainly of inflammatory monocytes, F4/80+ macrophages and ***T. cruzi*** tetramer-specific CD8+ T lymphocytes capable of producing IFNγ and TNFα, but not IL-17. ***T. cruzi***-specific IgG was detected in serum from infected mice, but antibody deposits were

absent from the lesions. Thus, ***T. cruzi*** infection in mice may be a specific infectious trigger of severe PAN-like chronic systemic vasculopathy driven by pathogen-specific Type I immune responses.

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MODULATION OF MACROPHAGE FUNCTION VIA METABOLISM BY *TRYPANOSOMA CRUZI*

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Chagas cardiomyopathy is a neglected disease that develops in approximately 30% of those infected with the blood-borne parasite, ***Trypanosoma cruzi***. Over 8 million people are infected with ***Trypamosoma cruzi;*** however, no vaccine or safe therapeutics are available. Macrophages constitute the early host defense; however, are unable to clear ***T. cruzi*** resulting in parasite dissemination and disease progression. Differential metabolic states, i.e., anaerobic glycolysis and mitochondria-dependent oxidative phosphorylation, respectively, are suggested to be associated with pro-inflammatory (M1) and anti-inflammatory (M2) functional activation of macrophages. Reactive oxygen species (ROS) have been shown to be an intracellular signal for glycolysis while peroxisome proliferator-activated receptors (PPARs) that enhance fatty acid oxidation provide transcription control of macrophage functional state. In our studies using diverse ***T. cruzi*** isolates, we showed that SylvioX10 (virulent), but not TCC (non-virulent), isolate of ***T. cruzi*** was able to control extracellular and intracellular ROS levels in macrophages. We found in macrophages infected with SylvioX10, the nuclear expression of PPAR-α was increased by 18 h post-infection, and mitochondrial metabolic activity was similar to that noted in normal controls; these events are indicative of anti-inflammatory function of macrophages therefore prohibiting ***T. cruzi*** clearance. In ongoing studies, we are examining the impact of PPAR-α inhibitors in modulating the metabolic gene expression profile, functional phenotype and parasite survival in macrophages. Our data will provide the first indication that host macrophages have deficient pro-inflammatory capacity due to sub-optimal glucose oxidation and enhancing the metabolism that supports ***T. cruzi*** clearance will provide a valuable basis for a strategy to arrest Chagas disease progression.

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CASPASE-1/ASC INFLAMMASOME-MEDIATED ACTIVATION OF IL-1B-ROS-NF-KB PATHWAY FOR CONTROL OF *TRYPANOSOMA CRUZI* REPLICATION AND SURVIVAL IS DISPENSABLE IN NLRP3-/- MACROPHAGES

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Chagas disease, caused by ***Trypanosoma cruzi***, is endemic in Latin America and an emerging disease in US and other developed countries, globally affecting 11-18 million people. Studies in experimental models have shown that macrophages (mφ) among other immune cells play an important role in control of ***T. cruzi*** infection. The interaction of ***T. cruzi*** with mφs and other cell types involved in the innate immune response are mediated by pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and Nucleotide binding oligomerization domain (NOD) like receptors (NLRs).Multi-meric protein macromolecules formed by NLRs, named inflammasomes activate caspase-1 leading to secretion of IL-1β and IL-18. In this study, we utilized wild-type (WT), ASC-/- and NLRP3-/- macrophages and inhibition approaches to investigate the mechanisms of inflammasome activation and their role in ***T. cruzi*** infection. ***T. cruzi*** infection elicited a subdued and delayed activation of inflammasome-related gene expression and IL-1β production in macrophages in comparison to LPS-treated controls. When WT and ASC-/- mφ were treated with inhibitors of caspase-1, IL-1β, or NADPH oxidase, we found that IL-1β production by inflammasomes required reactive oxygen species (ROS) as a secondary signal. Moreover, IL-1β regulated NF-κB signaling of inflammatory cytokine gene expression and, subsequently, intracellular parasite replication in macrophages. NLRP3-/- mφ, despite an inability to secrete IL-1β and elicit inflammatory cytokine gene expression, exhibited a 4-fold decline in intracellular parasites w.r.t. WT controls. NLRP3-/- mφ were not refractory to ***T. cruzi*** and exhibited a high basal ROS level, maintained in an IL-1β-independent manner that contributed to efficient parasite killing. We conclude that caspase-1/ASC inflammasomes play a significant role in the activation of IL-1β/ROS and NF-κB signaling of cytokine gene expression for ***T. cruzi*** control in human and mouse macrophages. However, NLRP3-mediated IL-1β/NF-κB activation is dispensable and compensated for by ROS-mediated control of ***T. cruzi*** replication and survival in macrophages.

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MODULATIONS IN HLA-DR EXPRESSION IN VISCERAL LEISHMANIASIS INFECTION

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HLA class II is a highly polymorphic cell surface glycoprotein, crucial for regulating host immune responses and thereby determining the disease outcome in visceral leishmaniasis by their ability to present the foreign antigens to CD4+ T lymphocytes and activating the T-cells. The highly complex regulation of HLA class II in different cellular compartments determines the triggering and maintenance of immune responses and has vital role in determining the parasite clearance. This study determines the differences in the expression of HLA-DR at the gene as well as the functional level in visceral leishmaniasis cases. The study was performed on whole blood and splenic aspirates from VL patients pre-/post-treatment and endemic control groups at the gene level real time-PCR and the functional expression by flow cytometry. There was significantly lower expression of DRB1 (p=0.047) in active cases compared to endemic healthy controls but there were no significant differences in DRB1 expression in active cases compared to paired cured cases in either PBMC (p=0.848) or splenic aspirates (p=0.628). At the cellular level, HLA-DR was significantly increased on CD14, CD16 and CD19 positive populations while CD4+ cells were having lowered kinetics of expression upon drug cure in splenic as well as the whole blood. We found that the HLA-DR expression by cell types was similar in splenic aspirates as well as the whole blood assays but there were functional differences in the levels of HLA-DR expression at the gene as well as the protein level. The variable levels of expression of HLA-DR on macrophages and B-cells indicates for targeting these cell populations for modulating the immune responses by altering the antigen processing and presentation abilities hence designing the therapeutic/prophylactic options for VL.

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CONTRIBUTION OF NK CELLS TO THE IMMUNOPATHOLOGY OBSERVED IN CUTANEOUS LEISHMANIASIS PATIENTS

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Cutaneous leishmaniasis (CL) due to ***Leishmania braziliensis*** infection is characterized by the development of ulcerative skin lesions. A cross-sectional study with CL patients from Corte de Pedra and healthy subjects was performed. Peripheral blood and lesion biopsies were collected to characterize, determine the frequency of cytotoxic sub-populations, determine the expression of granzyme and perforin in the cytotoxic sub-populations and to determine the percentage of degranulating cells. We identified four sub-populations of cytotoxic cells in the peripheral blood based on the expression of CD56, CD8 and CD3. Then, we determined the frequency of these sub-populations in peripheral blood from healthy subjects and CL patients. We found that the frequency of NK cells were increased in CL patients when compared with healthy subjects while the

frequency of CD8 T cells were decreased in CL patients when compared with healthy subjects. We evaluated the expression of granzime and perforin in cytotoxic sub-populations and we found that NK cells CD8- and CD8+ express more granzyme than CD8+ T cells in peripheral blood from CL patients and the same way, the two sub-populatios of NK cells CD8- and CD8+ express more perforin than CD8+ T cells in peripheral blood from CL patients. Cytotoxic lymphocytes presented granzyme and perforin proteins. The center is covered by a lysosomal membrane glycoprotein, CD107a. During degranulation, CD107a is exposed on the cell surface. Thus, CD107a can be a marker of degranulation on cytotoxic lymphocytes. We found that NK cells expressed more CD107a than CD8+ T cells. NKG2D is an activating receptor found on NK cells and CD8 T cells. The ligands for NKG2D are induced during infections making the cell susceptible for the lysis by NK cells and CD8 T cells. In experiments performed by microarray we found that MICB is expressed in the lesions of CL patients. Flow cytometry experiment showed that MICA/B is expressed in CD11b cells from the CL lesions and immunostaining for MICB showed this ligand in CL lesion. In conclusion, our data suggest a role of NK cells in the pathogenesis of cutaneous leishmaniasis.

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TYPE I INTERFERON SIGNALLING SUPPRESSES ANTI-PARASITIC CD4+ T CELL RESPONSES DURING VISCERAL LEISHMANIASIS

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Many pathogens, including viruses, bacteria, and protozoan parasites, suppress cell mediated immune responses through activation of type I IFN signalling. However the role of type 1 IFN's during ***Leishmania donovani*** infection causing visceral leishmaniasis (VL) is not well known. Here we report that type-1 IFN's play an important role in the pathogenesis of VL by impairing parasite clearance and suppressing pro-inflammatory cytokine production. Mice lacking type-1 IFN signalling (B6.IFNαR1-/- mice) had better control of parasite growth and an enhanced Th1 cell response. Studies in VL patients supported these findings and showed enhanced accumulation of mRNA encoding type I IFN signature genes in peripheral blood mononuclear cells (PBMCs) that were reduced following successful drug therapy. Critically, we also showed, using a whole blood assay, that blockade of type-1 IFN signalling enhanced antigen specific IFN-γ production, and that this response was HLA-II restricted. Together, these results identify the type-1 IFN signalling pathways as a potential therapeutic target to treat VL by stimulating anti-parasitic CD4+ T cell responses.

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CYTOTOXICITY INDUCED BY CD8+ T CELL IN *LEISHMANIA BRAZILIENSIS* LESIONS RESULTS IN INFLAMMASOME ACTIVATION, IL-1B RELEASE AND SEVERE DISEASE

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The host immune response plays a critical role not only in protection from human leishmaniasis, but also in promoting disease severity. We have recently described a pathological role for CD8+ T cells in cutaneous leishmaniasis caused by ***Leishmania braziliensis***. Using experimental models of infection and skin samples from infected patients, we found that cytotoxicity induced by CD8+ T cells is a major mediator of immunopathology in cutaneous leishmaniasis. However, the downstream mechanisms of cytolytic activity that cause disease severity remain unclear. We hypothesized that CD8+ T cells release damage associated molecular patterns that activate the inflammasome with the consequent release of the pro-inflammatory cytokine IL-1β, amplifying the inflammatory response. Using experimental models of cutaneous leishmaniasis, we observed that cytotoxic CD8+ T cells induce inflammasome activation as measured by IL-1β protein production and active caspase-1 expression. We also found increased production of IL-1β by cells from the lesions of ***L. braziliensis*** patients. Importantly, blocking IL-1β signaling prevented pathology induced by CD8+ T cells mice. In summary, we show that IL-1β is detrimental in ***L. braziliensis*** infection and therefore targeting this pathway should be considered for immunotherapy in patients infected with ***L. braziliensis***.

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ROLE OF IL-17 IN THE PROTECTION INDUCED IMMUNITY BY LIVE ATTENUATED *LEISHMANIA DONOVANI* CENTRIN DELETED PARASITES AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL), a neglected Tropical Disease, is the fatal form of the leishmaniasis-disease complex. The estimated disease burden of VL varies from 1,969,000 to 2,357,000 Disability Adjusted Life Years. Existing drugs are toxic, and the emergence of drug-resistant parasites makes ***Leishmania*** treatment challenging; till date there is no licensed vaccine available. Previously our laboratory reported on the protective role of live attenuated centrin gene-deleted ***L. donovani*** (LdCen1-/-) parasites through induction of Th1 type immune response in mice, hamsters and dogs. Previous and recent studies showed that along with Th1 cytokines, IL-17 plays a complementary role in human protection against ***L. donovani*** infection, and acts synergistically with IFN-γ to promote protection against L. infantum Infection in C57BL/6 mice, respectively. In the present study we explored role of IL-17 in protection induced by LdCen1-/- in C57BL/6 mice. We measured, mRNA level of IL-6, Il-1β and TGF-β (inducers of IL-17) from Bone Marrow Derived DCs infected with LdCen1-/- and wild type parasites (LdWT) ***in vitro***. LdCen1-/- infection induced IL-6, Il-β and TGF-β at 96 h suggesting LdCen1-/- parasites induce DC to produce IL-17 promoting cytokines. Next we evaluated levels of Th17 cytokines ***in vivo*** at 2 and 5 wk after infection with LdCen-/- and wild type parasite. RT-PCR was performed with RNA extracted from splenocytes and ELISA was performed with leishmanial-antigen stimulated spleen cell culture supernatants, respectively. Both mRNA and protein levels of IL-17A were significantly increased in LdCen1-/- immunized C57BL/6 mice in comparison to LdWT. There was also concomitant increase in IL-17 inducers such as IL-6 mRNA at 2wk post immunization and IL-23 mRNA at 5wk post immunization. Role of IL-17 induced protective immunity by LdCen-/- will be further investigated using IL-17-/- C57BL/6 mice. In addition, we will investigate the role of IL-17 in protection against wild type ***L. donovani*** challenge. To our knowledge this is the first attempt addressing the role of IL-17 in vaccine induced protection in VL.

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MONOCYTE SUBSETS CONTRIBUTING TO DELETERIOUS INFLAMMATORY RESPONSE IN CUTANEOUS LEISHMANIASIS PATIENTS

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Skin ulcer development in cutaneous leishmaniasis (CL) patients is associated with exaggerated inflammatory response with high levels of

TNF. ***Leishmania braziliensis*** is the most prevalent species causing CL in Brazil and also the one responsible for the most severe skin forms of the disease. Lesion infiltrate is mainly composed by lymphocytes and mononuclear phagocytes, and few parasites are observed. To investigate the presence of inflammatory mediators in ulcer from CL patients we first conducted an unbiased microarray analysis of lesion biopsies and compared with healthy skin transcripts. Among the upregulated genes in CL lesions we found the inflammatory genes Metalloproteinase-9, CXCL9, CXCL10, TNF and IL-1β. We than confirmed these findings by looking at protein of these mediators in supernatants of peripheral blood mononuclear cells culture and also in supernatants of lesion biopsies, in response to soluble Leishmania antigen. Interestingly, these chemokines and cytokines were present in groups of patients with ulcerated lesions and those with pre-ulcerative lesions. Peripheral monocytes in human are known to be heterogeneous and based on CD14 and CD16 expression they are subdivided in classical, intermediate and non-classical monocytes. We found that while all monocyte subsets produce Metalloproteinase-9 and CXCL9, the intermediate monocyte population is the main source of CXCL10, TNF and IL-1β. The production of IL-1β can happen upon activation of inflammasomes. To test the pathway by which ***L. braziliensis*** triggers IL-1β production we infected C57BL/6 mouse macrophages lacking NLRP3, AIM2, Caspase1, ASC and IL-1R. We found that ***L. braziliensis***-induced IL-1β production is dependent on NLRP3, Caspase1 and ASC. Altogether our data show that intermediate monocytes are the main population producing inflammatory cytokines and hence may be a potential therapeutic target candidate.

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ALTERED NUMBER AND MIGRATION OF DENDRITIC CELLS IN MALNUTRITION CONTRIBUTES TO VISCERALIZATION OF *LEISHMANIA DONOVANI*

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Malnutrition affects about 800 million people worldwide and is a risk factor for visceral leishmaniasis (VL) caused by ***Leishmania donovani***. Most people infected with ***L. donovani*** have a subclinical infection, but malnourished children are more likely to develop fatal VL. The underlying mechanisms of immune deficiencies driven by MN are not clearly defined. We found that the increased risk of VL was recapitulated in a mouse model of malnutrition. Dissemination of ***L. donovani*** was associated with a deficit of dendritic cells (DCs) in the skin-draining lymph node in the malnourished host. Following infection in the skin, ***L. donovani*** infects phagocytes, including DCs, which then traffic parasites to the draining lymph node. We examined possible causes of the reduced DCs in malnutrition and the effect on parasite dissemination. In the bone marrow of malnourished mice we found a slight reduction in common myeloid progenitors but higher proportions of CCR2+ inflammatory monocytes. A lower frequency of CCR2+ DCs and inflammatory monocyte precursors was also found in the lymph node of malnourished mice. These results suggested malnutrition leads to impaired egress and/or subsequent death of inflammatory monocytes, which are potential precursors of DCs. We also found that the expression of CCR2 and CCL2, which are required for emigration of inflammatory monocytes from the bone marrow, were increased in the skin but reduced in the draining lymph node. Furthermore, transwell assays showed DCs from malnourished mice had impaired migration toward the CCL2 chemokine. Fluorescent labeling of DCs in the infected skin revealed more rapid migration and increased accumulation of infected DCs in the spleen of malnourished mice. This was associated with increased expression of CCR7 and its ligands in the lymph node and spleen. Collectively, these data indicate that (1) impaired DC development from inflammatory monocytes contributes to the deficit of DCs in the lymph node in malnutrition, and (2) increased migration and/or reduced retention of dermal DCs in the lymph node promotes visceralization of ***L. donovani*** in the malnourished host.

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IMMUNE RESPONSE IN PATIENTS WITH ACUTE CHAGAS DISEASE: POSSIBLE IMPLICATIONS FOR DISEASE SEVERITY DURING THE CHRONIC PHASE

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Chagas disease is caused by the intracellular protozoan ***Trypanosoma cruzi*** that affects16 to 18 million people, with 80 million considered at risk. Infection with ***T. cruzi*** via oral route has recently been an important focus of attention, currently being amongst the most frequent modes of transmission in Brazil, even in localities where vector transmission has been eradicated. Identification of the acute phase is critical for treatment administration; however, due to the difficulty to detect this phase, especially in endemic areas where the symptoms can be mild or inexistent, patients may progress to the chronic phase. During the chronic phase, while most patients remain asymptomatic, about 30% of the infected individuals develop a deadly cardiac disease. It is well accepted that the host's immune response is critical in determining disease evolution. Our hypothesis is that early immunological events that take place in the acute phase of Chagas disease will be critical to determine clinical outcome during the chronic phase. We compared the immunological profiles of patients in the acute phase and after they entered the chronic phase, at an early stage. We evaluated the frequency of CD4+, CD8+ and TCD4-CD8- (double negative - DN) cells in acute Chagasic patients (ACT) and non-infected individuals (NI). Similar frequencies of these cell populations between ACT and NI groups were observed. However, we observed that ACT displayed higher levels CD4+HLA-DR+ compared to NI. Moreover, we observed that expression of the inflammatory cytokines TNF-α and IL1-β by CD8+Granzyme A+ cells was higher in ACT as compared to NI. In our follow-up study, we compared the immunological profile of chagasic patients in the acute (ACT) and chronic (CHR) phase. As disease progresses to chronic phase, we observed a lower frequency of CD8+IFN-γ and an increase in IL-10 expression. Our data show an activated immune profile of patients in acute phase and, as disease progresses, cell populations with distinct functional profiles could be controlling an exacerbated inflammatory response.

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DENDRIMERIC ACID-LABILE LINKED OLIGOSACCHARIDES OF *LEISHMANIA* SPP. LIPOPHOSPHOGLYCAN ALTER INNATE AND ADAPTIVE IMMUNE RESPONSES

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Lipophosoglycans (LPG) of ***Leishmania spp***. are known to alter innate immune responses and be critical for parasite binding to the vector gut wall. However, the ability of the oligosaccharide sugars capping the apex and terminal branches of LPG to specifically alter adaptive T cell responses is unclear. To study cap sugar-T cell interactions, pathogen mimics—namely tri-sugar dendrimer-coated latex beads with acid labile linkers were synthesized. Upon lysosomal acidification, linker breakdown releases the sugar dendrimers for possible loading on antigen presenting molecules to induce T cell growth. To determine the role of ***Leishmania*** oligosaccharides in altering adaptive immune responses, we measured T cell subset proliferation via FACS and cytokine production via ELISA. T cell proliferation was significantly increased after co-culture with macrophages exposed to covalently and acid-labile-linked di- and tri-mannose beads as compared to T cell proliferation from cells co-cultured with macrophages

exposed to non-functionalized beads. Inhibition of phagolysosomal acidification only reduced T cell proliferation within T cells co-cultured with macrophages exposed to acid-labile-linked beads and not to covalently-linked beads. These sugar-modified reagents show that oligosaccharides alone can drive T cell proliferation by acidification-requiring presentation, most significantly in NKT receptor (CD160)-restricted T cells.

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URINARY BLADDER BILHARZIASIS IN A SOMALI REFUGEE: AN IMPORTED CASE FROM THE JUBBA RIVER VALLEY IN SOMALIA

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Schistosomiasis (Bilharziasis) is a neglected tropical disease which is associated with significant morbidity which infects an estimated 200 million people globally. The burden is highest in Sub-Saharan Africa: the disease is closely linked with poverty, and lack of access to basic resources such as clean water and sanitation. Transmission to human occurs through freshwater contaminated with feces or urine, via infected snails, which are the intermediate hosts. Schistosoma is endemic in Southern Somalia, of note in the Shabeelie and Jubba river valleys which are the farming regions. Herein we present a case of chronic granulomatous urinary bladder Schistosomiasis in a male refugee from Somalia, who lived in the Juba River valley, which is one of the endemic areas in Somalia. A few months after he came to the U.S, he presented with diffuse abdominal and rectal pain: colonoscopy and endoscopy were non-revealing. A CT scan of the abdomen showed a small, circumferentially thickened urinary bladder with calcifications, and subtle calcification of the distal right ureter. A cystoscopy with bladder biopsy showed focal granulomas, chronic inflammation and numerous calcified Schistosoma eggs: some of the eggs had terminal spines consistent with ***Schistosoma hematobium***. Even though the patient had presented with chronic urinary bladder disease, he was treated with the standard WHO dosing regimen of oral Praziquantel at a dose of 40 mg/kg/day in two doses for one day. He continued to be symptomatic, and was retreated 4 weeks later, with mild improvement in symptoms. Chronic infection with ***S. hematobium*** is known to lead to granulomas, fibrosis, and eventually cause obstructive uropathy and renal failure. This case illustrates the importance of being aware of the high prevalence of this infection and its sequelae in immigrants from endemic areas.

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FIRST REPORT OF *BIOMPHALARIA PFEIFFERI* SHEDDING *SCHISTOSOMA* CERCARIAE IN NKALAGU, SOUTHEASTERN NIGERIA

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Published studies have shown that ***Biomphalaria*** species found in south-eastern Nigeria did not shed ***Schistosoma*** cercariae, which has led to the total absence of ***Schistosoma mansoni*** infection in the area. This study was birthed from a research aimed at studying the distribution of snails of medical and veterinary importance in Nkalagu, a rural community endemic for ***S. haematobium***. ***Biomphalaria pfeifferi*** were collected from water contact site from October 2011-September 2012 and made to shed cercariae by exposure to light. ***Schistosoma*** shedding snails were collected between April and May, with overall infection rate of 4.35. Other morphological types of cercariae found in ***B. pfeifferi*** were armatae xiphidocercariae, echinostome cercariae, cercariaum cercaririae and cystophorous cercariae. Mice were infected with ***Schistosoma*** cercariae and at 12 weeks post infection, they were perfused to recover adult worms while sections of liver and spleen were examined for histopathologic changes. Worms recovered had characteristic adult ***Schistosoma*** morphologic features while histopathologic changes were ***S. mansoni***-like. A purposive sampling technique was employed to select primary school children living close to the infected water contact site. From 286 children sampled, no ***S. mansoni*** egg was seen in stool samples. Consequently, rats were caught along the water contact site and examined for ***S. rhodaini*** eggs but none was seen. In conclusion, this is the first report of ***Schistosoma*** cercariae in ***B. pfeifferi*** in south-eastern Nigeria. However, the results obtained highlight the need for further studies on the Schistosoma cercariae encountered in ***B. pfeifferi*** in the study area to confirm the species. If confirmed to be ***S. mansoni***, control measures will be recommended to prevent human infection.

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ESTABLISHING THE EVIDENCE OF SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHIASIS INFECTIONS IN BENIN

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There is a scarcity of data about the prevalence of Schistosomiasis (SCH) and Soil-Transmitted helminths (STH) in Benin. Initial surveys conducted decades ago showed highly focal and intense SCH infections around the mountainous regions of Atacora and Borgou. In 2013 the national NTD control program started an ambitious exercise of mapping all the communes for SCH and STH. The results of the new surveys will allow to launch mass drug campaigns for the control and or the elimination of SCH and STH in Benin. The mapping was carried out from March to April 2014 in 30 communes. In each commune, 5 villages/schools were selected purposely based on proximity with water bodies and anecdotal reports and clinical records from health facilities. In total, 7500 school children were surveyed in 150 primary schools throughout the 30 communes. In each school, 25 girls and 25 boys aged 8 to 14 years were randomly selected and both urine and stool samples collected from each child were analyzed using urine filtration and Kato Katz. Results were compared with historical data from WHO Atlas of SCH issued in 1987. ***S. haematobium*** was found in all 30 communes with a prevalence ranging from 0.80% (95% CI: 0 - 1.9) to 56.40% (95% CI: 50.3-62.6) while ***S. mansoni*** was found in only 16 communes with a prevalence from 0.40% (95% CI: 0-1.2) to 46.0% (95% CI: 39.8-52.2). At least 10 communes were co endemic for both urinary and intestinal SCH. The commune of Cobli showed the highest co-infection rate of 14% (95% CI: 7.2-20.8). All four common STH were found in stool samples with a predominance of hookworms followed by roundworm, whipworm. The overall prevalence of STH varied from 9.2% (95% CI: 5.6-12.8) to 60.0% (95% CI: 53.9-66.1). In comparison with historical data the results demonstrated a persistence and almost stagnant level of SCH endemicity located in the Atacora region where urinary SCH prevalence up to 60% were recorded 28 years ago. In all communes surveyed, children are suffering more for diseases related to the lack of sanitation and hygiene than water related disease. The prevalence remains stable for many years if no major environmental change occurs and if no mass treatment is undertaken.

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LAMP: POINT-OF-CARE DIAGNOSIS FOR *SCHISTOSOMA MANSONI* AND *S. HAEMATOBIUM*

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Schistosomes are easily transmitted and multiply considerably so if control strategies based on targeted mass drug administration (MDA) are to succeed it is essential to have a simple to operate sensitive and accurate test. As the control programs operating become more and more effective in reducing the parasite burden in the individual, the issue of diagnostic sensitivity will become more critical in the assessment of program

effectiveness. We have demonstrated that species-specific DNA can be detected in human urine by PCR when some specimens are apparently egg negative. This method is effective in detecting and amplifying DNA from urine residue on Whatman No. 3 filter paper that is dried after filtration and can be stored for several months without freezing and easy to transport. In the current study done in a low to moderate transmission area in Ghana, we assessed the efficacy of detection of either or both ***Schistosoma mansoni*** and ***S. haematobium*** specific DNA from 86 urine residues both by PCR and loop mediated isothermal amplification (LAMP). We also compared the DNA extraction techniques by standard extraction kit and field usable LAMP PURE kit and have evaluated these procedures on species-specific DNA detection. With ***S. haematobium*** all three methods showed similar sensitivity and specificity when compared with PCR amplification (100%). For ***S. mansoni*** sensitivity was highest for LAMP amplification (100%) than PCR and LAMP PURE (99% and 94%). The LAMP PURE extraction produced false negatives, which require further investigation for this field usable extraction kit. Overall high positive and negative predictive values (90% - 100%) for both species were indicative of a highly robust approach. The same pattern was observed when stratified for sex specific analysis. LAMP approach is close to point of care use and more sensitive than detection of parasite eggs in urine or stool. Our approach with LAMP can be an effective means to detect low intensity infection and would enhance the effectiveness of surveillance and MDA control programs of schistosomiasis.

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SCHISTOSOMIASIS SEROPREVALENCE IN THE GAMBIA

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Schistosomiasis or bilharzia remains a chronically debilitating and potentially lethal parasitic disease, affecting an estimated 200 million people globally, mostly in Sub Saharan Africa. The disease is caused by the blood fluke (trematoda) of the genus ***Schistosoma***, with both S***. haematobium*** and ***S. mansoni*** endemic in the Gambia. Country prevalence estimates for both species are based on historical data and stand at 0.5% for ***S. mansoni*** and 17.6% for S. ***haematobium*** respectively. With no comprehensive survey conducted on schistosomiasis in the Gambia in the past 30 years, there is little understanding of how the prevalence of this neglected tropical disease might have changed. We conducted a preliminary survey of 3277 school-aged children in the eastern part of the Gambia in 2011 which revealed S. ***haematobium*** prevalence of 7.5% (range 0.9 - 44.8%) based on urine egg counts from a single time point. Although likely to be an underestimation, it nevertheless, showed that the disease is localized to a number of ***foci*** in the region and remains a public health problem in the Gambia. A better understanding of the epidemiology of Schistomiasis across the entire country and in all age groups is required to inform effective control interventions. We therefore carried out a nationwide seroprevalence survey of Schistosomiasis in the Gambia using ELISA screening of 4000 dried blood spot (DBS) samples collected from all 6 regions (32 villages) across the length of the Gambia in 2012. This is the first comprehensive survey in recent years to analyse the distribution of infection within the country and provides a rapid and cost effective screening tool for surveillance of infection capable of supporting focused intervention in the Gambia.

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ELEVATED P-GLYCOPROTEIN AND MDR1 LEVELS CORRELATE WITH REDUCED PRAZIQUANTEL SUSCEPTIBILITY IN *SCHISTOSOMA JAPONICUM*

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Schistosomiasis ***japonica*** remains a major public health concern in China. Currently, praziquantel is virtually the only drug of choice for the treatment of human ***Schistosoma japonicum*** infections. Following long-term extensive use, there is a worry about the emergence of praziquantel-resistant parasites. It has been proved that ***S. japonicum*** may develop resistance to praziquantel under drug pressure. P-glycoprotein and MDR1 have been shown to be involved in the resistance to praziquantel in ***S. mansoni***. We investigated and compared the expression of P-glycoprotein and MDR1 at various developmental stages of praziquantel-susceptible and -resistant isolates of ***S. japonicum*** using quantitative real time PCR (qRT-PCR) and Western blotting assay. Significantly elevated P-glycoprotein and MDR1 levels were detected in praziquantel-resistant isolate than in susceptible isolate at the developmental stages of miracidia, cercariae, and adults at both protein and mRNA levels ($P < 0.01$). It is concluded that elevated P-glycoprotein and MDR1 levels may correlate with reduced praziquantel susceptibility in ***Schistosoma japonicum***

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EPIDEMIOLOGICAL STUDY OF ZOONOTIC SCHISTOSOMIASIS AMONG WATER BUFFALOES IN DIFFERENT ENDEMIC AREAS IN THE PHILIPPINES

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The contribution of animals in the transmission of the zoonotic schistosomiasis caused by ***Schistosoma japonicum*** has been already established in several studies. However despite this significant role, animals were not given much importance in the control of this parasitic disease. Among these animals, water buffaloes are perceived to be the most important as they are continuously and constantly exposed to the parasites in the transmission sites. In this study, a cross-sectional study was done among water buffaloes in endemic areas in the Philippines. Study sites include municipalities with varying degrees of endemicities for schistosomiasis ranging from near elimination areas (Talibon and Trinidad) to moderate (New Corella and Gonzaga,) to highly endemic area (Calatrava and Catarman). Samples were tested using microscopy, stool PCR, SEA-ELISA, SjTPx-1 ELISA and Sj1TR ELISA. Results showed significant positivities for schistosome infection in all the municipalities with the highest prevalence at 45.7% seen in Catarman and 40.5% in Gonzaga. Water buffaloes also tested positive in near elimination areas of Talibon (15.7%) and Trinidad (20.6%). This proved that high prevalence in water buffaloes does not really reflects the human prevalence but represents the threat of human transmission. Water buffaloes are good indices for human transmission of ***S. japonicum*** parasite and should therefore be included formulating elimination guidelines to prevent emergence and re-emergence of zoonotic schistosomiasis.

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UTILIZATION OF COCKTAIL-ANTIGEN ELISA FOR THE DETECTION OF ZOONOTIC SCHISTOSOMIASIS IN MULTIPLE HOST SPECIES

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Due to the zoonotic nature of the ***Schistosoma japonicum*** parasite, the development of a unified surveillance in multiple host species is necessary to strengthen the current schistosomiasis control. Among these animals, dogs and water buffaloes were identified as the most important reservoirs contributing to ***S. japonicum*** human transmission. Previous studies showed that thioredoxin peroxidase-1 (SjTPx-1) and tandem repeat proteins (Sj1TR, Sj7TR) were good diagnostic antigens individually in humans, water buffaloes and dogs. This study aimed to test the diagnostic potentials of mixing these recombinant antigens in different combinations in a cocktail-ELISA against the samples from the three host species obtained from endemic areas in the Philippines. As compared with the diagnostic potential calculated for each of the three recombinant antigens used, their combination has presented improved specificities, positive predictive values and kappa values. Results showed that the cocktail-ELISA having the combination of SjTPx-1/Sj7TR/Sj1TR has the highest sensitivity (84.1% in humans, 80% in water buffaloes and dogs) and specificity (100% in all host species). The results of this study indicate the potential of the optimized cocktail-ELISA used in the development of a common diagnostic tool that will improve the surveillance for zoonotic schistosomiasis in multiple host species.

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DEFINITIVE DIAGNOSTICS ARE CRITICAL FOR ELIMINATION OF SCHISTOSOMES INFECTIONS

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As our understanding of parasite epidemiology expands and to meet the needs of large scale and repeated mass drug administration to control or even eliminate the problem, there is a critical need for accurate definitive diagnostics. While point-of-care tests are useful in planning interventions, their lack of sensitivity overlooks a large proportion of cases either miss-diagnosed or inadequately treated but yet sufficiently productive with parasite eggs to repopulate the intermediate host snails with parasites. This is particularly important with schistosomes that are transmitted by massive intramolluscan reproduction. Irrefutable evidence from numerous sources indicates that relying on detection of eggs in urine or fecal specimens will miss a large proportion of infected persons. This has been shown in two recent studies of ***S. japonicum***, in China, that mentioned a six fold increase of prevalence using DNA detection in serum and more recently in the Philippines which showed an increase in prevalence from 22.9% to 90.2% in a cohort examined by Kato-Katz method and rtPCR of stool. Studies in Zambia showed that PCR detection of ***S. mansoni*** specific DNA in urine residue on filter paper yielded a prevalence of 89% in a heterogeneous group of people, whereas Kato Katz detected 51% and CCA detected 60% of infections. In her original work with urine based DNA detection found in Nigeria that of a cohort of 89 adults, 64.0% were found to carry parasite specific DNA in urine, while eggs were found in only 48.3%. Diagnostic tests with poor sensitivity will be misleading and will miss partial cures and low intensity infection which may not be a serious problem for the patient, but it will certainly not eliminate the infection from communities or from regions. Available point of care schistosomiasis tests are insufficient to assess the epidemiology of schistosomiasis associated with mass drug administration programs currently under way.

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DIAGNOSTIC PERFORMANCE OF SCREENING METHODS FOR URINARY SCHISTOSOMIASIS IN A SCHOOL-BASED ANTIHELMITHIC PROGRAM IN RURAL ENDEMIC DISTRICT, TANZANIA

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Diagnostic performance of screening methods varies in different endemic zones, age groups and sexes especially when there is low prevalence. This study aimed at evaluating the diagnostic performance of screening methods for urinary schistosomiasis after wide scale use of praziquantel in a school based antihelminthic control programme in Mbozi District. A cross-sectional study was conducted from March to June, 2013. A total of 429 participants from standard I - VII were chosen proportionally from twelve schools which were obtained through multistage cluster random sampling. The diagnostic performance of screening tests were compared with microscopic examination of urine for ***Schistosoma haematobium*** egg (filtration method, which was regarded as the gold standard) Chemical reagent strip for haematuria was the most sensitive of all screening methods assessed with sensitivity of (78.8%) also with high NPV (97.8%), followed by self-reported haematuria (51.9%), and visual examination of urine was least sensitive of all (42.4%). In terms of specificity, self-reported haematuria was the most specific (91.7%) with positive predictive value (34.9%). In conclusion, chemical reagent strip and history of haematuria are still useful diagnostic tools for targeting Praziquantel-MDA to school children in schistosomiasis endemic areas.

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PRODUCT INNOVATION IN SCHISTOSOMIASIS AND ITS POTENTIAL ROLE IN PREVENTING CO-INFECTIONS

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At Merck Serono, R&D Global Health aims at delivering innovative, integrated and affordable solutions to tackle unmet medical needs of poverty-related diseases, focusing on pediatric health solutions for schistosomiasis and malaria. In this context, a recently formed public-private partnership has been established with the goal to develop, register, manufacture and launch a pediatric formulation of praziquantel (PZQ). Younger children (< 6 yr) suffering from schistosomiasis are currently left untreated due to a missing pediatric formulation of PZQ, resulting in a manifestation of the disease during early childhood. An international consortium has been formed which consists of Merck KGaA, Astellas, the Swiss TPH Institute, TI Pharma, Farmanguinhos and Simcyp. This consortium showcases the importance of working in partnership to leverage and synergize expertise, know-how and resources and is currently completing Phase I of clinical development. Amongst all different consequences, schistosomiasis may cause significant damage to the human genitalia: in particular, Female Genital Schistosomiasis (FGS) represents a common complication mainly caused by ***Schistosoma haematobium.*** The consequences of FGS are not widely acknowledged and could be more significant than suspected until now: recent studies have substantiated the plausibility that FGS may elevate general transmission of HIV and that control of schistosomiasis might have an impact on HIV/AIDS. Access to anti-schistosomal treatment in the battle against HIV, sexually transmitted infections (STIs) and other reproductive tract morbidities is therefore of high interest, and partnerships are being built to define a research platform for further clinical testing. In this context, a new pediatric formulation of PZQ plays a very active role, not

only in the effort towards elimination of schistosomiasis, but also with respect to regular treatment of young girls which may potentially prevent HIV, STIs and/or other diseases affecting the reproductive tract.

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MEASURING THE PERFORMANCE AND IMPACT OF THREE ANNUAL NATIONAL TREATMENTS FOR SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHES IN MALAWI

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Since 2012, the Malawi Ministry of Health with the technical support of the Schistosomiasis Control Initiative has successfully carried out three national treatment campaigns totalling 8,400,000 treatments with praziquantel to treat schistosomiasis and albendazole for soil transmitted helminth infections (STH). We present results from two different surveys assessing the programmatic performance – impact monitoring and coverage surveys. Twenty-two schools were randomly selected from districts with moderate and high prevalence, as sentinel sites for impact evaluation. Cross-sectional parasitological data was collected to evaluate the reduction in prevalence and high-intensity infection of ***S. mansoni*** and STH, and ***S. haematobium*** using Kato Katz technique and urine filtration, respectively. At baseline in 2012, 2642 children were surveyed, 2304 in 2014, and an additional 2,640 children in the same schools were surveyed during the second follow up in 2015. Reductions in prevalence and high-intensity infection were observed for both ***Schistosoma*** species and STH in all but one school with varying impact. To validate the performance in terms of treatment coverage, two multi-stage cluster surveys were used. The first survey was conducted in 2 districts after the 2012 treatment campaign and a second was done in 6 districts after the 2014 campaign. Findings highlighted the need to increase treatment coverage of non-enrolled school-age children and adults particularly in areas known for high transmission. Alternative information channels, as well as the quality and frequency of treatment messages to communities also needs to be reviewed to improve community participation. Results from the latest survey show that the national NTD control programme has improved its performance, shown an impact on prevalence and intensity, improved its process monitoring and sensitisation within the districts, all of which lead to a more effective program; however, many areas still need to be improved in order to maximise the performance of Malawi's treatment campaigns if they are to achieve elimination.

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COMPARISON OF FULL AGE-INTENSITY PROFILES FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTH INFECTION FROM A TWO-YEAR STUDY IN UGANDA

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Many large-scale schistosomiasis and soil-transmitted helminth (STH) control programmes are now operational. Most of these also incorporate a monitoring and evaluation component which aims to estimate the impact of the control programme on levels of infection in a population, often using data collected from school-aged children (who are usually the target for treatment). The age-intensity (AI) profiles developed from this information could be used to help provide an accurate picture of the current infection patterns in endemic countries. In the present study data were collected from 7500 individuals across a wide age-range (1 - 103 years) from 10 different sites in Uganda which displayed a range of underlying endemicities and treatment histories. Data were collected from 3 different representative prevalence and treatment history groups: 1. "low prevalence and treated" - areas that have supressed transmission as a result of MDA; 2. "low prevalence and untreated" -areas that are at low-prevalence endemic equilibrium; 3. "high prevalence and treated" - areas still experiencing high levels of infection after multiple treatment rounds. The AI profiles of each of the major helminth species infecting humans in these regions were then developed. The analysis has been separated by parasite species, sex of host, and prevalence/treatment history group. Results showed that the age-infection profile for S. mansoni followed similar patterns as found in previous studies. However, group 1 and group 2 sites followed this pattern more closely. For the STHs, the overall prevalence was low and a trend could only be seen in the AI profile for hookworm infection, where infection intensities increased with age and reached a plateau. The same information in this study will be collected at one year following treatment which will allow a fuller evaluation of the impact of treatment in the different settings and parts of the community. The opportunity will be taken to estimate the age-specific force of infection and the basic reproductive number, R0, using these AI profiles. The findings from these will be discussed as well as the conclusions from the full AI analysis.

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USING SURFACTANTS TO DISPERSE NICLOSAMIDE ON SCHISTOSOME INFESTED WATERS

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Schistosomiasis, a parasitic disease with socioeconomic burden that rivals malaria, is prevalent in developing areas in Africa and Asia and affects upwards of 200 million people worldwide. Snails are the intermediate carriers for the Schistosoma (the infecting parasite), which emerge as cercariae, the larval form which infects humans. Niclosamide, an FDA approved molluscicide, is used to control snail growth and has shown cercariacidal effects as well. Niclosamide is usually hand sprayed onto infested water bodies using mechanical pumps. Suspensions of niclosamide have surface tensions of approximately 71 mN/m, which are close to that of water. This limits the surface transport of these suspensions once they land on water, and hence they have to be sprayed over the whole water body to cover all of the area. Niclosamide use has also resulted in fish and tadpole die-off, which has been attributed to non-uniform distribution of niclosamide when sprayed onto bodies of water. We are attempting to develop the use of surfactants for spreading of niclosamide and to maintain niclosamide concentrations that are lower than the LD50 for fishes and tadpoles so as to reduce die-off but still control snail and cercarial growth. Surfactants are surface active agents that can reduce the surface tension of formulations. Due to reductions in surface tension when sprayed onto water bodies, these suspensions can spread and disperse across the surface via surface tension gradients. This is beneficial as it can allow for the suspension to be sprayed onto a smaller area and still cover the whole affected area. With microliter scale droplets, we have observed that these suspensions can cover areas much larger than those covered by their surfactant-free counterparts at similar volume. We suspect that niclosamide travels along with the spreading drop, which will be confirmed with resonance light scattering (RLS). RLS has previously been used to measure very low niclosamide concentrations with precision. After establishing that surfactants allow for surface transport of niclosamide, it will be important to test and implement this novel technology using sprays and aerosols.

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UNDIAGNOSED ACUTE SCHISTOSOMIASIS AND DEVELOPMENT OF NEUROSCHISTOSOMIASIS IN ONE OF FIVE TRAVELERS AFTER FIVE YEARS POST-EXPOSURE

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Unsuspected Schistosoma infection after exposure to contaminated waters represents a challenge in travelers returning from endemic areas. Acute manifestations may be inconspicuous in addition to risk of disability-related outcomes. Neuroschistosomiasis (NS) is one of the most severe clinical forms of ***Schistosoma mansoni*** infection, presenting with no or low parasite load. Also, NS may occur without concomitant intestinal and/or hepatic disease. Herein, we report five cases of S.mansoni infection undiagnosed during acute phase and progression to NS in one of the cases after five years post-exposure. Five adult males (ages: 29 to 36 y) became infected with S.mansoni during a trip to Simonésia (Minas Gerais, Brazil) in 2009. One week post exposure, four out of five reported symptoms like malaise, local transient itching in two, epigastric discomfort in one and a three week duration fever in another. In 2014, the later individual complained of acute back pain, weakness and pain on lower limbs. Also, progressive paraparesis and paraplegia with loss of urinary and bowel sphincter control and erectile dysfunction were reported. MRI showed a thickening of cauda equina roots and specific anti-Schistosoma antibodies were detected in cerebrospinal fluid. Ultrasound excluded hepatic disease. Albeit coproscopy was negative, IgG and IgE anti-***Schistosoma*** were detectable and real-time PCR demonstrated DNA amplification in serum and fecal samples, respectively. Corticoid pulse therapy and praziquantel were initiated. In another traveler of the group, epigastric discomfort was the solely symptom persisting for the past 2-3 years while no manifestations were reported by the others. Among the four cases, active Schistosoma infection was confirmed by coproscopy and/or DNA amplification and high levels of specific IgG and IgE in three travelers and only by serology in one. Single dose of praziquantel was prescribed. Diagnosis and treatment of returning travelers from schistosomiasis endemic areas may require not only clinical and epidemiological suspicion but also the use of accurate diagnostic tools such as immunodiagnosis and DNA - based assays.

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IMPACT OF THE DESIGN OF PREVENTIVE CHEMOTHERAPY STRATEGY ON CONTROL AND ELIMINATION OF SCHISTOSOMIASIS

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The World Health Organisation's (WHO) 2020 Roadmap on NTDs has targeted schistosomiasis for control and elimination in the Americas and selected countries in Africa by 2020. WHO's guidelines recommend control through the repeated administration of praziquantel to school-aged children (SAC) in affected communities, at levels defined by baseline infection prevalence. We assess the expected impact of these guidelines using a deterministic model of parasite dynamics within the host community in combination with a description of the demography of the host population and its contact with reservoirs of infection. The model is fitted to baseline datasets for host infection intensity with age from high, moderate and low risk areas. We examine the impact of the current treatment guidelines on schistosome infection prevalence and intensity with age in 2020 and beyond. Additionally, we investigate under what conditions elimination might be achieved and whether additional drug supplies are better spent extending treatment to adults compared to increasing the treatment frequency among SAC.

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MASS DRUG ADMINISTRATION STRATEGIES TO CONTROL SCABIES IN A HIGHLY ENDEMIC POPULATION

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Recently added to the World Health Organization list of neglected tropical diseases, scabies is an under-recognised case of morbidity in many developing countries, due to secondary bacterial infection of the skin leading to septicaemia, kidney disease and potentially rheumatic heart disease. Countries of the Pacific region have a particularly high burden of scabies and its complications. Control of scabies based on treatment of individual cases is difficult due to frequent re-infestation. We implemented a large-scale intervention trial of Mass Drug Administration (MDA) for endemic scabies to ascertain the efficacy and safety of two alternative regimens (topical permethrin and oral ivermectin MDA) compared with current standard care. We identified 3 isolated island communities to which we randomly assigned one of the 3 treatment regimens. The study enrolled 2051 people: 803 participants in the standard care arm, 716 in the ivermectin arm and 532 in the permethrin arm. In each site, the proportion of the total island population enrolled was >85%. At baseline, scabies prevalence was found to be high in all arms, with the highest prevalence in the permethrin arm (41.7%) followed by 36.6% in the standard care arm and 32.1% in the ivermectin arm. A year after the intervention we observed a considerably greater reduction in scabies prevalence in the ivermectin arm with a prevalence of 1.9% corresponding to a relative risk reduction (RRR) of 94% (95% CI 83-100). The prevalence of scabies was reduced in the 2 other arms, however with lower effect size: permethrin RRR 62% (95% CI 49-75) and standard care RRR 49% (95% CI 37-60). The prevalence of impetigo was also high at baseline, ranging from 20% to 24%. The effect of the intervention followed a similar trend to that of scabies, with greatest reduction in the ivermectin arm (RRR 67%, 95% CI 52-83) compared to the permethrin arm (RRR 54%, 95% CI 35-73) and the standard care arm (RRR 32%, 95% CI 14-50). These results show that ivermectin MDA is a highly effective strategy for reducing community scabies prevalence and demonstrate the potential role of MDA in addressing a serious cause of illness in many developing countries.

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HOW TO OPTIMALLY DIAGNOSE *ORIENTIA TSUTSUGAMUSHI* OR *RICKETTSIA TYPHI* INFECTIONS IN PATIENTS WITH CENTRAL NERVOUS SYSTEM DISEASE?

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The importance of ***Orientia tsutsugamushi*** (scrub typhus, ST) and ***Rickettia typhi*** (murine typhus, MT) not only as causes of fever but also severe infections with central nervous system (CNS) involvement has recently been highlighted. In order to reduce morbidity and mortality, early diagnosis is important to guide anti-rickettsial therapy. The aim of this

study was to investigate the value of different diagnostic tests in diagnosis of rickettsial CNS disease. As part of a hospital-based study in Laos (n=1011), quantitative PCRs (qPCR) and antibody-based rapid diagnostic tests (RDTs; ST: IgM/IgG Standard Diagnostic, Korea; MT: IgM GeneBio, USA) were used with different sample types (CSF, buffy coat, plasma, serum) and compared to a combined reference group (Immunofluorescent assay (IFA), qPCR). Bacterial loads and positivity rates in relation to epidemiological characteristics and sample type were analyzed. Approximately half of the patients had paired CSF and blood available with 3.1% of blood and 1.2% of CSF samples positive for O. tsutsugamushi by qPCR. For Rickettsia spp. the same proportion of CSF and blood samples was positive by qPCR (0.8%; n=509). No significant correlations between bacterial loads and sample type, age, fever days and severity could be identified. The majority of ST was detected, by qPCR or IFA, between 4 and 8 days post-disease onset while MT was detected between day 1 and 4. In this pilot study the RDTs for ST exhibited a sensitivity of 71.4% (10/14) with plasma and 35.7% (5/14) with CSF compared to qPCR or IFA. For MT the sensitivity of RDTs using both sample types was low with 7.7% (1/13) and 0% (0/13) for plasma and CSF, respectively, in comparison to qPCR or IFA. Specificities for all sample types and test were between 85-100%. Planned latent class modeling will allow a more in-depth analysis of the test characteristics. These data illustrate the myriad difficulties of rickettsial diagnosis, even in severe disease. We will discuss new approaches to improve diagnosis based on epidemiological clues and combined molecular and serological methods to guide innovative developments in the future.

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GEOGRAPHIC VARIATION IN THE MICROBIOTA OF *IXODES* TICKS FROM THE EASTERN UNITED STATES

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Ixodes scapularis is the principle vector of Lyme disease in the East coast and upper Midwest regions of the United States, yet the tick is also present in the Southeast, where Lyme disease is absent or rare. A closely related species, I. affinis also carries the pathogen in the South but does not seem to transmit to humans. In order to better understand the microbial community structure and its geographic differences, we analyzed the microbiomes of 89 adult ***I. scapularis*** and affinis ticks captured in 15 locations in South Carolina, North Carolina, Virginia, Connecticut and New York. Initially ticks from 4 sites were analyzed by 454-pyrosequencing. Subsequently, ticks from these sites plus 11 others were analyzed by Illumina MiSeq. Data were analyzed using the QIIME-based pipeline. By both analyses, female tick microbiomes were significantly less diverse than those of male ticks. The dissimilarity between tick microbiomes increased with distance between sites. The genus Rickettsia was prominent in all locations. ***Borrelia*** was also present in most locations, and was especially high in one site in Western Virginia. In contrast, Enterobacteriacea was very common in North Carolina ***I. scapularis*** but uncommon in North Carolina I. affinis and in ***I. scapularis*** from other sites. These data suggest substantial variations in the ***I. scapularis*** microbiome associated with geography.

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DEER REDUCTION REDUCES THE FORCE OF ENZOOTIC TRANSMISSION OF *BABESIA MICROTI*

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The public health burden of Lyme disease and the associated guild of deer tick-transmitted infections has increased in the northeastern U.S. in the last decade. The only intervention that may stably reduce vector density is deer reduction. However, evidence demonstrating a long term change in enzootic transmission due to deer reduction remains to be presented. We hypothesized that the agent of human babesiosis, ***Babesia microti***, may be less efficiently transmitted than those of Lyme disease or granulocytic ehrlichiosis and thus may be sufficiently sensitive to detect evidence of reduced transmission within a short ecological time frame after intervention. Accordingly, we compared the force of B. microti transmission on Great Island (GI), MA, where the seminal deer reduction experiment was initiated in 1984; deer tick densities were reduced by 80% as a result and have been maintained at the same low level ever since. Our comparison site was Nantucket Island, where no such intervention has occurred. A main reservoir host for B. microti, Peromyscus leucopus, was intensively sampled from 1984 to 1997 and on an ad hoc basis since. Archived blood samples were analyzed for the presence of B. microti and positive samples genotyped using a variable number tandem repeat (VNTR) assay. The prevalence of infection in mice on GI decreased significantly from 72% (95% confidence interval 62-81) in the late 1980s to 44% (35-53) in the 1990s. The prevalence of infection in mice from Nantucket did not differ between the two time periods, 64% (52-75) and 59% (47-70), respectively. The diversity of B.microti VNTR genotypes, as measured by Shannon indices, tended to diminish from the 1980s to the 1990s on Great Island (P=0.08), while the diversity increased on Nantucket. We conclude that deer reduction reduced the force of transmission of B. microti on Great Island as a result of reduced infestation of mice by subadult deer ticks.

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PROBING THE GENOMIC DIVERSITY OF THE TICK-BORNE PATHOGEN, *BABESIA MICROTI*

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Babesia microti is an emerging pathogen causing human babesiosis, a malaria-like febrile illness whose geographic range and human incidence are on the rise in the United States. Despite the epidemiological relevance of ***B. microti***, little is known about its standing genetic variation, population dynamics, and the genetic basis of human virulence. A major bottleneck in our understanding of the ***B. microti*** genetic diversity stems from the difficulty to efficiently obtain sufficient number of whole genome sequences of this parasite from vector or clinical samples, without the need to propagate strains in rodents. To start probing ***B. microti*** genomic diversity we adopted hybrid capture techniques to enrich for and sequence ***B. microti*** strains directly from 22 tick and clinical samples originated form 12 geographic sites in US. Hybrid capture enabled efficient sequencing across these samples, yielding a minimum of 15X coverage across >92% of the target ***B. microti*** genome (6.4Mb) (mean coverage: 320X). We identified up to 2,574 single nucleotide polymorphisms across these samples, enabling a first assessment of patterns and levels of ***B. microti*** strain genomic variation in ticks and humans.

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ELUCIDATING THE ROLE OF INFILTRATING IMMUNE CELLS AT THE TICK-HOST INTERFACE DURING POWASSAN VIRUS INFECTION

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Tick-borne diseases continue to emerge in the United States, as demonstrated by the identification of three new tick-borne pathogens (***Heartland virus***, ***Ehrlicha muris***-like agent, ***Borrelia miyamotoi***) and the expanding geographic range of ***Powassan virus*** (POWV) in the past few years. POWV is a neuroinvasive virus transmitted to humans by infected tick bites. Skin is the interface between an attached, feeding tick and a host; consequently, it is the first line of defense against invading pathogens that are delivered to a vertebrate host together with tick saliva at the bite site. POWV can be transmitted to a host as early as one hour after attachment and initiation of tick feeding. To our knowledge, no research has systematically examined the host immune response during the earliest stages of tick-borne virus transmission at the tick-host interface (i.e. the skin). The objective of this study was to immunophenotype infiltrating immune cells and to identify POWV-infected cells at the tick bite site during early feeding time points. We expect that this data will provide us with a better understanding of the role of cutaneous and infiltrating immune cells during the early stages of POWV-infected tick feeding. In the present study we fed POWV-infected and uninfected ***Ixodes scapularis*** nymphs on naïve mice for 3, 6, 12, and 24 hours (1 tick per mouse). 4-µm sections were taken near the mouthparts of each feeding tick. Infiltrating immune cells and POWV-infected cells at the tick bite site were identified by immunohistochemistry. Our data clearly demonstrates that the hypostome of ***I. scapularis*** nymphs penetrate to the subcutaneous layer of the skin during the feeding process. After 12 and 24 hours of tick feeding we have identified macrophages and fibroblasts that stained positive for POWV at the tick bite site. Complete immunophenotyping of infiltrating immune cells for all time points of tick feeding (3, 6, 12, and 24 hours post-infestation) is underway. The location, timing, identity, and quantity of immune cells determined via histology will enable us to define the quality and kinetics of the host response to tick-borne POWV infection.

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DISTRIBUTION AND SURVIVAL OF *BORRELIA MIYAMOTOI* IN HUMAN BLOOD COMPONENTS: RELATIONSHIP TO TRANSFUSION TRANSMISSION

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Borrelia miyamotoi (Bm), the agent of relapsing fever is a tick-borne spirochete, first isolated in Japan in 1994. Since then, the spirochete has been detected in ticks globally, generally in the same vectors as the Lyme disease agent. Human infection has been reported in Russia, Europe, Japan and the U.S., as influenza-like febrile illness. Two cases of meningoencephalitis caused by ***Bm*** have been reported in immunocompromised patients. We explore the distribution and infectivity of the spirochete in human blood components after separation and storage, using standard blood bank conditions. Freshly collected human whole blood was spiked with ***Bm***-infected mouse plasma and separated into red blood cells (RBCs), plasma and platelets. Bacterial counting in the components after separation determined that the majority of the bacteria were found in the RBCs fraction. Components were injected into immunocompromised (SCID) or wild type mice (***Peromyscus leucopus***), either after collection of after storage at 4°C for the RBCs and -20°C for plasma. Platelet samples were not stored. Infection was determined by increasing spirochetemia in the mouse blood collected at different time points. Spirochetemia was detectable in all the SCID animals (30 total) injected with RBCs, either before or after storage for 21 and 42 days. Similarly, all SCID mice (20 total) injected with plasma and platelets derived from ***Bm***-spiked whole blood before storage developed spirochetemia. In the wild-type mice, 9 out of 10 developed infection when challenged with RBCs before storage or after 21 days in both groups, while RBC samples stored for 42 days infected 5 of 10 wild-type mice. Plasma and platelet samples before storage infected 5 of 10 and 6 of 10 challenged ***P. leucopus,*** respectively. Also, SCID mice were unable to clear the infection by the last observation (day 30) while, in the wild- type mice, spirochetes were not detectable after day 20. None of the mice developed the infection when injected with frozen plasma. This study demonstrates that ***Bm*** survives standard storage condition of most human blood components, suggesting the possibility of transmission by blood transfusion.

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MAPPING OF DENGUE VIRUS SEROTYPE 2 NEUTRALIZING ANTIBODIES HIGHLIGHTS A QUATERNARY STRUCTURE EPITOPE

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Dengue fever and dengue hemorrhagic fever are caused by the four serotypes of dengue virus (DENV), which circulate globally. Dengue serotype 2 (DENV2) is widespread and frequently responsible for severe epidemics. DENV2 infections stimulate durable, serotype-specific neutralizing and protective antibodies in people. The leading live tetravalent dengue vaccine was protective against DENV1, DENV3 and DENV4, but not DENV2. The epitope of protective antibodies that develop in people following DENV2 infections have not been defined. Here we show that DENV2 specific neutralizing antibodies target a quaternary epitope that is only displayed on intact virus particles. By mapping human DENV2 type-specific monoclonal antibodies and using recombinant viruses, we demonstrate that the majority of DENV2 specific neutralizing antibodies in immune sera use a complex epitope. The epitopes recognized by DENV2 neutralizing antibodies include envelope domain III (EDIII) of one E monomer and additional regions on adjacent E proteins. Thus, the display of this epitope requires the assembly of E protein into higher order structures. Our results reveal the identity of a major neutralizing epitope of DENV2 that is targeted by natural infection and a live virus vaccine induced antibodies. The identification of DENV2 type-specific neutralizing antibody epitopes could be harnessed to evaluate antibody response of vaccines under development and develop next generation vaccines.

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AN ATOMIC-LEVEL FUNCTIONAL MODEL OF DENGUE VIRUS ENVELOPE PROTEIN INFECTIVITY

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A number of structures have been solved for the Envelope (E) protein from Dengue virus (DENV) and closely related flaviviruses, providing detailed pictures of the conformational states of the protein at different stages of infectivity. However, the key functional residues responsible for mediating the dynamic changes between these structures remain largely unknown. Using a comprehensive library of point mutations covering all 390 residues of the DENV-3 E protein ectodomain, we identified residues that are critical for virus infectivity, but that do not affect E protein expression, folding, virion assembly, or budding. The locations and atomic interactions of these critical residues within different structures representing distinct fusogenic conformations help to explain how E protein 1) regulates fusion loop exposure by shielding, tethering, and triggering its release, 2) enables hinge movements between E domain interfaces during triggered structural transformations, and 3) drives membrane fusion through late stage zipper contacts with stem. These results provide new targets for drug and vaccine

development and integrate the findings from structural studies into a cohesive functional model that explains how specific residues in this class II viral fusion protein enable virus infectivity.

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DISSECTING THE SPECIFICITY OF THE NEUTRALIZING ANTIBODY RESPONSE *TO* A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (TDV) CANDIDATE

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Dengue is a major medical and public health problem worldwide. The four DENV serotypes (DENV-1 to -4) can cause a range of pathologies from mild fever to severe hemorrhagic fever and shock. Currently, there are no vaccines available for dengue. As dengue specific antibodies can enhance infection and disease under certain conditions, vaccine candidates must stimulate responses that simultaneously protect against all four serotypes. We have developed a live attenuated Tetravalent Dengue Vaccine candidate (TDV) that consists of an attenuated DENV-2 strain (TDV-2) and three chimeric viruses containing the prM and E protein genes of DENV-1, -3 and -4 expressed in the context of the attenuated TDV-2 genome backbone (TDV-1, TDV-3, and TDV-4, respectively). TDV has been shown to be immunogenic and efficacious in animal models, well tolerated in Phase I trials in humans, and is currently in Phase II clinical trials in dengue endemic regions. In the present study we sought to analyze the qualitative features of the neutralizing antibody response induced in naïve and DENV immune individuals who received TDV. Using a structural-based epitope exchange technology of transplanting conformational epitopes from one DENV serotype to another as well as human monoclonal antibody blockade assays, we demonstrate that TDV elicits type-specific DENV-1 and DENV-2 neutralizing antibody responses in many vaccinated individuals. In the case of TDV-1, the neutralizing antibody response was focused on a quaternary epitope surrounding the DENV-1 EDI/II hinge region. In the case of TDV-2 the specificity of the antibody response was directed towards a quaternary epitope centered on EDIII of DENV-2. Studies are currently in progress to determine the specificity for the neutralizing response induced by TDV-3 and TDV-4. These early findings highlight the potential of using novel technologies to determine relationships between antibody epitopes targeted by natural DENV infections and dengue vaccine candidates, and may also pave the path for defining correlates and specific mechanisms of TDV induced protection.

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ENGINEERING OF A FULL SPECTRUM POTENT ANTIBODY FOR THE TREATMENT OF DENGUE

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Dengue is an illness caused by one of four dengue virus (DENV) serotypes. The disease is endemic to the tropics and subtropics and the global incidence of dengue has increased gradually over recent years due to increased air travel, population, and urbanization with reduced vector control. There are currently no approved anti-viral therapies to treat dengue. Utilizing our computational amino acid interaction network technology, we have engineered VIS513, a humanized antibody targeting domain III of the E protein that is able to efficiently bind to and neutralize all four serotypes of the virus. We demonstrate that the antibody is highly potent, neutralizing virus in the low nM (ng per mL) range across a wide range of isolates, encompassing diverse genotypes within the four serotypes, and including challenge strains that represent significant diversity within the VIS513 epitope. In comparative ***in vitro*** studies, we show that VIS513 is more highly neutralizing than other recently discovered antibodies, including fusion loop or E dimer epitope (EDE) antibodies. Furthermore, serial ***in vitro*** passaging of virus in the presence of VIS513 demonstrates that VIS513 escape variants arise less frequently than serotype-specific antibodies that engage quaternary epitopes. In various mouse models, we extend the above findings and show that a single administration of VIS513 up to 48 hours following DENV infection is able to rapidly decrease viral titers, prevent thrombocytopenia of human platelets and mitigate disease progression. Furthermore, we demonstrate that VIS513 is produced at high levels in a CHO cell line, appropriate for manufacturing, and thus is able to reduce costs associated with production of a biologic. Taken together, these data demonstrate that VIS513 is a potent, efficacious, and broad binding immunotherapy for dengue. Given the proven track record of safety associated with antibodies targeting foreign antigens, we anticipate that VIS513 has the potential to be an important agent for the treatment or prophylaxis of DENV infection and mitigation of disease complications. We anticipate clinical study of VIS513 to begin in 2016.

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DENGUE VIRUS INFECTION ELICITS HIGHLY POLARIZED CX3CR1+ CYTOTOXIC CD4+ T CELLS

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The frequency of infection with any of the four dengue viruses (DENV 1-4) has increased dramatically in the last few decades, and the lack of a vaccine has led to significant morbidity and mortality worldwide. Dengue virus is a pathogen with unusual pathogenesis and protective immunity is poorly understood. While DENV-specific CD8+ T cell responses have been extensively studied, the breadth and specificity of CD4+ T cell responses remains to be defined. In effort to define HLA-restricted CD4+ T cell responses resulting from natural infection with DENV, we endeavored to map T cell responses in individuals from Sri Lanka where DENV is hyper-epidemic. Characterization of the phenotype of the responding CD4+ T cells revealed a DENV specific T cell subset that is specifically expanded in donors carrying an allele associated with protection from severe DENV disease. Analysis of DENV-specific CD4+ T cells revealed that the virus specific cells were highly polarized, with a strong bias towards a CX3CR1+ Eomes+ Tbet+ perforin+ granzyme B+ CD45RA+ CD4 CTL phenotype. Importantly, frequency of these cells correlated with a protective HLA DR allele and we demonstrate these cells have direct DENV-specific cytolytic activity. We speculate that cytotoxic dengue-specific CD4+ T cells may play a role in control of dengue infection ***in vivo***, and this immune correlate may be a key target for dengue virus vaccine development.

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IMMUNOLOGICAL INVESTIGATIONS TO UNDERSTAND THE OUTCOME OF THE PHASE III EFFICACY STUDIES OF THE SANOFI PASTEUR CANDIDATE DENGUE VACCINE

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Two pivotal large scale Phase III studies have been conducted in Asia and Latin America to assess the efficacy of the Sanofi Pasteur candidate dengue vaccine. These trials are now in the hospital phase, and long-term follow up data are being accrued, in addition to those obtained during the first two years of the active phase. Scientific investigations have

been launched to deepen our understanding of the impact of different host parameters, and address their potential link to the observed results. New immunological assays have been developed and applied, aiming at evaluating in vaccinees and controls the homotypic and heterotypic nature and affinity of the vaccine and/or dengue-induced immune responses. The immunological signature present in the acute samples of breakthrough dengue cases has also been characterized in both hospitalized vaccinees and controls. The results of these ongoing investigations will be presented, which combined analyses may help understand the observed short term and long term efficacy and safety profile of the vaccine.

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DENGUE VIRUSES GROUP AS DIFFUSE CLUSTERS ON ANTIGENIC MAPS MADE WITH PRIMARY HUMAN VACCINATION AND NATURAL INFECTION ANTISERA

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The four dengue virus types, DENV1-4, are mosquito-borne flaviviruses that infect an estimated 390 million individuals each year. Isolates of DENV1-4 are thought to group into four discrete serotypes, in large part because the enduring, protective neutralizing antibody response following primary infection is observed to be serotype-specific. We previously found that primary African green monkey (AGM) antisera recognize DENVs as diffuse antigenic clusters. Here we use antigenic cartography to further test: 1) if primary human monovalent vaccine and natural infection antisera recognize a genetically diverse panel of DENV isolates as discrete serotypes, and 2) if the neutralizing responses of these antisera are type-specific. We generated two antigenic maps of the DENV panel: one using antisera from 40 individuals, each inoculated with a monovalent vaccine component of the NIH live vaccine, and a second using antisera from 21 Nicaraguan children drawn in the year following their first DENV infection. On maps made with monovalent vaccine and natural infection antisera, similar to what we observed with AGM antisera, the antigenic distance within a DENV type was the same as the antigenic distance between types. We then tested if the neutralizing antibody responses were type-specific. On both the monovalent vaccine and natural infection antigenic maps, some individuals had type-specific responses, but just as often, individuals had neutralizing antibody responses that fell between DENV types. Further, we found that the monovalent vaccine antigenic map changed very little if made with only the most central, cross-reactive antisera or only the most peripheral, type-specific antisera. We thus found that human monovalent vaccine and natural infection antisera reacted to the DENV panel as diffuse antigenic clusters, that both type-specific and cross-reactive neutralizing antibody responses were observed following primary DENV exposure, and that these neutralizing responses identified similar underlying antigenic relationships among genetically diverse DENV strains.

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DELIVERY OF A MALARIA PRE-ERYTHROCYTIC PEPTIDE VACCINE FORMULATED IN TLR AGONIST ADJUVANTS TO SKIN ASSOCIATED LYMPHOID TISSUE (SALT)

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Long-lived protective immunity against malaria can be elicited in human volunteers by immunization with sporozoites, however, mass vaccination with cryopreserved parasites or by mosquito bite presents logistical challenges for delivery to the one third of the world's population currently at risk of ***Plasmodium*** infection. The "gold standard" for large scale vaccination remains the Smallpox Eradication campaign which demonstrated the utility and cost effectiveness of vaccine delivery by skin scarification (SS) using trained community health workers. SS delivery of malaria vaccines may more closely mimic the natural route of sporozoite injection by mosquito bite which is known to elicit neutralizing antibodies that immobilize sporozoites in the skin and protective cellular immunity in the skin draining lymph nodes. Our recent studies demonstrate that mice immunized SS with a ***P. falciparum*** circumsporozoite peptide formulated in adjuvants containing synthetic TLR agonists can elicit neutralizing antibody that protect mice against sporozoite challenge. Inclusion of TLR agonists was critical, as SS immunization with peptide administered in oil adjuvants without TLR agonists elicited a Th2-type antibody response that was not protective. Immune responses in the skin reflect a complex dermal network of innate and adaptive immune cells that potentially include keratinocytes, Langerhans cells, dendritic cells and NK cells, as well as resident memory T cells recently shown to be elicited by various viral vaccines. In an effort to elucidate the spacio-temporal response in SALT to an SS delivered malaria vaccine, we have utilized reporter mice and intravital imagining, confocal microscopy, and flow cytometry to define the TLR agonist- dependent mechanisms functioning in the induction of sporozoite neutralizing antibodies. These studies identify elements of the innate and adaptive immune response that can be used to guide the rational development of improved SS adjuvant formulations for vaccines against ***Plasmodium*** parasites as well as other skin invasive pathogens.

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VACCINE MEDIATED PROTECTION WITH SINGLE AND DOUBLE SPECIES CHALLENGE AFTER WHOLE PARASITE CHEMICALLY ATTENUATED BLOOD STAGE VACCINES

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Mixed species malaria infections are common in parts of the world and can result in more severe malarial disease than infection with a single species. Understanding the impact mixed infections have on vaccine mediated protection is important for developing an effective malaria vaccine. To model this we assessed protection against single and double species challenge in C57BL/6 mice immunized with a chemically attenuated blood stage vaccine containing ***Plasmodium chabaudi AS, Plasmodium yoelii 17X,*** or a combination vaccine containing both species. Mice received three doses of vaccine containing 10^6 centanamycin attenuated pRBCs of a single species or each species within a single inoculum or an equivalent number of centanamycin-treated normal red blood cells (control) from a naive mouse. Mice were challenged five weeks post vaccination with 10^5 pRBC of each species or 10^5 pRBC of both species in a single inoculum. Utilizing differences in the MSP1 gene sequence between ***Plasmodium chabaudi AS*** and ***Plasmodium yoelii 17X*** quantitative real-time PCR was used to measure species-specific parasite levels. Blood samples were collected prior to challenge and post challenge for PCR. Physiological data including internal temperature, body weight, glucose, and hemoglobin were collected on alternate days from day 1 and compared to baseline. Additionally, the severity of disease in mice was evaluated using our system of clinical scoring based on criteria such as pallor, urine color, fur texture, activity, and posture. All data were collected blinded post challenge. None of the vaccines (single or double) conferred complete protection against parasite burden (as measured by qPCR). However, single and combination vaccines provided clinical protection against severe disease following a single parasite challenge. Double (mixed) challenges resulted in increased disease severity (clinical score) in all groups of vaccinated and control mice relative to single challenge. Nonetheless, a combination vaccine provided enhanced survival against a double challenge compared to control or single vaccines. The full results of these new studies are presented here.

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ENABLING THE VACCINE POTENTIAL OF DUFFY BINDING PROTEIN (DBP): BROADLY NEUTRALIZING EPITOPES, RECEPTOR BINDING, AND ANTIGEN ENGINEERING

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The Duffy Binding Protein (DBP) plays a critical role in ***Plasmodium vivax*** invasion of reticulocytes. Three features have hampered the progression of DBP as a vaccine candidate: 1) The polymorphic nature of DBP induces strain-specific immune responses; 2) the epitopes of broadly-neutralizing versus non-protective antibodies are not known; and 3) a limited understanding of the structure of DBP bound to its receptor DARC. We will present structural, computational and functional data on DEKnull, a synthetic DBP based antigen that has been engineered through mutation to enhance the induction of strain-transcending, blocking inhibitory antibodies. We will also present high-resolution epitope mapping data of multiple anti-DBP antibodies. The mapping data clearly identifies broadly neutralizing epitopes that, in conjunction with our structural determination of the DBP:DARC complex, lay a clear path forward for the rational design of the next generation of DBP-based antigens. This work highlights new approaches to target the molecular mechanism of invasion and increases the inhibitory epitope repertoire to be incorporated into future vaccine designs. Vaccine efficacy may be improved by targeting critical functional regions and broadly-neutralizing epitopes of parasite proteins, while avoiding decoy-epitopes identified by these studies.

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MIDGUT FIBRINOGEN-RELATED PROTEIN 1 (FREP1) AS A NOVEL TARGET FOR MALARIA TRANSMISSION-BLOCKING VACCINES

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Plasmodium development in mosquito midguts is imperative for malaria transmission. Recently, fibrinogen-related protein 1 (FREP1) has been demonstrated to be critical for parasite infection in mosquitoes. FREP1 is expressed in the mosquito midgut where it mediates ***P. falciparum*** ookinete invasion assisting parasite through anchoring to the peritrophic matrix and penetration of the midgut epithelium. Here, we determined that FREP1 protein could also bind ***P. berghei***-infected mice red blood cells and ookinetes using indirect immunofluorescence assays. We propose that anti-FREP1 antibodies disrupting the interaction between FREP1 and ***Plasmodium*** will interfere with parasite infection in mosquitoes. To test this hypothesis, we expressed FREP1 in ***E. coli***, using the purified protein to generate polyclonal anti-FREP1 antibody in rabbits. We demonstrated that feeding mosquitoes with the purified anti-FREP1 rabbit polyclonal antibody (200μg/ml) mixed with ***P. berghei*** infected mouse blood significantly reduced the number of oocysts in ***A. gambiae*** compared with pre-immune rabbit IgG ($p<0.0001$). The number of oocysts per midgut decreased from 10 in the control group to 3 in experimental group. To further examine whether FREP1 can be a potential vaccine antigen against parasite transmission to mosquitoes, we immunized mice with purified recombinant protein and boosted twice with a 16-day interval to elicit a high titer antibody response in outbred mice. Two weeks after the third boost, we infected the immunized mice with ***P. berghei*** that were used to infect mosquitoes 10 days later. Results showed that 80% of mosquitoes in experimental groups were free from infection compared to 12.5% of mosquitoes from control group. We repeated the experiments >3 times, and obtained consistent results, suggesting that antibodies that recognize FREP1 is able to inhibit the ***Plasmodium*** invasion in midguts and then interfere with parasite infection in mosquitoes. Collectively, we confirmed FREP1's role in the ookinetes invasion of mosquito midgut and demonstrated that FREP1 can be used as a midgut antigen target for mosquito-based transmission blocking vaccine.

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DISCOVERY OF NOVEL TRANSMISSION BLOCKING VACCINE CANDIDATES USING GAMETOCYTE PROTEIN MICROARRAY

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Natural and vaccine-induced immune responses can reduce the transmission of malaria from man to mosquitoes. The development of malaria transmission blocking vaccine (MTBV) candidates has so far been limited by difficulties achieving correctly folded immunogenic proteins for vaccination. Two leading candidates, pre-fertilization antigens Pfs48/45 and Pfs230, are expressed on the surface of mature gametocytes and elicit transmission blocking immunity (TBI) in a fraction of individuals naturally exposed to malaria infection. It has been shown repeatedly that full TBI may be present in absence of immune responses to these well characterised proteins, yet no attempts have been made to comprehensively identify the immune signatures of naturally acquired TBI. To this end we compiled a list of 319 ***P. falciparum*** proteins whose transcription is upregulated in mature gametocytes and which possess characteristics of surface-located proteins (e.g. transmembrane domains, GPI anchor). These proteins were expressed in an E. coli based ***in vitro*** transcription-translation system and printed onto a nitrocellulose microarray. To assess the relationship between functional TBI and reactivity to the array of gametocyte proteins, we collected a library of 587 sera from naturally malaria exposed expatriates and individuals from the Gambia, Cameroon, and Burkina Faso. Serum IgG was purified from all samples and provided to mosquitoes with cultured gametocytes in the standard membrane feeding assay (SMFA), revealing that 3.6% (21/587) of individuals possessed strong, reproducible TBI. Probing the sera on the array identified 10 proteins that individuals with TBI reacted to more intensely, and more commonly. One of the reactive proteins, PF3D7_1103800 ($p=0.014$), has not previously been associated with TBI but is highly expressed in stage V gametocytes and ookinetes and was associated with TBI in multiple endemic settings. Our analysis substantially increases the number of ***Plasmodium*** antigens implicated in the development of immune responses that inhibit parasite development in mosquitoes, and provides multiple novel targets for MTBV development.

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INTERACTIONS BETWEEN TRANSMISSION BLOCKING VACCINES AND NATURAL IMMUNITY AGAINST *PLASMODIUM FALCIPARUM*

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Malaria transmission-blocking vaccines (TBV) are promising candidates for disrupting the ***Plasmodium*** life cycle within mosquitoes. Using membrane feeding assays, pre-clinical studies identified vaccine candidates able to impede ***Plasmodium*** development in the mosquitoes. This vaccine-induced immunity could complement observed natural human immunity, which also reduces ***Plasmodium*** human to mosquito transmission. In this study, we investigated the efficacy of two major TBV candidates and the interaction between natural and vaccine-induced immunity. Mosquito Membrane Feeding Assays were performed by exposing ***Anopheles coluzzii*** females to gametocyte-infected blood from naturally infected patients. Total IgG purified from mice immunized with viral-vectors expressing Pfs25 or Pfs230-C were mixed with gametocyte-infected blood, with and without serum replacement. Female mosquitoes were then fed on the mixture. Fully fed mosquitoes were dissected 7 days-post feeding and oocysts were counted. The effect of natural immunity on TBV efficacy was tested using blood from 6 different gametocyte carriers. We observed a reduced TBV efficacy when using whole blood compared to blood with serum replacement. In the context of the translational investigations for implementation of transmission blocking vaccines, this finding highlights the need to consider the natural immunity of the patients when evaluating TBV candidates.

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IN VITRO PRODUCED *PLASMODIUM FALCIPARUM* SPOROZOITES ARE AS INFECTIOUS AS MOSQUITO PRODUCED SPOROZOITES IN HEPATOCYTE ASSAYS

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Development of an effective ***in vitro*** methodology for ***Plasmodium falciparum*** (Pf) sporogony and the production of infective sporozoites (SPZ) without the use of mosquitoes will radically alter Pf biology research paradigms and vaccine development program similar to the revolutionary discovery of Pf asexual ***in vitro*** culture methodology, as reported. This enabling technology will also dramatically reduce the cost of production of Sanaria® PfSPZ products; PfSPZ Vaccine (radiation attenuated PfSPZ), PfSPZ Challenge (infectious PfSPZ for controlled human malaria infection [CHMI]) and PfSPZ-CVac (PfSPZ Challenge given with antimalarial drugs as a vaccine) all of which have shown excellent results in clinical trials, as previously reported. Using the ***in vitro*** culture protocol developed at Sanaria, we consistently achieved 2.4 to 12.5% (mean 8.3%) transformation of stage V gametocyte to 8 day oocysts. This was 39-fold higher than that observed in mosquitoes. ***In vitro***-produced PfSPZ were harvested from culture supernatants and we produced between 180,000 and 350,000 mature PfSPZ in 7 independent experiments. Mature ***in vitro***-produced PfSPZ were motile, 10-13 µm long, and highly reactive to anti-PfCSP mAb, thus identical to salivary gland produced PfSPZ. ***In vitro*** produced PfSPZ were tested for their infectivity in 6 day hepatocyte potency assays. In 4 independent 6 day hepatocyte assays, ***in vitro*** produced PfSPZ seeded at 56,598 ± 7,294 PfSPZ/well produced 28.6 ± 7.0 PfMSP-1 expressing 6 day parasites greater than 10 µm in diameter. While mosquito-produced, fresh aseptically purified PfSPZ seeded at 50,000 per well, produced 25.7 ± 4.1 mature 6 day parasites expressing PfMSP-1. The size of the 6 day parasites developed in HC04 cells from ***in vitro*** and ***in vivo*** (mosquito) produced PfSPZ were similar. These data demonstrate that ***in vitro***-produced PfSPZ were as infectious in hepatocyte cultures as fresh mosquito-produced PfSPZ, and provide the foundation for ongoing efforts to optimize ***in vitro*** PfSPZ production.

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DEPLOYMENT AND USE OF MOBILE PHONE TECHNOLOGY FOR REAL-TIME REPORTING OF FEVER CASES AND MALARIA TREATMENT FAILURE IN AREAS OF DECLINING MALARIA TRANSMISSION IN MUHEZA DISTRICT NORTHEASTERN TANZANIA

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Early detection of febrile illnesses at community level is essential for improved malaria case management and control. Currently, mobile phone based-technology is common used in developing world for collecting and transferring health information and services. This study assessed the applicability of mobile phone technology for real-time reporting of fever cases and management of malaria by village health workers (VHWs) in northeastern Tanzania. The Community mobile phone-based Disease Surveillance and Treatment for Malaria (ComDSTM) system combined mobile phones and web applications was developed and implemented in three villages and one dispensary in Muheza district from November 2013 to October 2014. A baseline census was conducted in May 2013; data were uploaded on a web-based database and updated during follow-up visits by VHWs. Active (ACD) and passive case detection (PCD) of febrile cases was done by VHWs. Cases found positive by malaria rapid diagnostic test (mRDT) were treated by artemether-lumefantrine (AL). The first dose was given by nurse while other doses were taken under supervision of VHW at home. Each patient was visited on day 7 to observe recovery process. Data from each stages of the surveillance life cycle were captured and transmitted to the database using mobile phones.The baseline population in the three villages was 2934 in 678 households. A total of 1907 febrile cases were recorded by VHWs. Out of these, 1828 (95.9%) were captured using mobile phone. At the dispensary, 1778(93.2%) febrile cases were registered where 84.2% captured through PCD. Positivity rates were 48.2% and 45.8% by mRDT and microscopy, respectively. Nine cases had treatment failure reported on day seven post-treatment and adherence to treatment was 98%. In conclusion, the study showed that mobile phone based-technology can be successfully used by VHWs for surveillance and timely reporting of fever episodes and monitoring of treatment failure in remote areas. Further optimization and scaling-up of tools will be required to improve malaria case management and drug resistance surveillance

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SPATIAL CLUSTERING OF MALARIA INFECTIONS IN NORTHERN NAMIBIA: IMPLICATIONS FOR SURVEILLANCE AND RESPONSE STRATEGIES FOR ELIMINATION

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Reactive case detection (RACD), whereby household members and neighbours of passively detected index cases are tested and treated when positive, is a widely used method to identify and target malaria hotspots. Typically RACD is carried out using rapid diagnostic tests (RDTs) or microscopy, both of which are likely to miss low density (sub-patent) infections. This represents a potential barrier to interrupting transmission as these sub-patent infections are still able to infect mosquitoes. Using data from a larger epidemiological study in Engela district in northern Namibia between January 2013 - June 2014, we explore the occurrence and spatial clustering of sub-patent infections around passively detected cases. Individuals testing RDT positive at any health facility in Engela district were followed up to their home. Household members and individuals of four neighbouring households were asked to provide a blood sample for RDT and dried blood spot (DBS) for later molecular analysis. Blood samples for RDT and DBS were also taken from randomly selected individuals within the district. If they tested negative by RDT, members of their household and individuals of the four nearest neighbouring households were also screened. Households of study participants were geolocated using GPS devices. Sub-patent infections were identified by subsequent analysis of DBS samples by Loop-mediated isothermal amplification (LAMP). In total, 1,759 individuals from 62 case neighbourhoods and 944 individuals from 56 control neighbourhoods provided DBS samples. Results show that sub-patent infections were more prevalent in the neighbourhoods of cases than controls (OR 7.0, p=0.002). Furthermore, there appeared to be a decay in risk around case households, with the odds of finding additional infections far higher within the index household than in neighbouring households (OR 3.8, p=0.005). These results suggest that RACD with RDTs is insufficient to target the infectious reservoir and more aggressive interventions, such as targeted presumptive treatment, may be required.

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HETEROGENEITY IN MALARIA PROPAGATION POTENTIAL: QUANTIFYING REAL-LIFE EXPOSURE TO *ANOPHELES* VECTORS USING DNA FINGERPRINTING

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The number of mosquito bites individuals infected with malaria receive is a major determinant for onward transmission of infection. In this study, we quantified individual-specific exposure to Anopheline mosquitoes throughout the transmission season. In a village in Burkina Faso, 189, 326 and 620 blood-fed mosquitoes were collected from 40 households at the end of the transmission season (November-December 2013), and from 20 households at the start (July 2014) and peak (September 2014) of the following wet season, respectively. Finger prick blood samples were collected from individuals living in these households to allow for the identification of blood meal source by DNA fingerprinting. 171/189 mosquitoes collected during the first survey (2013) had their blood meal genotyped. 82.9 % of Anopheles vectors with single blood meals were linked to individuals living in the household where they were caught. A non-negligible proportion of mosquitoes (22.1 %) took their blood meal from multiple human hosts. Most bites (69.6%) were on adults even though they represented only 38.1% of the population. The mean number of mosquito bites per study participant and its variance were 1.2 and 7.4. 11.1 % of the population living in households where fed mosquitoes were collected received 71.8 % of mosquito bites. Mosquitoes collected during the start and peak of the wet season are currently being analysed and data will be presented. Our preliminary results provide evidence that encounters between malaria vectors and human hosts are heterogeneously distributed and that a high proportion of mosquitoes feed on more than one human host. This heterogeneous effective exposure to mosquitoes has profound consequences for our interpretation of the human infectious reservoir for malaria.

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MOLECULAR INVESTIGATION OF A MALARIA OUTBREAK IN CUSCO DEPARTMENT OF PERU LINKS IT TO A STRAIN OF *PLASMODIUM FALCIPARUM* GENETICALLY SIMILAR TO A RECENT TUMBES DEPARTMENT OUTBREAK STRAIN

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In November 2013, a malaria outbreak occurred in Cusco department, southern Peru. Eleven cases were detected, four of which persisted after presumptive treatment for ***Plasmodium vivax*** infection. Eventually,microscopic examination confirmed ***P. falciparum***, which had been reportedly absent in Cusco since 1946. In order to define the genotypic characteristics of the ***P.falciparum*** isolates from this outbreak and their possible origin(s), we utilized a panel of seven neutral microsatellites (MS) that had been used previously to characterize parasite populations in Peru and other countries. We had employed the same MS markers to investigate a malaria outbreak that occurred in Tumbes department, northern Peru, from 2010-2012; ***P.falciparum*** transmission had been halted for five years prior to that outbreak.The Tumbes investigation identified the origin of the outbreak as being a B-variant falciparum strain (B_{V1}) from Loreto department. In the current investigation, we found that all four Cusco isolates were genetically identical to the B_{V1} strain linked to the Tumbes outbreak. These isolates had drug resistance marker alleles that matched those of the B_{V1} strain at ***Pfcrt, Pfmdr1***, ***Pfdhfr*** and ***Pfdhps*** genes. The isolates had also deleted ***Pfhrp2*** and ***Pfhrp3.*** Epidemiological data suggested that this outbreak was caused by road construction workers who migrated from Loreto department. Our molecular data was consistent with the epidemiological findings. Previous laboratory investigations revealed that the B_{V1} strain was circulating in Loreto department as early as 2009. Given that the B_{V1} strain is multi-drug resistant, can escape detection by PfHRP2-based RDTs and has contributed to two outbreaks in different geographical regions where ***P. falciparum*** transmission had been halted for several years prior, it is important for countries in the Amazon Basin to monitor for its potential expansion. Molecular epidemiological investigations are valuable for tracking the potential source of outbreak parasite strains and their drug and diagnostic resistance genetic characteristics.

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ASSESSMENT OF COMMON GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY ALLELIC TYPES IN ETHIOPIA

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Ethiopia aims to eliminate malaria from selected low transmission settings by 2020. As ***Plasmodium falciparum*** and ***P. vivax*** are co-endemic in Ethiopia, the use of primaquine is indicated for both transmission interruption and radical cure, respectively. However, primaquine use has been limited in Ethiopia due to the limited data available on G6PD deficiency. Dried blood spot (DBS) samples were collected in 2011 as part of the national Malaria Indicator Survey-a multi-stage nationally representative survey of all malaria-endemic areas of Ethiopia. A randomly selected subset of DBS was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Considering the geographical position and ethnic mix of the country, three common G6PD variants, i.e. G6PD*A (A376G), G6PD*A- (G202A) and Mediterranean (C563T), were investigated in 2000 DBS samples. Of the 2000 samples selected, 1585 (76.8%) were available for genotyping, among which 54% were from females. G6PD*A (8.99%) was the only genotype detected, with no samples positive for A- or Mediterranean variants. Regional variation in G6PD*A prevalence was observed, with the highest prevalence reported in samples from the Southern Nation and Nationalities Peoples' Region (11.33%), followed by Tigray (10.94%), Oromia (9.48%),Gambella / Benishangul Gumuz (9.4%), Afar / Somali (7.03%), and Amhara (5.73%); no G6PD deficiency was detected in samples from Harari. Of the A mutation 31% were found in males; 62.1% and 6.8% of mutations found in females were heterozygous and homozygous, respectively. The results support the limited historical evidence of low prevalence of G6PD deficiency in Ethiopia. The A mutation observed is a mild deficiency causing around 85% of the normal enzymatic activity and is of little clinical significance. The more severe G6PD deficiency allelic types, A- and Mediterranean, common in other parts of Africa were not observed. Our results support the safe use of primaquine especially the single low-dose (0.25 mg/kg) as an elimination strategy for falciparum malaria in Ethiopia.

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SPATIAL ANALYSIS USING SEROLOGICAL MARKERS FOR THE IDENTIFICATION OF AREAS OF HIGH MALARIA TRANSMISSION IN THE LOW ENDEMIC NORTHERN COAST OF PERU

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As transmission becomes increasingly focal in low transmission settings, an accurate identification of malaria hotspots is crucial to better design targeted interventions. In this study, we evaluated the ability of a spatial analysis using serological markers for identifying cluster areas with high incidence rates of clinical ***P. vivax*** malaria in a low endemic district (Bellavista) of the Peruvian Northern Coast. After a full census and georeferencing of houses in the study setting in May 2010, a cross-sectional survey was conducted to collect blood samples on filter paper from 1925 individuals. Antibodies for specific IgG responses to ***P. vivax*** merozoite surface protein-1 —terminal region 19-kDa C— (PvMSP-119) and apical membrane antigen-1 (PvAMA-1) were detected by ELISA. A mixture model was used to determine the cut-off of antibody responses for each antigen. An individual with antibody responses exceeding the cut-off value for at least one of the two antigens was considered seropositive. QGIS was used to map all surveyed individuals and SaTScan (applying a Bernoulli model) to detect spatial clusters of individuals with ***P. vivax*** seropositivity. The most likely spatial cluster was a 0.22 km radius cluster including 694 individuals (19.7% of total censored individuals) in 163 households (21.9% of total censored houses) (RR=2.3, p<0.001). After the survey, an enhanced passive case detection (PCD) at local health facilities from June 2010 to May 2012 allowed the detection of 35 clinical ***P. vivax*** episodes in the study setting. A logistic regression analysis showed that individuals living inside the most likely seropositivity cluster (identified in May 2010) were four times more likely to present clinical ***P. vivax*** episodes detected by PCD in the following two years than those living outside the cluster (OR=3.92, 95%CI 2.01-7.64). Study findings confirmed that the spatial analysis using serological markers allowed identifying future areas with high risk of clinical ***P. vivax*** malaria. These tools may support the risk stratification and prioritization of targeted interventions to better reduce transmission and move towards malaria elimination.

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MICRO-EPIDEMIOLOGY OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* INFECTIONS IN AN AREA OF LOW TRANSMISSION OF SOLOMON ISLANDS: STRIKING CONTRAST WITH IMPLICATIONS FOR FUTURE INTERVENTIONS

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Historically, Solomon Islands has endured considerable ***Plasmodium falciparum*** and ***P. vivax*** burden. In the last 20 years, it has achieved 90% reduction in malaria cases and is aiming for elimination. In 2012, we conducted a cross-sectional survey in Ngella (n=3501), an area of low transmission of Solomon Islands to investigate the natural reservoir and local epidemiology of ***P. vivax*** and ***P. falciparum***. The contrast was striking. Whilst only five clonal ***P. falciparum*** infections were identified from a single village, ***P. vivax*** prevalence remains moderately high (qPCR 13.4%), with predominantly afebrile, submicroscopic infections, which display genetic complexity and considerable spatial heterogeneity. We typed 360 ***P. vivax*** infections at nine microsatellite loci and subsequent analyses of diversity (F_{ST}, G_{ST}, Jost's D) and structure (Bayesian clustering, multidimensional scaling and phylogenetics) revealed a genetically diverse ***P. vivax*** population, but spatially fragmented, even among villages 10-30km apart. This indicates that whilst ***P. vivax*** may be more difficult to eliminate than ***P. falciparum***, local parasite populations have been affected by control interventions. Living in a household with at least one other ***P. vivax*** carrier increased the risk of ***P. vivax*** infection, indicating possible intra-household transmission. Genetic relatedness of ***P. vivax*** clones will be measured and compared at household, village and island levels to decipher fine scale spatial clustering of infection and thus presence of transmission hotspots. Association of ***P. vivax*** infection with human genetic factors known to confer protection against infection (α-thalassemia and Southeast Asian ovalocytosis) will also be integrated in this analysis. The implication of these findings will be discussed in the context of factors which may impact on the efficacy of follow-up elimination strategies: i) enhanced and targeted surveillance, ii) source of ***P. vivax*** importation, and iii) prevalence of glucose-6-phosphate dehydrogenase deficiency and the use of primaquine mass drug administration to eliminate malaria.

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NATURAL HISTORY OF PERILESIONAL EDEMA IN CALCIFIC NEUROCYSTICERCOSIS

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Perilesional edema around calcified ***Taenia solium*** granulomas (calcifications) is commonly detected in patients with seizures in endemic regions, occurring in 50% of recurrent seizures in persons who have calcifications, a history of seizures and a positive cysticercosis serology. We characterized the long-term course and consequences for persons experiencing one or more documented episodes of perilesional edema (episodes) in 21 patients followed for a median of 8.5 years (range 0.4-28.8 yrs) at the National Institutes of Health (NIH). A perilesional event (event) was defined as a transient fluid-attenuated version recovery (FLAIR) magnetic resonance imaging (MRI) signal at least twice the diameter of the involved calcification, whose presence was confirmed by computerized tomography (CT) examination and/or on SWI MRI sequences on or before the first episode. A sizable minority of episodes (15.4%) encompassed multiple events involving separate calcifications. Repeat MRI examinations were done when new symptoms occurred, at intervals after documented episodes when possible, when evaluating other lesions or complications and semiannually or annually. The study group had a median age 30.5 yr (range 19.7-69.8), two thirds were female and 71.5% were Hispanic. In all there were 77 episodes, which included 104 events involving 50 of 728 calcifications, which corresponds to 5 calcifications/person (1-280), 2 involved calcifications/person (1-11) and 3 events/persons (1-32)[(data are median (range)]. In 64 evaluable episodes, symptoms were seen in 70.3% and were mostly focal and/or generalized seizures. No symptoms were reported in 29.7% of episodes, a high proportion that may be the result of the relatively large numbers of MRIs performed in asymptomatic patients. Other findings will be presented. Our data demonstrate that recurrent episodes involving multiple calcifications can occur over decades causing significant morbidity. Many episodes are asymptomatic suggesting that the number of episodes may be significantly greater than estimated from symptomatic cases.

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NEUROCYSTICERCOSIS AT A LARGE ACADEMIC CENTER IN SOUTH FLORIDA

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Neurocysticercosis (NCC) is a neglected parasitic disease with an estimated mortality rate of 2-9.8% in the United States of America (USA). Florida accounted for 6.3% of NCC deaths in the USA between 1990 and 2002, yet there are no major NCC studies for the region. This is a retrospective study with the aim of characterizing the population diagnosed with NCC at 2 large tertiary medical centers in South Florida within Miami-Dade County, where 51.3% of the population is foreign born. Seventy-seven patients met criteria for study: 62.3% female, and 67.5% uninsured. The largest region of origin was Central America (50.6%), followed by the Caribbean (32.5%). The largest nationality was Haitian at 28.6% in the study, yet 7% of the foreign born population are Haitian in Miami-Dade county, and only 5% of cases were Mexican. 93.5% met diagnostic criteria for definitive and probable NCC, 67-78% had no serology or stool study to support radiologic evidence of NCC. 68.8% had parenchymal NCC, 31% had extraparenchymal NCC, and only 52% of extraparenchymal cases underwent resection of cyst. Five cases had mention of treating close contacts of patients or giving case patient praziquantel to target possible intestinal disease. There was a correlation between symptom improvement and increasing length of therapy, lesion type and NCC lesion resolution, but not symptom improvement and NCC lesion resolution. Nationalities represented differ from what has been reported in other studies, and disproportional to the foreign born populations in Miami-Dade County. This study shows that South Florida has conditions for the autochthonous acquisition of NCC, identifies Haitians has a high-risk group for NCC in the USA, variations in care and the need for treatment guidelines.

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PREVALENCE AND FACTORS ASSOCIATED WITH INTESTINAL TAENIASIS IN PATIENTS WITH CYSTICERCOSIS AND THEIR RELATIVES

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Neurocysticercosis, common parasitic disease of the central nervous system, is a leading cause of seizures worldwide. Identification and treatment of tapeworm carriers is key to block further infections and reduce the burden of disease of this zoonosis. In this study we evaluated the prevalence and associated factors of taeniasis in patients with cysticercosis and their close relatives (people living in the same house). Patients with a serological diagnosis of cysticercosis (positive on enzyme-linked immunoelectro transfer blot [EITB, western blot] assay) attending the Cysticercosis Unit in the Institute of Neurological Sciences, a referral center in Lima, Peru, and their relatives were routinely asked to provide at least one stool sample to rule out teniasis by microscopy using a tube sedimentation technique. This retrospective analysis had as its primary outcome the proportion of taeniasis in patients and relatives. Additionally age, sex and place of residence (living or not in Lima, capital of Peru) were evaluated in a logistic regression model to assess their role as associated factors. A total of 4,563 new patients with serological diagnosis of cysticercosis were identified in our unit between June-1999 and December-2010. From them only 3,405 patients and 4,217 relatives provided stool samples. The average age in patients was 36.2 years (SD 18.8) and 26.4 years (SD 18.0) in relatives. The proportion of males among patients was higher than relatives, 49.3% (95%CI ±1.68) and 44.55% (95% CI±1.5) respectively. The prevalence of microscopically confirmed taeniasis among patients was 2.3% (95%CI ±0.5) and 0.8% (95% CI±0.3) in relatives. In the logistic regression including sex age and place of residences as covariates we found that only younger age (OR 0.98 p<0.01) and living outside of Lima (OR 1.83, p=0.059) are associated to taeniasis. The prevalences found both in cysticercosis patients and in their relatives are similar to levels reported in highly endemic areas. Taeniasis should be actively ruled out in patients with positive results to western blot to cysticercosis especially in young population and in people who live outside of Lima.

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ASSOCIATION BETWEEN EDEMA PERI-CALCIFICATION AND EPILEPTIC SEIZURES: A CASE-CONTROL STUDY IN ENDEMIC AREA FOR CYSTICERCOSIS IN THE NORTHERN COASTAL OF PERU

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Neurcysticercosis (NCC) is a helminthic infection of the Central Nervous System due to larval stage of ***Taenia solium*** and one of the most important contributor of late-onset epilepsy in resources poor-region around the world .Between 30% and 42% of overall epileptic seizures can be attributed to NCC in rural Peru. A previous study performed in patients with calcified NCC and seizures showed that 34.5% presented peri-lesional edema at MRI evaluation. The main objective of this case-control study was to evaluate the association between peri-calcification brain edema and seizures in field conditions in an endemic region for cysticercosis This study was performed in health centers in rural villages of the northern coastal of Peru (Tumbes) where people with epilepsy (PWE) receive monthly their antiepileptic treatment. Fifty-eight PWE with calcified images associates with NCC were included, 29 PWE (case group, CG) were matched by age (+/- 4 years) and sex with their control group (CC), PWE and calcified NCC with more than 02 year without seizures In a window-time of seven days after the last seizure an MRI (axial slides of T1, T2 and Flair protocol) was performed in cases and controls. Females were 17/29 (58.62%), history of partial seizure were more frequent CG compare to CC, 27/29 (93.1%) vs. 17/29 (58.62%), p= 0.0237), Peri-lesional edema was found in 34.48% (10/29) vs. 10.34% (3/26), p=0.028; other variables were not significative. The OR 6.71 [(95%CI 1.18-38.05), p=0.031] to develop an epileptic seizure was higher in PWE with calcified lesions associated with edema in CG compare to CC. In conclusion, edema Peri-calcification is strongly associate to epileptic seizures in PWE and Calcified NCC in field conditions.

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ASSOCIATION BETWEEN INDIVIDUAL- AND VILLAGE-LEVEL FACTORS AND THE PREVALENCE OF CURRENT INFECTION WITH *TAENIA SOLIUM* CYSTICERCOSIS IN 60 VILLAGES OF BURKINA FASO

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Taenia solium cysticercosis is an infection with great potential to be controlled using One Health. The goal of this study was to use the baseline data from a community-based randomized controlled trial conducted in

60 villages of Burkina Faso to explore the association of environmental- and individual-level variables and human cysticercosis prevalence. Between February 2011 and January 2012, 60 individuals living in 60 villages located in three provinces of Burkina Faso were selected using clustered random sampling. One individual was sampled per compound. The chief of the household, housewife and the participant answered questionnaires about socio-demographic characteristics, sanitation, hygiene, pig management and pork consumption. Blood samples were drawn by venipuncture and the sera were tested for circulating antigens of ***T. solium*** metacestodes. Between March and November 2014, soil texture and pH were measured in each village. A Directed Acyclic Graph was developed to assess the structure of variables affecting environmental contamination and exposure to eggs. Bayesian hierarchical log-binomial models were fitted with individual and village level variables, with village- and province-level random-effect intercepts. Diffuse priors were used. Circulating antigens were detected in 120 of 3609 participants. The prevalence of current cysticercosis infection varied considerably across provinces and ranged from 0% to 11.5% across villages. At the individual level, being aged between 18 and 49, male, and eating pork outside the household were associated with increased prevalence while having access to a latrine and living in a more wealthy household decreased prevalence. At the village level, restraining pigs some of the time, a higher percentage of silt texture and higher pH in the soil decreased the prevalence. This study demonstrates that at the individual level young males who eat pork outside of the home show higher prevalence of cysticercosis. The highly clustered nature of cysticercosis could be explained in part by the soil texture which may impact survival of taeniid eggs in the environment.

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TH-17 CELLS ARE ASSOCIATED WITH DISEASE ACTIVITY AND PATHOLOGY IN HUMAN ALVEOLAR ECHINOCOCCOSIS

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Echinococcus multilocularis infections, which usually cause alveolar echinococcosis, are often clinically silent, but some individuals develop severe pathological reactions. In several disease processes, T-helper 17 (Th17) cells have been linked to tissue injuries, while regulatory T cells (Tregs) are thought to down modulate inflammatory reactions. We assessed whether disease activity and pathology in human ***E. multilocularis*** infection is related to the balance of Th17 cells and Tregs. We used a murine model of ***E. multilocularis*** infection to further investigate whether the peripheral profiles reflected ongoing events in tissues. We characterized T-helper cell subsets in the peripheral blood and different hepatic tissue of alveolar echinococcosis patients in an endemic area and in the peripheral blood, spleen, and abdominal cavity of ***E. multilocularis***–infected C57BL/6 mice. Hepatic alveolar echinococcosis patients with active stage had a significantly higher percentage of Th17 cells than those without pathology. Moreover, the Th17 related cytokines and transcription factor were highly elevated in infected hepatic tissue compare with normal tissue. Percentages of interleukin 17–producing cells and related transcription factors were significantly higher in spleen and abdominal cavity of infected mice compare to non-infected mice. This difference was also reflected in the peripheral blood. Our study indicates that Th17 cells may be involved in the pathogenesis of human alveolar echinococcosis.

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PERFORMANCE COMPARISON OF THREE RAPID DIAGNOSTIC TESTS FOR THE SERODIAGNOSIS OF HEPATIC CYSTIC ECHINOCOCCOSIS IN HUMANS

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The diagnosis of cystic echinococcosis (CE) is based on imaging, in particular ultrasound (US) for abdominal CE, complemented by serology when US features are unclear. In rural endemic areas, where expertise in US diagnosis of CE may be scant and conventional serology techniques are unavailable due to the lack of laboratory equipment, Rapid Diagnostic Tests (RDTs) are appealing. However, the performances of immunoassays are heterogeneous and influenced by many variables, and the interpretation of results may be difficult. We evaluated the performances of 3 commercial RDTs for the diagnosis of hepatic CE. Sera from 59 patients with single hepatic CE cysts (38 active, 21 inactive) and 25 patients with non-parasitic cysts were analysed by RDTs VIRapid HYDATIDOSIS (Vircell, Spain), Echinococcus DIGFA test (Unibiotest, China), ADAMU-CE (ICST, Japan), and by RIDASCREEN Echinococcus IgG ELISA (R-Biopharm, Germany). Sensitivity, specificity and ROC curves were compared with McNemar and t-test. For VIRapid and DIGFA, correlation between semiquantitative results and ELISA OD values was evaluated by Spearman's coefficient. Reproducibility was assessed on 16 randomly selected sera with Cohen's Kappa coefficient. Se and Sp of VIRapid (74%, 96%) and ADAMU-CE (57%, 100%) did not differ from ELISA (69%, 96%) while DIGFA (72%, 72%) did (p=0.045). ADAMU-CE was significantly less Se in the diagnosis of active cysts (p=0.019) while DIGFA was significantly less Sp (p=0.014) compared to ELISA. All tests were poorly Se in diagnosing inactive cysts (33.3% ELISA and ADAMU-CE, 42.8% DIGFA, 47.6% VIRapid). ROC curves of VIRapid (AUC 0.851) and DIGFA (AUC 0.722) were significantly different (p=0.042). The reproducibility of all RDTs was good to very good. Band intensity of VIRapid and DIGFA correlated with ELISA OD values (r=0.76 and r=0.79 respectively, p<0.001). RDTs may be useful in resource-poor settings to complement US diagnosis of CE in doubtful cases. In this regard, VIRapid test appears to perform best among the examined kits, but all tests are poorly Se in presence of inactive cysts, which may pose considerable problems of differential diagnosis.

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GEOPHAGY IS ASSOCIATED WITH ENVIRONMENTAL ENTEROPATHY AND IMPAIRED GROWTH IN CHILDREN IN RURAL BANGLADESH

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Undernutrition is estimated to be an underlying cause of over half of all deaths in young children globally. There is a growing body of literature indicating an association between stunting and environmental enteropathy, a disorder thought to be caused by repeated exposures to enteric pathogens. Environmental enteropathy is thought to arise from unsanitary environmental conditions leading to repeated exposure to enteric pathogen causing infections. To investigate the relationship between exposure to enteric pathogen through geophagy, consumption of soil, environmental enteropathy, and stunting, we conducted a prospective cohort study of 216 children under five years of age in Bangladesh. Geophagy was assessed at baseline using direct observation and caregiver reports. Stool was analyzed at baseline for fecal markers of intestinal inflammation: alpha-1-antitrypsin, myeloperoxidase, and neopterin combined to form an environmental enteropathy disease activity (EE) score, and calprotectin. Eighteen percent of children had observed geophagy events and 28% had caregiver reported events in the past week. Nearly all households had ***Escherichia coli*** (97%) in soil, and 14% had diarrheagenic ***E. coli***. Children with caregiver reported geophagy had significantly higher EE scores (0.72 point difference, 95% confidence interval (CI): 0.01, 1.42). Furthermore, at the 9 month follow-up the odds of being stunted (height for age z-score <-2) was double for children with caregiver reported geophagy (OR: 2.27, 95% CI: 1.14, 4.51). These findings suggest that geophagy may be an important unrecognized risk factor for environmental enteropathy and stunting in susceptible pediatric populations.

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ESCHERICHIA COLI CONTAMINATION OF COMPLEMENTARY FOODS AND ASSOCIATION WITH DOMESTIC HYGIENE IN RURAL BANGLADESH

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Consumption of contaminated food is particularly harmful for children <2 years who have immature immune systems and are vulnerable to infection. Fecal indicator organisms and pathogens have been detected in children's complementary food in Bangladesh. This study aimed to identify the frequency and concentration of ***Escherichia coli*** in child complementary food and its association with domestic hygiene practices in rural Bangladesh. It was nested within the control arm of a large-scale randomized-controlled trial of water, sanitation, hygiene and nutrition interventions. Field workers collected stored complementary food samples from 608 households with children <2 years, performed spot checks on domestic hygiene and measured the ambient temperature in the food storage area using AcuRite 00325 monitor. Food samples were analyzed using the IDEXX most probable number (MPN) method with Colilert-18 media to detect ***E. coli***. We calculated adjusted prevalence ratios (APR) to assess the relationship between ***E. coli*** and domestic hygiene using modified poisson regression, adjusting for mother's education, household wealth, number of children and number of households in the compound. 58% of stored complementary food was contaminated with ***E. coli*** and high levels of contamination (>100 MPN/dry g food) were found in 12% of samples. High levels of ***E. coli*** contamination was more prevalent in food that was stored uncovered rather than covered (APR=2.0, 95% CI=1.2-3.2), transferred from the storage pot to serving dish by hand rather than utensil (APR=2.0, 95% CI=1.3-3.2), stored at higher ambient temperatures (26-40°C vs. 14-25°C; APR=2.7, 95% CI=1.5-4.7) and stored for longer periods of time (>4 hours vs. ≤4 hours; APR=2.5, 95% CI=1.5-4.2). Food container location, presence of flies and feces in the food storage area, presence of animals in the compound and utensil cleanliness were not significantly associated with high levels of ***E. coli*** contamination. Interventions to keep stored food covered, reduce storage temperatures, avoid hand contact while serving food and reduce storage time would be expected to reduce the concentration of ***E. coli*** in complementary foods.

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PARASITIC CONTAMINATION OF VEGETABLES FROM SELECTED ORGANIC AND CONVENTIONAL FARMS IN THE PHILIPPINES: ITS IMPLICATIONS TO PUBLIC HEALTH

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This is a study on parasitic contamination of vegetables from selected farms in the Philippines. A total of 248 freshly harvested vegetable samples from organic and conventional farms were collected by systematic random sampling. The vegetable samples were processed by means of sedimentation technique. Results showed that 35 out of 248 (14%) vegetable samples were contaminated with eggs/cysts of parasites. The parasites found were ***Ascaris*** sp., ***Toxocara*** sp., ***Trichuris*** sp., ***Balantidium coli***, ***Isospora*** sp. ***Taenia*** sp. and hookworm/strongylid. ***Ascaris*** sp. was considered as the most prevalent parasite in raw vegetables (5.95%) while ***Toxocara*** sp. had the highest mean density of 16 eggs/kg of vegetables. Also, contamination rate was higher in organic farms (15%) than in conventional farms (11%). Lettuce, showed the highest contamination rate among the sampled vegetables in both types of farms. Farming practices and texture of vegetables were some of the factors that contributed to parasite contamination. These findings have important implications on food safety and on public health. The goal of the report is not to scare consumers but to aid regulatory agencies in their efforts to prevent contamination and improve food safety. Hence, proper authorities should address and review policies in certifying organic farms to address these issues.

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USAGE OF CARBON-FINANCED ("LIFESTRAW") WATER FILTERS BY RURAL KENYAN HOUSEHOLDS

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In early 2011, 877,505 Lifestraw family water filters were distributed freely to >4.5 million people residing in Kenya's Western Province by Vestergaard Fransen. The company registered the "Carbon for Water" project under the Gold Standard voluntary market; carbon credits are awarded based on semi-annual audits. This program overlapped a large RCT ("WASH Benefits") evaluating the impacts of water, sanitation, hygiene, and nutrition interventions among households with newborns in rural villages in Kakamega and Bungoma counties. To characterize baseline water management practices and drinking water quality in the study population, we conducted independent household surveys of Lifestraw ownership and usage at 6 (n=499), 12 (n=344) and 18-24 (n=8,224) months post filter distribution; we also sampled stored drinking water to assess levels

of E. coli contamination. We found 90.8% of the surveyed households had received a filter and 93.6% reported that a local Lifestraw behavior promoter had visited in the past 6 months. Reported filter use on currently stored drinking water declined progressively (31.8% at 6-mo, 12.0% at 12-mo, and 8.8% at 18-24mo). Although the percent of households that reported any filter use was higher, it also declined over time (62.7% at 6-mo, 44.0% at 12-mo, and 23.8% at 18-24mo). Few respondents obtained water directly from the filter when asked to fetch a glass of water for a young child (1.1% at 6-mo, 0.7% at 12-mo, 0.4% at 18-24 mo). Microbial quality was improved in Lifestraw filtered vs. unfiltered stored water, but typically still reflected ***Escherichia*** coli contamination (geometric mean 21 vs. 28 ***E. coli*** per 100mL, p=0.021). Half (49.5%) of households reported filters were not functioning after 18-24 mo. Our data suggest carbon-financed water filters have not contributed to improved water quality among households in Kenya's Western Province. While carbon credit financing has been promoted as an innovative means to finance water treatment for low-income populations, our data suggest the program is consistent with other studies showing poor adoption of household water treatment interventions provided programmatically.

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EFFECTS OF COMPLEX HANDWASHING INSTRUCTIONS ON ADHERENCE AMONG SCHOOL CHILDREN IN A LOW INCOME URBAN COMMUNITY OF DHAKA, BANGLADESH

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Interventions to improve handwashing include instructions on how and when to wash hands. Instructions vary in complexity, with some recommending multiple steps. To assess whether increasingly complex handwashing instructions changed adherence among school-aged children, we conducted a randomized trial in a low-income area in Dhaka, Bangladesh. We randomly assigned each child to one of three different handwashing instruction sets: simple, moderate, or complex. The simple instructions had 3 steps: wet, lather, and rinse hands, moderate included the simple instructions plus steps to scrub palms, backs of hands, and dry hands by waving in the air. Complex instructions included the moderate instructions plus steps to scrub between fingers, under nails, and lather for 20 seconds. Fieldworkers observed baseline handwashing practice and then taught participants the handwashing instructions. Participants demonstrated handwashing immediately after intervention, then after two days, and after two weeks. Adherence was defined as the demonstration of all steps prescribed to the assigned group. Fieldworkers collected information from 134 (31%) children in simple, 148 (35%) in moderate and 147 (34%) in complex instruction groups. The mean age of the children was 7.5 years. At baseline, 68% children performed steps included in the simple, 6.7% in moderate and <2.7% in complex instructions. After the intervention, adherence to simple instructions increased to >99% at 3 follow-up time points (immediately after the intervention, after two days and after two weeks). Adherence to all 3 sets of handwashing instructions significantly increased at each follow-up compared to baseline (mean difference: baseline vs. after two weeks= 31% [p<.001] for simple, 32% [p<.001] for moderate and 36% [p<.001] for complex instructions). Although adherence to moderate and complex handwashing instructions was low at baseline, children in all groups adhered to the assigned set of instructions two weeks after the intervention. These results suggest that complex handwashing instructions can be included in school handwashing programs for better hand hygiene.

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MOBILITY UP AND DOWN THE SANITATION LADDER IN RURAL COMMUNITIES OF ZAMBIA FOLLOWING COMMUNITY-LED TOTAL SANITATION TRIGGERING

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The sanitation ladder consists of different stages of sanitation that correlate with increasing or decreasing risk of the transmission of diarrheal disease and soil-transmitted helminthes. Open defecation lies at the bottom rung of the ladder and is the most detrimental to health; the ladder progresses upward toward flush toilets. Between open defecation and flush toilets different types of latrines and latrine attributes are all associated with decreasing risk of disease transmission: a lid covering the hole; a smooth, cleanable floor; and a hand washing station with water and soap or ash. Using mobile-to-web community-led total sanitation (CLTS) data we explored the temporal stability of the three parameters (lid, smooth cleanable floor and hand washing station) across 13,000 communities over 1.5 years and determined factors that lead to ascending and descending the sanitation ladder. Before CLTS implementation an estimated 69.5% of households had access to any latrine; following CLTS implementation an estimated 89.1% of households had access to any latrine. Approximately 5.1% of communities (592) were open defecation free at the beginning of mobile-to-web CLTS, and 31.2% of communities (3650) were open defecation free 1.5 years later. Among villages with any type of latrine coverage the different components of adequate sanitation per latrine in use went from 66.2%, 87.5%, 56.4% before intervention roll-out to 86.9%, 95.0%, 81.4% following intervention rollout for lid, smooth cleanable floor, and hand washing station, respectively. As communities climbed the sanitation ladder toward open defecation free temporal instability was noted in latrine access and all three parameters. Approximately half of villages reported backsliding down the sanitation ladder at some point in their push toward ODF. Progress up the sanitation ladder appears to be unstable, with gains made in climbing the sanitation ladder subject to temporary setbacks. Continuous monitoring is helpful for the reinforcement of latrine construction and ensuring that adequate latrine components are present.

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BEHAVIORAL IMPACT OF PROMOTING WATERLESS HAND CLEANSING WITH CHLORHEXIDINE DURING THE PERINATAL PERIOD: RESULTS FROM A RANDOMIZED CONTROLLED TRIAL

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One-quarter of neonatal deaths are attributable to infections. Observational data suggest that maternal handwashing with soap prevents neonatal mortality but maternal handwashing is difficult to increase in the neonatal period. We conducted a randomized controlled trial to test the impact of a chlorhexidine-based waterless hand cleansing promotion on behavior of mothers and other household members. In a demographic surveillance area in Bangladesh, we randomized consenting pregnant women at 32-34 weeks gestation to intervention or control. In the intervention arm, behavior change communicators promoted chlorhexidine

hand cleansing to pregnant women and their household members, and provided a bottle of 4% chlorhexidine lotion. Using data from structured observations, we compared hand cleansing with chlorhexidine or handwashing with soap before baby care among mothers and household members in the intervention arm to those in the control. Chlorhexidine was observed in the baby's sleep space in 97% of 130 intervention homes, compared to soap in 59% of 128 control homes. Hand cleansing before baby care was observed 5.6 times more frequently among mothers in the intervention arm, compared to controls (95% CI 4.0 - 7.7). Hand cleansing was observed at 34% of baby care events among women other than the mother in the intervention arm and 3% in the control arm (RR 10.9, 95% CI 5.1 - 23.1). Girls in the intervention arm cleansed hands before baby contact 37.0 times more frequently than those in the control arm (95% CI 5.2 - 263.7). Men and boys in the intervention cleansed hands before 29% and 44% of baby care events, compared to 0% for each in the control arm. A waterless hand cleansing option using chlorhexidine resulted in substantial increases in hand cleansing behavior among mothers who have greatest contact with neonates. Other household members, particularly children who may introduce new organisms to the neonate's environment, had far greater increases in hand cleansing in the intervention arm than mothers. The impact of chlorhexidine hand cleansing among mothers and other household members on neonatal infections should be evaluated.

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RETROSPECTIVE LONGITUDINAL GENOMIC SURVEILLANCE OF *PLASMODIUM FALCIPARUM* MALARIA PARASITES DOCUMENTS THE EMERGENCE OF ARTEMISININ RESISTANCE IN THAILAND

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Artemisinin-based combination therapies (ACT) are the first line treatment for ***Plasmodium falciparum*** infections worldwide, but resistance to artemisinin (ART) has risen rapidly in prevalence in Southeast Asia over the last decade. Mutations in the gene encoding the kelch protein (PF3D7_1343700) located on chromosome 13 of ***P. falciparum*** have been associated with ART resistance. However, this locus eluded several efforts to identify resistance genes using genome wide association studies and scans for signals of natural selection, and it is still unclear if mutations in other genes also contribute to ART resistance. To explore the power of a longitudinal genomic surveillance approach to detect a resistance signal in ***kelch*** and other loci that potentially contribute to ART resistance, we retrospectively sequenced and analyzed the genomes of 148 ***P. falciparum*** isolates collected from patients from two sites in Northwest Thailand between 2001 to 2012, bracketing the era in which there was a rapid increase in ART resistance in this region. We employed hybrid selection to enrich the fraction of parasite DNA in samples collected in 2008 or earlier, which were not white cell depleted prior to DNA extraction. This dataset enabled us to observe changes of SNP allele frequencies genome-wide during the process of acquisition of resistance to ART, including alleles in ***kelch*** now known to be associated with this phenotype and other candidate biomarkers. We evaluated multiple statistical metrics of temporal change in the frequency of individual SNPs or the diversity profile of genes. Assuming that SNPs associated with resistance should exhibit a change in frequency proportional to the increase in ART resistance over this time period, the resistance-associated C580Y mutation in ***kelch*** exhibits the strongest signal of any variant in the genome. However, other loci exhibit temporal signatures nearly as strong, and warrant further investigation. This analysis demonstrates the potential of a longitudinal genomic surveillance approach to detect resistance loci and improve our mechanistic understanding of how resistance develops.

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MODELING MALARIA GENOMICS REVEALS TRANSMISSION DECLINE AND REBOUND IN SENEGAL

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We examined a set of 1,007 ***Plasmodium falciparum*** samples collected in Thiès, Senegal between 2006 2013 in order to study the effects of malaria-control interventions on parasite population genomics. We genotyped these parasite samples using our 'molecular barcode' of 24 single-nucleotide polymorphisms and observed that about 35% of the single-genome samples grouped into subsets with identical barcodes, varying in size by year and even persisting across dry seasons. The barcodes also formed networks of related groups that were similar to those also identified by analysis of 164 completely sequenced parasites that revealed extensive sharing of genomic regions. In at least two cases we found first-generation recombinant offspring of parents whose genomes were similar or identical to genomes also present in the sample. We applied an epidemiological model that tracks parasite genotypes and reproduced these patterns of barcode subsets. Quantification of likelihoods in the model strongly suggests reduced transmission from 2006-2010, with a significant rebound in Thiès in 2012-2013. The reduced transmission and rebound was directly confirmed by incidence data from Thiès and was not found in Senegal overall. These findings imply that intensive intervention to control malaria results in rapid and dramatic changes in parasite population genomics. The results also suggest that genomics combined with epidemiological modeling may afford prompt and continuous tracking of progress toward malaria elimination.

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DIFFERING POPULATION STRUCTURE AND GENOMIC SIGNATURES OF SELECTION IN SYMPATRIC WESTERN CAMBODIAN *PLASMODIUM VIVAX* AND *P. FALCIPARUM* POPULATIONS: IMPLICATIONS FOR ELIMINATION AND VACCINE DEVELOPMENT

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Plasmodium vivax and ***P. falciparum*** populations circulating in Cambodia are being shaped by intense selective forces. To provide a comprehensive comparison of the genes under selection in sympatric

Cambodian ***P. vivax*** and ***P. falciparum*** populations, we whole-genome sequenced (WGS) 70 ***P. vivax*** and 75 ***P. falciparum*** clinical isolates, which were collected from three provinces during 2009-2013 as part of an ongoing antimalarial drug-susceptibility study. Based on our WGS data, ***P. vivax*** infections were commonly multiclonal with no population structure, while ***P. falciparum*** infections were monoclonal and demonstrated strong population structure within and between provinces. This suggests that recent demographic events - eg. selection and migration - have not affected admixture within the ***P. vivax*** population to the same degree as ***P. falciparum***. To better understand how these demographic events have shaped antigenic selection within Cambodian ***P. vivax*** and ***P. falciparum*** populations, we used coalescent simulations to model the effect of these forces for >90% of all ***P. falciparum*** and ***P. vivax*** genes. ***P. vivax*** and ***P. falciparum*** populations showed overall negative gene-wise Tajima's D values, consistent with a purifying selective event followed by population expansion. Among the minority of genes with signatures of balancing selection, erythrocytic stage vaccine candidates in both species were over-represented (eg. AMA-1, MSP-1). However, the protein domain under selection frequently varied between orthologs, suggesting that critical antigenic moieties vary between species. In addition, vaccine-candidate antigens expressed during non-erythrocytic stages - eg. CSP and TRAP - showed species-specific differences in selection, with ***P. falciparum*** antigens exhibiting higher diversity and stronger balancing selection. As the first genome-wide comparison of sympatric ***P. falciparum*** and ***P. vivax*** populations, our data demonstrate profound differences in population structure and orthologous vaccine candidates while providing insights into how control interventions and vaccine design must be tailored to address each species.

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IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF AN ESSENTIAL RNA-BINDING PROTEIN IN *PLASMODIUM FALCIPARUM*

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Malaria remains a major global health problem for which new drugs and effective vaccines are urgently needed for prevention, treatment and disease eradication. These efforts are significantly hindered, however, by a poor understanding of the parasite's biology. About 50% of ***Plasmodium falciparum*** genes are of unknown function, as few tools previously existed for doing robust functional genetics studies in ***P. falciparum.*** RNA-binding proteins (RBPs) are an important, yet poorly understood, class of proteins that are putatively involved in regulating various aspects of RNA metabolism in the parasite. However, most of the annotated RBPs have no described function. Since RBPs participate in processes essential for parasite survival, they may represent a novel class of therapeutic targets. To test this idea, we used a highly efficient CRISPR/Cas9 genome editing method to achieve conditional regulation of a putative RBP candidate gene by an anhydrotetracycline-inducible translation control system we previously developed. Protein expression profiling revealed tight regulation of expression. In growth assays, we find that knocking down expression of this RBP severely impairs parasite asexual growth after two generations; strongly indicating this protein is essential for intraerythrocytic development. We are presently carrying out various biochemical, cell biology and RNA-Seq experiments to characterize this protein and elucidate its mechanism of action. We believe that the integrated approach we have taken here will enable efficient identification of targets for potential antimalarial therapies and vaccines.

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BRIDGING EPIGENETIC AND GENETIC CONTROLS IN MALARIAL ANTIGENIC VARIATION WITH EVOLUTIONARILY CONSERVED NONCODING ELEMENTS

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Antigenic variation in ***Plasmodium falciparum*** depends on the tight transcriptional regulation of var multi-copy gene family. To escape host immune detection, restricted subset of var genes are expressed few at a time. This mutually exclusive expression pattern is primarily controlled by modifications of chromatin structure as well as DNA regulatory elements associated with each var gene. However, the relationship between epigenetic and genetic control remains to be established. Here, we showed the intron region of each var gene always keeps a 'poised' epigenetic state, which have high potential to bind transcriptional factor (TF). The presence of stable transcripts for different var gene family is associated with this 'poised' potential. We further delineated a set of specific sequence elements located on the edge of intron region that play a key role for 'anchoring' this poised state. Interestingly, this Conserved Noncoding Element (CNE) is conserved in the introns of kir genes in ***P. knowlesi*** - which is another malaria parasite exhibiting antigenic variation. Taken together, our work showed the regulatory element binding potential in intron region is involved in the control mechanism of var gene expression and is differentially regulated among different var genes mediating antigenic variation.

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CYP2D6 GENOTYPES AND *PLASMODIUM FALCIPARUM* GAMETOCYTE CLEARANCE AFTER SINGLE-DOSE PRIMAQUINE IN BURKINA FASO

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Genetically determined cytochrome P450 2D6 (CYP2D6) metabolizer phenotype may determine primaquine (PQ) efficacy by influencing its biotransformation into active metabolite(s). While there is evidence that ***CYP2D6*** genotype influences primaquine efficacy against ***P. vivax*** hypnozoites, it has not been shown that the same holds true for the gametocytocidal activity of PQ on ***P. falciparum***. As part of a dose-finding study, Burkinabè children (aged > 2 and < 15 years) with asymptomatic malaria infection were randomized to receive artemether-lumefantrine (AL) with primaquine (0.25 or 0.4 mg/kg single dose) or placebo at the time of the fifth dose of AL. Gametocyte carriage at days 0 and 7 was determined by ***Pfs25*** quantitative nucleic acid sequence based amplification (QT-NASBA) on nucleic acids from 50µL blood samples. DNA was extracted from EDTA-anticoagulated whole blood samples and genotyped for 19 ***CYP2D6*** sequence variants using custom TaqMan genotyping OpenArrays on a QuantStudio 12K Flex Real-Time PCR system. ***CYP2D6*** copy number was determined using two TaqMan copy number variation assays. In preliminary analyses 90/109 samples were successfully genotyped for ***CYP2D6***. CYP2D6 metabolizer status was inferred from the genotypes with the classical method, yielding predicted phenotypes for all but one of the genotyped individuals. The number of poor metabolizers (PM) and ultrarapid metabolizers (UM) in this Burkinabè cohort was low (PM=3%, UM=4%), but 34% (30/89) of the children were intermediate metabolizers (IM). Data for gametocyte prevalence on both days 0 and 7 was available for 77/89. Preliminary data for both PQ treatment arms were combined

and indicated that 72% (36/50) of extensive metabolizers (EM) and UMs had cleared gametocytes by day 7 compared to only 48% (13/27) of IMs and PMs (p=0.038). Data collection is currently ongoing; our preliminary data suggest an important role for CYP2D6 metabolizer phenotype in determining the ***Plasmodium falciparum*** transmission-blocking properties of primaquine.

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GENOME WIDE ASSOCIATION AND COPY NUMBER VARIATION IN CHILDREN WITH SEVERE MALARIAL ANEMIA

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Infectious diseases, such as malaria, exert strong natural selection on the human genome as evidenced by genetic variability and altered susceptibility to diseases in different ethnic groups in Africa. To date, studies examining the influence of genetic variability on malaria have largely taken a candidate gene approach. The use of genome-wide association studies (GWAS) is rapidly becoming the favored approach to unravel genetic variations involved in the complex biology of malarial immunity. Although GWAS typically utilizes phenotypically well-defined homogenous populations with large sample sizes, we utilized an approach with a reduced sample size in which polarized extremes were enriched from a homogenous population with ***Plasmodium falciparum*** in Western Kenya (n=1,654). To identify markers associated with susceptibility to severe malarial anemia [SMA, hemoglobin (Hb)<5.0 g/dL)], we investigated genetic variation in children with malaria who had exceedingly low Hb levels (n=22, avg. Hb=4.1, case) versus and those with high Hb levels (n=26, avg. Hb=10.8, control). Children with HIV-1, bacteremia, HbAS, and G6PD deficiency were excluded from selection. The GWAS was performed using Illumina®'Human(Omni2.5) beadchip' with >2.45M markers to profile both SNPs and copy number variants (CNVs). Variants were selected using the GenomeStudio® genotyping module. A full regression model was created (controlling for covariates and Bonferroni correction) using SNP and Variation Suite (SVS). SNP analysis identified 1,007 variants across 339 genes with ***P*** values between 4.01×10^{-7} to 9.99×10^{-4}. CNV analysis identified 315 genes with increased copy numbers (>2) and 940 genes with decreased copy numbers (≤1). Additionally, functional canonical pathway networks generated using GeneGo® identified 7 networks with high significance ($P < 1.0 \times 10^{-19}$) for genes associated with the host-immune response, signal transduction, and hematopoiesis. This strategy demonstrates that individuals representing polarized extremes from a larger population can be utilized to identify novel variants associated with malaria susceptibility.

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ASYMPTOMATIC HUMANS TRANSMIT DENGUE VIRUS TO MOSQUITOES

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Dengue is a self-limiting, systemic infection caused by RNA viruses transmitted by ***Aedes*** mosquitoes. Close to 400-million dengue virus (DENV) infections are estimated to occur each year throughout the tropics, of which about 75% are clinically inapparent. Symptomatic DENV infections cover a wide range of disease manifestations from mild febrile illness to severe and fatal disease. People with mildly symptomatic and clinically inapparent DENV infections are generally assumed to inefficiently infect mosquitoes and, therefore, to not be important contributors to DENV transmission. We used cluster sampling around confirmed cases to identify 182 people with natural, active DENV infections and quantify human-to-mosquito transmission across a wide spectrum of disease manifestations, including 14 people with no detectable symptoms and 42 viremic people prior to the onset of symptoms. Despite their lower average level of plasma viremia, the contribution of asymptomatic people to mosquito infection was similar to those with symptomatic infections, due to their higher relative infectiousness. At a given level of viremia, people with asymptomatic and presymptomatic DENV infections are markedly more infectious to mosquitoes than when a person's infection was symptomatic. Because DENV infected people with mild or undetectable symptoms may be exposed to more mosquitoes than sick people through their undisrupted daily routine and they represent the majority of DENV infections, our data indicate that mild and inapparent infections contribute significantly more to DENV transmission than previously recognized. This finding fundamentally changes the current paradigm of DENV transmission dynamics, which is based on people with apparent infections.

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TRENDS IN CASES OF DENGUE IN INFANTS: INSIGHTS INTO SEROTYPE DIFFERENCES IN DISEASE IN NAÏVE AND NON-NAÏVE INDIVIDUALS AND POPULATION TRANSMISSION DYNAMICS

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Infants with maternally acquired antibody to dengue provide unique insight into dengue pathogenesis in immune modulated infections. This age group may also provide important information on population level transmission dynamics. Using serotype specific dengue case data from Queen Sirikit National Institute of Child Health over 40 years (1973-2012), we compared infant (<1 year old) dengue cases to other paediatric dengue cases (both primary and secondary). The serotype distribution of cases in infants (proportions DENV1-4: 0.37, 0.32, 0.27, 0.04) was more similar to the serotype distribution of secondary cases (0.35, 0.31, 0.22, 0.12), than the distribution of primary cases (0.57, 0.05, 0.37, 0.01). This is consistent with intrinsic differences between serotypes in the ability to cause disease in immune-naïve and non-naïve individuals. For cases of all serotypes, the mean age in the under 1s was 6.7 months (95% CIs; 6.6, 6.9). The mean age was 7.3 months (6.9, 7.5) for DENV1 and DENV3, 6.7 months (6.5, 7) for DENV2 and 5.9 months (5 to 6.5) for DENV4. These differences between serotypes could be explained by previously reported serotype differences in maternal antibody decline (with DENV1 the slowest and DENV4 the fastest), leading to differences at which protection ends and enhancement starts. We show that trends over time in the mean age of under 1s mirror trends in mean age of cases in the general paediatric population, suggesting transmission dynamics at a population level are reflected in trends in age of infant cases. We observe that as the mean age of cases increases, a smaller proportion of cases occurs in the under 1s. This is consistent with a decrease in the force of infection leading to an increase in the mean age, and more infections after the age of 1 leading to a reduction in the risk of severe disease upon first infection. We conclude that cases in infants give insight into dengue pathogenesis and transmission dynamics. An understanding of disease in infants will be of increasing importance after the introduction of a dengue vaccine, particularly a vaccine that will not be given to this group, or one which reduces disease but not infection.

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INTRAHOST SELECTION PRESSURES DRIVE RAPID DENGUE VIRUS MICROEVOLUTION IN ACUTE HUMAN INFECTIONS

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Dengue, caused by infection with one of the four serotypes of dengue virus (DENV-1 to DENV-4), is the most prevalent mosquito-borne viral disease in humans. Yet, the selection pressures driving DENV evolution within human hosts ('intrahost') remain unknown. We employed a whole-genome segmented amplification approach coupled with deep sequencing to profile DENV-3 intrahost diversity in 68 peripheral blood mononuclear cell (PBMC) and 31 plasma samples from 77 dengue patients from a hospital-based pediatric dengue study in Nicaragua. DENV-3 intra-human diversity in acute dengue appears to be directed by the immune repertoire, with reduced variation observed in individuals with pre-existing immunity (secondary infection) in contrast to patients with primary DENV infection. In addition, we detected distinct DENV variant populations in plasma compared to PBMC samples, suggesting that cell-free circulating virions were derived from replication sites distinct from PBMCs. We also identified one hotspot for variation in the Envelope gene (E-315) and two linked hotspots in the pre-Membrane gene (prM-100,101 and prM-101) that were present in 60-78% of the patients. These variants arose via convergent viral microevolution, as determined by the manifestation of these hotspots in four distinct haplotype backgrounds spanning the prM-100,101 and E-315 hotspots. Furthermore, we constructed infectious clones with the E-315 or prM-100,101 mutations and demonstrated that the dominant intrahost variants exhibited reduced replicative fitness and altered epitope accessibility profiles. Dengue is thus a salient example of an acute human infection in which selection pressures within infected individuals drive rapid intrahost virus evolution. The immune pressures driving DENV microevolution could provide additional insights into dengue pathogenesis and disease outcome, as well as the efficacy of vaccines and therapeutics.

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FORMULATION AND EVALUATION OF A REAL-TIME CLIMATE-BASED DENGUE FEVER FORECAST MODEL FOR THAILAND IN 2015

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Dengue is a mosquito-borne infectious disease that places an immense public health and economic burden upon Thailand. Annual outbreaks of varying sizes provide a particular challenge to the public health system because treatment of severe cases requires significant resources. Advanced warning of increases in incidence could help public health authorities allocate resources more effectively and mitigate the impact of epidemics. In collaboration with the Thai Ministry of Public Health (MoPH) and Bureau of Epidemiology, we have developed a statistical model for infectious disease surveillance that uses data from across Thailand to give early warning of developing dengue epidemics in real time. For each province, the forecast is based on (1) seasonal dynamics of dengue in the focal province, (2) observed case counts at recent time-points from the focal province and neighbors demonstrated to be relevant through model selection using historical data, and (3) short-term weather patterns provided by NOAA weather grids and stations. Beginning in April 2014, we created updated forecasts every two weeks based on the most current data from the Thai MoPH database. We will present the results of this real-time forecasting exercise, including evaluating the performance of different forecasting models in predicting different features of the 2014 and 2015 dengue season in each Thai province. Additionally, we will introduce a suite of evaluation metrics designed to measure the accuracy of different aspects of the prediction model, including the model's ability to detect the timing of and magnitude of outbreaks.

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ESTABLISHING THE WMEL STRAIN OF WOLBACHIA IN *AEDES AEGYPTI* POPULATIONS PREDICTED TO REDUCE THE DISEASE BURDEN FROM DENGUE BY AT LEAST TWO-THIRDS

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In recent work we have shown that stably infecting ***Aedes aegpyti*** mosquitoes with the wMel strain of Wolbachia substantially reduces their competence as vectors for dengue viruses, such that establishment of wMel across the ***A. aegypti*** population of an area is predicted to reduce the reproduction number, R0, of dengue by at least 2/3 within the treated zone. Here we examine the potential impact of widescale use of wMel as a vector control measure on the burden of disease arising from dengue infection. We developed a mathematical model of the transmission dynamics of the four dengue viruses and calibrated the model to dengue surveillance data from South East Asia and to the phase III trial results of the Sanofi dengue vaccine candidate. The model was designed to model a wide range of potential interventions measures, including release of Wolbachia-infected mosquitoes, other vector control measures and the introduction of vaccination. We explored the potential impacts of large-scale release and establishment of wMel-infected ***A. aegypti*** on dengue transmission dynamics and disease burden. In all transmission settings, the sudden reduction in the reproduction number of dengue induced by wMel release is predicted to interrupt dengue transmission for a minimum of 5 to 10 years. After that time, in high transmission intensity settings (R0>3), dengue transmission is predicted to resume as a result of the gradual build-up of susceptible individuals (through births) in the population. However, transmission rates are predicted to be substantially lower than prior to the introduction of wMel, leading to a long term reduction in disease burden from dengue of at least two-thirds. In low to moderate transmission settings (dengue R0<3), wMel introduction reduces the reproduction number of dengue below 1, leading to elimination of endemic transmission. A higher intensity of density dependent regulation of larval mosquito populations than typically seen is predicted to reduce the impact of wMel on dengue transmission, but impact predictions are robust to uncertainty regarding the efficiency of vertical transmission of the wMel strain.

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THE GLOBAL BURDEN OF SEVERE DENGUE

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Dengue is a tropical disease of global public health importance with symptomatic manifestations that range from mild fever to severe haemorrhage and death. In 2013, the first evidence-based attempt to estimate the global burden of dengue suggested 390 million (95% credible interval 284-528) infections occur each year. Only a small proportion of these infections, however, will manifest as severe dengue cases that are at the highest risk of mortality and place a

disproportionately high burden on the healthcare system. Since the publication of these first estimates, the number of cohort studies that measure dengue incidence have been nearly doubled through on-going research efforts and the addition of the control arm results of the multi-centre Sanofi Pasteur Asian and Latin American vaccine efficacy trials. By monitoring over 10,000 individuals in 34 sites using a common experimental design these cohort studies provide the first reliable estimates of the proportion of severe dengue cases among symptomatic dengue virus infections. Here we provide updated estimates of the global burden of dengue infections and, for the first time, include estimates of severe dengue burden. These estimates reflect significant updates to the database describing dengue's global distribution and incidence with new covariates in an improved and expanded modelling framework. Global and national estimates of severe dengue burden are essential for measuring progress towards the WHO's global strategy goal of reducing dengue morbidity by 25% between 2010 and 2020. Furthermore, these estimates will form an important part of the evidence base for national and international funders to support important resource allocation decisions for dengue control.

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ESTIMATING WITHIN-HOST DYNAMICS OF DENGUE VIRUS SEROTYPES USING TWELVE YEARS OF INDIVIDUAL-LEVEL LONGITUDINAL SEROLOGICAL DATA

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Dengue, which is caused by any of four related but antigenically distinct virus serotypes, has increased its incidence and geographic range considerably in the past 50 years. Infection with a DENV serotype induces lifelong immunity to that serotype and a short-term, temporary cross-immunity (TCI) to the other serotypes. Despite a century of research, the strength and duration of TCI remains uncertain because it is difficult to estimate using disease surveillance data. With data from large, longitudinal serological studies, on the other hand, we can evaluate the relative change in infection risk immediately following a DENV infection. We used a 12-year dataset from Iquitos, Peru to estimate the strength and duration of TCI in an endemic population. The dataset contained information from 14,335 individuals whose blood was assayed by PRNT every 6-9 months (38,416 total samples). There were 3,854 interval-censored, serotype-specific infections. We applied a novel modeling approach to simultaneously estimate interactions between serotypes as well as the time-varying intensity of transmission for each serotype (force of infection). We identified significant variation in the duration of TCI and the strength of heterologous protection_ depending on data cleaning assumptions and the functional form of TCI. The presence of individuals that were infected several times over the course of the study (422 with 2 infections, 39 with 3 and 5 who were infected with all 4 serotypes during their time in the cohort) provides strong evidence indicating that TCI is either short-lived or imperfect. The average time between seroconversions for these individuals was 449 days, but 250 occurred in between three sequential assays. We are conducting sensitivity analyses to evaluate model assumptions. Additional analyses are assessing the possibility of within-host interactions that could boost or dampen infectiousness during second, third or fourth infections. Assumptions about TCI in dengue virus transmission play a critical role in disease models. Our findings inform the design of appropriate models, which will be used to guide disease intervention strategies.

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IMPACT OF INTERMITTENT MASS SCREENING AND TREATMENT (IMSAT) FOR MALARIA ON INCIDENCE OF INFECTION IN AN AREA OF HIGH MALARIA TRANSMISSION IN WESTERN KENYA

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Malaria burden remains high in western Kenya (population parasite prevalence=39%) despite widespread ITN coverage. Interventions targeting asymptomatic infections, at least one-third of all infections in this area, could help reduce transmission. We implemented the first multi-year community-based, cluster-randomized controlled trial of intermittent mass screening and treatment (iMSaT) for malaria in an area of high transmission. Participants aged 1–75 years were recruited from 10 intervention and 10 adjacent control areas within 3 km of 10 study clinics. Participants in the intervention area (n>27,000) received three rounds of iMSaT annually; they were tested with combination HRP2/pLDH rapid diagnostic tests (RDTs,and those testing positive were treated with dihydroartemisinin-piperaquine. Malaria infection incidence by RDT was estimated through passive case detection at study clinics beginning 6 months prior to iMSaT through 12 months after implementation. Data were analyzed as an interrupted time series. A total of 514 participants were enrolled in the incidence cohort in year 1. Coverage (proportion of intervention population screened at each round) was 73% in September 2013, 80% in January 2014, and 75% in April 2014. Malaria infection incidence at baseline in the intervention and control areas was comparable, 28.1/1000 and 24.2/1000 person-months, respectively (p=0.35). Compared with baseline, there was a 14% (incidence rate ratio [IRR]=0.39 vs 0.46, p=0.046), 16% (IRR=1.17 vs 1.39, p=0.02), and 10% (IRR=1.04 vs 1.15, p=0.22) relative reduction in incidence of malaria infection after the first, second, and third round, respectively. In the first year of follow-up, the crude median time to first infection was 232 days (7.6 months) in the intervention arm and 200 days (6.5 months) in the control arm; hazard ratio of 0.86 (95% CI 0.69-1.07, p=0.16). These preliminary data suggest iMSaT is not associated with a decline in incidence of malaria infection and time until first infection among recipients of iMSaT compared with participants in control villages. Age-specific and second year data will be available in August, 2015.

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IMPACT, INTERACTIONS AND LIMITATIONS OF LARVICIDING, WINDOW SCREENING, BED NETS AND HUMAN BEHAVIOR FOR PREVENTING MALARIA IN AN AFRICAN CITY WITH READILY AVAILABLE ARTEMISININ-BASED COMBINATION THERAPY AND RAPID DIAGNOSTIC TESTS

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In the city of Dar es Salaam in Tanzania, programmatic distribution of insecticide-treated nets and application of mosquito larvicides, as well as provision of artemisinin-based combination therapy and rapid diagnostic

tests, have been complemented by commercial sale of window screening. After a decade of progressively improving coverage with all these interventions, vector populations which remained pyrethroid-susceptible became sparse and lower malaria infection risk became evenly distributed across all ages but stable transmission persisted. High estimated proportion of potential vector exposure occurring indoors (ϖh,i) was protective against malaria among the majority of residents (85.8%) living in houses with window screens (OR [95%CI]= 0.73[0.60,0.90], P=0.0040 and 0.64[0.49,0.83],P=0.00094 for highest and middle versus lowest terciles, respectively) but not in unscreened houses ($P \geq 0.51$). Residents of houses with window screening who spent most night hours indoors (middle and high terciles of ϖh,i) were protected against malaria infection (OR [95% CI]=0.71 [0.59,0.85],P=0.00013) but not those spending even a few hours outdoors in the evening and early morning (P=0.433). Malaria risk among residents with unscreened houses (14.2%) increased with vector density (P=0.0093) and was reduced by larvicide application (OR[95% CI] = 0.42 [0.23,0.74], P=0.0033), but was unaffected by either factor among residents with screened houses (P=0.23 and 0.19, respectively). Only small fractions of persisting malaria infections could be attributed to houses lacking window screens (4.1%), spending time outside screened houses (5.8%), or gaps in larviciding coverage, (15.8%), respectively. Despite high coverage of effective nets, screening and larviciding in this urban context with very sparse populations of pyrethroid-susceptible vectors, malaria transmission persists and further improving coverage and adherence for these interventions are therefore only likely to prevent a minority of remaining cases. New or improved transmission control measures are required to enable elimination of malaria from Dar es Salaam.

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FACTORS ASSOCIATED WITH INSECTICIDE TREATED NET USE AND PROTECTION FROM MALARIA INFECTION AMONG SCHOOL-AGED CHILDREN IN MALAWI: A MULTIPLE CROSS-SECTIONAL STUDY

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Recent data from Malawi suggests that school-aged children (SAC), aged 5 to 15 years, have the highest prevalence of ***Plasmodium falciparum*** parasitemia among all age groups. They are the least likely group to utilize bed nets, the most commonly used intervention to prevent malaria in Africa. This study examined the effects of a universal mass net distribution campaign in Malawi on net usage among SAC and the duration of the impact. We identified factors that influence net usage among SAC and the protective effect of bed nets in this age group. Six cross-sectional surveys using cluster random sampling were conducted in the rainy and dry seasons in southern Malawi from 2012 to 2014. Mass net distribution occurred between the first and second surveys. Data were collected on household ITN usage as well as demographic variables. Blood samples for detection of ***P. falciparum*** infection were obtained from all household members who were present. Statistical analyses used generalized linear mixed models to account for clustering at the household and neighborhood level. There were 7,296 observations from SAC. SAC used ITNs significantly less frequently than the rest of the population (odds ratio=0.26 [0.24, 0.28]). The OR did not change significantly following mass net distribution. The campaign did lead to a statistically significant population-wide increase in net use, however net use returned to near baseline within 3 years. Lower ratio of people to nets in a household and higher proportion of nets that were hanging at the time of survey were the most important predictors of ITN usage. Older SAC (11-15 yrs) were significantly less likely to use nets than younger SAC (5-10 yrs) (OR=0.24 [0.21, 0.28]). Net use was highly protective against ***P. falciparum*** infection during the dry seasons (OR=0.42 [0.31, 0.59]), but had no significant effect during the rainy seasons. This study suggests that one time net distribution campaigns are not sufficient to increase net usage among SAC. New and targeted interventions are needed to address the high prevalence of infection in SAC.

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RISK FACTORS ASSOCIATED WITH MALARIA EPIDEMIOLOGY IN ZANZIBAR: A PRE-ELIMINATION SETTING

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Imported cases by travelers to and from Zanzibar tend to make up the majority of reported cases. Travel therefore, poses a great challenge in malaria epidemiology and must be addressed if the country is to achieve malaria elimination. This study aimed to identify the risk factors associated with malaria epidemiology in Zanzibar. Malaria surveillance data were collected between August, 2012 and September, 2014. For each case, a message was sent from health facility to a Central server which generated an SMS alert to the District Malaria Surveillance Officer's (DMSO) mobile phone and tablet. Data included patients' names and villages, which enabled the DMSO to follow up patients to their households. All household members of the index cases were tested for malaria using rapid diagnostic tests (mRDT). Household members testing malaria positive were treated with artemisinin-based combination therapy (ACT). Data was subjected to univariate and multivariate analysis, logistic regression model was used to identify the risk factors. Among 18,640 patients followed, 6.2% (1,158) were malaria positive, of which 17.9% (207) were aged below 5 years. Among positive cases significantly increased odds of malaria positivity were found in people aged 5-14 years (odds ratio [OR] 1.6; 95% CI: 1.4-1.9) and who didn't sleep under treated net previous night (OR 1.4; 95% CI: 1.2-1.7). In addition, those with history of fever in the last 2 weeks (OR: 23.2; 95% CI: 19.7-27.4), who had travelled outside Zanzibar in the past one month (OR: 9.8; 95% CI: 8.3-11.6) and patients without nets (OR: 1.3; 95% CI: 1.1-1.6) were found to have significantly increased odds of malaria positivity. Failure to use nets and travel are major drivers of malaria cases in both high and low transmission periods. Identification of gaps in gaps in net use, knowledge and relevant types of human movement, and development of strategies addressing travel is highly recommended.

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SEROLOGICAL EVIDENCE OF A SUSTAINED REDUCTION IN MALARIA TRANSMISSION 2004-2012 IN BIOKO ISLAND, EQUATORIAL GUINEA

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Following comprehensive malaria control interventions since 2004, malaria prevalence in Bioko island, Equatorial Guinea, has reduced substantially. However, the effects of the interventions have been heterogeneous across the island, resulting in residents of some regions remaining at relatively high risk of infection. To determine which regions were experiencing the highest burden of infection and to investigate changes in transmission over time, parasitological and serological measures were compared from two island-wide surveys undertaken in 2008 and 2012. Parasite prevalence, as measured by rapid diagnostic test, decreased from 18.0% to 11.1% between 2008 and 2012 ($p<0.001$), with wide variations by sentinel site.

The change in parasite prevalence between 2008 and 2012 correlated strongly with the change in seroprevalence in children aged 5 and under (r2 0.6, p<0.001). The 2012 data indicated that the seroconversion rate, which is a proxy for the force of infection, has reduced by approximately 80% since interventions began. Spatial analysis suggested transmission had become less heterogenous between 2008 and 2012 but indicated new ***foci*** of transmission had appeared in the South East region. Whilst it appears that interventions were still relatively effective in Bioko, the highlighted hotspots in the South East region, coupled with a lack of further reduction in parasitological and serological measures between 2008 and 2012 in this area, were a cause for concern and indicated that interventions needed to be adapted. This resulted in a concentrated response by the program which has led to a reduction in parasite prevalence in the South East in the subsequent malaria indicator surveys.

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MEASURING THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION AS PART OF ROUTINE MALARIA CONTROL IN KITA, MALI

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Seasonal malaria chemoprevention (SMC), the administration of complete therapeutic courses of antimalarials to all children during the malaria transmission season, is a new strategy recommended by WHO in areas of highly seasonal transmission. Although randomized controlled trials (RCTs) have shown SMC to be highly effective, evidence and experience from routine implementation of SMC are limited. This study aimed to evaluate reductions in malaria and anemia when SMC is delivered through routine programs and existing community health workers. A non-randomized pre-post design was used, with one intervention district (Kita), where 4 rounds of SMC with SP+AQ took place in August-November 2014, and one comparison district (Bafoulabé). Children aged 3-59 months from 15 selected localities per district, sampled with probability proportional to size, were surveyed and blood samples collected for malaria blood smears and hemoglobin (Hb) measurement in two cross-sectional surveys, one prior to SMC (July 2014) and one after SMC (December 2014). Difference-in-differences regression models were used to assess and compare changes in malaria and anemia in the intervention and comparison districts. During round 1, 84% of targeted children received at least the first SMC dose, but coverage declined to 67% by round 4. Across the 4 treatment rounds, 54% of children received 4 complete SMC courses. Prevalence of parasitemia and malaria disease (fever+parasitemia) was similar in intervention (23.4%) and control (29.5%) districts prior to SMC (p=0.34). After SMC, parasitemia prevalence fell to 18% in the intervention district and increased to 46% in the control district (Difference-in-differences (DD) OR=0.35; 95% CI: 0.20-0.60). SMC also significantly reduced the odds of malaria disease (DD OR=0.20; 95% CI: 0.04-0.94) and moderate anemia (Hb<8 g/dL) (DD OR=0.26, 95% CI: 0.11-0.65). Routine implementation of SMC in Mali substantially reduced malaria and anemia, with reductions of similar magnitude to those seen in previous RCTs. Improving coverage could further strengthen SMC impact.

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CHANGES IN MALARIA TRANSMISSION AND IMMUNITY AND THE EMERGENCE OF ARTEMISININ RESISTANCE IN THAILAND FROM 2001-2011

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Over the past decade a series of anti-malaria interventions and control efforts have led to a significant decline in malaria morbidity and mortality globally. A major contributor to this reduction was the introduction of artemisinin derivatives as first line treatment for malaria. Despite this success, resistance to artemisinin derivatives is now well established in western Cambodia, western Thailand, southern Myanmar and southern Vietnam. Resistance to artemisinin, taken as an increase in blood-stage parasite clearance time, has recently been linked to mutations in the ***kelch13*** giving us a potential molecular marker for resistance. However, little is known about how much naturally acquired immunity to blood-stage malaria parasites contributes to the observed clearance times, especially in a scenario of declining transmission and potentially declining immunity. The aim of this study was to quantify the impact of changing immunity in a population from Thailand where transmission is declining, and further assess its impact on parasite clearance and to assess the emergence of resistance to artemisinin derivatives. We obtained dried blood spots and plasma samples from 1,585 hyperparasitaemic patients admitted to clinics of the Shoklo Malaria Research Unit along the northwestern border of Thailand between 2001-2011. Levels of antibodies to the ***Plasmodium falciparum*** merozoite antigens AMA1, $MSP1_{42}$, MSP2 and $EBA140_{RII}$ were measured by ELISA. Polymorphisms in the ***kelch13*** gene were obtained after PCR amplification of the full sequence of the gene. We show that immunity declines between 2001-2004 and is followed shortly by the appearance of mutations in the ***kelch13*** gene which increase in prevalence from 0% to 67.4%. Levels of immunity were lower in patients with slower parasite clearance times, compared to those with faster parasite clearance times. Immunity is therefore an important confounder in the evaluation of resistance to artemisinin when using parasite clearance, particularly in the context of changing malaria transmission.

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DEVELOPMENT OF AN TRICHURIASIS VACCINE; STARTING FROM THE BEGINNING

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Estimations say that 604 to 795 million people are infected worldwide with ***Trichuris trichuria,*** which causes Trichuriasis. ***T. trichuria*** infects the large intestine and disease transmission occurs by ingestion of the eggs, which are found in feces of infected patients. Symptoms vary from minor to severe with painful passage of the stool, while children may suffer from growth retardation and impaired cognitive development. Although anti-helminthic drugs are available, to many poor people these remain inaccessible. An affordable preventive treatment is needed to prevent infections and reduced transmission of the disease. By using the mouse ***T. muris*** laboratory model, ES antigens from live ***T. muris*** adult worms were extracted and AKR mice were vaccinated with the ES antigens and ISA720 adjuvant subcutaneously. Mice cohorts were as follows: cohort

A was only vaccinated once, cohort B was vaccinated twice, cohort C was vaccinated once and challenged with 300 ***T. muris*** eggs, and cohort D was vaccinated twice and challenged with 300 ***T. muris*** eggs. After sacrificing the animals, immunogenicity studies included antibody analysis of ES specific IgG, IgG1 and IgG2a by ELISA, and cytokine responses of splenocytes which were re-stimulated with ES antigens are currently analysed. It was observed that both one and two vaccinations boosted the ES specific total IgG and IgG1 titers, while the ES specific IgG2a titer remained low. Results also suggested that when challenged with ***T. muris*** eggs, two vaccinations were required to maintain high levels of ES specific IgG and IgG1 and a low-titer of IgG2a. Results from the worm counts in the GI tract showed a reduction of worms in animals vaccinated twice compared to non-vaccinated animals (mean 4.8 vs 50.2 respectively). Data from these studies suggest a T_H2-mediated immune response was induced by the vaccine, and correlated with a decreased worm burden. These results suggest that vaccination can be successful means of eliciting a protective immune response against ***Trichuris.*** Recombinant protein candidate antigens are being identified for vaccine development and their immunogenicity and efficacy evaluated.

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GROUP 2 INNATE LYMPHOID CELLS ARE EARLY RESPONDERS THAT SUPPORT TH2 EFFECTORS AFTER HELMINTH INFECTION

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Helminth infections are associated with a modified type 2 immune response, characterized by T helper type 2 (Th2) cell-derived IL-4, IL-5, IL-13, and IL-9. In inflamed tissues, these cytokines recruit and activate innate effector cells, which in turn alter the local environment to repel intruders and repair damage. Modulating immunity to treat and prevent disease requires a more complete understanding of the basic pathways involved. We and others have identified Group 2 Innate Lymphoid Cells (ILC2) as an important novel source of type 2 cytokines in mice and humans. In models of acute and chronic intestinal nematode infection, ILC2 increase in number and in cytokine production, corresponding with eosinophilia and Th2 cell induction. Analysis of cytokine expression in an IL-5/IL-13-dual reporter strain showed different expression patterns in different effector cell types. Deletion of ILC2 and live imaging of inflamed tissue revealed a unique role for these cells in recruitment of other effector cells that persist in chronic infection. We surmise that ILC2 are "first responders," uniquely poised from early development to detect tissue stress and shape ensuing T cell responses. Our data support a highly cooperative innate-adaptive relationship at the initiation of type 2 immunity. Further characterization of these interactions is ongoing, with the goal of identifying novel targets in treating human disease.

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PHASE 1 TESTING OF THE NA-GST-1/ALHYDROGEL HOOKWORM VACCINE IN BRAZILIAN AND AMERICAN ADULTS

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Necator americanus glutathione S-transferase-1 (***Na***-GST-1) is a 24-kDa protein produced by adult hookworms that is thought to play a role in detoxifying heme and other breakdown products of the hookworm blood digestion pathway. Vaccination of laboratory dogs and hamsters with recombinant GST-1 resulted in reduced hookworm fecal egg counts and reduced adult worm burden following challenge with infective larvae. Recombinant ***Na***-GST-1 was expressed in ***Pichia pastoris*** and formulated on Alhydrogel. Two Phase 1 trials were conducted: one in the USA (n=40) and a second in Brazil (n=102). In both studies, healthy adults were vaccinated with 1 of 3 different dose concentrations of ***Na***-GST-1 (10, 30 or 100 µg) either with or without the point-of-injection addition of an aqueous formulation of glucopyranosyl lipid A [GLA-AF], a synthetic Toll-like receptor-4 agonist. Subjects received 3 intramuscular injections at 2-month intervals. In Brazil, the trial was conducted in both hookworm-unexposed volunteers in an urban center and in hookworm-exposed adults (including recently treated) at a rural trial site. In both the US and Brazil studies, the vaccine was well tolerated: common adverse events included mild to moderate injection site pain and tenderness, headache, and nausea. No differences were observed in adverse events between dose groups or GLA formulations. Anti-***Na***-GST-1 IgG antibody levels as measured by qualified indirect ELISA were modest after the 2nd vaccination, but increased significantly from baseline after the 3rd vaccination in those who received 30 or 100 µg ***Na***-GST-1. For each dose concentration of ***Na***-GST-1, the increase in IgG levels was not significantly different in those who received formulations containing GLA-AF. In the Brazilian subjects, the antigen-specific IgG response consisted mainly of the IgG1 subclass whereas in the US subjects, both antigen-specific IgG1 and IgG3 were detected. These first-in-human trials of the ***Na***-GST-1 hookworm vaccine demonstrate that it is well tolerated and immunogenic in both unexposed and exposed adults and justifies further clinical testing of this vaccine.

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RNAI COMPETENCY IN ADULT *ASCARIS SUUM* - POTENT, PERSISTENT AND REPRODUCIBLE KNOCKDOWN

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The development of anthelmintic resistance threatens the primary mode of helminth parasite control. Whilst the utility of RNA interference (RNAi) for the validation of drug targets in nematode parasites is widely accepted, issues with their variable sensitivity to RNAi have undermined the development of robust gene silencing platforms. ***Ascaris suum*** is an important veterinary-parasite, a zoonotic pathogen and a close relative of the prevalent human parasite, ***Ascaris lumbricoides***. The large size of adult ***A. suum*** has facilitated its development as a tractable model for nematode parasite biochemistry, neurobiology and physiology research. The recent completion of the ***A. suum*** genome has prompted the development of functional genomics tools in this species. Whilst RNAi has been reported in the L3 larval stages of ***A. suum***, it has not been demonstrated in adults, limiting the utility of this gene silencing technique. Here we report efforts to develop an RNAi platform for adult ***A. suum*** through the direct delivery of double stranded (ds)RNAs into the pseudocoelomic cavity of adult female worms. RNAi success was determined through qPCR transcript analysis, and by monitoring encoded protein expression and worm phenotype. The data presented highlight: (i) the dynamics associated with the rate of induction of RNAi in ***A. suum***; (ii) the ability to induce RNAi in tissues distant from the site of dsRNA injection; and (ii) the consistency of RNAi across a range of gene targets expressed in a variety of therapeutically relevant tissues. Despite achieving specific, robust and reproducible transcript knockdown for all target genes, no post-RNAi phenotypes were recorded over the course of these investigations. This was in spite of efforts to: (i) target putatively essential genes; (ii) improve the RNAi trigger delivery methodology; and (iii) employ a highly quantitative electrophysiology-based phenotypic assay. The data described here highlight an opportunity for the development of a functional genomics platform that supports organism-, tissue- and cell-based biology in a model nematode parasite.

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METHYLPREDNISOLONE ACETATE INDUCES *STRONGYLOIDES STERCORALIS* HYPERINFECTION IN NSG MICE

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Strongyloides stercoralis infected patients, immunosuppressed with corticosteroids, develop hyperinfection, which if left untreated, has a mortality rate approaching 90%. While immunocompetent mice are resistant to infection with ***S. stercoralis***, we have demonstrated that highly immunodeficient NSG mice support the development of all life stages of this parasite. Interestingly, hyperinfection is not observed in NSG mice, even though they lack macrophages, B, T- and NK cells. In this study we tested the hypothesis that treating NSG mice infected with ***S. stercoralis*** with methylprednisolone acetate (MPA), a synthetic glucocorticoid, would lead to hyperinfection. NSG mice were infected with 5,000 infective third-stage larvae and treated with MPA for 6 weeks. Approximately 50% of infected mice treated with MPA died prior to the conclusion of the experiment. MPA treatment caused a significant increase in the number of parasitic female ***S. stercoralis*** recovered from 61±33 in untreated mice to 708±684 in treated mice. In addition, auto-infective third-stage larvae, which initiate hyperinfection, were not observed in NSG mice but were found in high numbers in MPA treated mice. Despite their severe immune deficiencies NSG mice retain neutrophils and eosinophils. Consequently, we hypothesized that MPA treatment exerted its effect by decreasing granulocyte function, which eliminated immune control of hyperinfection. To test this, NSG mice were treated with monoclonal antibody RB6-8C5 to eliminate granulocytes, however, although the granulocytes were abolished, hyperinfection did not develop in these mice. In conclusion, using a ***S. stercoralis*** hyperinfection model in NSG mice treated with MPA that we developed, we show that hyperinfection is not due to the action of glucocorticoid on B, T, NK cells or granulocytes, leaving open the possibility that these compounds may be acting directly on the parasite.

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A NOVEL PHARYNGEAL NICOTINIC ACETYLCHOLINE RECEPTOR FORMED BY EAT-2 AND EAT-18 AS A POTENTIAL DRUG TARGET

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There is a compelling need for research to develop new anthelmintic targets and drugs. Nicotinic agonists are effective and widely used agents. The nicotinic agonists target nicotinic acetylcholine receptors (nAChRs) found at the nematode neuromuscular junction, causing depolarisation and hyper contraction of the parasite. Previous studies have demonstrated the nAChRs on the nematode pharynx are insensitive to current cholinomimetic drugs. This study was aimed at identifying the potential of EAT-2, a pharyngeal nAChR subunit and EAT-18, a small transmembrane protein, as chemotherapeutic targets. EAT-2 and EAT-18 have been reported to be involved in pharyngeal pumping in ***C. elegans***, hence affecting feeding behaviour. Using molecular techniques, we expressed EAT-2 and EAT-18 from ***C. elegans*** in ***Xenopus laevis*** oocytes. To study the pharmacology of the receptor we used the two electrode voltage-camp technique. Expression of either EAT-2 or EAT-18 alone did not produce a functional nicotinic ion channel. When expressed in combination, EAT-2 and EAT-18 produced a functional receptor that responded robustly to acetylcholine in a concentration-dependent manner. The rank order potency of agonists on this receptor was acetylcholine ≈ nicotine > methacholine > carbachol ≈ butyryl choline > epibatidine > oxantel. However, there was no response to levamisole, cytisine, pyrantel, and tribendimidine. Various nicotine analogues including alpha-cotinine, nornicotine, anabasine, altinicline failed to produce any agonistic activity. Also, alpha-bungarotoxin did not show any significant antagonistic effect on the expressed nAChR. It is worthy of note that EAT-2 is not an nAChR alpha subunit. In conclusion, we report the ability of a non-alpha subunit to express as a functional homomer in oocytes for the first time. The pharmacology of this previously uncharacterised receptor appears to be different from other nAChR's characterized in nematodes. Thus, this subunit offers a possibility for being used as a potential target site for developing new anthelmintic compounds.

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SPECIES-SPECIFIC ASSOCIATIONS BETWEEN HELMINTHS AND MICRONUTRIENTS IN VIETNAMESE SCHOOLCHILDREN

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Several different associations between helminth infections and micronutrient status in children have been reported. We aimed to study associations between specific STH species and micronutrients in schoolchildren. Vietnamese children (n=510) aged 6-9 years were recruited from two primary schools. STH infections were determined in stool samples. In blood samples, hemoglobin, ferritin, retinol and zinc were measured, as well as CRP to control for inflammation. Iodine excretion was measured in urine. Associations of single and multiple ***Ascaris lumbricoides, Trichuris trichiura,*** or hookworm infections and micronutrients were estimated by regression techniques. ***Ascaris*** infections showed a specific and dose-dependent relationship with vitamin A. Trichuris and hookworm infections were associated with lower hemoglobin concentration, but not with plasma ferritin. Trichuris-infected children had zinc deficiency less often than uninfected children. The different life cycles of STH species might have specific effects on the absorption or loss of specific micronutrients.

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VARIATION IN RELAPSE FREQUENCY, SEASONALITY, AND THE TRANSMISSION POTENTIAL OF *PLASMODIUM VIVAX* MALARIA

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There is substantial geographical variation in the relapse frequency of ***Plasmodium vivax*** malaria with fast relapsing phenotypes observed in tropical areas with year round transmission and relapses with long-latency observed in temperate areas with seasonal transmission. We hypothesise that much of the global phenotypic diversity in ***P. vivax*** relapses has been generated by the evolution of time to next relapse to optimise transmission potential in a given environmental niche. We develop a mathematical model of ***P. vivax*** relapses incorporating competition between strains with different relapse frequency, and the evolution of strains to optimise their transmission potential. The model was validated using data on the seasonal suitability for malaria transmission (determined by temperature, rainfall and mosquito abundance) and datasets on time to first relapse collected from a large number of geographical locations. In high transmission tropical zones, transmission potential is optimised by strains with time to first relapse of 1 to 3 months - slower relapsing strains will be out-competed. In low transmission tropical areas, transmission

potential is optimised by strains with time to first relapse of 4 to 5 months - long enough for the primary infection to clear. In highly seasonal settings characteristic of temperate zones, transmission potential is optimised by strains with time to first relapse of 8 to 10 months - long enough for hypnozoites to survive winter in the human liver. These findings will have consequences if ***P. vivax*** transmission is lowered through sustained control where we may see the emergence of slower relapsing strains to optimise transmission potential in the new lower transmission setting. In settings where ***P. vivax*** elimination is being pursued, the potential increase in the time to next relapse may introduce additional challenges for the detection and treatment of the last few cases of malaria.

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PREDICTING MALARIA REINTRODUCTION RISK IN AN AGENT-BASED MODELLING FRAMEWORK TO UNDERSTAND THE IMPACT OF GLOBAL, PATCH-LEVEL, AND INDIVIDUAL VARIANCE

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Recent research highlights spatial targeting of malaria elimination resources as a potential way to ensure limited resources are used most efficiently. Targeting intervention efforts to reduce costs and achieve regional elimination requires an accurate picture of heterogeneity in transmission intensity and parasite spread across the landscape. Spatially resolved estimates of transmission intensity are often uncertain, however, as source data can be highly clustered or noisy. Quantifying human-facilitated parasite spread is also difficult, as people move for a variety of reasons, vary in movement patterns, and data on movement can be difficult to obtain. Understanding how these uncertainties affect predictions of parasite spread is critical for assessing what data must be obtained for effective spatial targeting, and which factors are the most important predictors. In this study, we use an agent-based modelling approach to quantify the effects of these uncertainties on predictions of malaria spread. We simulate malaria transmission within Namibia using a map of transmission intensity with corresponding estimates of uncertainty, and simulate parasite spread between patches using anonimized mobile phone call data records to inform human movement patterns. Our simulations incorporate uncertainty in the transmission intensity map, demographic stochasticity, and individual-level variation in movements. We partition the variance in malaria spread using a global sensitivity framework to yield the relationships between global uncertainty, patch-level transmission characteristics, and individual-level movement patterns in driving number of cases from a reintroduction event over time. We also found that patch-level transmission intensity alone was a poor predictor of parasite spread, and that network context must be considered with transmission intensity to accurately predict whether particular patches are at high risk for a malaria outbreak.

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MODELING THE IMPACT OF TRANSMISSION-BLOCKING ACTIVITY ON THE COMPOSITION AND DYNAMICS OF THE MALARIA INFECTIOUS RESERVOIR

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In malaria endemic settings, only infectious gametocyte carriers are responsible for mosquito's infection allowing subsequent transmission of the disease. This fact strongly suggests that a comprehensive understanding of the human infectious reservoir becomes a key prerequisite for malaria control and elimination efforts although limited resources make it challenging to generate required data in several of endemic countries. Anti-gametocyte immunity can influence gametocyte infectiousness and may allow predictions of the infectious reservoir as well as future transmission blocking vaccine testing. The ultimate impact of this type of immunity on the composition and dynamics of the infectious reservoir has never been studied. Here, we use data from an area of intense malaria transmission to calibrate the EMOD agent-based mechanistic model and assess the impact of naturally acquired anti-gametocyte immunity on the composition and dynamics of the infectious reservoir. Repeated measurements were carried out at distinct transmission seasons (start-wet, peak-wet and dry season) and provided data on Pfs48/45 and Pfs230 specific antibody responses as anti-gametocyte immunity. Data on low-density gametocyte infections and their infectiousness were obtained using an ultra-sensitive molecular method and mosquito feeding assays respectively. To predict the composition (high and low-density infection) and the dynamics of the infectious reservoir, model simulations were run accounting for anti-gametocyte immunity or not. A model solution of the age and temporal changes in the infectious reservoir could be obtained when anti-Pfs48/45 and anti-Pfs230 immune responses parameters were accounted for; and not when they were turned off. Our mathematical simulations indicate that anti-gametocyte immunity may be as representative of transmission blocking activity and a key factor for predicting and understanding the composition and the dynamics of the infectious reservoir.

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IMPACT OF IRS ON MALARIA BURDEN WHEN COMBINED WITH LLIN IN ETHIOPIA: MODELLING THE 2015-2017 MALARIA NATIONAL STRATEGY

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The Ethiopian malaria strategy is stratified on transmission level with full coverage of long lasting insecticidal nets (LLINs) in all malarious woredas (sub-districts), supplemented in some woredas by indoor residual spraying (IRS) using carbamate. Simultaneous LLIN and IRS deployment is planned only in epidemic-prone woredas and those with high transmission. Fine scale simulation of multiple scenarios can help to assess the likely impact of such approaches, especially where local data are limited. We simulated malaria transmission, prevalence and burden for each of these woredas using the OpenMalaria platform. Transmission levels were derived from recent annual parasite incidence and parasite prevalence data. National estimates of access to healthcare, historical intervention coverage and malaria seasonality were used to parameterize the simulations further. The national strategic plan for 2015-2017 was simulated accounting for variable levels of effective LLIN usage, access to healthcare and pyrethroid resistance. The predicted incremental benefit of IRS decreases with levels of effective usage of LLINs and of pyrethroid resistance. Changes in mosquito resting/biting behaviour, prevalence of ***Plasmodium vivax*** and LLIN deployment delays would contribute to a higher impact of IRS. The simulations of the different scenarios indicate the expected impact of the interventions in Ethiopia and can be used during decision making process, for example in cost effectiveness analysis. They can also help to understand what would happen when interventions are deployed if all factors influencing malaria transmission intensity could be controlled. Deviations from the predictions could be used to identify where implementation differs from what was anticipated in the plan and hence where additional efforts are required.

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MODELLING THE POTENTIAL INCREMENTAL VALUE OF INTERMITTENT SCREENING AND TREATMENT IN SUB-SAHARAN AFRICA

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We combine estimates from recent trials of the effectiveness of Intermittent Screening and Treatment during pregnancy (ISTp) relative to that of Intermittent Preventative Therapy with sulfadoxine-pyrimethamine (IPTp-SP) with data on how the accuracy of the rapid diagnostic test used in ISTp is likely to vary throughout pregnancy and according to differing levels of immunity, in order to assess the relative effectiveness of ISTp and IPTp-SP across the range of transmission settings in Africa. This work suggests that, as a result of the imperfect sensitivity of the diagnostic and the additional prophylactic value of presumptive therapy, ISTp will not be as effective a strategy for reducing the burden of malaria in pregnancy in areas where the parasite remains sensitive to sulfadoxine-pyrimethamine (SP). Then, using data on the geographical distribution of molecular markers of SP resistance and their relationship to the reduced effectiveness of SP, we mapped how resistance to SP is likely to impact upon the relative effectiveness of the two interventions. Our estimates suggest ISTp is only likely to be more effective than IPTp in areas of "super-resistance", those with substantial prevalence of the A581G sextuple mutation. We estimate that in 2010 these areas represented only 10% of pregnancies across Africa, and only 8% of those pregnancies estimated to have a malaria-attributable LBW baby in the absence of intervention. In areas of intermediate levels of SP resistance, representing much of East Africa, we estimate that an alternative strategy of presumptive therapy with an effective artemisinin combination drug would successfully provide better protection for pregnant women from infection.

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MODELING THE DYNAMICS OF IMMUNOLOGICAL MEMORY TO MALARIA

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Each year nearly 200 million people are infected with the malaria parasite, ***Plasmodium falciparum***. One of the most notable features of infection with malaria is the inability of individuals to acquire sterilizing immunity to the parasite. Even with multiple exposures as is common in areas of high endemicity, individuals remain semi-immune avoiding disease but not the presence and persistence of parasites. The failure of protection is almost certainly in part due to parasite strategies to avoid and limit host immune response including antigenic variation. Recent evidence, however, suggests that in addition to parasite-mediated strategies there may be deficiency in the ability of the immune system itself to create long-lived protection against ***P. falciparum***. Conflicting reports of the levels of long-lived malaria-specific antibodies may be the result of the method and timing of measuring antibodies compared to the timing of infection. Here, we develop a mathematical model of the generation and maintenance of B cell and antibody response to ***P. falciparum***. We analyze simulated output to understand the origin of protective as well as ineffective immune responses in the presence of a multitude of varied proteins as well as antigenically varying proteins. We find that the level of persistent antibodies depends upon assumptions on the relative production of different type of antibody producing cells. Understanding the development and maintenance of protective immune responses to malaria is key in the on-going push towards elimination and eradication.

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OPTIMAL POPULATION-LEVEL DEPLOYMENT OF ARTEMISININ COMBINATION THERAPIES

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Artemisinin combination therapies (ACTs) are used worldwide as first-line treatment against confirmed or suspected ***Plasmodium falciparum*** malaria. ACTs together with vector control, individual protective measures against mosquitoes, prophylactic drug use, and improvements in health care capacity form the basis of modern malaria control and its elimination. Despite the recent success of these strategies at reducing the global burden of malaria, emerging resistance to artemisinin threatens those gains. Countering the onset of resistance may require deliberate tactics aimed at slowing the decline in ACT effectiveness. Using an individual-based microsimulation of regional malaria transmission, we revisit a classical dilemma in evolutionary epidemiology: how to apply a therapy as widely as possible without also accelerating the erosion of its efficacy by drug resistance. We compare the simultaneous distribution of multiple first-line therapies (MFT) against strategies where ACTs would be cycled or used sequentially, either on a fixed schedule or when population-level efficacy reaches the WHO-threshold level of 10% treatment failure. We show that deploying multiple first-line therapies reduces the long-term number of treatment failures when compared to strategies where a single first-line ACT is recommended. We show that this result is robust to various epidemiological, pharmacological, and evolutionary features of malaria transmission. Additionally, we analyze the benefits of including a single non-ACT therapy in an MFT strategy and predict that this approach would have significant benefits in reducing the pressure on artemisinin-resistance evolution, delaying its emergence and slowing its spread. Adjusting national antimalarial treatment guidelines to encourage the simultaneous use of multiple first-line therapies is likely to extend the useful therapeutic life of currently available antimalarials resulting in long-term beneficial outcomes for patients.

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ACCEPTABILITY OF THEORETICAL IMPLEMENTATION OF MINIMALLY INVASIVE AUTOPSIES IN DIFFERENT CULTURAL AND GEOGRAPHICAL CONTEXTS

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Complete diagnostic autopsies (CDAs), the gold standard method for cause of death (CoD) ascertainment, are not routinely performed in low and middle income countries for several reasons, including scarcity

of human and technical resources, and poor acceptability. Minimally invasive autopsies (MIAs), a fine-needle based approach to obtain post-mortem tissues, are being investigated as an alternative to CDA. For MIA implementation, its feasibility and acceptability assessment in diverse cultural, religious and geographical backgrounds is essential. We studied the willingness to know the CoD and the theoretical acceptability of MIAs in Mozambique, Mali, Gabon, Kenya and Pakistan. Five-hundred interviews with key informants (151), health workers (158) and next of kin of recently deceased people (191) were conducted. A thematic analysis was performed. Seventy five percent of interviewees desired to know the CoD of their relative. Theoretical MIA acceptance rates from family members of the deceased at different time-points after death were high, at 71% (15/21) within 24 hours after death, 73% (56/77) in those interviewed 1 to 7 days; and 66% (61/93) between 30 and 40 days. MIA was perceived as acceptable by the majority of participants because of its simplicity, rapidity and avoiding the mutilation of the body. Participants believed that MIA could help to prevent contagious diseases and to address hereditary diseases. Respondents also noted that knowing the CoD could avoid witchcraft accusations and conflicts within the family. Concerns were raised regarding confidentiality for the CoD, fear of organ removal and MIA's compatibility with religious beliefs. A few participants questioned MIA's usefulness, as the person was already dead. Health professionals were more reluctant, with concerns about MIA´s accuracy, or fears related to the questioning of their previous clinical diagnoses. In conclusion, MIA's real acceptability will depend on community's engagement and consideration of potential barriers: confidentiality, delivery of clear messages about the need of knowing the CoD, and sensitization and collaboration of health workers.

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INTEGRATING TRANSMISSION DYNAMICS IN THE MODELLING OF VACCINATION IMPACT AGAINST YELLOW FEVER IN AFRICA

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Yellow fever is a vector-borne flavivirus infection, whose major burden is concentrated in the African inter-tropical area. Eradication is not possible due to the existence of a wildlife reservoir, but a high level of control is achievable as an efficacious and safe vaccine conferring long-lasting immunity is available. After a period of resurgence due to low vaccination coverage, preventive mass vaccination campaigns for yellow fever have been implemented since 2007 in the 12 most affected countries in Africa to curb the rising burden and control future outbreaks. In order to evaluate the burden of Yellow Fever and to assess the impact of recent vaccination campaigns, we developed a comprehensive estimation method based on generalized linear regression models accounting for location of reported outbreaks, surveillance quality, environmental variables and parameters of transmission intensity estimated from serological surveys. We have now further developed this model by integrating a transmission dynamic component. This allowed us to estimate the basic reproduction number R0 at the province level and to account for the indirect protective effect of vaccination due to "herd immunity" enabling us to estimate, at the province level, critical vaccination coverages (CVC) needed to prevent outbreaks. We estimated very low R0 values in numerous provinces in Eastern Africa, but values up to 3.9 [95% CI 1.9-6.9] in Senegal, corresponding to CVC ranging from 0% to 74% [95% CI 48 - 85%]. As of 2012, 19 of the 34 African countries endemic for Yellow Fever had already achieved critical vaccination coverage in ≥90% of their provinces, including 10 of the12 countries with recent mass vaccination campaigns. We identified several provinces with substantial risk of outbreak (R0>1.25) but where <50% of the CVC was achieved, particularly in Mauritania and Guinea-Bissau neither of which have benefited from the recent vaccination activities. Integrating dynamic transmission processes in an existing model allows more comprehensive impact estimates of past and future vaccination campaigns, producing parameters directly useful for disease control strategies.

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ASSESSMENT OF MOBILE PHONE SHORT MESSAGE SERVICE (SMS) AS A POST-TRAINING APPROACH IN UGANDA

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In Uganda, the population coverage for mobile telephony is close to 100%, while geographical coverage is about 65% according to the Uganda Communication Commission. The Infectious Diseases Institute, through the Stop Malaria Project, assessed the effectiveness of using mobile phone SMS to measure retention of knowledge amongst 95 laboratory personnel from rural health centers. These personnel had previously attended a three-day training on laboratory diagnosis of malaria using microscopy and rapid diagnostic tests (RDT). Six weeks after the training, all 95 alumni began receiving follow-up quiz questions via SMS. The alumni were asked to reply to a toll free SMS platform which instantly acknowledged receipt of response and provided feedback on whether the answer was correct or not. Alumni were asked a total of 25 questions – 21 multiple choice and four free response – over the course of 13 weeks. The questions covered the topics of phlebotomy; preparing, examining, and reporting blood smears; differentiating malaria species; and performing RDTs. A toll free phone line was also used to provide reminders and receive technical queries in relation to the SMS. 71% of the trainees responded to the SMS quiz questions. Multiple choice questions had an average response rate of 74% while free response questions were at 51%. Of the trainees that responded, 75% submitted the correct response. Challenges that hindered responses included busy work schedules, poor network coverage, and lack of battery power. The high percentage of responses in general, and specifically the high percentage of correct responses, shows that SMS is effective as a post-training approach to measure knowledge retention. The results also emphasized that multiple choice questions are more appropriate for SMS post-training follow-up compared to free response questions.

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CROWDSOURCING HIV TESTING: A PRAGMATIC, NON-INFERIORITY RANDOMIZED CONTROLLED TRIAL IN CHINA

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Crowdsourcing, the process of shifting individual tasks to a large group, may enhance HIV testing interventions. We conducted a non-inferiority, pragmatic randomized controlled trial to compare first-time HIV testing rates among men who have sex with men (MSM) and transgender individuals who received a crowdsourced HIV test promotion intervention to a group who received a health marketing intervention. Participants were recruited through three large Chinese MSM web portals. We randomly assigned 721 MSM and transgender individuals (≥16 years old, never before tested for HIV) to one of two video interventions. The crowdsourced video was developed using an open contest and formal transparent judging while the evidence-based health marketing video was designed by experts. Study objectives were to measure HIV test uptake within four weeks and cost per new HIV test and diagnosis. Overall, 624/721 (87%) participants completed the study from 31 provinces in 217

Chinese cities. HIV test uptake was similar between the crowdsourced arm (37%, 114/307) and the health marketing arm (35%, 111/317). The risk difference between crowdsourced and health marketing intervention was 2.1% (95% confidence interval, -5.4 to 9.7%). Sensitivity analysis using imputation supported the similarity of the two interventions. Among those tested, 31% (69/225) reported a new HIV diagnosis. The crowdsourced intervention cost substantially less than the health marketing intervention per first-time HIV test ($131/person vs. $238/person) and per new HIV diagnosis ($415/person vs. $799/person). Crowdsourcing may be a cost saving tool to enhance community engagement and improve tropical medicine campaigns.

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VIGILANCIA COMUNITARIA: IMPROVING COMMUNITY-BASED INFECTIOUS DISEASE SURVEILLANCE IN NICARAGUA USING A LOW- COST MHEALTH TOOL FOR DATA COLLECTION AND DECISION SUPPORT

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In Nicaragua, a critical data source for early detection of infectious disease outbreaks comes from the community level. Nicaragua has an extensive network of 20,000+ community-based volunteers, trained by the Ministry of Health (MoH), who provide important community-level data to the public health system. Over several years working in close collaboration with the Information Systems Office and the Office of Epidemiology and Surveillance at the central and state levels of the MoH, we developed, tested, fine-tuned and helped to implement a community-based mobile surveillance tool called Vigilancia Comunitaria (VC) for use on low-cost Android mobile phones. VC is based on the open source software platform Open Data Kit and was designed as a disease tracking and case referral tool for community health workers (CHWs). The goal of this tool is to help increase efficiency and quality of infectious disease surveillance at the community and municipal levels, while also providing near-real time information via an interactive web-based dashboard alert system to supervisors and disease surveillance personnel in prevention and control activities at the municipal, state and national levels. VC allows CHWs to document and maintain a dynamic community census and to track and monitor the health of individuals (as well as aggregate information at the household level) for probable cases of dengue, leptospirosis, leshmaniasis, malaria, cholera, acute diarrheal and respiratory diseases, poisoning and intoxication events. VC also includes modules for infant mortality and high-risk pregnancies. By late 2014, >1200 CHWs were trained on the use of VC on phones in 4 Northern states in Nicaragua. In 2015, an additional module with family planning methodology and reproductive health outreach information will be added. We present a mixed methods impact evaluation of the VC tool and its capacity as it scales up nationally to 1) increase the efficiency and quality of CHW data collection, reporting and referrals, and 2) improve access by state and national decision-makers to timely and reliable information about disease outbreaks.

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THE BOSTON COMBINED RESIDENCY PROGRAM PEDIATRIC GLOBAL HEALTH FELLOWSHIP: EDUCATING PEDIATRICIANS FOR CAREERS IN GLOBAL HEALTH RESEARCH

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In recent years, demand for global health (GH) training and experience has risen dramatically in medical education programs in the United States. There has been a proliferation of GH residency tracks and post-graduate fellowships, few of which are designed to prepare medical trainees for careers in global health research. In 2009, a unique GH track for residents was established at the Boston Combined Residency Program in Pediatrics (BCRP), a pediatrics residency co-sponsored by Boston Medical Center and Boston Children's Hospital. One pediatric resident, after completion of their intern year, is selected to extend training by one year, in order to undertake mentored GH research in partnership with the Center for Global Health and Development at the Boston University School of Public Health. The goal is to prepare pediatricians for careers in global health research or service delivery. Since inception, there are four graduates of the BCRP GH program, but their experiences and track record of the fellowship are illustrative: 2 Directors of Pediatric Global Health at their respective academic institutions, 1 Bill & Melinda Gates Foundation Program Officer, and 1 Director of Transitional Medicine at a managed care organization who is also a Director of the Welbodi Partnership NGO in Sierra Leone. With 6 years' experience administering the fellowship, challenges have included ensuring funding, meeting diverse needs of residents entering training for GH activities, and an evolving future job market. Future directions include solidifying funding for this distinctively integrated, GH research skills-focused fellowship, and partnering with other clinical departments to leverage the collective interest in GH training. The BCRP Pediatric Global Health Research Fellowship remains unique in that it is the only training program integrated with an established pediatric residency that provide trainees the skills necessary for a career in pediatric global health research, with potential lessons to provide to other leaders in GH education.

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PREDICTING AND MITIGATING OUTBREAKS OF INFECTIOUS DISEASE BY USING NASA SATELLITE REMOTE SENSING TECHNOLOGY AND MODELS

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The presentation is designed to present progress in the effort to predict and mitigate infectious disease using remote sensing parameters. The speaker will discuss models developed by NASA and their partners for application of the research results for improved prevention and prediction of outbreaks. The presentation will focus on using satellite remote sensing of to fill the gaps of environmental, spatial, and temporal data for tracking disease. Satellite earth observations provide a wealth of health applications for the imaginative investigator. The session is directly related to Global Environmental Health Surveillance and will present research results of the remote sensing environmental observations of earth and health applications, which can contribute to the tropical medicine research. This presentation will show the advancements that have occurred over the past years and new research that has not been presented. The use of NASA technology to benefit society on earth is a very important topic. For years NASA had been at the forefront of technology in space now we are at the forefront on studying science here on Earth. The will presentation will

show how remotely sensed data has been used to predict and mitigate diseases such as Dengue Fever, Malaria, Oyster Norovirus Outbreaks, West Nile Outbreaks and other Vector Borne Diseases.

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IRON DEFICIENT RBCS ARE RESISTANT TO GROWTH AND INVASION BY *PLASMODIUM FALCIPARUM*: A FIELD STUDY

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Iron Deficiency Anemia (IDA) and malaria are interconnected public health concerns and both cause significant morbidity in Sub-Saharan Africa. Anemia, predominantly IDA, affects up to 50% of pregnant women and 40% of preschool children in the developing world, significantly impacting perinatal and developmental health. The World Health Organization has previously recommended iron supplementation for at risk populations in areas with prevalent malnutrition. However, clinical studies have revealed that iron deficiency protects against malaria, and that administration of iron to iron-deficient individuals increases the risk of malaria. This has complicated recommendations for universal supplementation in malaria endemic regions. Our previous ***in vitro*** work, which has focused on the red blood cell (RBC) stage of the malaria infection, has confirmed these clinical observations: we have shown malaria parasite growth in and invasion of IDA RBCs is impaired, using iron deficient blood donors from our U.S.-based clinic and standard laboratory ***P. falciparum*** strains. Now we have now extended these studies to a real world setting where malaria and iron deficiency are prevalent. Specifically, we show that ***P. falciparum*** growth is directly proportional to hemoglobin status in RBCs from iron deficient Gambian children and pregnant women; ***P. falciparum*** invasion is reduced in iron deficient RBCs from Gambian children; and Gambian field isolates of ***P. falciparum*** exhibit the same phenotype of reduced invasion and growth in IDA RBCs from Gambian children. Our overall goal is now to understand the mechanisms by which iron deficiency protects individuals from malaria, in order to develop safe iron supplementation guidelines in malaria endemic areas.

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UPSA AND DC8 PFEMP1 VARIANTS ARE ASSOCIATED WITH SEVERE ADULT MALARIA IN INDIA

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Expression of a particular subset of ***Plasmodium falciparum*** var genes/PfEMP1 proteins, termed DC8 and DC13, has been linked to severe pediatric malaria. This finding has important implications in disease pathogenesis because DC8 and DC13 PfEMP1 bind Endothelial Protein C Receptor (EPCR), a receptor that plays a key role in regulating endothelial cells permeability, coagulation and blood vessel inflammation. Although the clinical presentation of severe malaria differs significantly between children and adults, almost nothing is known about the var expression profile in severe adult malaria. To better understand the origin of these differences, we analyzed the var profile of infected adult populations in India. Parasites from severe patients significantly overexpressed UpsA var transcripts and DC8 variants, but not DC13s. In addition, an identical DC8 var tag was detected in multiple patients with distinct severe malaria complications. By expressing the DC8 CIDRα1 adhesion domain from patients with high DC8 expression, we found that Indian CIDRα1 domains differed in EPCR binding strength and presented phenotypic differences in the extent of APC blockade. Our findings demonstrate that DC8 PfEMP1 are associated with adult severe malaria and reveal considerable heterogeneity in the CIDRα-EPCR binding phenotype that may contribute to the different disease outcomes observed in adult patients with severe malaria.

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INCREASED TOTAL BODY ARGININE FLUX AND DECREASED NITRIC OXIDE SYNTHESIS IN SEVERE FALCIPARUM MALARIA

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We have previously demonstrated that nitric oxide (NO) is protective against the development of severe falciparum malaria (SM). Our prior work showed that plasma arginine, NO metabolites, PBMC NO synthase (NOS) protein and RNA, and the NOS cofactor tetrahydrobiopterin are low in children and adults with SM compared to healthy controls (HC), while plasma arginase is elevated. To determine whether low nitric oxide in malaria is due to increased degradation of arginine by arginase (arginine urea + ornithine citrulline) or due to decreased synthesis via NOS (arginine NO + citrulline), in this study we measured arginine metabolic flux and calculated total body NO synthesis by administering primed infusions of $^{13}C_6$,$^{15}N_4$-labeled arginine to 10 children with SM and 10 HC children (age 4 to 8 years). Isotopic-enrichment of arginine and citrulline was measured at 0, 90, 120, 150, and 180 minutes post-infusion. Plasma amino acid levels were measured using an amino acid analyzer, and isotope ratios using UPLC-MSMS with selected ion monitoring. By tracing the isotopic enrichment and measuring total plasma citrulline levels, the concentrations of the enriched forms of citrulline were determined, which enabled us to distinguish citrulline produced from arginine via arginase vs. citrulline produced from arginine via NOS. Plasma arginine levels were lower in SM than in HC [mean (range) 43 (25-78) vs. 74 (32-112) μM; p=0.001]. Isotopic equilibrium in arginine was attained within 180 minutes. We noted that arginine flux was higher in children with SM than in HC children [mean (range) 106 (83-167) vs. 81 (65-101) μmol/kg/hour at 120 minutes; p=0.026]. A+9 citrulline (derived from NOS action) was significantly lower in children with SM than in HC children [mean (range) 0.019 (0.008-0.037) vs. 0.040 (0.019-0.129) μM; p=0.045]. Overall, our results definitively demonstrate that children with SM have increased arginine flux and decreased total body NO production (based on the low measurement of citrulline derived from actions of NOS). This new knowledge should help guide development of adjunctive therapies that increase NO in patients with SM.

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POLYMORPHIC MOLECULAR SIGNATURES IN *PLASMODIUM FALCIPARUM* VAR2CSA ARE ASSOCIATED WITH VIRULENCE IN PLACENTAL MALARIA

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Plasmodium falciparum-infected erythrocytes accumulate within the placenta by expressing VAR2CSA, a variant of the highly polymorphic erythrocyte membrane protein-1 (PfEMP1) family that has unique specificity for a low-sulfated form of chondroitin sulfate A (CSA) found

only in this tissue. The resulting syndrome, placental malaria (PM), has important repercussions for the health of both mother and fetus, with the first and second pregnancies being at highest risk for placental pathology and maternal and fetal morbidity and mortality. Partial immunity against VAR2CSA, and therefore protection against pathogenesis, is achieved after multiple PM-exposed pregnancies, making this protein a leading vaccine target. However, the extent to which VAR2CSA diversity contributes to immune evasion and parasite virulence remain poorly understood. In this study the genetic complexity of the DBL3X domain of var2csa in ***P. falciparum***-infected placental blood samples from Kenyan women was characterized. Higher copy numbers of var2csa were found in multigravidae, which may indicate gravidity-associated selection for parasites with inherently greater capacity for immune evasion. Deep sequencing revealed a remarkably high number of unique DBL3X sequences in some individuals, with histologically-confirmed chronic placental infection, but not gravidity, being a significant predictor. Scrutiny of the sequences, however, unveiled divergent, gravidity-biased sequence patterns, with unique types that are more prevalent among primigravidae being associated with high density parasitemia and low birth weight. These results provide for the first time compelling evidence that previous exposure to PM induces selection for multiple copies of unique VAR2CSA types that have reduced virulence and should redirect vaccine efforts to further identify and target antigen types associated with poor birth outcomes.

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ACUTE KIDNEY INJURY IN UGANDAN CHILDREN WITH SEVERE MALARIA: A PROSPECTIVE COHORT STUDY

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Acute kidney injury (AKI) is a common complication of severe malaria in adults, but rarely reported in the context of pediatric populations. Using a new consensus definition of AKI (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group), we evaluated the incidence of AKI in a cohort of Ugandan children with severe malaria.180 children aged 1 to 10 years with a rapid diagnostic test positive for both ***Plasmodium falciparum*** histidine rich protein 2 and lactate dehydrogenase, and at least one of the following criteria for severe malaria: repeated seizures, impaired consciousness, and respiratory distress were enrolled in the study. Renal function was monitored daily for four days using serum creatinine measured by iSTAT. AKI was defined retrospectively by KDIGO. Baseline data were available for 178 children. The day 14 mortality rate was 9.0% (n=16), but AKI was only assessed in 168, as 10 children died before a repeat creatinine measurement. Overall, 63.7% of children (n=107/168) met the criteria for AKI with 55.1% (59/107) staged as "risk", 28.0% (30/107) as "injury", and 16.8% (18/107) as "failure". There was an increase in mortality across stages of AKI (p=0.004) with the mortality rate reaching 16.7% in children with KDIGO-staged renal failure. Overall, 78.6% of children had their creatinine levels peak within the first two days of admission. To evaluate the nature of renal dysfunction in children with AKI we assessed the BUN:creatinine ratio and found 84.2% of children had a BUN:creatinine>20, suggestive of prerenal injury. Since the majority of study deaths occurred before a repeat creatinine measure on day 2, we evaluated the association of renal biomarkers at admission with outcome. There were no differences in creatinine levels between survivors and non-survivors (p=0.1858), but there was an increase in BUN in non-survivors (p=0.0066). These data suggest that renal dysfunction may be more common in pediatric populations with severe malaria than previously assumed and is associated with increased mortality.

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METABOLOMICS OF *PLASMODIUM VIVAX* RELATED ANEMIA FROM THE BRAZILIAN AMAZON

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Anemia is one of the most common complications of ***Plasmodium vivax*** malaria. Multiple host and pathogen factors are involved, as well as nutritional and environmental exposures. Currently, the field of metabolomics uses sophisticated machine learning approaches able to analyze small number of samples while accounting for multiple possible confounding variables. We hypothesized that metabolomic analyses of plasma samples from patients would help us better understand the complex metabolic host-pathogen interactions occurring in ***P. vivax*** anemia in the Brazilian Amazon. We recruited 150 patients (age ≥18 years) with PCR confirmed ***P. vivax*** monoinfection at the Fundação de Medicina Tropical Heitor Vieira Dourado in Manaus, Brazil during 2011-2012. Samples underwent randomization followed by Liquid Chromatography/ Mass Spectrometry. Each sample was run in triplicate and data was extracted using xMSanalyzer. 16,791 unique ***m/z*** and retention times were obtained in all patients. Analyses were restricted to ***m/z*** present in at least 70% of all samples with adjustment for multiple hypothesis testing by the Benjamini-Hochberg false discovery rate method ($p<0.05$). A regression model for hemoglobin levels as a continuous variable was created using partial least squares (PLS) regression with variable importance in projection scores ≥2. We obtained 141 significant ***m/z*** from PLS regression and used them for pathway enrichment analyses with mummichog, an algorithm for pathway-level annotation of metabolomics data. Top altered pathways included butyric acid metabolism (p=0.02) and the carnitine shuttle (p=0.03); metabolite confirmation is under way. Butyric acid and carnitine have been previously implicated in sickle cell and end stage renal disease related anemia, respectively, and both are considered alternative treatments for each of these conditions. These pathways are likely altered as a result of the host-pathogen interaction rather than arising from the host or the pathogen alone. Although not evidence of causality, our efforts yielded potential biochemical pathways associated with ***P. vivax*** related anemia from the Brazilian Amazon.

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STRUCTURE OF PLASMEPSIN V COMPLEXED WITH AN INHIBITOR THAT BLOCKS PROTEIN EXPORT AND MALARIA TRANSMISSION TO MOSQUITOES

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Every day around the world, over 1 million people are infected with malaria parasites. These infections are caused when female Anopheles mosquitoes feed on the blood of infected humans and themselves become infected with gametocytes, followed weeks later by biting another individual and depositing sporozoites into the new host. For the global burden of malaria to be reduced, this transmission cycle needs to be blocked. We have developed a new small molecule that potently inhibits the malarial export protease, plasmepsin V, with an IC_{50} 1 nM. The compound blocks the ability of the most virulent parasite of humans, ***Plasmodium falciparum***, to export proteins that would normally commandeer the infected erythrocyte for parasite survival, and the parasites subsequently die. Treatment of gametocytes, the parasite

form that exits humans and infects mosquitoes, with the compound kills them and a sub-lethal dose blocks their transmission to Anopheles mosquitoes. This establishes plasmepsin V as an essential protein in gametocytes, in addition to the asexual stage, and an attractive drug target for reducing malaria transmission. We have used the inhibitor to obtain diffractable crystals and solved the structure of liganded plasmepsin V to 2.37 Angstroms. This provides a clear basis for the strict requirements for substrate and inhibitor binding of this protease, and unveiled both a plant-like fold and a malaria-specific helix-turn-helix motif that are unique to plasmepsin V and likely to be important in its function in cleavage of effector substrates for export.

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THE PIRNA PATHWAY AND STRESS IN *ANOPHELES STEPHENSI*

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Stress-induced mobilization of transposons is well-documented in many organisms. The piRNA pathway is an RNA interference pathway responsible for repressing transposon mobilization in germ-line tissues of the fruit fly, ***Drosophila melanogaster***. The genes encoding the components of the piRNA pathway, Piwi, Aubergine (Aub) and Argonaut 3 (Ago3), were identified and characterized in the malaria vector mosquito, ***Anopheles stephensi***. Preliminary experiments show that they are induced in embryos following short-duration heat stress. Current experiments are designed to assay the effects of prolonged heat and cold stress on the expression levels of the mosquito orthologs of the heat-shock protein genes, hsp70 and hsp90, as well as Piwi, Aub and Ago3 and putative endogenous transposon transcripts identified from ***An. stephensi*** RNA sequencing data. Additionally, mosquitoes mutant for Piwi, Aub and Ago3 are being generated using Cas9-mediated site-specific genome targeting that will be tested for a phenotype affecting the temperature stress response. The results of this work are expected to inform the development of transposon-based gene-drive systems for introgressing beneficial traits into vector mosquitoes.

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A KEY REPRODUCTIVE GENE INFLUENCES *PLASMODIUM* DEVELOPMENT IN THE MAJOR MALARIA VECTOR *ANOPHELES GAMBIAE*

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In the major malaria vector ***Anopheles gambiae***, male-female molecular interactions following mating are important determinants of fertility and fecundity. Intriguingly, an increasing amount of evidence points to reproductive processes playing an important role in ***Plasmodium*** parasite development. Male transfer of the steroid hormone 20-hydroxyecdysone (20E) during mating activates the transcription of a ***Mating-Induced Stimulator of Oogenesis*** (***MISO***) gene that transduces the mating signal into an increase in egg development. Silencing ***MISO*** by RNA interference reduces egg development to levels observed in virgin females. This phenotype is caused in part by improper release of 20E from the mating plug in the absence of MISO, leading to disregulation of genes important for oogenesis. In particular, silencing ***MISO*** reduces expression of yolk protein precursors (YPPs) and impairs lipid accumulation in the oocyte. Previous research shows that the same YPPs essential for lipid accumulation in the developing mosquito egg help parasites escape the immune system. We show evidence that MISO depletion impacts both ***Plasmodium falciparum*** and ***P. berghei*** infection in ***A. gambiae;*** however, the effects in these ***Plasmodium*** species differ, enabling us to further reconstruct the molecular pathways linking egg development and ***Plasmodium*** infection. Our studies suggest ***MISO*** may modulate aspects of mosquito biology that are relevant to anopheline vector competence.

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INVESTIGATING MOSQUITO MOLECULAR FACTORS THAT CONTROL GUT MICROBIOTA VARIABILITY IN *AEDES AEGYPTI*

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The ***Aedes aegypti*** mosquito midgut microbiota can alter mosquito susceptibility to dengue virus, and understanding factors that shape it could explain field transmission dynamics and contribute to development of novel dengue control strategies. In the current study, we aimed to identify mosquito molecular factors that control bacterial load of the midgut and contribute to within-species variability in gut microbial load. We reared multiple strains of ***A. aegypti*** in a controlled laboratory environment and used culture-dependent and -independent techniques to assess bacterial load in the midguts of sugar and blood fed females from each strain. We then compared genome-wide gene expression in response to blood feeding and bacterial ingestion between two strains showing the greatest difference in microbial load. We identified genes that showed strain-specific patterns of up- or down-regulation and used Gene Ontology and KEGG analyses to identify pathways enriched in a strain-specific manner. Finally, we used RNAi to knock down candidate genes to validate their role in influencing gut microbial load. We found significant variation between strains in gut bacterial load. Our transcriptome analysis revealed that an unexpectedly high number of metabolism-implicated genes were differentially expressed between strains. We also identified strain-specific variation in the mRNA abundance of multiple immunity genes. Preliminary data from RNAi knock down experiments suggests that the immunity gene galectin 1 as well as genes involved in valine, leucine and isoleucine degradation are implicated in controlling proliferation of gut bacteria in a strain-specific manner. Taken together, these data suggest that metabolic activity in the mosquito gut may act to control bacterial load and that variability in metabolic activity has the potential to control within-species variation in gut microbial load.

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A PROTEOMIC-BASED "SHOTGUN" APPROACH OPENS NEW PERSPECTIVES TO MOSQUITO AGE-GRADING

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Information on age structure of wild mosquito populations is fundamental to assess impact of control measures and vectorial capacity of species implicated in transmission of malaria or arboviroses. Transcriptional studies on major disease vectors, such as ***Anopheles gambiae*** and ***Aedes aegypti***, have highlighted age-related variations in some genes which have been exploited to develop quantitative reverse transcriptase-PCR age-grading methods. However, due to low RNA stability, these qRT-PCR approaches require a careful manipulation and preparation of samples and relatively high-tech and expensive equipment. In this work we applied a proteomic-based "shotgun" approach to identify and quantify proteins from carcasses of laboratory reared Aedes albopictus belonging to six different age-groups and different physiological stages. Proteins were extracted from heads and thoraxes and the same total protein content of each group was processed for a nanoLC-nanoESI-MS/MS analysis on an Ultimate 3000 HPLC coupled to a LTQ Orbitrap mass spectrometer. Protein intensities across samples was evaluated using a LFQ (label-free quantitation) method. Approximately 600 proteins/age-group were identified, 4 of which (i.e. an hemocyanin protein; an insect cuticle

protein; a glutathione S-transferase; and a not yet annotated protein) were shown to strongly decrease with age in all biological and technical replicates carried out to exclude possible technical biases. Antibodies against two of these proteins have been produced and are being exploited to develop ELISA or Western Blotting age-grading protocols to be applied on Ae. albopictus, a species whose longevity is difficult to assess due to lack of specific molecular approaches. In fact, these approaches are expected to be more manageable and cheaper than the currently used qRT-PCR approaches, thus allowing a larger scale assessment of longevity in epidemiological studies as well as in the evaluation of the efficacy of control strategies. This study represents a proof-of concept for the development of similar methods to tropical vectors of malaria and arbovirosis.

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MOLECULAR MECHANISMS MEDIATING INNATE IMMUNE PRIMING IN *ANOPHELES GAMBIAE* MOSQUITOES

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An innate immune priming response is triggered when ***Plasmodium*** ookinetes invade the mosquito midgut and the microbiota comes in direct contact with injured cells. This is a long-lasting response that confers the mosquito enhanced ability to control subsequent ***Plasmodium*** infections. The immune priming response involves hemocyte differentiation, in particular an increase in the granulocyte population. A hemocyte differentiation factor (HDF) is released into the hemolymph and transfer of cell-free hemolymph from challenged mosquitoes can induce hemocyte differentiation and enhanced immunity in recipient naïve mosquitoes. In this study, we have characterized the biochemical nature of HDF. We discovered that this factor consists of a Lipoxin / Lipocalin complex. RNAi-based silencing of the lipocalin (Evokin) component abolished the release of HDF activity in the hemolymph extract, indicating that it is a critical component of the immune priming response. In turn, LC/MS/MS analysis showed that the HDF lipid component is an eicosanoid (Lipoxin). Injection of synthetic lipoxin recapitulates the phenotype observed in ***Plasmodium***-infected mosquitoes as well as those observed when transferring cell-free hemolymph from challenged to naïve mosquitoes. In summary, we show that innate immune priming involves a persistent increase in expression of Evokin, and in the ability of mosquitoes to convert arachidonic acid to lipoxins, predominantly Lipoxin A4.

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AN ENDOSYMBIONT-REGULATED TSETSE ODORANT BINDING PROTEIN MEDIATES HOST IMMUNE SYSTEM MATURATION PROCESSES

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Endosymbiotic bacteria serve important functions within their animal hosts, including maintenance of immune system homeostasis. Little is known about the mechanisms that enable these bacteria to induce host immunity-related phenotypes during development and into adulthood. Tsetse flies (***Glossina*** spp.) house 3 distinct endosymbiotic bacteria that are vertically transmitted from mother to offspring during this insect's unique viviparous mode of reproduction. When intrauterine tsetse larvae mature in the absence of their endogenous microbiota (referred to as 'aposymbiotic'), subsequent adults present a highly compromised cellular immune system that is characterized by the absence of phagocytic hemocytes. We demonstrate that an odorant binding protein-encoding gene (***obp6***) is expressed at 22x higher levels in wild-type versus aposymbiotic larvae. Expression of this gene is reduced significantly in wild-type larvae when their lactating moms are microinjected with gene-specific short interfering RNAs (siRNAs). Offspring that imbibe anti-***obp6*** siRNAs exhibit phenotypes consistent with the presence of a reduced or dysfunctional population of crystal cells, which are responsible for initiating the melanization cascade via the release of prophenoloxidase. Our findings provide valuable insight into molecular pathways that underlie symbiont-mediated immune system maturation in tsetse.

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IMPACT OF INCREASED INSULIN SIGNALING IN THE FAT BODY OF *ANOPHELES STEPHENSI* AND *AEDES AEGYPTI* MOSQUITOES ON INNATE IMMUNITY, LIFESPAN AND REPRODUCTION

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The World Health Organization (WHO) estimates that 2.5 billion people live in dengue-endemic areas and are at daily risk of infection. Thus, there is an urgent need for new innovative strategies to ease the burden of dengue transmission. One strategy is to genetically engineer the primary mosquito vector, ***Aedes aegypti***, to be resistant to dengue infection and with improved fitness to replace wild, susceptible mosquito population. In mosquitoes, the insulin/insulin growth factor 1 signaling (IIS) cascade regulates lifespan, reproduction, and innate immunity. To better understand the impact of IIS in mosquitoes, we induced IIS in the fat body of transgenic ***Ae. aegypti*** mosquitoes by expressing an active form of ***Ae. aegypti*** Akt (AeagAkt), a key component of the IIS cascade. The mosquito's fat body is the main tissue responsible for antimicrobial production and has been shown to serve as "signaling center" for the IIS pathway. In early studies we found that active AeagAkt transcript and protein expression occurred in a fat body and blood meal specific manner, as expected for a transgene regulated by the vitellogenin promoter. Furthermore, we were able to demonstrate increased activation of downstream IIS molecules indicating activation of the IIS cascade. We also observed changes in vitellogenin protein levels, but surprisingly did not observe any change in egg production. Most importantly, we observed a significant extension in the lifespan of the myr-AeagAkt transgenic ***Ae. aegypti***. This increase in lifespan opens intriguing possibilities for manipulating the fitness of transgenic virus resistant mosquitoes.

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COMPARISON OF DOXYCYCLINE, MINOCYCLINE, DOXYCYCLINE PLUS ALBENDAZOLE AND ALBENDAZOLE ALONE IN THEIR EFFICACY AGAINST ONCHOCERCIASIS

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In search of new macrofilaricidal drugs against onchocerciasis that lead to regimens shorter than the current macrofilaricidal "gold standard" doxycycline (Doxy) 200mg/d given for 4 weeks, preclinical studies within the A·WOL consortium showed that minocycline (Mino) had an efficacy superior to Doxy. Further studies suggested that a combination treatment with Doxy and albendazole (Alb) increased the effect of Doxy alone. Therefore a randomized, open-label, pilot trial was carried out in an area in Ghana endemic for onchocerciasis, comprising 5 different regimens: the standard regimen Doxy 200mg/d for 4 weeks (Doxy 4w), the experimental regimens Mino 200mg/d for 3 weeks (Mino 3w), Doxy 200mg/d for 3 weeks plus Alb 800mg/d for 3 days (Doxy 3w + Alb 3d), Doxy 200mg/d for 3 weeks (Doxy 3w) and Alb 800mg for 3 days (Alb 3d). Of 156 volunteers, 136 (87.2%) completed the treatment according to protocol. Of these, 99 (72.8%) were present for the surgical extirpation of their onchocercomata 6 months after treatment onset. Histological analyses of the adult worms in the extirpated nodules revealed absence of Wolbachia in 98.8% (Doxy 4w), 81.4 % (Doxy 3w + Alb 3d), 72.7% (Mino 3w), 64.1% (Doxy 3w) and 35.2% (Alb 3d) of the female worms. All 4 treatment regimens showed superiority to Alb 3d ($p < 0.001$, $p < 0.001$, $p = 0.002$, $p = 0.008$, respectively). Additionally, Doxy 4w showed superiority to all other treatment arms (vs. Doxy 3w + Alb 3d $p = 0.005$, vs. Mino 3w $p = 0.002$, vs. Doxy 3w $p < 0.001$). The observed differences regarding the absence of Wolbachia between the 3-week regimens did not reach statistical significance. Furthermore Doxy 4w and Doxy 3w + Alb 3d showed a higher amount of female worms with degenerated embryogenesis compared to Alb 3d ($p = 0.028$, $p = 0.042$, respectively). These results confirm earlier studies that Doxy 4w is sufficient for Wolbachia depletion and the desired parasitological effects. The data further suggest that there is an additive/synergistic effect of Alb (3 days) on top of that of Doxy 3w alone, and that Mino 3w has a stronger potency than Doxy 3w. These latter two results are preliminary and need confirmation in a full randomized controlled phase 2 trial.

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DEVELOPMENT OF NOVEL SEROLOGIC TOOLS TO STUDY *ONCHOCERCA VOLVULUS* POPULATION GENETICS

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The ongoing public health and socioeconomic impact of onchocerciasis requires a better understanding of ***Onchocerca volvulus*** biology that can be translated into innovative methods for improved surveillance, diagnosis, and treatment. In particular, studies of ***O. volvulus (Ov)*** population genetics may help elucidate its transmission, spread, emergence of drug resistance, and persistence despite control measures. Currently, studies of ***O. volvulus*** population genetics are significantly limited because the extraction of parasite tissue from hosts requires invasive and painful procedures. Therefore, we are developing a novel approach to help advance our understanding of ***O. volvulus*** allele diversity and spatial distribution using well-characterized serum and available parasite material. We have identified non-synonymous single nucleotide polymorphisms (SNPs) in the coding sequences of 9 of the 14 most highly immunogenic ***Ov*** proteins: Ov7, Ov16, OvASP1, OvCHI1, OvM3, OvALT1, OvB8, OvFAR1, and OvRAL1. Moreover, we have confirmed these SNPS, using PCR-based genotyping and applied these genotyping assays to individual skin snip samples from patients with onchocerciasis (e.g. Ov7 S29F and Ov16 R180P). Using KLH-linked synthetic peptides that each contain one or the other SNP variant for each of these antigens, we have developed serum-based immunoassays to genotype ***O. volvulus*** infecting strains using Ov-infected patient sera. We plan to investigate the diversity and distribution of ***O. volvulus*** populations over geographical space and time without the need for parasite material. The results are expected to shed light on features of ***O. volvulus*** population genetics that may facilitate elimination efforts.

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CAN WE USE SIMPLE PHYSICAL MEASURES TO DETECT COVERT LYMPHEDEMA IN ADOLESCENTS AND YOUNG ADULTS INFECTED WITH LYMPHATIC FILARIASIS?

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Lymphedema after infection with lymphatic filariasis (LF) and lymphedema after treatment for cancer have differing causes but similar chronic manifestations. In both conditions only a portion of those at risk will develop chronic disease and in both cases early detection and intervention can result in reversal of symptoms or even complete recovery, whereas progression to later stages prevents any possibility of cure and reduces opportunity for improvement. Devices used to detect and assess cancer related lymphedema were used in an LF early detection study in a highly endemic area in Central Myanmar (Amarapura Township). Participants aged between 10 and 21 years were screened by ICT (n=315) and positive cases were age and gender matched with negative cases and invited into a longitudinal study. Physical measures were taken on 104 participants before and after the annual MDA campaign. Infection rate was 17.8% in younger people (aged 10-17) and 23.08% in older participants (aged 18-21). Of the 95 participants who returned for follow up measures there was conversion from negative to positive and vice versa in 5 and 1 participants respectively. Physical measures included limb circumference and tissue tonicity at the mid-point of the anterior and posterior thigh and the calf. Bio impedance spectroscopy was used to assess segmental fluid load in the whole leg, thigh and upper thigh. The results will assess the usefulness of physical measures for early detection of covert lymphatic changes in young people infected with LF and the impact of MDA on early lymphatic dysfunction.

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MEASURING THE IMPACT OF LYMPHOEDEMA ON PATIENT MOBILITY USING GPS DATA LOGGERS: A CASE-CONTROL STUDY OF PATIENTS IN CHIKWAWA, MALAWI

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Lymphoedema, one of the most common clinical manifestations of lymphatic filariasis, is known to have a significant impact on a patient's quality of life by restricting mobility both day-to-day due to the swelling of the limb, and during acute attacks during which the patients is often completely incapacitated. This can impact both their economic and mental

stability, and perpetuates poverty in endemic areas. This purpose of this study was to directly measure the physical and socio-economic impact of lymphoedema on affected individuals and their carers in an endemic area of Malawi by comparing a group of lymphoedema patients (cases) to a group without the condition (controls). There were three components to this project: (i) a self-assessment questionnaire, including questions on mobility, self-care, assistance received and daily activities, (ii) observed mobility tests and (iii) a diary and GPS data logger study. Components (i) and (ii) involved 30 cases and 30 controls, whereas a subset of 10 cases and 10 controls were asked to complete a daily activity sheet over a three week period, and further to wear a GPS logger to track their movements over the same period. The self-assessment questionnaire required participants to score each question. Participants indicated their score using a graphical representation of stacked blocks was used, as opposed to a more traditional written scale in order to produce more accurate self-assessed responses. Individual scores were summed to produce an overall disability score. Observed mobility tests included a timed 10 metre walk, and a timed 'up and go' test during which the time required to stand up from a chair, walk 3 metres, then return to a seated position was calculated. The GPS logger component allowed the average distance and travelling speed to be calculated over the three week period for each case and control, and further to identify mobility patterns. The disability score, observed mobility test times, daily activities and mobility patterns were then compared between cases and controls to obtain a more accurate understanding of the relative impact of lymphoedema on affected individuals.

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ABL-KINASE TARGETING OF FILARIAL PARASITES BY IMATINIB IMPAIRS EMBRYOGENESIS AND SHEATH/CUTICLE INTEGRITY: STRUCTURAL STUDIES IN *BRUGIA MALAYI*

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Unanticipated severe side effects (including death) in ***Loa loa*** (Ll)-endemic regions of Central Africa caused by ivermectin use in mass drug administration (MDA) campaigns for onchocerciasis and lymphatic filariasis elimination has resulted in cessation of MDA. The severity of these post-ivermectin treatment reactions has been found to be proportional to the number of Ll microfilariae (MF) and is thought to result from rapid MF killing. Thus, there is a need for new drugs that either kill MF more slowly or only target adult worms. We have previously shown a high degree of sequence identity between the human c-Abl protein and Abl-like proteins expressed by ***Brugia malayi*** (Bm), ***Onchocerca volvulus***, ***Wuchereria bancrofti***, Ll, and ***Mansonella perstans*** at the imatinib binding site. Imatinib also kills Bm ***in vitro*** at concentrations achievable in humans after a single oral dose. Prior to full scale human trials, it is important to understand the mechanism by which imatinib is acting on the MF and the other parasite stages to anticipate possible efficacy and safety. First, we used RNAseq data to quantify the relative expression of Abl-like proteins. These data showed that the highest Abl-like expression was found in the adult female (AF)(246 RPKM), followed by MF (125 RPKM), and lastly by L3 (68 RPKM) and by adult males (AM)(63 RPKM). Confocal microscopy was next performed and demonstrated that fluorescently labeled rabbit anti-human c-Abl showed extensive staining of the cuticle in both AM and AF and in the uterine lining of AF. Moreover, the MF sheath stained brightly. Compared to untreated control parasites, transmission electron microscopy of imatinib-treated AF showed an arrest in development of embryogenesis, with only immature ova/embryos visible with pyknotic nuclei. The cuticles of the AM and AF were markedly disturbed, and the MF revealed complete loss of internal architecture. Scanning electron microscopy of MF demonstrated a thinning and loss of sheath integrity. These data suggest that imatinib inhibits filarial embryogenesis, and effects mobility and survival of MF and AF/AM by its disruption on the sheath and cuticle, respectively.

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EXPERIMENTAL ANTI-WOLBACHIAL THERAPY OF FILARIASIS WITH COMBINATIONS OF REGISTERED ANTIBIOTICS SUGGESTS TREATMENT SUCCESS IS ACHIEVABLE WITHIN SEVEN DAYS

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Filarial parasites can be targeted by antibiotic treatment due to their unique endosymbiotic relationship with Wolbachia bacteria. This finding has led to successful treatment strategies in both, human onchocerciasis and lymphatic filariasis. A 4-6 week treatment course using doxycycline results in long-term sterility and ultimately death of the parasite. However, current treatment times and doxycycline contraindications in children and pregnant women preclude administration of doxycycline in mass chemotherapy within public health control programs; therefore, establishing of shorter anti-wolbachial regimens is a focus of ongoing research. We have used a sensitive rodent model of ***Litomosoides sigmodontis***, for compound screening, which allows us to quickly provide initial evidence for ***in vivo*** efficacy. In this model, combinations of registered antibiotics were tested. Administration of rifamycins (Rifampin or Priftin® /rifampicin or rifapentine) in combination with doxycycline for 7 days successfully depleted Wolbachia by > 2 log (99% reduction) and thus resulted in a significant reduction of the treatment duration. Using a triple combination of a tetracycline (doxycycline or minocycline), a rifamycin and a fluoroquinolone (moxifloxacin) given ip led to an even greater shortening of the treatment time to approx. 4 days. Both alone or in the triple combination rifapentine showed superiority to rifampicin. Reduction of treatment time was similar when administration was by oral gavage instead of ip. Testing all double combinations that could be built out of the triple combinations revealed that the combination of rifapentine (15mg/kg) and moxifloxacin (2 x 200mg/kg) showed the best efficacy. For both administration routes (oral or ip) it could be proven that the rifapentine plus moxifloxacin combination was equivalent to the triple combination (>99% Wolbachia reduction). These investigations suggest that it may be possible to shorten anti-wolbachial treatment times to 7 days or less in humans, in accordance with the currently favoured TPP for macrofilaricidal drugs.

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TYLOSIN ANALOGS AS ANTI-FILARIAL AGENTS, PART B: PRE-CLINICAL EVALUATION IN RODENT MODELS OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS"

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Onchocerciasis and lymphatic filariasis (LF) are priority neglected tropical diseases targeted for elimination. The causative agents of LF and onchocerciasis harbor a symbiotic endobacterium, Wolbachia. Targeting Wolbachia with doxycycline has delivered curative outcomes and is safe to administer in loiasis co-infection. Contraindications and long treatment courses required for efficacy limit doxycycline scale-up for widespread programmatic use. Orally bioavailable analogs of the veterinary antibiotic, tylosin A (TylA): A-1535469 and A-1574083, are potent anti-Wolbachia

agents ***in vitro***, with promising pharmacokinetic profiles. We tested the anti-Wolbachia effect of TylA analogs in rodent models of LF and onchocerciasis. During ***Brugia malayi*** intraperitoneal filarial infections of mice, oral A-1535469 and A-157083 effectively depleted Wolbachia from larval tissues. Testing at 50 mg/kg, A-1535469 dosing for 14 days mediated comparable anti-Wolbachia efficacy to parenteral TylA and superior activity compared to doxycycline (99.3% vs 98.3%, P<0.001). Seven day dosing of A-1535469 or A-1574083 induced 99.3% and 98.8% reductions in Wolbachia, both superior to 7-day doxycycline treatment (27.6%, P<0.001). Against pre-fecund ***B. malayi*** female worms, A-1535469 administered for 14 days at 50 mg/kg or 7 days at 250 mg/kg mediated equipotent Wolbachia depletion compared with 28-day dosing with the second generation tetracycline, minocycline, (90% vs 93% and 98.3% vs 95.5%, respectively). In gerbils, A-1574083 dosed for 14 days at 10 or 50 mg/kg were equipotent to 21 day high-dose doxycycline (200 mg/kg) in depletion of Wolbachia from mature female ***B. malayi*** (99.4 / 99.8% vs 99.1%). Further, 50 mg/kg dosing of A-1574083 significantly depleted microfilariae compared with control infections (99.64% reduction, P=0.039). In conclusion, orally bioavailable TylA analogues can achieve >90% Wolbachia depletion ***in vivo*** following 7-14 day dosing, a threshold validated as predictive of curative outcome in both human LF and onchocerciasis. 'TylAMac' compounds are now being advanced into full-scale pre-clinical development.

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BUSINESS TRAVEL AND ILLNESS: ANALYSIS OF DATA FROM THE GEOSENTINEL SURVEILLANCE NETWORK

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There are ≥150 million international business trips annually; business travelers represent 14-21% of travelers reported in the literature. Illness in business travelers has economic consequences for both employee and employer. We used data from the GeoSentinel Surveillance Network to describe health problems in business travelers seen at GeoSentinel clinics in 26 countries. Specifically, we describe the demographics of business travelers evaluated after travel, review their illnesses and severity based on hospitalization, and determine prominent diagnoses. From 1997 through 2014, a total of 14,041 business travelers presented with a travel-related illness to a GeoSentinel clinic (16,697 total diagnoses). The vast majority (94%) were adults aged 20-64 years; most (75%) traveled from Western Europe or North America, and two-thirds were male. Most (86%) were seen as outpatients. Fewer than half (44%) reported a pre-travel encounter. Frequent regions of exposure were sub-Saharan Africa (33%), Southeast Asia (13%), South Central Asia (13%), South America (6%), and North East Asia (6%). The most frequent diagnoses were acute unspecified diarrhea (7%), viral syndrome (5%), acute bacterial diarrhea (4%), chronic unknown diarrhea (4%), and falciparum malaria (4%). Species was specified for 933 (84%) of 1116 malaria patients and included 657 ***Plasmodium falciparum***, 177 ***P. vivax***, 52 ***P. ovale***, 29 ***P. malariae***, 1 ***P. knowlesi***, and 17 with two species. Of 598 (54%) with chemoprophylaxis information, 92% took none or incomplete courses. Also underestimated, 17 malaria cases were drug resistant. Over half of malaria patients were hospitalized. There were 116 patients with severe malaria. Sixteen deaths occurred in business travelers, of which 8 were from malaria. Our analysis identifies gastrointestinal diagnoses as the most common problems related to business travel and raises concern for malaria in business travelers, including death and acquisition of resistant strains. These results indicate the need to promote pre-travel health through public health messages as well as for improved messages regarding safe food and drinks, and malaria prevention.

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TRENDS IN IMPORTED INFECTIONS IN THE UNITED KINGDOM: 2000-2015

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We established a prospective database to capture all admissions with tropical infections at a large tertiary referral infectious diseases and tropical medicine unit in the United Kingdom. The database allows analysis of cause and length of admission, region of travel and time between travel and admission. We will present data on over 2,200 consecutive admissions, from almost 2,000 patients between August 2000 to February 2015. Between them these admissions resulted in 12,962 bed days. Falciparum malaria was the single commonest reason for admission (n = 1,115, 50.1%) but contributed only 30.5% of the total number of bed days. Conversely leishmaniasis and leprosy were the reason for admission in only 269 (11.9%) and 106 (4.7%) patients respectively but contributed 27.4% and 12.3% of the total number of bed days. Although the total number of cases of malaria in the United Kingdom remained steady, the number of admissions at our site fell significantly over the study period from an average of 120 cases per year between 2001 to 2004 to only 34 cases a year between 2011-2014, Africa was the most common region of travel (n=1,303, 57.4%), followed by Asia (n=419, 18.5%) and the Americas (n=242, 10.7%). As expected the majority of falciparum malaria arose following travel to West Africa (n=726) followed by East Africa (n=247). Falciparum malaria (84.9%), enteric fever (5.3%) and dengue (6.5%)were the most common presentation in individuals presenting within 3 months of travel. In patients presenting more than 3 months after travel, chronic conditions, in particular leishmaniasis (57.8%), non-falciparum malaria (12.6%), leprosy (9.4%), and neurocystericosis (8.2%) were more common. These data, collected prospectively over almost 15 years, represent the most comprehensive data available on the trends in imported infections seen in the United Kingdom and will help be of value to all clinicians in high income settings involved in delivering care to returning travellers.

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EFFECTIVENESS OF LOPERAMIDE MONOTHERAPY FOR SELF-TREATMENT OF MODERATE OR SEVERE ACUTE WATERY DIARRHEA

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Standard guidelines recommend the use of antibiotics and loperamide for self-treatment of moderate or severe travelers' diarrhea (TD). However, recent data suggests that the use of antibiotics during travel may be associated with acquisition of extended spectrum-beta-lactamase producing gram negative rods, suggesting the need for judicious use of antibiotics for self-treatment. We evaluated the effectiveness of loperamide alone, versus antibiotics with or without loperamide for self-treatment of moderate or severe acute watery diarrhea (MS-AWD). Travelers presenting to 4 US military travel clinics were prospectively enrolled. Participants completed a diary, post-travel survey and follow-up surveys documenting any diarrhea during travel, self-treatment taken, duration of symptoms, and symptoms of irritable bowel syndrome (IBS). Standard definitions were used to assess for MS-AWD and IBS. We compared the duration of diarrhea in days and clinical cure at 72 hours among travelers who took loperamide alone vs. antibiotics alone or antibiotics plus loperamide for MS-AWD. 2232 participants were enrolled between 2010 and 2015, of whom 204 met criteria for MS-AWD and completed the diary/survey. The median number of stools during a 24 hour period was 4 stools (IQR: 3-7 stools); 48 participants used no self-treatment, 48 used loperamide alone, and 108 used either antibiotics alone or in combination with loperamide for self-treatment. No difference in the duration of diarrhea (1.1 days vs. 1.5 days) or 72 hour cure rate (90% vs. 90%) was observed among participants who used loperamide alone, vs. antibiotics alone or in combination with loperamide. There was no significant difference in the incidence of PI-IBS at 12 months (7.7% [2/26] vs. 5.3% [3/57]) between the two treatment groups. Loperamide alone was as effective as antibiotics with or without loperamide in shortening the duration of MS-AWD, in this observational pilot study based on self-reported symptom duration. This suggests that using loperamide alone for MS-AWD may be a reasonable option. Further effectiveness studies aimed at judicious use of antibiotics during travel are needed.

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GASTROINTESTINAL POLYPARASITISM: POTENTIAL IMPACT ON HOST RESPONSE AND PERSISTENCE OF INFECTIONS

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Over 30% of the human population is infected with gastrointestinal parasites. Quantitative Real-Time PCR (qPCR) has allowed for easier detection of helminth and intestinal protozoa co-infection. Co-infection can alter the host immune response. Protozoa stimulate a T-helper cell-1 (Th1) polarized response and helminths stimulate a polarized Th2 response. The immune response to one parasite may directly influence the immune response to another parasitic infection and alter clearance of infection. We hypothesized that co-infected children with intestinal helminth (***Ascaris lumbricoides***) and protozoa (***Giardia lamblia***) would have evidence of Th2 cytokine polarization with a reduction in Th1 cytokines compared to singly infected children with either ***Ascaris*** or ***Giardia***. Fecal and plasma samples were collected from 39 randomly selected 3 year-old asymptomatic children living in Ecuador of whom 11 children infected only with ***G. lamblia***, 7 with only ***A. lumbricoides***, 13 with both ***A. lumbricoides*** and ***G. lamblia***, and 8 with no parasites (controls). Parasitic infections were detected using qPCR of stool samples. Plasmas were used to measure cytokines using BioRad Luminex MagPix. Children with co-infections had a significantly increased IL-10/IFN-γ ratio compared to uninfected controls (0.48 vs. 0.16, $p < 0.05$) and compared to ***Ascaris*** only infections (vs. 0.17, $p < 0.05$) and a non-significant increase compared to ***Giardia*** only infections (vs. 0.26, $p = 0.09$). Children with co-infections compared to those with ***Giardia*** only infections had significantly reduced levels of Th1 cytokines (IL-2 [3.83 vs. 8.08 pg/mL; $p < 0.05$], IL-12 [4.72 versus 14.89 pg/mL; $p < 0.05$], TNF-α [1.52 versus 2.58 pg/mL; $p < 0.05$]). Our data provide evidence that children with co-infections (i.e. ***A. lumbricoides*** and ***G. lamblia***) have reduced plasma levels of Th1 cytokines and relatively increased levels of the immune regulatory cytokine, IL-10. An increase in immune regulation in the context of reduced Th1 responsiveness associated with ascariasis may be permissive to chronic carriage of intestinal protozoa such as ***Giardia***.

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DISCREPANCIES BETWEEN TEST RESULTS, DIAGNOSIS, AND TREATMENT OF FEBRILE ILLNESS IN KENYAN CHILDREN

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In low resource settings where malaria is prevalent, accurate diagnosis of the etiology of febrile illness in children can be challenging. The WHO currently recommends laboratory-confirmed diagnosis of malaria, if available, prior to starting antimalarials in patients who are stable. Given the increasing prevalence of other febrile illnesses such as dengue and chikungunya viruses, plus ongoing high risk of bacterial infections such as diarrheal illness and pneumonia, we chose to investigate trends in clinical practice in four Kenyan hospitals. These hospitals rely on clinical assessment and blood smears for malaria to differentiate illness and determine management in children who present with febrile illness. This study examined factors present on presentation in a cohort of acutely febrile Kenyan children at 4 hospital sites over a 33 week period. Clinical officers at each site completed a comprehensive intake form for each child including components of history, physical exam, malaria smear results, diagnosis and treatment. We examined the correlation of malaria smear results and diagnosis with treatment. In a cohort of 598 febrile children, preliminary data indicates that 56.8% (95% CI 51.2-62.2%) of those with a negative malaria smear were given a primary diagnosis of malaria. Of patients who were malarial positive with no additional viral or bacterial diagnosis, 30% (95% CI 23.2-37.8%) were given antibiotics. Of patients who were malaria smear negative and diagnosed with viral illness, 70.6% (95% CI 64.1-76.3%) were given antibiotics. In a cohort of acutely ill Kenyan children, malarial test results and diagnosis did not correlate over half of the time. In addition, antibiotics were given to a large percentage of cases diagnosed with either malarial or viral illness. More thorough investigation, including qualitative analysis on the principles, practices, and knowledge of these clinical officers is needed to understand if there are discrepancies between evaluation and appropriate treatment of these children in a resource-poor setting. Further analysis is needed to examine various factors that influence management decisions.

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DETERMINING THE SAFETY OF LUMBAR PUNCTURE IN COMATOSE MALAWIAN CHILDREN: A RETROSPECTIVE CASE-CONTROL STUDY

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Coma is a common clinical presentation for critically ill children in sub-Saharan Africa, where major differential diagnoses include cerebral malaria, viral encephalitis, and bacterial and tuberculous meningitis. Lumbar puncture (LP) is a crucial diagnostic test to distinguish between these etiologies and determine optimal treatment. However, clinicians may be hesitant to perform an LP due to concerns of precipitating herniation and death, particularly in environments where pre-procedure neuroimaging is unavailable. This concern may be compounded by a recent major study demonstrating that substantial brain swelling occurs in children with cerebral malaria and is strongly associated with fatal outcome. We performed a retrospective case-control study of the safety of LP in comatose Malawian pediatric inpatients recruited over consecutive rainy seasons from 1999-2013. Our goal was to assess whether performing an LP changed the odds of mortality within the first 12 hours after the procedure. Propensity score matching was used to estimate the independent effect of LP on outcome. Nine hundred thirty-three children were in coma and had data on all key covariates, of which 718 (77%) had an LP and 215 (23%) did not. Following propensity score matching, all baseline characteristics were balanced between matched pairs of children who did and did not have LPs. After matching, 12 hour mortality was not significantly different between children who did and did not receive lumbar punctures (average treatment effect of receiving an LP was to reduce mortality by 5.3%; 95% CI: -1.1% to 11.6%; P=0.11). Logistic regression, stratification on propensity scores, and inverse probability weighting analyses all showed a trend towards a lower mortality in the group that received an LP. In our study population of African children in coma, performing a diagnostic LP in the absence of prior neuroimaging was not associated with increased mortality risk.

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CURRENT BURDEN OF LIVER ABSCESS AND FACTORS AFFECTING PATIENT SURVIVAL IN THAILAND: NATIONWIDE STUDY

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We aimed to evaluate the burden of liver abscess in Thailand, using information from the 2009-2013 Nationwide Hospital Admission Data, the National Health Security Office (NHSO), Thailand. All patients with a primary diagnosis of pyogenic liver abscess and amoebic liver abscess (ICD10-K750, A064) were included. Epidemiological data, baseline characteristics, hospital course and survival were analyzed. 11,296 admissions comprised of 8,423 patients from 844 hospitals across Thailand were eligible for analysis. The mean age was 52±17 years and 66.1% of patients were male. Most pyogenic liver abscess patients were from the northeastern part of the country (3,157/7,975, 39.6%), and patients with amoebic liver abscess were mostly from the south (205/448, 45.8%). The highest incidence of liver abscess occurred in the rainy season (June to November, p<0.01). The median length of hospital stay was 8 days (IQR 4 to 13 days), and mean cost of hospitalization was 846±1,574 USD. The overall in-hospital mortality rate was 2.8%. Etiology of pyogenic liver abscess could be determined in 1,029 cases. Most common pathogens causing pyogenic liver abscess were ***Burkholderia pseudomallei*** (56.5%), ***Klebsiella*** pneumonia (22.2%), and ***Escherichia coli*** (10%). Of these, ***E. coli*** posed the highest risk of in-hospital mortality (HR 3.38; 95% CI, 1.69-6.76; p=0.001). HIV infection was found to be one of the most significant comorbidities associated with increasing in-hospital mortality (HR 6.57; 95% CI, 3.81-11.33; p<0.001). Overall long-term survival was significantly higher among patients with amoebic liver abscess compared to pyogenic liver abscess (HR 0.54; 95% CI, 0.42-0.68; p<0.001). Incidence of amoebic liver abscess decreased over the five-year study period, while incidence of pyogenic liver abscess increased (p<0.01). Treatment outcomes did not significantly change (p=0.68) but the cost of treatment gradually increased over the five-year period (p<0.01). Direct cost of hospitalization was estimated to 10 million USD. The burden of liver abscess remains a public health problem in Thailand, with an overall mortality rate of 2.8%.

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COMMUNITY BARRIERS FOR THE IMPLEMENTATION OF A MASS DRUGS ADMINISTRATION FOR MALARIA IN THE GAMBIA

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With some countries in Sub-Saharan Africa documenting a decline in malaria transmission, the feasibility of Mass Drug Administration (MDA) for malaria elimination is being reconsidered. For this strategy to work, participation of the target population in excess of 80% is needed. Hence, understanding community perceptions of MDA and response to the intervention is fundamental to ensure its effectiveness. This anthropological study is ancillary to a study aiming to understand the heterogeneity of malaria transmission in the Gambia. Within this study, MDA took place before the rainy season in 12 villages throughout the Gambia. Research was carried out during the MDA and its direct aftermath. Several barriers were identified that influenced the uptake of and adherence to MDA:(i) long and short-term mobility of individuals and groups; (ii) perceived adverse drug reactions and related rumors, (iii) size and taste of the medication, (iv) waiting time and (v) public pregnancy tests to exclude pregnant women. MDA refusers often did not attend the sensitization meetings and misinterpreted essential information about the MDA. Personal characteristics of the distribution team and the level of trust between this team and locals were key factors for participation and adherence. Although community involvement was aimed for during this trial, the lack of active involvement in the set-up of the trial extravagated internal frictions within communities and may have led to refusal to participate or lack of adherence to the medication Formative research may be a useful approach when developing and implementing new interventions as it allows for the identification of potential constraints and enabling factors at community level. By taking into account local perceptions about the roll-out of MDA, treatment preferences and rumors caused by perceived side effects and internal divisions within local communities, the implementation of trials may be improved and internal conflicts enhanced by MDA procedures prevented.

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ANTIMALARIAL TREATMENT-SEEKING AND USE IN SOUTHERN MALAWI: CHALLENGES TO INTERRUPTION OF *PLASMODIUM* TRANSMISSION

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Antimalarial treatment is key for clearing ***Plasmodium*** infections, which can persist for months. We studied treatment-seeking in southern Malawi to assess the proportion of the infectious reservoir that seeks and obtains treatment. School-aged children (6-15 yrs) have the highest infection prevalence in this area. Each of four cross-sectional surveys over two years included ~3,000 people from 900 households. All members were asked about fever and treatment-seeking in the past two weeks; those present had blood sampled for PCR testing for ***P. falciparum***. Multilevel logistic regression was used to analyze predictors of whether febrile people sought treatment, did so at government/private clinics vs. other sources (shops, community health workers, etc.), and reported taking antimalarials. Of 12,652 interviewed, 87.1% reporting recent fever sought treatment, but the proportion decreased with older age. School-aged children and adults (≥16 yrs) were less likely to attend government/private clinics than young children (≤5 yrs). Antimalarial use was more common among febrile people who visited government/private clinics (42.5%) than those going to other sources (7.9%), with diagnostic testing performed on 47.5% of people at government/private clinics and only 2.8% at other sources. These treatment patterns may contribute to high infection prevalence in school-aged children. Afebrile people rarely sought treatment (5.5%); of 1,616 people who were PCR-positive for ***P. falciparum*** when interviewed, only 24.4% sought treatment in the past two weeks. Of people who sought treatment but did not receive antimalarials, 6.9% from government/private clinics and 16.8% from other sources were PCR-positive for ***P. falciparum*** when surveyed. Treatment of symptomatic malaria is common in southern Malawi, but current practices miss a substantial portion of human infectious reservoirs. Encouraging treatment at government/private clinics, especially among school-aged children, could help reduce the high prevalence in this group and potentially decrease transmission; additional intervention will be needed to address the asymptomatic reservoir.

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EVALUATING HIGH-RISK VENUE-BASED MALARIA SURVEILLANCE USING TIME-LOCATION SAMPLING IN NAMIBIA

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While Namibia has experienced a rapid decline in reported malaria cases, from 538,512 cases in 2001, to 3163 cases in 2012, low transmission persists in the north of the country. As part of efforts to eliminate malaria by 2020, targeted active surveillance strategies are being evaluated to identify risk factors and undiagnosed and asymptomatic infections, which may serve as a reservoir driving ongoing transmission. We developed a novel active, venue-based surveillance strategy to target individuals who congregate outdoors or partially outdoors at specific locations ("venues") during evening and morning mosquito biting hours. Venues were selected within the catchment areas of 6 randomly selected health facilities in the Zambezi Region, which borders Zambia and Angola. Focus groups and interviews were conducted with community members to develop an exhaustive map of venues in the selected areas, estimate peak attendance times, and elicit recommendations on study design. Mapping identified 24 shabeens (bars), 6 churches, and one fishing camp with regular evening or morning attendance. Drawing on these findings, a sample frame of venue-day-time intervals (VDTs) was constructed. Local field teams visited randomly selected VDTs and recruited a random selection of 750 venue-goers over a 2 month period. Study participants completed an interviewer-assisted survey, provided dried blood spot samples, received testing by RDT, and treatment if positive. In this presentation we discuss the feasibility of this active surveillance strategy by exploring participation rates, self-reported venue attendance patterns, prevalence of infection by RDT and PCR, and socioeconomic and behavioral risk factors. Estimates were adjusted for probability of selection and clustering by VDT. Prevalence of infection and risk factors are compared to a contemporaneous household survey of 10% of households in the same catchment areas. These methods provide a potentially simple surveillance strategy for accessing individuals at high risk of malaria transmission who may be missed by household level surveillance.

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MALARIA ELIMINATION IN ACEH, INDONESIA: USING LAMP IN REACTIVE CASE DETECTION TO INCREASE INFECTION DETECTION

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Reactive case detection (RACD), whereby household members and neighbors of passively-detected malaria cases are screened for infection, is a WHO-recommended strategy for malaria elimination, however there are limited data to guide practice, particularly from mixed ***Plasmodium*** species settings. We introduced the use of molecular detection for RACD of malaria in Aceh Besar District, Aceh Province, Indonesia and compared infection detection to currently used RACD practices based on microscopy. Microscopy-confirmed index cases identified at five sentinel health facilities received a follow-up investigation in their home, and family members and neighbors were targeted for blood testing by microscopy and the collection of dried blood spots for testing by high sensitivity ***Plasmodium***-specific loop-mediated isothermal amplification (LAMP). From June 2014 to March 2015, we enrolled 21 index cases which led to the screening of 778 household members and neighbors (median 38/index case, mean 35/index case). Positivity rate by microscopy was 0.4% (3/778) versus 0.6% (5/778) by LAMP (p=0.16), with LAMP detecting 1.5 fold more infections than microscopy alone. Compared to passive surveillance alone, whereby 21 cases were identified, RACD using microscopy increased the detection of infections by 1.1 fold, and RACD using LAMP increased the detection of infections by 1.2 fold. Of the five additional infections found by slide and/or LAMP from RACD, speciation by species-specific PCR showed 1 ***Plasmodium falciparum***, 3 ***P. vivax***, and 1 ***P. knowlesi***. The speciation for three infections (1 Pf, 1 Pv and 1 Pk) matched that of the index case infection and two did not (1 Pv was associated with a Pf index case and 1 Pv was associated with a Pk index case). Although the difference was not significant, RACD using LAMP, compared to RACD using microscopy, increased the detection of infections in a low transmission, mixed ***Plasmodium*** species setting. The study is ongoing and further enrollment of subjects may strengthen findings and potentially show declines in incidence.

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WHAT MAKES A MALARIA HOTSPOT A HOTSPOT? EXAMPLE FROM ZANZIBAR

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Health facility based, passive case detection surveillance systems have been in place in Zanzibar since 2007. Spatial analysis of this data revealed ***foci*** of clinical malaria cases which were present every transmission season. In order to investigate why these areas consistently record higher numbers of malaria cases, a cross-sectional Knowledge, Attitudes, Practices and Behaviour (KAPB) study was undertaken in all 30 hotspot villages (N of households=1,036), as well as from 30 randomly selected control villages (N of households=1,117). In order to determine current prevalence all household members were tested using rapid diagnostic test (RDT) or RDT and loop-mediated isothermal amplification (LAMP), to assess the proportion of low-density parasitemias present. Entomological indices were also collected. Analyses are still ongoing. Prevalence of infection was low (0.3% by RDT, 1.6% by LAMP) and did not differ in hotspots and controls. Preliminary results show that 60% of study participants reported sleeping under a net the previous night. Coverage and usage of nets did not differ between hotspot villages and controls. Households in hotspot villages were more likely to have been sprayed in the previous 6 months (55% compared to 31%, p<0.001), highlighting the targeted nature of spray campaigns in Zanzibar. People living in hotspot villages were more aware of malaria (transmission season, symptoms, causes) but appeared to have less faith in the protective effect of sleeping under nets and were more likely to wait more than 2 days before presenting to a health facility with a fever (p=0.02). More Anopheline mosquitoes were found in hotspot households than controls, with outdoor biting prominent in both. Examining KAPB of residents living within malaria ***foci*** may help to elucidate reasons for the higher burden of infection and subsequently enable interventions to be adapted or targeted as required.

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WHEN SUSTAINABILITY FAILS: THE IMPACT ON ELIMINATION

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As many countries now target elimination of malaria, whole regions are harmonising policies and regimens in order to present a united front in pushing back the frontiers of malaria distribution. This is most apparent in southern Africa where a number of multi-country initiatives are in place to increase the pace for elimination. The Lubombo Spatial Development Initiative (LSDI) was one such initiative. It aimed to accelerate socio-economic development in parts of South Africa, Swaziland and Mozambique but malaria was an impediment to this goal and the LSDI malaria control component was established. During its lifetime, the LSDI achieved remarkable results but unfortunately the programme was not sustainable and terminated after 12 years, affecting the elimination efforts in South Africa and Swaziland. To examine the impact of this failure in sustainability, retrospective data from the LSDI era was compared to current data (when no LSDI initiatives were implemented). Indoor Residual Spray (IRS) coverage gave an indication of the effectiveness of the vector control intervention in place. Impact was measured as the parasite prevalence rate in Mozambique and the incidence rate in South Africa and Swaziland. During the life of the LSDI, there was a rapid decline in prevalence in Mozambique from an average prevalence of 70% in 2000 to 5% in 2010. The area under control increased and the IRS implemented was at a high rate of coverage. In South Africa and Swaziland, the incidence of malaria also decreased by 98% within 3 years. When IRS was no longer implemented in Mozambique, the malaria cases rose from 5% up to 14% in three years and the incidence increased to >1% in districts of Mpumalanga and Limpopo Provinces. In South Africa and Swaziland, imported malaria increased and resulted in focal outbreaks of malaria. Malaria cases decreased when IRS was implemented in a co-ordinated manner. However when the interventions were no longer applied, the malaria cases reached the same level as pre-intervention prevalence. Failure of control in one country can hamper the elimination agenda of surrounding countries.

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REACTIVE CASE DETECTION WITH TARGETED MASS DRUG ADMINISTRATION FOR MALARIA ELIMINATION IN NORTHWESTERN PERU

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Reactive case detection (RCD) with targeted mass drug administration (tMDA) has been proposed as an effective strategy for malaria elimination, although little is know about its effectiveness. In this study, we estimated the effectiveness attributable to RCD/tMDA as compared to passive case detection (PCD) on reducing the incidence of malaria to zero in Tumbes, Peru. During years 2009-2010 we piloted a malaria-elimination programme based on RCD/tMDA in the two most malaria-endemic districts in Tumbes; then, during years 2011-14 we scaled up to the other 11 districts in Tumbes. Under RCD/tMDA every malaria case that was detected passively was followed within the first 24 hours to census and treat each of his/her household contacts, excluding elders, pregnant women, and chronically-ill subjects. Then each eligible subject was treated with chloroquine (25mg/kg, total dose) over 72 hours plus primaquine (0.5mg/kg) taken orally for 7 days. The primary study endpoint was a 50% reduction in the annual parasite incidence (API = total malaria cases/ 1,000 inhabitants) at the surveillance-reporting units level, analyzed by intention to treat. The Peruvian Ministry of Health sponsored the study and the Ecuadorian government donated 20,000 vivax malaria treatments. During the pilot study we treated a total of 8,243 subjects, including 7376 household contacts. The estimated reduction in the mean API across intervened and non-intervened reporting units was 86.2% (95% CI 72–100) at 12-mo and 97.9% (93–100) at 24 months, and -230.9% (95% CI -395–-66) at 12-mo and -19% (-87– 49) at 24-mo, respectively. When comparing those mean API reductions we found that in both cases, at 12-mo (p=0.02) and at 24-mo (p=0.04) the differences were statistically significant. Any antimalarial adverse events were reported during study period. After scaling up, the APIs dropped to 3.1 and 0.4 in years 2011 and 2012, with zero cases in year 2013, and one imported case in year 2014. Hereby, we concluded that RCD/tMDA represent an effective strategy to support malaria elimination initiatives in region with high predominance of vivax malaria such in northwestern Peru.

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RATS FOR SALE: INVESTIGATING THE SCOPE AND PUBLIC HEALTH CONSEQUENCES OF RODENT CONSUMPTION IN NORTHERN VIETNAM

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In Vietnam, rodents are frequently trapped on rice fields before and after harvesting and are sold live in wet markets for food consumption. The rodent trade in the southern Mekong Delta is vigorous, with an estimated 3300-3600 tonnes of rodent meat sold per year. We hypothesize the presence of significant regional differences in rodent trade between northern and southern Vietnam, reflecting differences in rodent density, rodent species diversity, seasonality, culinary traditions, and variable risk perceptions. As part of a larger study on zoonotic disease transmission at the human-animal interface in Vietnam, we have conducted a systematic survey of wet markets in the northern province of Hanoi, to identify sites where rodents are sold for human consumption; determine the common species sold in markets, and estimate the volume of trade; characterize seasonality and fluctuations in rodent trade over the course of one year; and to obtain samples from epidemiologically linked humans and rodents for further molecular and serological analysis of microbial ecology. To date, although our results from various study sites in Vietnam confirm low-to-moderate levels of endemic circulation of rodent-borne viruses and bacterial agents (e.g. hantaviruses, flaviviruses, parechovirus, Bartonella, Leptospira), there is little evidence to suggest a public health risk associated with rodent meat consumption. These ongoing investigations and analyses will be discussed.

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ANTHROPOGENIC DISTURBANCE, SPECIES DIVERSITY AND RISK OF RODENT-BORNE DISEASES IN MADRE DE DIOS, PERU

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Anthropogenic disturbance of natural ecosystems may result in biodiversity loss with altered rodent population dynamics and risk of rodent-borne disease to humans. Influencing factors include rodent population density, environmental conditions and food availability, and human behavior. Here we describe the preliminary findings of a 5-year study on the effects of anthropogenic habitat perturbation and risk of rodent-borne diseases in 4 communities along a highway recently built through the Madre de Dios Region in the southern Amazon Basin of Peru. At each site, live traps were placed in six 7x7m grids (49 trap stations per grid) selected, with the assistance of satellite imagery, to represent varied habitat disturbance: 2 in non-disturbed forest, 1 in disturbed areas (crop field, pasture or other) and 3 in edge areas. Trapping was conducted every 4 months to cover dry, rainy and mid-seasons. Blood was taken before marking and releasing rodents, with the exception of the last day of trapping, when necropsies were performed and organs obtained. From the first 4 trapping sessions, a total of 732 animals were captured, of which 129 were recaptures. The 603 individual rodents captured comprised 10 genera and 16 species, including ***Oligoryzomys microtis*** (43.4%), ***Necromys lenguarum*** (10.4%), ***Euryoryzomys nitidus*** (10.0%) and ***Hyaleamys perenensis*** (8.9%). We found ***E. nitidus*** more frequently in edge areas (51/75), similarly to ***N. lenguarum*** (40/78) and ***O. microtis*** (250/324), in contrast to ***H. perenensis*** which was more frequently found in non-disturbed areas (42/67). Simpson's biodiversity indices were higher for edge areas. The highest trap success was in the dry season, when scarce food availability likely led rodents to extend foraging into traps. Laboratory testing for pathogens is underway but, notably, three of the captured rodent species have been previously reported to carry hantaviruses. Full results will be presented at the meeting.

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THE EPIDEMIOLOGY OF Q FEVER IN HUMANS AND CATTLE, WESTERN KENYA

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Evidence suggests that the intracellular bacterial pathogen Coxiella burnetii (which causes Q fever) is widespread, with a near global distribution. While there has been increasing attention to Q fever epidemiology in high-income settings, a recent systematic review highlighted significant gaps in our understanding of the prevalence, spatial distribution and risk factors for Q fever infection across Africa. This research aims to provide a One Health assessment of Q fever epidemiology in western Kenya in cattle and humans. A cross-sectional survey was conducted: serum samples from 2113 humans and 983 cattle in 416 homesteads were analysed for C. burnetii antibodies. Questionnaires covering demographic, socio-economic and husbandry information were also administered. These data were linked to environmental datasets based on geographical locations (e.g. land cover). Multilevel regression analysis was used to assess the relationships between a range of socio-economic, demographic and environmental factors and sero-positivity in both humans and animals. The overall sero-prevalence of C. burnetii was 2.5% in humans and 10.5% in cattle. Multilevel modelling indicated the importance of several factors for exposure to the organism. Cattle obtained from market (as opposed to those bred in their homestead) and those residing in areas with lower precipitation levels had the highest sero-prevalence. For humans, the youngest age group had the highest odds of seroprevalence, variations were observed between ethnic groups, and frequent livestock contact (specifically grazing and dealing with abortion material) was also a risk factor. These results illustrate endemicity of C. burnetii in western Kenya, although prevalence is relatively low. The analysis indicates that while environmental factors may play a role in cattle exposure patterns, human exposure patterns are likely to be driven more strongly by livestock contacts. The implication of livestock markets in cattle exposure risks suggests these may be a suitable target for interventions.

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MAPPING LEPTOSPIROSIS HOTSPOTS IN FIJI USING ENVIRONMENTAL, SOCIOECONOMIC AND LIVESTOCK DATA: AN ECO-EPIDEMIOLOGICAL APPROACH TO EMERGING INFECTIOUS DISEASE CONTROL

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Leptospirosis is an emerging infectious disease in the Pacific Islands. In Fiji, two successive cyclones and severe flooding in 2012 resulted in outbreaks associated with 10% case-fatality. A seroprevalence study was conducted in 2013 to better understand environmental drivers of leptospirosis emergence in Fiji. Antibodies indicative of previous leptospirosis infection were found in 19.4% of 2152 participants (82 communities on the 3 main islands). Questionnaires and geographic information systems data were used to assess risk factors related to individual behavior, exposure to animals, socioeconomics, land use, and natural environment. Significant individual-level risk factors include male, ethnicity, working outdoors, swimming in rivers, and physical contact with rodents. A multivariable logistic regression model was able to correctly classify the presence/absence of previous infection in >80% of participants using only six community-level variables: rainfall in the wettest month (OR 1.004 per mm, p <0.01); living <100m from a major river (OR 1.46, p <0.01); poverty rate (OR 1.46 for the poorest 25%, p 0.03); living in rural area (OR 1.75 compared to urban, p <0.01), pigs in community (OR 1.64, p <0.01); and cattle density in district (OR 1.03 per head/sqkm, p 0.02). The model was used to predict community-level seroprevalence, and produce a predictive risk map of leptospirosis for Fiji to identify hotspots and inform public health interventions. Predicted community seroprevalence was highly correlated with levels observed in the 2013 field study (cor coeff 0.73). Leptospirosis transmission in Fiji is complex and multifactorial, disproportionately affecting the poorest. Predictive accuracy of the model highlights the importance of environmental and socioeconomic drivers of transmission. With global climate change, extreme rainfall and cyclones are expected to intensify in the South Pacific. Livestock farming and commercial agriculture are also expected to increase with population growth. These factors could combine to drive increasing incidence of leptospirosis if risks are not properly managed and mitigated against.

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CHAGAS DISEASE EPIDEMIOLOGY AND OUTREACH IN HIGH-RISK HUMAN AND CANINE POPULATIONS ALONG THE U.S.-MEXICO BORDER

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Chagas disease is a cardiac disease of humans and dogs caused by the parasite ***Trypanosoma cruzi***. The disease is most prevalent across Latin America in areas where the Triatomine 'kissing bug' vectors colonize the home. Despite the recognition of established enzootic cycles of parasite transmission involving wildlife in the southern United States, the human and veterinary health burden of Chagas disease in this area is largely unknown, although there are increasing diagnoses in immigrants and locally-exposed persons as well as privately-owned, stray, and working dogs. With a One Health framework involving collaborators from the medical, veterinary, agriculture, architecture, geoscience, and science colleges, we studied the epidemiology of Chagas disease in medically-underserved human and canine populations in the south Texas colonias. Colonias are impoverished communities along the US-Mexico border that lack some of the most basic living necessities, such as sewer systems, electricity, and sanitary housing, and may therefore be at high risk for vector exposure. During the summer activity season of kissing bugs, we conducted a cross-sectional study to determine the associations among human and animal infection, vector occurrence, and socioeconomic and environmental features. We collected human and canine blood samples in four colonias using a door-to-door approach that also allowed the opportunity for characterization of the peridomestic environment. Additionally, we implemented a kissing bug citizen science program including a new kissing bug mobile app, given the widespread use of smart phones in these communities, to empower the public with Chagas disease knowledge while allowing them to submit kissing bugs for identification and testing. Preliminary data from the area indicate over 6% of local dogs arriving at the major animal shelter in the region were exposed to ***T. cruzi***. Our citizen science program has resulted in over 2,000 bugs submitted across Texas, characterized by >60% infection prevalence. Key findings, including risk factors for human and canine ***T. cruzi*** infection will be presented.

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HAS RIFT VALLEY FEVER VIRUS EVOLVED TO INCREASED VIRULENCE IN HUMAN POPULATION AFTER EXISTENCE FOR A CENTURY IN EAST AFRICA?

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Rift Valley Fever (RVF) outbreaks have occurred across East African countries from 1912 to 2010 in cycle of 4-15 years. RVF virus becomes enzootic/endemic once it is introduced into a certain permissive ecologies, causing periodic outbreaks with the potential to spread further to non-endemic/enzootic environments with favorable conditions. In Kenya, of 22 RVF outbreaks from 1912 to 2007, 14 were national affecting 3 -38/69 districts while 8 were localized to 1 - 2/69 districts. Unlike other parts of Africa, most of these outbreaks were epizootics in early 1900s and were not accompanied by significant epidemics in human populations in East Africa. However, in 1998, RVF outbreaks in the horn of Africa led to 478 deaths, followed by further epidemics a decade later, in 2006-2007, during which 1,107 human cases were reported with 350 deaths. In 2008, comparable epidemics occurred in Sudan (698 human cases; 222 deaths) and Mozambique (412 human cases, 17 deaths). Comparisons between isolates from different outbreaks reveal specific genetic mutations and reassortments that have diversified RVF virus genomes over the past century. Although genetic diversity of RVF virus appears to be low (~5%), these changes in combination with the accumulation of mutational changes over the years could have influenced RVF virus host preference and virulence in human populations. Factors such as underreporting, lack of appropriate diagnostic kits, under-recognition of clinical signs, changes in case definitions could have influenced paucity of information on human RVF in early 1900s. However, the explosive nature and pronounced RVF epidemics in humans in recent decades became so overwhelming to attribute the obvious changes to improvement in the above mentioned factors. We speculate that the evolutionary diversification of the virus could have resulted in distinct lineages with increased and possibly increasing virulence and pathogenicity in humans. The emerging infectious disease threats posed by the modern RVF virus strains to humans increases the potential public health and socio-economic impacts of future RVF epidemics.

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RETROSPECTIVE ANALYSIS OF HUMAN-BONOBO ZOONOTIC PATHOGEN TRANSMISSION IN THE DEMOCRATIC REPUBLIC OF CONGO

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Forest destruction and the rampant bushmeat trade in the Democratic Republic of Congo (DRC) have increased proximity between human and African great ape populations. Because humans and great apes are genetically similar, known and emerging pathogens can opportunistically transmit between hosts in close contact, further endangering remaining ape populations and potentially instigating human epidemics that could weaken the DRC's already fragile healthcare infrastructure. Zoonotic transmissions are well documented between chimpanzees and humans, but few data exist on transmission between humans and bonobos (Pan paniscus), To introduce bonobos, found only in the DRC and the least studied of the great apes, into the growing body of zoonotic disease research, we examined fifteen years of veterinary records from 125 rescued orphan bonobos rehabilitated at a sanctuary outside the densest city in the DRC. Common clinical syndromes described in the records included gastrointestinal illnesses, skin infections, and injury. Respiratory illness accounted for 52% of the 1,481 reports and 31% of all deaths (n=16) since the sanctuary's inception. In contrast, respiratory illness is infrequent among wild bonobo populations with limited human contact. Temporal correlations between bonobo and human respiratory outbreaks reported through Ministry of Health surveillance from 2006 to 2011 suggest transmission of respiratory pathogens from humans to bonobos. The results of this study have implications for increased safety and hygiene practices at rehabilitative sanctuaries to reduce the introduction and spread of respiratory pathogens, with larger implications for limiting the increasingly inevitable contact between humans and wild bonobo populations as the landscape of the DRC continues to change.

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INNOVATIVE 2-D AND 3-D *IN VITRO* CULTURING SYSTEMS FOR *ONCHOCERCA VOLVULUS*

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Elimination of Onchocerciasis where mass drug administration with ivermectin is not sufficient (loasis co-endemic regions or areas where suboptimal responses to ivermectin are observed) will require a drug that kills adult ***Onchocerca volvulus*** worms (macrofilaricidal drugs). The macrofilaricidal drug discovery efforts are limited in part by having access to only surrogate filarial adult worms isolated from infected animals (e.g. cows: ***O. ochengi***, ***O. lienalis***, ***O. gibsoni*** or ***O. gutterosa***; gerbils: ***Brugia malayi***, ***B. pahangi***). Thus far, the life cycles of ***Onchocerca*** and ***Brugia*** spp. have not been completed ***in vitro***. At most, partial molting of the infective third-stage larvae (L3) to the fourth-stage larvae (L4) has been attained ***in vitro;*** within a week for ***O. volvulus*** and 2 weeks for ***Brugia***. To address the lack of access to adult ***O. volvulus*** worms, we developed a 2-Dimensional (2-D) ***in vitro*** monolayer culture and an innovative 3-D culturing system that provided supportive microenvironments for the growth and development of ***O. volvulus*** L3 to the L5 adult female and male stages. Four human cell lines (HUVEC, NHEK, HDFB, and HSMC) supported the growth and development of ***O. volvulus*** L3 (~350 µm) to female and male L5 worms after 100 days in 2-D cultures. These worms ranged in length from 1400-1800 µm and most likely consisted of both sexes since male worms are generally shorter in length compared to female worms. The 3-D scaffold was made of PET fibers infused with NHNDF, MVE and LMVE dermal cells and supported the growth of L3s to L5s that were >2,000 µm in length after 100 days. Initial screening of repurposed drugs with such L5s demonstrated that these pre-adult ***O. volvulus*** could be used to identify novel macrofilaricidal drugs. Once the culture system is optimized, we anticipate that we will be able to provide large quantities of adult ***O. volvulus*** worms to routinely screen for novel macrofilaricidal drugs and thereby accelerating the drug discovery program aimed to support the elimination of onchocerciasis.

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TRANSCRIPTOMIC ANALYSIS FOR IVERMECTIN-TREATED ADULT *BRUGIA MALAYI*

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Lymphatic filariasis (LF) is a disabling and disfiguring neglected tropical disease caused by three filarial nematodes (***Wuchereria bancrofti***, ***Brugia malayi*** and ***B. timori***) which are transmitted by mosquitos. The presence of adult worms in the lymphatic system can lead to progressive damage and pathology. Presently, 120 million people are infected and 1.4 billion people are targeted for annual mass drug administration (MDA) in an attempt to eliminate this disease by 2020. The MDA strategy is to block parasite transmission by using combinations of albendazole and diethylcarbamazine or, in areas co-endemic for onchocerciasis, albendazole and ivermectin. Paralysis of pharyngeal pumping by ivermectin could result in deprivation of essential nutrients, inducing a wide range of starvation responses, including altered gene expression and biochemical activities, alternative developmental states in intrauterine larvae and altered physiological responses. Previous studies have shown that ivermectin treatment significantly reduces microfilariae release from females within four days of exposure; however, the mechanisms responsible for reduced microfilaria production are not well understood. In this study, we analyzed transcriptomic profiles from ivermectin-treated and -untreated ***B. malayi*** adult females using Next Generation RNA Sequencing technology at different concentrations (100 nM, 300 nM and 1 uM) and time points (24, 48, 72, and 120 hours). Our analysis revealed altered expression of multiple genes involved in fertility, embryogenesis and larval development, which were significantly down-regulated as early as 24 hours post-exposure. These changes reflect and provide insight into the mechanisms involved in ivermectin-induced reduction in microfilaria output and impaired embryogenesis. RNA interference phenotypes of the homologs of these genes in C. elegans include maternal sterile, embryonic lethal, larval arrest, larval lethal, reduced brood size and egg shape variable.

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RATIONAL DESIGN FOR OXAMNIQUINE DERIVATIVES THAT KILL SCHISTOSOMES

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Human schistosomiasis is a disease caused by species of the genus ***Schistosoma***, which globally affects over 200 million people. The major species affecting humans are ***S. mansoni***, ***S. haematobium***,

and ***S. japonicum***. There is currently only one method of treatment (monotherapy), the drug Praziquantel. Constant selection pressure through mass chemotherapy - this year alone will see the administration of over 250 million doses - has yielded evidence of resistance to PZQ. This has been observed in both the laboratory and field. The goal of this research is to develop a second drug for use in conjunction with PZQ. Previous treatment of ***S. mansoni*** included, among others, the use of oxamniquine (OXA), a prodrug that is enzymatically activated in ***S. mansoni*** but is ineffective against ***S. haematobium*** and ***S. japonicum***. The OXA activating enzyme was identified, described, and crystallized by our laboratories as being a sulfotransferase (SmSULT). The focus of this research is to reengineer OXA to be effective against ***S. haematobium*** and ***S. japonicum***. In this regard we isolated the ***S. haematobium*** (ShSULT) and ***S. japonicum*** (SjSULT) sulfotransferases. Thirty OXA derivatives were synthesized, of which eight showed schistosomicidal activity as good as or better than OXA that may potentially be used to treat schistosomiasis mansoni. ***In vitro*** tests demonstrated that some of these 8 derivatives had activity against ***S. haematobium*** and ***S. japonicum***. This iterative process of using structural data to inform chemical synthesis of derivatives, which are then tested ***in vitro***, continues to provide us with novel compounds with improved anti-schistosomal activity. The information gleaned from these early studies will be used to optimize OXA derivative design. The most active derivatives will be used in an ***in vivo*** model of schistosomiasis to evaluate efficacy before moving to safety and toxicity studies.

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STAGE-SPECIFIC PROFILING OF THE SOMATIC PROTEOMES AND SECRETOMES OF *ONCHOCERCA VOLVULUS*

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Stage-specific proteomes of filarial parasites have thus far been limited to ***Brugia malayi*** and ***Onchocerca ochengi*** as a surrogate for ***O. volvulus*** (Ov) - the causative organism of onchocerciasis ('river blindness'). Here we report the stage-specific somatic proteomes of the adult male and female worms, embryonic stages (EMB), microfilariae, infective L3 larvae, larvae undergoing molting and L4 larvae along with the secretomes of adult male (OvAM) and adult female parasites (OvAF). Global proteomic analyses were performed using Thermo Easy nLC 1000 UPLC interphased via a Thermo Easy Spray ion source to a Thermo Q-Exactive quadrupole-Orbitrap mass spectrometer. Spectral matching with combined databases of predicted proteomes of Ov, its endosymbiont Wolbachia (wOv) and humans resulted in the identification of ~56% (6821) of the predicted proteome of ***O. volvulus*** and ~69% (571) of wOv proteome. Though relative abundance varied, a small fraction ~ 5% (327) of the identified proteins were commonly found across all stages. Preliminary analyses suggest immunologically related, detoxification and protein modification associated proteins to be differentially expressed and enriched in the L3, adult females and microfilarial stages respectively. Surprisingly, despite EMB being derived from the adult female uterus, they had the highest degree of discrimination power among stages (384) with very few proteins unique to both EMB and OvAF being identified (58). Developmentally, stage-specific expression of 127 proteins were identified during the L3 to L4 molting process, some of which are well-characterized cysteine proteases, cystatins and serpins. More importantly, over 200 proteins were identified as specific to OvAF, proteins that were also identified in the secretome of OvAF. These data not only provide a comprehensive proteomic resource for understanding host-parasite interactions, but also provide a platform for the interrogation of candidate proteins that can support the ongoing quest for biomarkers (particularly for viable OvAF) during post-control surveillance in Ov-endemic areas.

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"EXTREME" QTL METHODS FOR GENETIC MAPPING FOR DRUG RESISTANCE AND HOST SPECIFICITY IN SCHISTOSOME PARASITES

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Classical quantitative trait locus (QTL) analyses, using controlled genetic crosses or natural pedigrees, together with phenotyping and genotyping of parents and progeny, has been used for QTL localization for almost a century and has been applied to multiple species ranging from yeast to humans. Extreme QTL (X-QTL) methods, developed by researchers working on yeast and malaria parasites, takes several short cuts to simplify QTL mapping. Instead of phenotyping, pooled F2 progeny are selected for the trait of interest, while instead of genotyping individual progeny, pools of selected or unselected progeny are quantitatively genotyped (or sequenced) to measure allele frequencies genome-wide. We first applied X-QTL to examine the genetic basis of drug resistance in a multicellular parasite, ***Schistosoma mansoni***, which infects an estimated 67 million people in Sub-Saharan Africa and South America. We conducted genetic crosses between oxamniquine resistant and sensitive parasites, and selected pools of F2 progeny with oxamniquine while controls were left untreated. Surviving drug-treated and control pools were then sequenced to high read depth (mean = 95-366×) across the genome using exome capture, Illumina sequencing and allele frequencies at 14,489 variants were compared between pools. We observed dramatic enrichment of alleles from the resistant parent in a small region of chromosome 6 in drug-treated male and female pools (combined analysis: Z = 11.07, p = 8.74 × 10-29). This region contains Smp_089320 a gene encoding a sulfotransferase recently implicated in oxamniquine resistance using classical linkage mapping methods, directly validating X-QTL methods for schistosomes. Encouraged by this success, we are currently applying the X-QTL approach to identify the genes underlying host specificity of larval schistosomes to the aquatic snail host.

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SCHISTOSOMA MANSONI EXPOSURE MODIFIES PLASMA EXOSOMAL MIRNAS INTERCELLULAR COMMUNICATIONS BETWEEN PARASITE AND HOST

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Schistosomiasis is a water-borne parasitic disease of major public health importance. More than 4.5 million disability adjusted life years (DALYs) are lost each year worldwide due to schistosome infection. More than 90% of reported cases are from sub-Saharan Africa where both ***Schistosoma mansoni*** and ***S. haematobium*** infections are endemic. The major of control programs use the antihelminthic drug praziquantel for mass drug administration. MicroRNAs are small non-coding RNAs transported in exosomes, and as such schistosome miRNAs may serve as an efficient cell-to-cell communication system linked to the host. miRNAs also have potential as novel therapeutic tools for diseases associated with inflammatory responses. Here we show differential expression of schistosome exosomal miRNAs in ***S. mansoni***-infected mouse plasma, including miR71, bantam, miR125 and miR-new 1, at various time point post exposure up to 7 weeks. Given that ***S. mansoni*** infection-associated inflammation is thought to potentially increase risk of colorectal cancer, we are currently evaluating the expression of putative miRNA biomarkers for inflammation/cancer, e.g. miR-let 7a, miR-150, and miR 21. We have

found that exosome-associated miRNA profiles shift significantly in mice at 2 weeks post exposure. Schistosome-specific miRNAs (miR-bantam and miR125) were increased after 2 weeks. On the other hand, miRNA let-7a and miRNA 150 were increased during chronic parasite infection. These results strengthen the notion that schistosomes secrete miRNA-containing exosomes into the host circulation, and that miRNAs may be highly sensitive biomarkers for infection status, including ***in vivo*** schistosome growth, development, and egg production. Our ongoing work may facilitate the development of highly sensitive diagnostics, miRNA-based blockade of schistosomiasis transmission, and even nanotechnology-centered therapeutics. Moreover, a deeper understanding of exosomal miRNAs as cancer markers would be useful for identifying new therapeutic targets for inflammation-induced carcinogenesis in chronic schistosomiasis and other helminth infection-associated malignancies.

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MEASURING POPULATION HEALTH: COSTS FOR ALTERNATIVE SURVEY APPROACHES IN THE NOUNA HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN RURAL BURKINA FASO

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To improve data quality and collection efficiency in the Nouna Health and Demographic Surveillance System (HDSS) in Burkina Faso, stand-alone data collection activities of the HDSS and the Household Morbidity Survey (HMS) were integrated, and the paper-based questionnaires were consolidated into a single tablet-based questionnaire, namely the Comprehensive Disease Assessment (CDA). This study aims to assess the comparative implementation costs of the two different survey approaches to measure health at the population level. Financial costs of stand-alone (HDSS and HMS) and integrated (CDA) surveys were estimated from the perspective of the implementing agency. Fixed and variable costs of survey implementation and key drivers of costs were identified, and costs per household visit were calculated for both survey approaches. While fixed costs of survey implementation remained similar across the two survey approaches, there were significant variations in variable costs, resulting in an estimated annual cost-saving of about US$45,000 with the integrated survey approach. This was primarily because costs of data management for the tablet-based CDA survey were significantly lower than the paper-based stand-alone surveys. The integrated survey approach was estimated to reduce the cost per household visit from US$25 to US$21 to collect the same amount of information from 10,000 HDSS households. In conclusion, the CDA survey appears to be a feasible and efficient method of data collection in the Nouna HDSS in rural Burkina Faso. The tablet-based data collection platform is likely to increase the quality of population and health data collected and this should be further explored.

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THE MILLENNIUM DEVELOPMENT GOALS AND DEVELOPMENT ASSISTANCE FOR HEALTH, 1990-2014

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Millennium Development Goals (MDGs) four, five and six focus on health in low- and middle-income countries. This presentation will present findings related to tracking the development assistance for health (DAH) and explore the relationships between the establishment of the MDGs, the scale-up in funding, and the attainment of the health targets. We extract data from the Institute for Health Metrics and Evaluation's Financing Global Health 2014 report. This report systematically tracks DAH from 1990 to 2014. We assess the allocation of DAH across 15 health focus areas, and compare the DAH received by countries likely to achieve each MDG to the DAH received by countries that are unlikely to achieve them. We use linear regression to test whether the onset of the MDGs was associated with a systematic shift in DAH disbursements. Since 2000, $227.9 billion of DAH has been provided for the MDG health focus areas, amounting to $24.3 billion in 2014 alone. DAH disbursements for the MDG causes grew by 13.9% annually between 2000 and 2010, while annual growth for these same health focus areas was only 7.4% from 1990 through 2000. DAH for HIV/AIDS grew 15.8% over 2000 to 2014, amounting to $10.9 billion in 2014. DAH for malaria and tuberculosis funding increased annually at 17.4% and 17.7% over the same period, reaching $2.4 billion and $1.4 billion, respectively. DAH for maternal, newborn, and child health grow at an annualized rate of 6.2%, with spending of $9.7 billion in 2014. Countries expected to achieve each goal have received on average more DAH for the associated health focus areas than those not expected to achieve a given MDG. Linear regression shows that in 2000 the annual increase of MDG-related DAH amounted to 324%, a statistically significant change. Still, growth slowed in 2010. If DAH had continued to climb from 2011 to 2014 as it had the decade prior, an additional $27.4 billion of DAH would be available for the MDG health focus areas. With the post-MDG era in sight, these trends and the concurrent evolution in burden of disease hold lessons for future global health ambitions.

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THE SOCIOECONOMIC IMPACT OF INTERNATIONAL AID: A QUALITATIVE STUDY OF AID AND HEALTHCARE RECOVERY IN POST-EARTHQUAKE HAITI

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International aid spending for healthcare has nearly quadrupled over the last two decades, but how these efforts are perceived by the recipient country in post disaster settings remains unclear. We assessed healthcare provider perspectives of international aid four years after the Haiti Earthquake to better understand the impact of aid on the Haitian healthcare system and learn best practices for recovery both in Haiti and future disaster contexts. We conducted 22 semi-structured interviews with the directors of local, collaborative, and aid-funded healthcare facilities in Leogane, Haiti, which was the epicenter of the 2010 Earthquake. We coded and analyzed the interviews using an iterative method based on a grounded theory approach of data analysis. We found that healthcare providers identified aid as benefitting the community via acute emergency relief, increased long-term healthcare access, and increased referral options for local healthcare providers. However, we also found that aid had many negatively perceived impacts, including episodes of poor quality care, internal brain drain, competition with local facilities, decrease in patient flow to local facilities, and forced emigration of Haitian doctors to abroad due to loss of economic revenue. As Haiti continues to recover, it is imperative for aid institutions and local healthcare facilities to develop a more collaborative relationship to transition acute relief to sustainable capacity building. Based on our findings, in future disaster contexts, we advocate for the use of both acute and long-term quality of care metrics, NGO Codes of Conduct, Master Health Facility Lists, and sliding scale payment schemes as policies to guide and improve future disaster response.

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"WOMEN IN CONTROL FOR TSETSE CONTROL"-COMMUNITY LED INTERVENTION INVOLVING INNOVATION

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Community-based approaches are widely employed in efforts to control a variety of neglected tropical diseases (NTDs). Some of the earliest attempts at community-based control of NTDs were concerned with controlling tsetse flies, the vector of human African trypanosomiasis (HAT). However, this approach was never widely adopted and no community-based interventions against tsetse have been reported in the past decade. Recent developments of cost-effective and simple-to-use tools, particularly 'tiny targets', provide a new opportunity for communities affected by sleeping sickness in Africa. The traditional roles of women (water collecting, washing) regularly bring them into the riverine habitats where vectors of the Gambian form of HAT concentrate. Women may therefore be able to play an important role in community-based interventions against tsetse. We used action research to implement and evaluate an intervention led by women in two villages in a sleeping sickness area of NW Uganda. Feasibility, sustainability, cost-effectiveness and participants' perceptions of managing this intervention were assessed. We ran mentoring meetings over three phases: preparation, action (target deployment) and evaluation. The community-led intervention was compared with a separate operation conducted by an 'expert' team in an adjacent area. The community deployed 194 targets of which 38% were still functional after 6 months: comparable to the professional operation. The women demonstrated good planning, leadership and management skills and their perception of ownership and empowerment increased during the process. Excluding the research component, a community intervention would cost $67 compared to $85 per km2 for the expert team operation. Women's attitudes towards the innovation were positive: they were motivated, confident and resourceful partners in interventions against tsetse. We suggest that promoting their involvement is feasible and will improve sustainability and cost-effectiveness of tsetse control. More broadly, women should be actively involved in the planning, execution and evaluation of disease control programmes.

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BETTER DATA INTEGRATION AND SHARING CAN ACCELERATE RESEARCH AND POLICY FOR GLOBAL POPULATION HEALTH

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The vast amount of data that is currently collected by researchers and health agencies around the world creates new opportunities to improve global population health. Most of these data are stored across thousands of different data systems and may never be used for new research because data cannot be easily accessed and integrated. Despite a global commitment to improve the use and sharing of global health data, this can be challenging in reality. We described the entire web of barriers to global health data sharing, and evaluated data availability and consistency for one example disease: dengue. We systematically reviewed documents that described barriers to global health data sharing, identified individual barriers, and grouped these in a taxonomy of major categories. We also integrated dengue surveillance data provided online by various WHO sources. We assessed the availability and consistency of WHO data across sources and compared WHO data to data provided online by two example countries: Brazil and Indonesia. We identified 20 potential barriers to global health data sharing and classified these in six categories: technical, motivational, economic, political, legal and ethical. The first three categories are deeply rooted in well-known challenges of health information systems for which structural solutions have yet to be found; the last three have solutions that lie in an international dialogue on policies and instruments for data sharing. Dengue data from 100 countries were available from WHO representing 23 million dengue cases and 82 thousand deaths reported to WHO since 1955. We found 84% agreement between WHO data sources, representing a discrepancy of almost half a million cases. The simultaneous effect of multiple interacting barriers greatly complicates access and integration of global health data for research and policy. The increasing complexity of this data landscape causes an urgent need for global coordination to standardize, integrate, and disseminate data. A new financial and operational framework should ensure the sustained availability of high quality data to improve global population health.

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BIOMEDICAL GRADUATE EDUCATION AND CDC WEBSITES PROMOTE INFECTIOUS DISEASES IN THE MEDICAL SCHOOL CURRICULUM AT THE JOHN A. BURNS SCHOOL OF MEDICINE, HONOLULU, HAWAII

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Biomedical scientists bridge the gap between the basic sciences and clinical medicine. Teaching medical microbiology to medical students in a Problem Based Learning curriculum can be challenging. Appropriate information must be conveyed at the appropriate time for maximal learning. Although major infectious organisms are covered in health care problems and in didactic lectures, many organisms of importance in travel and tropical medicine are not covered in the pre-clerkship curriculum (Years 01 & 02). To overcome this barrier, we introduced the concept of "Parasite of the Week" based on the CDC website: DPDx - Laboratory Identification of Parasitic Diseases of Public Health Concern (www.cdc.gov/dpdx).1 Introduction of relevant parasitic organisms was held during normally held pathology laboratory sessions. Before each session, a clinical case relevant to the parasitic organism is sent to the students. During the laboratory session informational material is offered covering biological and clinical aspects that included: 1) a large poster summarizing the main aspects of the life-cycle, pathology, diagnosis and therapeutic options, 2) informational handouts covering the material in greater depth for future reference and 3) microscopic and macroscopic specimens to solidify learning. Graduate students are present to explain the material and answer questions. The success of these sessions led to the graduate students in the Department of Tropical Medicine to developed informational material on bacterial infections of importance to travel and tropical medicine based on CDC's Yellowbook (http://wwwnc.cdc.gov/travel/page/yellowbook-home-2014/). We believe these sessions greatly increase medical student exposure to concepts of infectious diseases while providing productive interactions between medical and biomedical graduate students. 1. Use of visual displays to teach medical microbiology in the preclerkship years. WGEA conference, Honolulu, Hawaii. March 23-25, 2014

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PREDICTIVE STATISTICAL MODELLING TO INFORM TUBERCULOSIS PREVALENCE ESTIMATIONS

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A total of 22 nationally representative tuberculosis (TB) prevalence surveys have been conducted between 1990 and 2013, providing direct estimates of TB burden. In countries without surveys the World Health Organization (WHO) currently estimates prevalence indirectly from estimated incidence and disease duration. In order to provide WHO with alternative indirect estimates of TB prevalence, an ecological predictive statistical model was developed to predict prevalence in low and middle-income countries without survey data with an estimated prevalence over 0.1%. We included 13 nationally representative surveys, 2 district level surveys in India and subnational estimates for 5 surveys conducted between 2007 and 2013 resulting in 30 datapoints for model development (training set). Ecological predictors included TB surveillance and programmatic data, co-morbidities and socio-environmental factors extracted from online data repositories. We fitted a random effects multivariate binomial regression and predicted bacteriologically confirmed TB prevalence in 74 low to middle income countries across Africa, Asia and South America in 2013. Out of the 166 ecological predictors considered 37 were retained for model building (due to incompleteness or collinearity) and 7 were found significant in univariate analyses. The final multivariate model included 3 predictors: climate score, laboratory confirmed TB notification rates per 100,000 population and BCG vaccination coverage. Cross-fold validations in the training set suggested average fit (R-sq = 0.57). Out of sample predictions (all forms all ages) were on average consistent with WHO estimates (average difference -10 cases per 100,000), albeit with considerable scatter. Predictive ecological modelling can provide useful complementary estimates for TB burden and can be considered alongside other methods in countries with limited TB data. The predictive power of the model may be improved by including (sub)national estimates of 5 surveys which will become available in the coming year and more complex spatial correlation structures to the model currently presented.

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INSTITUTIONALIZATION OF QUALITY OF CARE IN HEALTH FACILITIES IMPROVES MANAGEMENT OF MALARIA IN PREGNANCY IN TANZANIA

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Malaria in Pregnancy (MiP) is one of the contributors to maternal mortality in Tanzania which persists at a ratio of 410/100,000 live births. Tanzania implements WHO's three-pronged approach to prevent MiP (use of insecticide treated bed-nets (ITNs), intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) and prompt diagnosis and treatment). Efforts are on-going to improve IPTp and ITN coverage which is 33% and 75%, respectively. Jhpiego, in collaboration with the Ministry of Health and Social Welfare, worked in 251 health facilities to improve the quality of maternal and neonatal health by building the capacity of health care providers through training, supportive supervision, mentoring and coaching. A total of 7,181 providers and 400 tutors were trained on MiP prevention and treatment. A quality of care study used the same methodology and sampling approach in 2010 and 2012, combining observations of women during antenatal care, inventory and record review as well as health worker knowledge. A team of MNH experts underwent clinical updates, training and orientation to the study tools. Data collection teams visited facilities, made observations and entered data into smart phones. The study was conducted in 12 regional hospitals and 38 lower level facilities in 12 regions including Zanzibar with a total of 391 and 366 ANC observations made in 2010 and 2012, respectively. Between 2010 and 2012, the percentage of women receiving an ITN increased by 26% (p-value = <0.0001); the change observed was due to a 33% increase in offering ITN vouchers at health centers and dispensaries. A slight improvement was seen in provision of IPTp-SP from 62% in 2010 to 65% in 2012. In Tanzania, application of the quality improvement approach contributed to improving MiP services. Moving forward, there is a need for the Ministry to continue strengthening ANC with effective monitoring and routine supervision to increase coverage of MiP prevention. Districts management teams and facilities need to ensure availability of SP and provide regular technical updates on the national service standards including counseling at ANC and birth preparedness.

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ASSOCIATION BETWEEN MALARIA CONTROL SCALE-UP AND MICRO-ECONOMIC OUTCOMES: EVIDENCE FROM A RETROSPECTIVE ANALYSIS IN ZAMBIA

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While substantial attention has been devoted to understanding the effectiveness of malaria control strategies on health outcomes, there has been less focus on understanding the economic impact of malaria control interventions. As malaria control interventions are scaled up and malaria episodes decrease, households may experience economic benefits such as improved household income and consumption, worker productivity, schooling attendance, and poverty status. This study assesses the associations between malaria control scale-up and micro-economic indicators in Zambia, where significant progress has been made in scaling up effective malaria control strategies, but also where malaria continues to be an important public health concern. Using data from 2006 to 2010 on the distribution of insecticide-treated nets (ITNs) and indoor residual spraying (IRS), this study examines whether the scale-up of these activities in Zambia is associated with improved micro-economic outcomes at the household level. Specifically, do these activities affect household spending on food, household spending on medical care, schooling attendance, agricultural production, and household savings and borrowing? To answer these questions, we will use secondary data collected by multiple Zambian ministries and the National Malaria Control Center (NMCC) to conduct retrospective, multivariate analyses to match districts at baseline in 2006. We will then compare micro-economic outcomes of nearly 20,000 households at endline in 2010, as a function of malaria control activities conducted during that year and preceding years. Study findings are expected by August 2015. Particularly in a context of limited resources, the results of the study will inform policymakers, donors, and development practitioners on the returns to investing in malaria control by considering the micro-economic benefits of these strategies.

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ONGOING NEONATAL MORTALITY AUDITS IN MALAWI HELP FOCUS MEDICAL AND NURSING EDUCATION

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The WHO estimates a critical shortage of 7.2 million healthcare workers globally. Malawi has 2 physicians and 34 nurses/midwives per 100,000 people, limiting quality of care for patients and quality of training and supervision for healthcare students. In Malawi, neonatal mortality accounts for 34% of deaths in children under 5 and are a vital target for reducing child mortality. Kamuzu Central Hospital (KCH), in Lilongwe, Malawi, is a teaching hospital for the University of Malawi College of Medicine and Kamuzu College of Nursing, and a placement site for volunteers with the Global Health Service Partnership (GHSP), a US-based public-private partnership that places US faculty in medical and nursing schools for one-year. At KCH, the neonatal unit admits more that 2000 sick newborns annually. Each month a pediatric intern, together with an intern from the obstetrics department, presents the perinatal morbidity and mortality data. These presentations have informed educational priorities and helped the team focus trainings and protocols on core needs. The most common admission diagnoses in 2014 were prematurity (37%), birth asphyxia/meconium aspiration (27%), neonatal sepsis (14%), and congenital anomalies (9%). The average neonatal mortality rate was 20%, with prematurity and asphyxia accounting for 75% of deaths. These findings highlight the need to emphasize staff training on protocols such as neonatal resuscitation, CPAP, kangaroo care, and antibiotic management. These skills are also emphasized in both medical and nursing students' curricula, and additional intern trainings have been introduced to ensure ongoing quality improvement in the care of neonates. By understanding current neonatal mortality statistics at KCH, physician and nurse educators can target classroom and clinical education to improve the care of neonates and reduce neonatal mortality at our teaching hospital as well as impact the care given by our graduates. Additionally, due to the heavy toll of birth asphyxia and prematurity, improved outcomes for neonates cannot be accomplished without the combined efforts of both the pediatric and obstetrics departments.

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ANTENATAL AND DELIVERY CARE RECEIVED BY WOMEN IN BUNGOMA COUNTY, WESTERN KENYA

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Maternal mortality remains high in sub-Saharan Africa. The Millennium Development Goal for maternal health aimed to reduce maternal mortality by three-quarters by 2015. However, most countries including Kenya fell short of achieving these targets. We describe the status of prenatal and delivery care for women in Bungoma county, western Kenya. Between September and November 2013, we conducted a household cross-sectional survey of women who had given birth within the last 4 months in Bungoma County, western Kenya. Using a structured questionnaire, data were collected on antenatal care (ANC) visits, delivery location and service satisfaction of a random sample of women from a demographic surveillance system. A total of 748 women had a recent birth; 89% (n=667) consented and were interviewed. Ninety-two percent (n=622) visited ANC clinic at least once, and 33% (n=223) made the recommended four ANC visits. Sixty-one percent (n=278) made the first ANC visit in the second trimester. Women who made the first ANC visit in the first trimester were more likely to complete ≥4 ANC visits (83.6%; 56/67) compared to those starting ANC visits in the second or third trimesters (27.3%; 151/557; p=0.0001). There was no significant difference observed in the percentage of women making ≥4 ANC visits among those who started ANC in the second or third trimesters (p=0.68). Only 45% (n=301) of women delivered at a health facility. Satisfaction with ANC services was 67% (415/622); satisfaction with health facility-based delivery services was 67% (203/301). Of the 205 women who made ≥4 ANC visits, 64.4% delivered in facilities compared to 37.9% of women who made fewer visits (p<0.0001). As the deadline for the Millennium Developmement goal for maternal mortality gets closer, Kenya did not achieve the stated targets. Over a third of women reported dissatisfaction with ANC and delivery services, which might contribute to low service utilization. Strategies to increase satisfaction with ANC and delivery services might positively impact utilization and improve maternal mortality so as to achieve the newly promulgated International health Development Goals.

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HEALTH FACILITY CASELOAD CHANGES DURING THE INTRODUCTION FOR COMMUNITY CASE MANAGEMENT FOR MALARIA IN SOUTH WESTERN UGANDA - AN INTERRUPTED TIME SERIES APPROACH

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Many malaria endemic countries are scaling-up community case management (CCM) programmes, which increase access to malaria testing and treatment to communities with limited access to health facilities. To date, evaluations of CCM programs have centred on community health workers (CHWs) compliance to guidelines, but broader effects on utilisation of health centres (volume and case mix) have not been documented. This analysis was conducted during a community case management intervention in Rukungiri District Western Uganda. Out-patient department (OPD) visit data for children under 5 was collected for one year before the trial started (pre-intervention) and during the 20-month trial (intervention-period) from three health centres serving the intervention area. An interrupted time series analysis with segmented regression models was used to compare the trends in all-cause and malaria-specific OPD visits during the pre-intervention and intervention periods. The introduction of the CCM intervention was followed by increases in the frequencies of diagnosis of diarrhoeal diseases, pneumonia and helminths and fewer malaria diagnoses. In the first month after the CCM intervention began all-cause OPD utilisation decreased by 63% compared to the pre-intervention period (p<0.001). Malaria-specific visits also saw a dramatic drop shortly after the intervention began, with 27 fewer visits per month during the intervention-period compared with the pre-intervention period (p<0.05). The declines in all-cause and malaria visits were sustained for the entire intervention period. In conclusion, introduction of a community-treatment programme for malaria reduced the total caseload seen at local health centres. The subsequent impact for health workers and patients in terms of reduced total working hours, increased time spent per consultation and/or other health centre duties, as well as required changes in stock management and financing, warrants further examination in order to fully document the effect of community case management programmes on the functioning of health systems.

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INTEGRATING GLOBAL HEALTH SURVEILLANCE DATA: AN EXAMPLE APPLICATION TO MALARIA SURVEILLANCE IN UGANDA

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The growing volume of data generated by systems around the world presents a tremendous opportunity for global health surveillance. These data, however, are fragmented in many ways, across diseases, countries, governmental and non-governmental organizations, and clinical institutions. This fragmentation poses a barrier to analyses that could benefit from using multiple data sources as the data must be integrated manually for each analysis, requiring a considerable amount of effort. Malaria surveillance, including the monitoring and evaluation of malaria control activities, is an example of how data fragmentation can hinder analyses. Matching effective interventions to specific factors in each area requires epidemiological analysis of data integrated across multiple sources. This type of data is generally not available to malaria control and elimination activities due to the challenges in identifying, accessing, and integrating data hosted within the wide range of organizations contributing to malaria control efforts. SDIDS (Scalable Data Integration for Disease Surveillance) is a software application designed to enable the integration and analysis of data across multiple scales to support global health decision-making. In this presentation, we present a prototype of SDIDS and show how it can be used to integrate malaria surveillance data collected by multiple organizations in Uganda. SDIDS is a web-based, ontology-driven software platform that automates the integration of heterogeneous data from multiple sources, and supports visualization, analysis, and sharing of these data. A central characteristic of SDIDS is its ability to scale-up and integrate data from other geographical regions and for other priority diseases. This scalability means that a wide range of data sources can be mapped once to SDIDS and then accessed and analyzed repeatedly by a wide range of global health users and applications.

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INTEGRATED COMMUNITY CASE MANAGEMENT DATA QUALITY IN MALAWI

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ICF conducts annual data quality assessments of WHO RAcE projects to review accuracy, availability, completeness, reliability, integrity, confidentiality and precision of iCCM data and to recommend ways to improve data quality. The first RAcE data quality assessment was conducted in Malawi in January, 2014. We focus here on findings from the data tracing part of the assessment with supporting information from the systems assessment and key informant interviews. ICF randomly selected 10 health facilities from all four RAcE project districts, and collected quantitative data with an adapted and comprehensive tool which included tracing selected indicators through the reporting system. We calculated averages of data agreement between reporting levels for three indicators. ICF also conducted key informant interviews with Health Surveillance Assistants (HSA), and facility, district and central Ministry of Health staff. Data availability was generally high except for supervision data. The data verification process identified gaps in completeness, integrity, and reliability, particularly in HSA's record keeping. Cross-checks revealed that data recorded by HSAs for new cases and referrals in their registers usually did not match the data they reported to health facilities. New cases are generally reported accurately from one level to the next in the system (e.g. within 10% difference between data at health facilities and districts), but referrals and stockouts are underreported from one level to the next. There were substantial differences between the number of new cases and referrals initially recorded by HSAs and the corresponding number reported through the system (ranges: -51 to 183 and -3 to 102, respectively). The system lacks some quality controls, including data entry verification, a protocol for addressing errors, and written procedures for data collection, entry, analysis and management. Staff at all levels would like more training in data management. We recommend prioritizing data management with documented protocols, additional training, and efficient supervision practices to improve iCCM data quality.

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BURDEN AND DISTRIBUTION OF MALNUTRITION IN GUAYAQUIL, ECUADOR: RESULTS FROM THE ECUADORIAN NATIONAL HEALTH AND NUTRITION SURVEY (ENSANUT-ECU)

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Guayaquil is the largest urban center in Ecuador (2.3 million), but there is little published nutritional data for this population. This study was conducted to examine the burden and distribution of malnutrition in Guayaquil, using data from the Ecuadorian National Health and Nutrition Survey (ENSANUT-ECU). Socio-demographic and anthropometric data (n=1,738) and venous blood samples (n=900) were collected. The prevalence of anemia, micronutrient deficiencies (zinc, vitamin A, iron, vitamin B12, folate), and stunting (LAZ<-2), underweight (WAZ<-2), wasting (WLZ1), and obesity (BMIZ>2) were calculated and mapped in ArcGIS. Binomial and linear regression models were used to examine the associations of socio-demographic characteristics with nutritional outcomes. Micronutrient deficiencies were common, including zinc deficiency (Zn<65.0 µg/dL; 50%) and anemia (Hb<11.0 g/dL; 13%), and the prevalence was significantly higher than the national level data ($p<0.01$). The prevalence of anemia was highest in children (29%; RR: 2.35, 95%: 1.55-3.59, $p<0.01$) and women of reproductive age (22%; RR: 2.50, 95%: CI 1.77-3.50, $p<0.01$), compared to all other age groups. Children also had the highest prevalence of vitamin A deficiency (serum retinol<20.0 µg/dL; 19%; RR: 3.53, 95% CI: 1.87-6.65, $p<0.01$), compared to all other age groups. Women of reproductive age in Guayaquil had lower hemoglobin, zinc, erythrocyte folic acid, and serum folic acid levels, compared to the national level data ($p<0.01$). The prevalence of overweight and obesity were high in adults; women were less likely to be overweight compared to men (46% vs. 37%; RR: 0.71, 95% CI: 0.55-0.90, $p=0.004$), however women were more likely to be obese (30% vs. 21%; RR: 1.60, 95% CI: 1.20-2.10, $p=0.001$). The burden of malnutrition is high in Guayaquil Ecuador, including anemia, zinc deficiency, and vitamin A deficiency, particularly among women of reproductive age and young children. Further understanding of the dual burden of malnutrition and its distribution in this population is needed to inform preventive interventions and public health approaches in Ecuador.

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HEALTHCARE CAPACITY BUILDING IN HAITI: TRAINING HEALTHCARE AND NON-HEALTHCARE PROVIDERS IN BASIC CARDIOPULMONARY RESUSCITATION

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Following the devastating 2010 earthquake in Haiti, the need for health care and support increased as medical schools and hospitals were destroyed or severely damaged. Outreach educational programs are crucial to decreasing the uneven geographical distribution of healthcare workers or skilled laypersons in this impoverished country. A pilot program was implemented in 2015 to provide necessary emergency training in the basics of adult and infant cardiopulmonary resuscitation (CPR). The purpose of this study is to describe our experience establishing an innovative educational opportunity for basic skills training in Haiti. A team of 4 U.S. providers developed a basic emergency provider program including a written guidebook with a French translation and utilized standard CPR manikins for training. This program was supported by the Haitian government and taught in 2 hour sessions. Training included adult and infant CPR and suffocation/ chocking treatment (Heimlich maneuver). Competency was assessed via direct observation and oral testing. Over 4 days, 80 healthcare providers, lay persons, and governmental security staff were trained by the international team. Local translation was available. At the completion of training, the participants were able to demonstrate skills competency and pass an oral quiz. Satisfaction ratings were high as well as performance confidence. The need for healthcare capacity building in Haiti is well recognized both locally and internationally. This pilot skills training program received positive evaluations from trainers and participants. Teaching basic healthcare skills and competencies to native Haitians will augment the availability of basic services and improve access to quality healthcare services to this most vulnerable of populations. Development of additional healthcare educational initiatives and curricula are underway as well as expansion of this program.

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SUITE FOR AUTOMATED GLOBAL ELECTRONIC BIOSURVEILLANCE (SAGES): SIMPLIFYING INSTALLATION AND IMPROVING USE AND SUSTAINABILITY

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SAGES is a collection of open source software tools designed to create or enhance existing electronic disease surveillance systems in resource-limited settings. The SAGES architecture uses mobile and web-based methods to collect structured data via SMS, Wi-Fi, and Internet connected devices. The primary data analysis tool, OpenESSENCE, provides a web-based interface for data analysis, visualization, and reporting for disease surveillance. Development of SAGES began in 2008, but significant advancements in open source software, data collection technology, and user needs instigated a re-engineering of the system. During initial development of SAGES customized systems were developed for each user. While this technique supplied a near-perfect fit for each installation, it also required significant technical support from the SAGES design team during installation. It also made independent configuration of SAGES tools by the users more difficult. An analysis of alternatives was done for both new front-end and back-end open source solutions for SAGES. The results suggested that creation of a base set of tools that would meet most users' disease detection needs and was ready 'out of the box' would increase the utility and usability of the SAGES software. However, this more generic version of SAGES also needed to leverage industry standard technologies to facilitate expansion of the generic system by users and by the SAGES development team. This presentation will describe the re-engineering of SAGES that began in 2013. The aim was to improve ease of installation, configuration, and use of the system, and to create a system that would meet the needs of 80% of users but be flexible enough to allow users to expand the system as needed. Particular attention will be paid to the trade-offs between designing customized systems versus designing 'out of the box' ready systems suitable for most users. The challenges of designing systems when technology is changing rapidly and where early design decisions have long term, and often unexpected, impacts will also be discussed, as will the challenges of designing tools for use in resource-limited setting.

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TRAUMA EDUCATION FOR MEDICAL STUDENTS: IMPACTING THE TRAUMA BURDEN IN ETHIOPIA WITH UNDERGRADUATE MEDICAL EDUCATION REFORM

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Ethiopia is a developing nation with a significant trauma burden and a lack of policy, infrastructure, and emergency medical services to address this burden. Though injury is one of the top causes of mortality in Ethiopia, trauma education and management training is not standardized across the country. Ethiopian medical school graduates are required to serve rural populations as general practitioners, and they often lack skilled hospital personnel to assist in managing critical trauma management. The purpose of our study is to determine the feasibility of implementing a basic trauma triage and resuscitation course for Ethiopian medical students, a population that will address trauma in locations where access to supplies is limited. Furthermore, we investigate the utility of this course as a teaching tool to expand the capacity to impact the trauma burden in low-resource settings. This modified ATLS-based course focused on the ABCD (airway, breathing, circulation, and disability) management of emergency care in low-resource settings. The educational session included a pre-test, educational intervention, and post-test. Two groups of participants, medical students (N=39) and laypersons (N=39), completed the entirety of the course. Both groups demonstrated an improvement in test scores following the education intervention. The medical school students had a mean post-intervention test score of 92% (SD = 8) and laypersons had a mean score of 75% (SD = 12). A t test for equal variances demonstrated significant difference between the post-intervention scores for the two groups ($p = 0.01$), and all 78 participants demonstrated significant difference between their pre- and post-test scores based on a paired t test ($p = 0.01$). Furthermore, all 78 participants were able to demonstrate the basic skills of the primary and secondary survey in ATLS. Based on this study, a basic trauma-training course provides new information for all participants and can be used to train a supply of teachers for less skilled hospital personnel and laypersons.

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PREDICTIVE STATISTICAL MODELLING TO INFORM TUBERCULOSIS MORTALITY ESTIMATES

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The global task force on tuberculosis (TB) impact measurement is mandated to periodically review and update methods to estimate TB incidence, prevalence and mortality. This study investigates the value of predictive ecological modelling to estimate TB mortality in low-income countries. A predictive ecological model was built using civil registration and vital statistics (CRVS) data with standard coding of cause of death

to World Health Organization (WHO) from 128 countries with quality CRVS systems collected between 2004-2012. Independent predictors of TB mortality, including TB program performance, TB co-morbidities, socio-environmental and climatic factors were extracted from online data repositories. A two-step procedure was used to select variables. Firstly, the association of each variable to TB mortality was assessed by fitting a univariate negative binomial model to the data. When predictor variables correlated > 80% the best fitting variable was retained. Secondly, a stepwise information based procedure was used to identify the multivariate model which best fit the data. Predictions were cross-validated by comparing model predictions to the WHO measurements. Out of the 166 ecological predictors initially considered, 19 were found to be uncorrelated and significantly associated to TB mortality in the univariate analysis. The multivariate model included 11 predictors including male life-expectancy, number of new sputum-smear positive cases, proportion population living in urban settings, proportion multidrug resistant TB cases among all TB cases notified, national health expenditure, prevalence of diabetes, HIV positivity rate among all TB cases notified, BCG vaccination coverage, percent of TB retreatments among all TB cases notified, treatment success rate among retreatments, annual precipitation. Predictive statistical modelling is shown to be valuable to estimate TB mortality in countries with limited availability of TB data. The predictive power of the model may be improved by considering more complex covariate correlation structures (random) to account for region specific variations to the model currently presented.

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GLOBAL QUEST FOR QUALITY AND MRIGLOBAL

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Laboratory and health care quality is becoming synonymous with competency and reliability; accreditation and certification are sought after by increasing numbers of facilities in many countries. In January 2008, representatives from World Health Organization, US PEPFAR, US Centers for Disease Control and Prevention, World Bank, several universities and 33 countries issued The Maputo Declaration recognizing the need to strengthen laboratory services and systems in developing countries. WHO and US CDC subsequently issued a statement calling for countries with limited resources to establish a path towards laboratory accreditation to ISO 15189 standard; as a result, in 2009, WHO AFRO launched Stepwise Laboratory Improvement Process Towards Accreditation in the African Region (SLPTA) program, making accreditation more accessible, affordable and sustainable for African countries. In other regions, Thailand and Argentina have been hailed as great examples of developing national laboratory quality programs. Republic of Kazakhstan in Central Asia is another example of a country that understands the need for accreditation and is making great strides in this arena. MRIGlobal, registered to the ISO 9001 and accredited to ISO 17025, with several ASQ Certified Quality Auditors, has contributed to the incorporation of QMS elements and ISO 15189 laboratory quality standard into the curricula of the Kazakhstani National Medical University in the Republic of Kazakhstan. After a thorough review of the Medical University curricula, MRIGlobal has worked with faculty and administrators to formulate a roadmap to achieve the integration of laboratory QMS with medical school coursework. This project has enabled the stakeholders to introduce international quality standards into clinical laboratory education, which will result in a sustainable laboratory quality in RoK.

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ENGAGING STUDENTS IN ADVOCACY, EDUCATION, AND RESOURCE MOBILIZATION TO SUPPORT THE EFFORT TO CONTROL AND ELIMINATE NEGLECTED TROPICAL DISEASES: THE END7 CAMPAIGN MODEL

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In January 2012, the Global Network for Neglected Tropical Diseases launched END7, an international advocacy campaign to raise the awareness and funding necessary to control and eliminate the seven most common neglected tropical diseases (NTDs) through targeted engagement with grassroots supporters. Undergraduate and graduate students at universities around the world have become a key constituency advancing END7's goals. Through its student engagement program, END7 seeks to develop the next generation of scientists, researchers, and advocates needed to see the NTD control and elimination effort to its completion. END7 works to engage students in the campaign, develop their leadership skills, and equip them with the resources necessary to plan advocacy, education, and fundraising events on campus. A specific theme, ranging from "NTDs and WASH" to "NTDs and Human Rights," is selected for each month of the academic year. The campaign blends digital organizing with traditional constituent engagement, complementing social media promotion, webinars, online petitions, and other digital tools with personal communication with student leaders, on-campus presentations, and support for student leaders to attend conferences and policy events. This online/offline approach has mobilized a large and growing community of students to support the campaign from universities across the United States, Europe, and the Middle East. Student groups at more than fifty universities have raised nearly $50,000 for NTD treatment programs and completed more than 2,500 targeted advocacy actions, ranging from online petitions urging the United Nations to include an NTD-specific target in the Sustainable Development Goals to meeting with members of Congress to advocate for an increase in funding for the United States Agency for International Development NTD Program in the U.S. federal budget. Through regular access to experts in the field, END7 student leaders develop a sophisticated understanding of the science underlying the WHO NTD Roadmap and the policy and resource mobilization targets that will need to be met in order to implement it - connecting science, policy, and program implementation.

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'A PEACEFUL PREGNANCY': THE INFLUENCE FOR CULTURAL SCHEMAS ON INTERMITTENT PREVENTION FOR MALARIA IN PREGNANCY IN MALI

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Research on intermittent preventative treatment of malaria in pregnancy has identified health system, cultural and individual barriers to uptake. Few studies have focused on links between such barriers and cultural schemas, culturally-shared cognitive systems that influence individual decision-making. Our study sought to understand Malian cultural schemas of pregnancy and their effect on local concepts of IPTp. We conducted interviews and focus groups with women, husbands, mothers-in-law and health workers in 4 rural villages in Sikasso and Koulikoro. We also observed antenatal care in health centers at each site. Women state that malaria is a significant threat to a healthy or "peaceful" pregnancy. For prevention, informants mention long-lasting insecticidal nets (LLINs), hygiene, nutrition and avoiding heavy work. Few mention IPTp spontaneously. When prompted, some recall receiving pills that fit the description of sulfadoxine pyrimethamine, but most are unsure of their purpose. During observations, SP was administered with little or no patient counseling. Some women asked about free LLINs, but none mentioned

IPTp or SP. In contrast nearly all recall receiving iron tablets and describe them as a key ANC element. Many link them to a Bambara ethnomedical concept about the importance of "strong" blood in pregnancy. In ANC consults, clinicians often refer to FeSO4 using a local Bambara term consistent with this concept. No analogous local term exists for SP or IPTp: Clinicians; if they mention it at all, use French. Existence of a linked Bambara term and construct may help explain why FeSO4 salience is so much higher than that of IPTp, despite women's view of malaria as a serious threat to pregnancy. Rebranding SP to tie it more closely with the cultural schema of "peaceful" pregnancy may help increase its salience and uptake. Strategies could include coining a meaningful local term for SP, administering it concurrent with LLIN distribution, and publicly promoting it as a free and valuable benefit. Raising SP's profile within the cultural narrative of "peaceful" pregnancy could increase demand for IPTp, thus reducing Mali's MIP burden.

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EPISAMPLE: AN ANDROID APP FOR POPULATION-BASED EPIDEMIOLOGICAL SURVEYS

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Household surveys are required for benchmarking many national and global indicators such as those used for Millennium Development Goals or national malaria control programs. Survey sampling is a critical component for household surveys to ensure representativeness but can be difficult and expensive for field implementation. EpiSample is an Android-based application that builds off of ODK 2.0 tools and offers additional census, sampling, and survey interviewing capabilities to facilitate the implementation of population-based epidemiological surveys using Android devices. Features are grouped into the following categories: census, sampling, navigation, surveying, and data aggregation. Census features include: (a) efficient census listings including the use of GPS coordinates of target points, (b) in-field aggregation of lists collected on multiple devices onto one device for sampling without the need for a laptop, (c) data connection, or cables, and (d) the ability to capture additional points of interest that can be excluded from the sampling. Sampling features include the application of a random sampling algorithm to a listing of points to select: (a) primary points to survey, (b) additional or oversampled points (if needed), and (c) replacement points that can be used to replace a primary or additional point that cannot be surveyed. The number of points selected can be defined in advance and locked down via a password-protected configuration or can be defined during field implementation. EpiSample allows surveying of points collected in the field and sample selection using a random sampling feature or points imported from a csv file. Points can be filtered or sorted in various combinations based on the type of point, distance from the device, time of data entry, interviewer name, Android device identification number, or point location. EpiSample can be used to open and manage ODK Survey-based questionnaires. All census and survey data collected can then be sent to a central server where it is aggregated and stored using ODK 2.0 tools. EpiSample will be used to conduct national malaria indicator surveys in Zambia and Ethiopia in 2015.

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GEOSPATIAL COVARIATES AVAILABLE IN GOOGLE EARTH ENGINE FOR DISEASE MODELING

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The Malaria Atlas Project (MAP), University of Oxford has compiled a suite of geospatial covariates useful for characterizing the spatiotemporal patterns of malaria prevalence via their relationships with vector ecology. The covariates consist of metrics that quantify environmental factors (e.g., temperature, moisture, and vegetation) known to affect the prevalence of numerous infectious diseases. To make these datasets available to the wider health research community MAP and Google have partnered to add them to the Google Earth Engine platform, which provides an online processing environment, data repository, and data distribution system that maximizes the utility and exposure of MAP covariates. Currently available covariates consist of global grids of land surface temperature (day, night, and diurnal flux), enhanced vegetation index, and brightness and wetness indices, with a 2.5 arc-minute (~5 km) spatial resolution and a monthly temporal resolution, for 2000-2014. These datasets were derived from MODIS satellite imagery and gap-filled to remove the effects of cloud contamination, thus producing wall-to-wall spatial coverage for the full 15-year time-series.

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TESTING DRUG QUALITY AT ALL POINTS IN THE SUPPLY CHAIN: INTEGRATION OF TECHNOLOGY AND HEALTH SYSTEM

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Substandard and counterfeit medicines are a major public health threat, not only because they cause loss of life and financial burden, but also because they lead to long-term drug resistance in vast areas affecting millions of people. With the growing global challenges in combat substandard and counterfeit medicines, there is an urgent and dire need to come up with tools that are not only affordable, but also quantitative, easy to use and are able to test drug ingredient and dissolution rapidly at multiple points within the health system. Current tools, unfortunately, are too cumbersome, expensive or qualitative and hence ill-suited for testing drugs at all points in the supply chain. We have developed, within our PharmaChk platform to test drugs, rapid luminescent and fluorescent assays to quantify Artesunate (ATS) and Amodiaquine in mono and combination therapies and are now extending our work to other life saving commodities including oxytocin. Here, we will discuss the design of the technology, laboratory tests, our field trials in Ghana focusing on both tablet and injectable formulations and our overall results which show excellent agreement with the gold standard method (HPLC) despite orders of magnitude cost difference in the two technologies. Overall, our lab results, combined with field studies show high performance and substantial improvement over field based methods, both in precision and accuracy, of our drug testing platform, PharmaChk. Finally, we will also identify areas of growth and improvement and also how we are working with partners both in and outside Ghana to integrate the technology within the healthcare system to substantially improve drug quality and save precious lives.

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GENOMICS APPROACH TO IDENTIFY THE IMMUNODOMINANT *BABESIA MICROTI* ANTIGENS FOR THE DIAGNOSTICS AND VACCINE USE

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Babesiosis, caused by infection of erythrocytes by parasitic protozoans of genus ***Babesia***, is a major public health concern in many parts of the world. In United States, ***B. microti*** is the dominant species and about 1200 clinical cases of babesiosis are reported annually, mostly in the endemic areas in Northeastern, Mid-Atlantic and upper Midwestern states. In addition, disease is highly underreported and misdiagnosed. There is no FDA-licensed diagnostic test or vaccine for ***B. microti***. We have applied a genomics approach to identify the novel immuno-dominant ***B. microti*** antigens to develop novel diagnostic tests and evaluate their efficacy as vaccine candidate. Using the ***B. microti*** genome sequence database (www.piroplasm.org) and ***B. microti*** Peabody sequence available in the Kumar laboratory, we have identified over 300 genes by bioinformatics analyses that meet the criteria for immuno-dominance (surface expression, repeat units, high copy number etc.) for recombinant expression, antigenic characterization and evaluation as diagnostic and vaccine candidate. In addition, in a parallel approach, we have developed more than 14 unique monoclonal antibodies using spleen cells from mice immunized by repeated infections with live ***B. microti*** parasites. The characterization of these mAbs for antigenic recognition by Western Blot and IFA, and finer epitope mapping by the phage display library approach is in progress. Whole-genome-fragment-phage-display libraries (GFPDL) expressing the open reading frames of ***B. microti*** genome were constructed. Each GFPDL contains more than 10^9 individual phages expressing ***B. microti*** sequences of 50-250 amino acids as fusion proteins with the pIII coat protein of the M13 bacteriophage. Anti-***B. microti*** monoclonal antibodies will be used to 1) identify the immuno-dominant ***B. microti*** antigens, and 2) develop a ***B. microti*** antigen detection assay for application in acute diagnosis and to screen blood donors with low-grade asymptomatic infections. Details of these studies will be presented.

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ECTOPARASITES FROM DOMESTIC ANIMALS DEVOID FOR RICKETTSIAL AGENTS, CUZCO, PERU

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In order to develop effective control measures against arthropod-borne diseases, it is very important to identify the pathogens and their vectors. In southern Peru, there have been few studies on domestic animal ectoparasites and their pathogens. However, there has been evidence of spotted fever group of rickettsial infections in patients from the same region. The purpose of this study was to detect and identify rickettsial agents of ectoparasites collected from domestic animals in southern Peru. Ectoparasite collections were performed in 14 sites in Cuzco, Peru during August 2013. The study sites were located between 2,938 and 4,014 m.a.s.l. On average, the min and max temperatures were registered between 2C and 24C. All visible ectoparasites were removed with fine forceps, preserved in 95% ethanol and transported to NAMRU-6 where they were identified using morphological keys. Each specimen was cut in half and one half was used for DNA extraction with PrepMan™ Ultra, followed by detection/identification using a genus -specific qPCR assay for ***Rickettsia*** targeting the 17-kD antigen gene. A total of 222 animals from twelve types of domestic animals (donkeys, burrows, horses, cows, pigs, chicken, goats, guinea pig, sheep, llama, alpaca, and vicuña) were sampled. From 122 animals, 1657 lice, 39 ticks, and 1 flea were collected. A total of 400 (24%) qPCR reactions were performed with DNA from: ***Melophagus ovinus*** (19.3%, 300), ***Haematopinus eurysternus*** (80%, 4), ***Bovicola bovis*** (83%, 10), ***Otobius megnini*** (89.7%, 35), ***Bovicola caprae*** (75%, 3), ***Linognathus stenopsis*** (89.5%, 17), ***Bovicola equinus,*** (80%, 8), ***Tunga penetrans*** (100%, 1), and ***Haematopinus suis*** (50%, 22). All samples tested were negative for rickettsial DNA. ***M. ovinus*** was the most common ectoparasite (98%, 1563), which was collected from sheep (93%, 1551) and donkeys (0.8%, 12), and the other nine species of ectoparasites made up only 2% of all the ectoparasites collected. There is a possibility that the austere environment (high elevation, and low temperature and humidity) may have played an important role in the lack of rickettsial agents encountered in the ectoparasites assessed from Cusco.

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EMERGENCE FOR LYME DISEASE IN NORTH DAKOTA: HOW DID IT GET THERE AND HOW IS IT BEING SUSTAINED?

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Northeast North Dakota is highly agricultural with large amounts of acreage devoted to industrial scale farming. But lying within the expanse of field crops are islands of forested areas. Since 2010, breeding populations of deer tick, Ixodes scapularis, have consistently been present in the larger (>200 hectares), disjunct 'forest islands'. So far, the field infection rates of ***Borrelia burgdorferi*** (agent of Lyme disease) and ***Anaplasmosis phagocytophilum*** in North Dakota deer ticks have been relatively low (3 to 5%) compared to endemic regions to the east. Analyses of the mitochondrial DNA of deer ticks collected from different forest islands revealed a high haplotype diversity (Hd=0.80), suggesting that the forest islands were being colonized continuously from multiple sources. The likeliest host candidate to explain such pattern would be birds. Therefore in 2014, birds were collected in northwest Minnesota - the geographically closest area of Lyme disease endemicity. Of 104 passerine birds examined, five (4.8%) tested positive by PCR and sequencing for ***B. burgdorferi***, supporting our hypothesis of avian introduction of infected ticks into northeastern North Dakota. Nearly half of the small mammals captured in forest islands were white-footed mice (***Peromyscus*** - known reservoir of Lyme disease) and half were red-backed voles (***Myodes gapperi***). Both voles and mice were parasitized by immature deer ticks. A small percentage (ca. 6%) of engorged ticks pulled from voles and mice tested positive by PCR for ***Borrelia*** and ***Anaplasma***. To determine whether voles served as reservoirs for Lyme disease or conversely, as dead-end hosts (i.e., dilution hosts), a laboratory colony of red-backed voles was established. F-1 voles were injected with ***B. burgdorferi*** s.s. and at 2 and 4 weeks, the voles were infested with larval deer ticks. Engorged ticks were allowed to molt to nymphs and the nymphs were re-fed on naïve laboratory mice to determine if the larval ticks got infected and, as nymphs, were able to transmit the borreliae. At the time of this writing (April 2014), final data are still forthcoming and will be presented at the meeting.

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CONTROL FOR VISCERAL LEISHMANIASIS: PERCEPTIONS, ACCEPTANCE AND WEAKNESSES FOR INDOOR INSECTICIDE SPRAYING (IRS) CAMPAIGN

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Visceral Leishmaniasis (VL) is a fatal vector born infectious disease transmitted by ***Phlebotomus argentipes*** sand flies. The disease is highly endemic in Muzaffarpur district of India. The elimination program targets

on vector control by indoor residual spraying (IRS) using DDT twice a year. The IRS coverage increased from 17% in 2010 to 70% in 2013. However, even in the villages with 100% coverage vector density did not reduce significantly. With the objectives of determining the perception and acceptance by the community and Identifying weaknesses in the current IRS practical execution, we conducted 16 focused group discussions (FGD) among villagers and spray team members in the five endemic villages: three with low coverage (<60%) and two with higher coverage (>85%). Male and female households' heads representing wider section of the community in terms of socio-economic status, education, and caste formed heterogeneous groups. Our FGDs identified several ditches between the planning and monitoring of IRS program including poor quality of insecticide, diluted solution, inadequate spraying of wall and peri-domiciliary areas and no spraying in remote houses. IRS was done only once in a year. Pungent bad odour, stain on the walls, contamination of food items and occasional illicit demands from spray team were the main factors for the non-acceptance in the low coverage villages. Denial of spraying in washrooms, no prior information, unknown team members and purdah by the rural women were other common reasons. Spray team complaints about refusal by the well-built houses, and resistance from Musahar caste. Both the community and the spray team advocated for awareness campaign and prior announcement, involvement of ASHAs/ ANMs in IRS activities, spraying at least twice a year and improving quality of spray solutions. The spray team demanded increased and timely payment and upgraded equipment. We did not observe major trouble and discomfort in the community towards the IRS program, however people felt IRS grossly ineffective. The program should immediately focus on improving IRS campaign as deficient coverage may develop resistance against the insecticide.

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PREVALENCE OF RICKETTSIAE IN FLEAS AND MITES COLLECTED FROM WILD RODENTS IN KENYA

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Rodent-borne diseases pose a public health threat and rodents serve as hosts for a number of parasites linked to zoonotic disease. Identifying and understanding the epidemiology of rodent-borne diseases associated with ectoparasites is important when instituting mitigation strategies. Rickettsiae cause significant acute febrile illnesses especially in developing countries. Ticks have been incriminated as vectors of rickettsiosis (scrub typhus, murine typhus, and tick typhus). Rodent parasites such as fleas and mites are also possible vectors that can transmit rickettsiae and could possibly play an active role in maintaining and transmitting rickettsiae in the absence of livestock. Rodents were collected across three sites with varying habitat conditions. Ectoparasites were removed by combing with a fine tooth nit comb. The parasites were identified and pooled per rodent and per species. Fleas and mites were pooled and total nucleic acids extracted from them. The DNA was screened for evidence of rickettsiae nucleic acid using two quantitative real time PCR assays that were specific to the Rickettsiae genus and Rickettsia felis. A total of 208 rodents and small mammals comprising 12 different species were collected. The rodents were groomed and 187 mites and 124 fleas were collected. Fleas were identified as ***Dinopsyllus lupus*** (85/124; 68.5%), ***Xenopsyllus cheopis*** (35/124; 28.2%) and Leptsylla spp. (4/124; 3.5%). Mites were identified as ***Haemolaelaps*** spp. Mites had the highest prevalence of rickettsiae (13.9%) compared to fleas (10.5%). Several species of rickettsiae have been identified in Kenya including ***Rickettsia typhi***, ***R. africae***, and ***R. felis***. It is possible that rodents provide stable reservoirs for rickettsiae. Detection of rickettsiae in rodents indicates that control of rodents could be a possible way of controlling rickettsiae. More genetic analysis needs to be conducted to characterize rickettsiae species in fleas and mites in addition to studies conducted during over overlapping seasons to establish seasonal patterns.

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UTILITY OF MATHEMATICAL MODELLING OF VECTOR-BORNE DISEASE

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Mathematical epidemiology provides a useful toolbox for understanding and modeling transmission of vector-borne diseases. By definition, the reproduction number, R_0, and vectorial capacity present the scientific community with easily identified critical thresholds for when cases of disease are expected to increase or decrease. However, these models make three key assumptions regarding the mechanisms by which transmission occurs that may fail to match reality: 1) a single host, single vector system, 2) consistency of model parameters over time, and 3) homogenous mixing of hosts and vectors. While most disease systems will not behave in manner that is as clear and easily interpretable as these mathematic equations, the degree to which any individual system breaks these assumptions varies. We utilize a complex vector-borne disease system, plague in mammalian populations, to demonstrate how each of these assumptions could prove invalid. This analysis shows that classic models of transmission are useful for making relative comparisons of the force of infection given specific differences in a disease system. However, the validity of applying these models rests strongly in the assumptions made. As a result, the interpretation needs to be carefully considered and explicitly presented within the limits of the assumptions. Despite these potential limitations, we present situations in which these models effectively represent the biological system being modeled and suggest alternative methodologies to model more complex disease systems.

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SEROPREVALENCE AND RISK FACTORS FOR *RICKETTSIA* AND *LEPTOSPIRA* INFECTION IN PUERTO MALDONADO, PERU

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Rickettsiae and ***Leptospira*** are common but often under-recognized causes of acute febrile disease. ***Rickettsiae*** are characterized into spotted fever group ***Rickettsia*** (SFGR) and typhus fever group ***Rickettsia*** (TGR). The distribution of the two groups is closely related to the distribution of their arthropod vectors. Conversely, ***Leptospira*** comprise a diverse genus with global distribution and a wide variety of animal hosts. To determine the burden of ***Rickettsia*** and ***Leptospira*** infections in the Madre de Dios Region in the Peruvian Amazon, we evaluated the seroprevalence of these two pathogenic genera among participants of an existing community cohort study of respiratory disease in Puerto Maldonado, the capital city of Madre de Dios. ***Rickettsia*** exposure was assessed by ELISA and ***Leptospira*** by microscopic agglutination testing. We also assessed risk factors for infection associated with seropositivity, collected serum samples and ectoparasites from domestic animals, and captured rodents in and around households. The antibody prevalence in the 353 humans tested was 13.9% (95% CI: 10.4, 17.9) for SFGR, 6.4% (95% CI: 3.9, 9.3) for TGR, and 11.3% (95% CI: 8.2, 15.1) for ***Leptospira***. Associated risk factors for SFGR were contact with birds, working in agriculture, older age and male gender while the risk factor for TGR was increasing age. No specific risk factors were noted for ***Leptospira***. In 65 households we sampled 137 domestic animals (59 dogs, 37 chickens, 25 ducks and 16 cats), captured 30 rodents, obtaining 130 serum samples and 432

ectoparasites (45% fleas, 35% ticks, 18% lice and 1% rodent mites). The most common ectoparasite species were ***Ctenocephalides felis*** (34.0%) and ***Rhipicephalus sanguineus*** (32.5%). Testing of the samples obtained from household animals and ectoparasites is in progress and will be presented at the meeting. The study provides valuable information on the burden of ***Rickettsiae*** and ***Leptospira*** in the region as well as shedding light on possible transmission route from domestic animals to humans.

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CRIMEAN-CONGO HEMORRHAGIC FEVER INVESTIGATION - GEORGIA, 2014

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By September 2014, the country of Georgia's National Center for Disease Control and Public Health reported 22 cases of Crimean-Congo hemorrhagic fever (CCHF), the highest annual case count since surveillance began in 2009. CCHF is a highly-fatal hemorrhagic illness naturally transmitted by infected ticks and animal blood. We investigated the 12 rural villages reporting at least one CCHF case in 2014; the goals were to assess CCHF risk factors (i.e. ticks, husbandry, and slaughtering/butchering), document CCHF-related knowledge, attitudes, and practices (KAP), and determine seroprevalance. A calculated sample size of 904 participants was proportionally allocated to each village by population size; 457 households were randomly selected. We enrolled all consenting adults (≥18 years old) residing in the households for the preceding two months. We performed a KAP/risk factor survey in conjunction with an anti-CCHF IgM and IgG serosurvey. We used Epi Info™ for analysis. Of 634 people eligible and available to interview, 618 (97%) completed the survey. Mean respondent age was 54 years (Range: 18-101); 214 (35%) were male. Most (57%) were agricultural workers; 102 (17%) were herders. Of the 389 (63%) who had tick contact, 286 (74%) handled ticks bare-handed; 95 (33%) knew the associated risk. Of 605 respondents, 355 (59%) reported animal blood exposure; 32 (9%) knew the associated risk. The 349 (57%) respondents with CCHF risk factor knowledge were more likely to have risk factors than others surveyed (OR=3.07, p=0.006). Of 440 serum samples collected, 13 (3%) were positive (one IgM, 12 IgG); seven (54%) were male. Mean seropositive subjects' age was higher than mean seronegative subjects' (71 vs. 52 years, p<0.001). Eight (62%) seropositive subjects had tick contact and four (31%) had fever in the preceding four months. In these rural villages, CCHF risk factors are highly prevalent but knowledge is limited and does not lead to preventative practice; educational campaigns should target these issues. CCHF seroprevalence in these villages is similar to the region's (~3%), thus improved surveillance may account for the increased case detection in 2014.

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CAN BED BUGS MEDIATE AN URBAN CHAGAS EPIDEMIC?

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Chagas disease, a zoonotic and vector-borne disease caused by the protozoan ***Trypanosoma cruzi***, is endemic throughout much of Latin America and affects tens of thousands of people in the United States. Recently, a study conducted with laboratory mice determined bed bugs to be a competent vector of ***T. cruzi.*** In light of these results and the dramatic rise in bed bug prevalence in many US cities, we examine through mathematical modeling whether bed bugs could mediate a Chagas epidemic in an urban neighborhood. The probability of transmission occurring within a bed bug infested household was modeled through simulations in a discrete stochastic model of bed bug feeding and bed bug-mediated ***T. cruzi*** transmission. This intra-household model was then combined with a discrete SEIS contact network model of inter-household bed bug infestations to determine the conditions under which a transmission event between households might occur. Key parameters of the inter-household model include network connectivity, visitation rate between contacts, and bed bug prevalence. When possible, parameter values were drawn from laboratory studies and field observations, and where little data was available – parameter ranges were examined via sensitivity analysis. A major limitation of this study was a lack of data on human movements between households. Our preliminary results suggest intra-household transmission is highly probable, and inter-household transmission is probable in a neighborhood with a clustered contact network, a high contact rate and high bed bug prevalence.

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INCIDENCE FOR TICK-BORNE INFECTIONS IN A COHORT FOR NORTH CAROLINA OUTDOOR WORKERS

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Numerous cross-sectional studies have shown that outdoor workers, such as park and forestry rangers, have high seroprevalences of tick-borne infections. However, the incidences of these diseases in outdoor workers are rarely reported. Here, we report the incidence of tick-borne diseases in a cohort of North Carolina park and forestry rangers followed over a 2-year period with repeated serologies. A cohort of 159 outdoor workers from the North Carolina State Divisions of Forestry, Parks and Recreation, and Wildlife were followed for 2 years as part of a randomized controlled trial evaluating the effectiveness of permethrin-impregnated clothing. Antibody titers against ***Borellia burgdorferi***, ***Rickettsia parkeri***, ***R. rickettsii***, ***R. amblyommi*** and ***Ehrlichia chaffeensis*** were measured by immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) at baseline (n=130), after one year 1 (n=82), and after 2 years (n=73). An incident infection was defined as a 4-fold increase in IFA titer over a 1 year period. At baseline, seroprevalence (IFA titers of 1:128 or greater) among the workers were ***R. parkeri***, 24%; ***R. rickettsia***, 19%; ***R. amblyomii***, 12%; and ***E. chaffeensis***, 4%. There were no subjects who were reactive to ***B. burgdorferi*** C6 antigen at any point in the study. Over 2 years, there was one clinically confirmed case of Ehrlichiosis and one of Spotted Fever Rickettsiosis. There were 50 total seroconversions among 37 individuals to ***R. parkeri*** (n=19), ***R. amblyomii*** (n=14), ***R. rickettsii*** (n=9), and ***E. chaffeensis*** (n=8). The risk of infection by any pathogen during the study period was 0.26. The risk of infection was lower in subjects wearing permethrin-impregnated clothing, but not significantly (RR=0.80; 95% CI: 0.47, 1.39). In summary, outdoor workers in North Carolina are at high risk of incident tick-borne infections most of which appear to be asymptomatic.

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MOLECULAR DETECTION AND CHARACTERIZATION OF *'CANDIDATUS RICKETTSIA ASEMBOENSIS'* IN ECTOPARASITES FROM DOMESTIC ANIMALS IN IQUITOS, PERU

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While studying rickettsial infections in Iquitos, Peru, we identified ***Candidatus Rickettsia asemboensis*** sequences in multiple fleas, ticks and lice collected from domestic cats, dogs and chickens. Candidatus

Rickettsia asemboensis was first isolated in samples from Kenya. Since then asemboensis-specific PCR diagnostics have been developed and the full bacterial genome has been sequenced. Following the Kenya isolation and its complete genomic characterization, there has been a single additional report of asemboensis in Ecuador. Specifically, partial fragments of the 16S rRNA (1387 bp), gltA (350 bp), and ompB (464 bp) genes were amplified by PCR and sequenced. We now report multiple asemboensis isolations from Peru that have been fully characterized by multilocus sequence typing (MLST) of the 16S rRNA (1506 bp), gltA (1308 bp), ompA (1047 bp), ompB (4305 bp), and sca4 (1035 bp) genes. We are using these sequences, along with additional genomic information derived from next-generation sequencing of the Peru asemboensis isolates, to infer phylogenetic relationships among asemboensis strains reported to date, and further, to infer the molecular timeline of asemboensis evolution in the context of other Rickettsial strains. This work should help us better understand Rickettsial infections, which are a leading cause of undifferentiated febrile illness commonly found throughout Latin America.

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USE FOR A NOVEL REAL-TIME FRET PCR ASSAY FOR DETECTION FOR TICK-BORNE RELAPSING FEVER GROUP *BORRELIA* FROM *IXODES SCAPULARIS* TICKS COLLECTED IN WISCONSIN (2013-2014)

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Tick-borne relapsing fever (TBRF) is caused by at least 15 members of the ***Borrelia*** genus including several species endemic to the United States. Although most species in this group are transmitted through the bite of a soft-bodied tick, ***Borrelia miyamotoi*** is transmitted by the hard tick ***Ixodes scapularis***, the same vector as ***B. burgdorferi***. Human infections with ***B. miyamotoi*** have been reported from Russia and the northeastern United States (US), and DNA has been detected in ticks from Japan, Canada and the northeastern and midwestern US. We describe a sensitive and specific real-time PCR assay for detection of TBRF ***Borrelia*** and the results from testing Ixodes scapularis field-collected ticks (2010, 2013, and 2014) from Wisconsin. The TBRF PCR utilizes primers and fluorescence resonance energy transfer (FRET) probes targeting a sequence of the GlpQ gene which is specific for ***Borrelia*** in the relapsing fever group and is not found in the ***Borrelia*** sensu lato complex. The assay utilizes post-amplification melting temperature (Tm) analysis to differentiate ***B. miyamotoi*** from other causes of TBRF. Assay validation experiments showed that the novel PCR is capable of detecting all TBRF agents tested (***B. turicatae, B. hermsii, B. parkeri***) and has a lower limit of detection of 1 gene target per microliter of extracted DNA. The assay did not detect 16 tested ***Borrelia*** sensu lato species, (including ***B. burgdorferi***) or multiple other bacterial, parasitic and viral tick-borne pathogens. Five of 257 I. scapularis tested (1.9%; 1 nymphs, 4 adults) were positive for ***B. miyamotoi*** which is similar to other reported rates of detection from the upper midwestern states. The presence of ***B. miyamotoi*** DNA in ticks collected in Wisconsin suggests that ***Borrelia*** species associated with Relapsing Fever may be endemic in Wisconsin in hard-bodied ticks and may be predictive of future disease in human populations. This newly described real-time PCR assay allows for sensitive detection of TBRF ***Borrelia*** species and is a useful diagnostic and research tool.

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ASSESSING DISPERSAL AND DIETARY PATTERNS IN BED BUGS (*CIMEX LECTULARIUS*) FOR VECTOR RISK IN PHILADELPHIA, PA

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Cimex lectularius, the common bed bug, is abundant across the globe, particularly in urban regions. ***C. lectularius*** feeds exclusively on blood, primarily from humans, posing a risk as a vector of disease. We conducted a door to door survey of bed bugs in row homes in Philadelphia. 596 residents participated, 66(11.1%) of whom reported recent bed bug infestations. We confirmed infestations in 15 of these homes by inspection and collected insects when possible. Spatial analyses including Ripley's L and Moran's I were conducted showing that bed bugs may spread to the immediate neighboring home (15m). The lack of significant clustering on city blocks, however, suggests that the active movement of insects between houses is limited, and the presence of insects throughout the study area suggests that the rate of active dispersal is overshadowed by the rate of passive dispersal. Because bed bugs are competent vectors of ***Trypanosoma cruzi***, the etiologic agent of Chagas disease, the high prevalence of the insect may facilitate disease transmission between human populations. Moreso, bed bugs may serve as a vector between animal reservoirs and human hosts by feeding on the various species in the same residence. We have used multiplex PCR to detect the presence of human, mouse, dog, and cat blood in the gut of each ***C. lectularius***. Using ***C. lectularius*** samples collected from houses in the survey in Southern Philadelphia we will identify the source of each bug's last blood meal. We will use the dispersal and dietary patterns to better assess and address the risk bed bugs pose as a vector of disease to Philadelphia residents.

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THE EFFECT FOR LARVAL EXPOSURE TO METAL POLLUTION ON THE LIFE HISTORY AND INSECTICIDE RESISTANCE PHENOTYPE FOR THE MAJOR MALARIA VECTOR *ANOPHELES ARABIENSIS (DIPTERA: CULICIDAE)*

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Anopheles arabiensis is one of the major malaria vectors of Southern Africa. This vector has been shown to shown to be strongly affected by agriculture, but in this study, the effects of metal pollution on ***An. arabiensis*** was assessed. In this study,laboratory strains of ***An. arabiensis*** strains were used to determine whether unselected and strains selected for insecticide resistant respond differently to metal pollution. The SENN strain is an unselected strain originating from Sennar, Sudan that displays baseline resistance. The SENN DDT is selected for DDT resistance and displays multiple resistance phenotypes, mediated by the kdr mutation and elevated metabolic detoxification enzymes. Hatchling larvae of both strains were raised at the maximum acceptable toxicant concentration (MATC) of cadmium chloride, copper nitrate and lead nitrate. Development, size and subsequent longevity was monitored. The comparative lethal doses of the three metals were determined for both strains. Larvae of both strains were reared at MATC and the LD50 values for malathion and deltamethrin of 4th instar larvae and compared to control larvae. Similarly, the effect of rearing SENN DDT larvae at MATC on the insecticide resistance phenotype was assessed. The effect of all three metals on detoxification and oxidative stress enzymes was assessed. These studies demonstrate a marked fitness advantage of the resistant SENN DDT strain over their non-susceptible counterparts. The relevance of these findings will be discussed in context of increased urban and industrial pollution in Africa.

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SUBSTANTIAL FITNESS COSTS FOR WOLBACHIA INFECTION ON THE STARVATION RESISTANCE FOR *AEDES AEGYPTI* LARVAE

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The mosquito ***Aedes aegypti***, the principal vector of the dengue virus, has recently been experimentally infected with ***Wolbachia***: intracellular bacteria that possess enormous potential as dengue biological control agents. In order for ***Wolbachia*** to spread throughout mosquito populations and supress dengue transmission, infected mosquitoes must be able to compete with the native inhabitants for access to limited resources. ***Wolbachia*** depend on their hosts for a wide range of nutrients which they are unable to synthesize themselves. Consequently, competition between ***Wolbachia*** and their hosts for nutrients could negatively affect host survival and development under the resource-limited conditions commonly experienced by ***Ae. aegypti*** larvae in the field. We test the tolerance of ***Ae. aegypti*** larvae to starvation when infected with one of three experimentally-generated ***Wolbachia*** strains: ***w***Mel, ***w***MelPop and ***w***AlbB, and compare their survival to wild-type uninfected larvae. We find that all three ***Wolbachia*** infections reduce the starvation resistance of larvae relative to uninfected. Additionally, the relative costs to starvation resistance for each of the three infections are concordant with previously characterized fitness costs in other life stages; highly virulent strains that reduce adult lifespan and egg viability also substantially reduce the starvation resistance of larvae, while benign infections only have a small deleterious effect. A reduced ability of ***Wolbachia***-infected larvae to survive in resource-limited habitats will limit the potential for ***Wolbachia*** infections to establish in highly competitive ***Ae. aegypti*** populations and control the spread of dengue.

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IS MALARIA VECTORS PEAK BITING TIME CHANGING? THE IMPLICATIONS FOR MALARIA CONTROL IN WESTERN KENYA

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Malaria is a major cause of morbidity and mortality in Kenya. Prevention strategies include vector control efforts such as ITNs and IRS which exploit the vectors behavior of feeding and resting indoors. However recent studies indicate malaria vectors biting and resting behavior may be changing in response to control measures. This study was set up to assess the biting pattern, species composition and genetic mutations associated with resistance amongst malaria vectors in western Kenya. Mosquitoes were collected at 2 hr intervals throughout the night using a rotating specimen collector attached to a CDC light trap set in a room with 2 sleeping spaces covered by ITNs. Collection continued for 11 months. Mosquitoes were collected every other week for 4 consecutive nights. The first cup collected mosquitoes from 6:30-8:30pm and rotated each 2 hr interval thereafter. The last cup remained open from 6:30am until 9:30am. A trap set before 6:30pm collected mosquitoes into 'Cup 0' until 6:30pm. Malaria vectors from each cup were identified and abdominal status determined. A total of 1082 mosquitoes were captured; 636 ***Anopheles*** and 446 ***Culex***. 627 of the ***Anopheles*** mosquitoes were female. 5% (31/619) of the females were fed and 39% (12/31) of those were gravid. Most mosquitoes were caught before 6:30pm and after 6:30am with a minor peak between 1030pm-1230am. 494 anopheles were identified as either ***Anopheles gambie*** ss (48%), ***An. funestus***(33%) or An. arabiensis(19%). >50% of ***An. gambiae*** ss and ***An. arabiensis*** were captured before 6:30pm and after 6:30am while 41% of ***An. funestus*** were captured between 1030pm-0430am. Sporozoite rate was 1%. 79% of An. gambaie were homozygous for kdr 1014S allele, associated with pyrethroid resistance. There was increased activity of malaria vectors between 6:30-8:30pm and 6:30-8:30am, which could suggest early and late biting as well as exophily and endophagy in malaria vectors. There are significant levels of pyrethroid resistance in ***An. gambiae***. This could greatly compromise the effectiveness of current vector control interventions and thus alternative efforts are needed to address this behavioural change.

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EXPLOITING THE 'KIDNEYS' FOR MOSQUITOES FOR THE DEVELOPMENT FOR NOVEL INSECTICIDES

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New insecticides are needed to improve our capabilities for mosquito control due to the emergence of resistance to conventional control agents (e.g., pyrethroids). Here we describe our efforts to develop new insecticides that disrupt the renal functions of mosquitoes by inhibiting the activity of inward-rectifier K^+ (Kir) channels expressed in the Malpighian tubules. We show that the Malpighian tubules of adult female mosquitoes (***Aedes aegypti***) express 3 Kir subunits (Kir1, Kir2B, Kir3) that exhibit cell and membrane specific localizations within the epithelium. Furthermore, we report the discovery of small molecule inhibitors of mosquito Kir1 channels that disrupt the capacity of isolated Malpighian tubules to secrete fluid in a manner consistent with the disruption of Kir-mediated K^+ secretion. Lastly, we show that the small molecule inhibitors of Kir1 elicit toxic effects on adult female mosquitoes in part by disrupting their excretory capacity and/or regulation of hemolymph K^+ homeostasis. Our data indicate that the mosquito Malpighian tubules and Kir channels are valuable physiological and molecular targets, respectively, for the development of novel insecticides.

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LABORATORY EVALUATION FOR A MIXTURE FOR CLOTHIANIDIN AND DELTAMETHRIN AGAINST TWO RESISTANT *ANOPHELES* STRAINS

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Resistance to currently available insecticides is a major threat to the continued success in the fight against malaria. There are few insecticides which exhibit desirable features for vector control in their own right; however insecticide mixtures present interesting alternatives to explore. A mixture containing the neonicotinyl, clothianidin, and the pyrethroid, deltamethrin was compared in bioassays against both insecticides applied alone at equivalent rates, on wood, concrete and mud. Efficacy was investigated with the ***Anopheles gambiae*** RSP-H strain (100% kdr pyrethroid resistant) and ***Anopheles funestus*** FUMOZ-R (elevated levels of P450 - resistant to pyrethroids and carbamates). Against FUMOZ-R; deltamethrin efficacy was between 60% and 80% on wood, concrete and mud at 7, 5, and 5 months, respectively. Clothianidin alone failed to fully control FUMOZ-R on any of the treated substrates and efficacy remained below 60% except for few readings at 9 months. The mixture achieved a higher level of mortality and better overall residuality across all tested surfaces with smaller variations between evaluations. The mixture achieved efficacy of 80%-100% for 9, 8, and 6 months on wood, concrete, and mud, respectively. Against RSP-H, deltamethrin performed surprisingly well, with efficacy above 80% up to 9 months on all surfaces; Clothianidin performed well initially on wood and remained between 60%- 80% (with high variability and few readings above 80%) for 7, 4 and 4 months on wood, concrete and mud, respectively however the mixture achieved 100% efficacy throughout 9 months on all surfaces. This study suggests that the mixture performs more reliably against pyrethroid resistant mosquito strains on a range of surfaces than either insecticide applied alone at equivalent rates. Further studies are underway to confirm

this finding in other mosquito strains and under field conditions. The implications and potential relevance of such a mixture for indoor residual spray application is discussed.

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CHARACTERIZATION FOR GLUTATHIONE-S TRANSFERASE EPSILON 2 (GSTE2) ISOFORMS IN *ANOPHELES STEPHENSI*

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Glutathione-***S*** transferase belongs to a multifamily gene responsible for metabolism and detoxification of endogenous and xenobiotic compounds by catalyzing the conjugation of glutathione (GSH) with electrophilic compounds resulting in products with greater water solubility easing excretion. Amongst the many cytosolic GST classes, Delta and Epsilon are specific to insects and show a relationship with resistance. Metabolization of DDT due to enhanced DDT dehydrochlorinase activity by enzyme glutathione-***S***-transferase (GST) is one of the mechanisms known in insecticide resistance, which is due to qualitative or quantitative changes, in particular, overexpression of GST epsilon2 (GSTe2) gene. Here we report the occurrence of multiple GSTe2 isoforms in the Asian malaria vector ***Anopheles stephensi.*** Cloning and sequencing of full GSTe2 gene from this vector revealed the presence of four isoforms. The two most abundant isoforms GSTe2_1 and GSTe2_2, differed by six amino acids arising from 12 bp insertion and four bases substitutions. These genes were expressed in ***E. coli*** for functional analysis. ***In vitro*** assays showed that these two recombinant isoforms are efficient in metabolizing DDT showing 83% and 68% DDT depletion, respectively, after 1 hour in the presence of cofactor glutathione. Other two isoforms (GSTe2.3 and GSTe2.4) are rare and found in DDT-susceptible mosquitoes.

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DISTRIBUTION OF *ANOPHELES GAMBIAE* S.L. AND ITS INSECTICIDE RESISTANCE PROFILE IN TANZANIA: IMPLICATIONS FOR MALARIA VECTOR CONTROL

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The ***Anopheles gambiae*** species complex exhibits an enormous diversity in its biology which impacts greatly on its importance as a vector of malaria. This study investigated the distribution of members of the ***An. gambiae*** complex and their insecticide resistance profile relative to the ecological differences found across Tanzania. Indoor-resting ***Anopheles*** mosquitoes were collected from 14 districts located across various ecological settings of Tanzania. These were morphologically identified and tested for their susceptibility to deltamethrin, lambdacyhalothrin, permethrin, propoxur, fenitrothion and DDT using standard WHO methods. Molecular diagnostics were used to genotype mosquitoes and screen for resistance mechanisms. A total of 7,596 mosquitoes were morphologically identified as ***An. gambiae*** s.l. of which 2,947 were identified to their species level. Out of these, 69% and 31% were ***An. arabiensis*** and ***An. gambiae*** s.s. respectively. Overall ***An. arabiensis*** predominated and was distributed widely across all ecological zones. The distribution of resistance was not homogenous. The species complex was resistant to the three pyrethroids tested (mortality rate < 90%) in Moshi, Arumeru, Muheza and Muleba. Resistance to DDT was recorded in Dar es Salaam and Muleba (mortality rate< 90%). There was no resistance to propoxur or fenitrothion. Knock down resistance mutations L1014S and L1014F were detected in some parts of the country in both species with varying allelic frequencies (4-41%). L1014S mutations were found most frequently in ***An. gambiae*** s.s. ($p<0.05$). The cytochrome P450s; cyp6p3, cyp6m2, cyp6z1 and cyp6z3 were significantly overexpressed in ***An. gambiae*** s.s. resistant to DDT. We have demonstrated the predominance and wide distribution of ***An. arabiensis*** in Tanzania. We have also demonstrated that ***An. gambiae*** s.l. is becoming resistant to pyrethoids and DDT in several parts of the country. This appearance of resistance mandates close monitoring and adoption of rational resistance management strategies in the country if the gains so far made in malaria control are to be sustained.

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BEHAVIORAL CHARACTERISTICS FOR MALARIA VECTORS IN A HIGH INSECTICIDE TREATED NET COVERAGE AREA FOR WESTERN KENYA

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The scale-up of ITN coverage to control malaria hasreturned mixed resultsdue to over exposure of malariavectors to insecticides. The aim of this study was to determine factorsresponsiblefor continued malaria transmission in spite of wide ITN coverage in western Kenya. Malaria vectors were captured usingCDC light trap, PSC and window exit traps. ***Anopheles gambiae*** group were identified to species using PCR while the sporozoites rate was determined by ELISA method, and kdr genotyping was used to determine L1014S and L1014F alleles. Window exit traps collected fewer mosquitoes; only 15 over 329 nights of collection compared to 1061 in 1598 nights of CDC light trap collections and 269 in 178 PSCcollections. Amongst anopheline vectors identified (n=1,044), 68.2% were ***An. gambiae***ss, 20.1% were ***An. arabiensis*** and ***An. funestus*** comprised 11.7%. The proportion of ***An. gambaiess*** was slightly higher in CDC light traps, althoughthese differences were not statistically significant (72% v 67%, p=0.27). 26.4% of anopheles mosquitoes were fed, but the sporozoite positivity was only 1%. There were significant differences in feeding status by type of collection technique; 70% of collections by PSC were fed compared to only 18% of those in the light trap. 255 ***An. arabiensis*** and 817 ***An. gambaie*** were genotyped at kdr allele as LL (wildtype), LS or LF (heterozygous resistant), and SS or FF (homozygous resistant). A small number of mosquitoes carried the F allele; 2.1% An. gambaie and 5.1% ***An. arabiensis*** were heterozygous LF and 0.4%***An. gambaiess***were homozygous FF. 67% of ***An. gambaiess*** were homozygous resistant (SS) compared to 14% ***An. arabiensis***. Homozygous resistant ***An. gambaiess*** were slightly more likely to have fed (30% vs. 23%, p=0.10), but homozygous resistant ***An. arabiensis*** were slightly less likely to have fed (18% vs. 40%, p=0.058). Low proportion of ***An. arabiensis*** captured indoors confirms this mosquito bites indoors but rests outdoors, which is a challenge to malaria control. We report both L1014S and L1014F kdr alleles; these could have negatively impacted vector competence and hence low sporozoite rate reported here.

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EVIDENCE OF MIXED FUNCTION OXIDASES MECHANISM IN *ANOPHELES GAMBIAE* S.L. WITH PYRETHROID RESISTANCE IN MALI

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There is a growing concern that the spread of vector resistance to pyrethroid insecticides (PYs) may compromise the effect of long lasting insecticidal nets (LLINs). The only alternative products available for use in areas of pyrethroid resistance (pyrR) are LLINs treated with PYs plus piperonyl-butoxide (PBO), a synergist that inhibits the activity of mixed function oxidases (MFOs) in the mosquitoes. Therefore the President's Malaria Initiative supported a field evaluation of LLINs with PYs plus PBO in Mali. One of the activities in this study was verifying the role of the MFO mechanism in ***Anopheles gambiae*** s.l. with pyrR. ***An. gambiae*** s.l. larvae were sampled in 38 villages from 2 Districts in southern Mali from August to September 2013. The distance of the villages in the same district is 5 to 10 Km. Local vector populations were screened with the Centers for Disease Control and Prevention bottle bioassay test for permethrin and deltamethrin resistance. Bottle assays with PBO as a synergist were used to determine the association between reduced survival and elevated levels of MFOs. Taxonomic identification to species as well as presence of the L1014F kdr (knock down resistance) mutation were determined by the polymerase chain reaction. According to WHO 2013 resistance classification there was evidence of high pyrR in all 38 villages tested. After exposure to PBO, the mortality increased significantly ($P<0.05$) in 18 out of 38 villages showing evidence of a MFO-based metabolic resistance mechanism involved in pyrR. There was high variability both in resistance and the effect of PBO on mortality from one village to another. The L1014F kdr allelic frequencies were also high: 25%-100% in ***An. coluzzii***, 69%-100% in ***An. gambiae*** s.s. This study confirmed the presence of high variability of the MFO mechanism in wild-caught mosquitoes with pyrR from one village to another in southern Mali.

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ENHANCING ENTOMOLOGICAL MONITORING FOR THE NATIONAL MALARIA CONTROL PROGRAM IN THE DEMOCRATIC REPUBLIC OF CONGO

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Since 2012, the United States President's Malaria Initiative (PMI) has supported the National Malaria Control Program (NMCP) in the Democratic Republic of Congo (DRC), one of the highest malaria burden countries, by financing malaria key interventions including entomological monitoring through the Africa Indoor Residual Spray (AIRS) Project. The NMCP has set the objective to regularly monitor vectors throughout the country, and be able to have in the near future a well-defined map with vectors classification, their behavior, density, and even resistance to insecticides. The (INRB), with the close support of PMI, has conducted this monitoring in sentinel sites throughout the Democratic Republic of Congo. In 2014, entomological monitoring was conducted in seven sites in five provinces: Kinshasa, Kisangani, Lodja, Tshikaji, Mikalayi, Fungurume, and Kapolowe. In each site, human landing catches and pyrethrum spray catches were conducted to determine biting rates and to find the species present in each site. Furthermore, resistance tests were conducted using WHO tube tests and mosquitoes were analyzed for presence of resistance mechanisms (specifically kdr). ***Anopheles gambiae*** s.l. was the primary malaria vector in DRC, but several other malaria vectors were collected (***An. funestus***, ***An. moucheti, An. nili***, and ***An. paludis***). The detection of large numbers of ***An. paludis*** has led to a separate study to evaluate its importance as a vector in Lodja. Resistance to dichlorodiphenyltrichloroethane (DDT) has been widely detected (as was resistance to permethrin in 2013), deltamethrin resistance was suspected in some cases, and little resistance to carbamates and organophosphates was found. The sentinel site system continues to be improved and harmonized with the epidemiological sentinel site system. The regular collection of entomological data provides useful information to the NMCP to inform its vector control decisions and research priorities. Results from the newly implemented surveillance system will be shown.

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A NOVEL LARVICIDE DELIVERY SYSTEM FOR ARTHROPODS

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For many of the world's most dangerous vector-borne diseases, larvicides are essential to effective disease control. Regrettably, larvicides in common use are limited by toxicity, degradation of aquatic resources, evolution of resistance, and cost. We have developed a novel approach to larval eradication designed to overcome these limitations: use of Baker's yeast (***Saccromyces cerevisae***) as a delivery vehicle for essential oils. Our method of loading essential oils produces a yeast cell continent to the entrapped oil but no longer viable. Essential oils are produced by plants to combat larval and adult insects and are not toxic to mammals. They are lethal to mosquito larvae at low concentrations (50 ppm), but have not been tested against the larvae of leishmaniasis vectors. We hypothesized that (a) larvae of the leishmaniasis vector ***Lutzomyia longipalpis*** would consume yeast loaded with an essential oil and (b) consumption of these loaded yeast cells would be fatal to the larvae. To test this hypothesis we fed 3rd instar ***L. longipalpis*** larvae yeast loaded with lemongrass oil, an essential oil with established cidal activity against mosquito larvae. We completed two trials, with experimental and control groups of approximately 30 larvae each. The experimental group was fed a mixture of loaded yeast cells and fish meal (95:5 w/w %). The control group was fed a mixture of yeast cells free of essential oils and fish meal in the same ratio. After 24 hours all larvae fed essential oil-loaded yeast had been killed, with only one death occurring in the control group. Although the sample size is small, these results argue lemongrass oil is cidal to ***L. longipalpis*** larvae and that essential oil entrapped in a yeast delivery vehicle merits investigation as a novel approach to disease control. It is worth noting that preparation of the essential oil-loaded yeast cells is low in cost, requiring no specialized equipment or technical skill. These attributes, combined with evidence that lemongrass oil is cidal to the larvae of multiple arthropods, suggest that the essential oil -yeast system may be a viable platform technology for control of multiple vector borne diseases.

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FINE SCALE HETEROGENEITY OF KDR FREQUENCIES AND DELTAMETHRIN RESISTANCE IN *AEDES AEGYPTI* IN MEXICO

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Insecticide resistance is a widespread concern in the control of vector borne diseases, including dengue fever. Insecticide resistance monitoring is most often carried out at sentinel sites, and outcomes are extrapolated to describe vector populations over a large geographical scale. The accuracy of these extrapolations can be called into question if insecticide pressures are heterogeneously applied on a finer scale, as is often the case with reactive insecticide spraying targeting ***Aedes aegypti*** around the houses of reported dengue cases in Mexico. To determine the scale at which heterogeneity could be detected in insecticide resistance phenotypes as well as kdr molecular markers, a cross-sectional entomological study was conducted in two small dengue-endemic towns located on the Yucatan peninsula of southern Mexico. Five blocks were sampled in each town. Ovitraps were placed in ten randomly selected houses on each side of the blocks, and a minimum of ten houses on each block were selected for adult mosquito collections. Adult female ***Ae. aegypti*** collected from inside houses were genotyped to determine kdr allele frequencies and insecticide bioassays were performed on mosquitos reared from field-collected eggs to determine phenotypic resistance. Significant differences between blocks were detected in deltamethrin susceptibility ($p<0.0005$) and F1534C allele frequency ($p<0.0001$). No significant evidence for the stability of heterogeneity was observed when comparing town level resistance data over the time period of a year ($p>0.1$). Fine scale variability of insecticide resistance is a particular concern from an operational perspective, since the ability to tailor vector control interventions at such a scale is limited for many vector control programmes. The operational implications of such fine-scale variability in ***Ae. aegypti*** insecticide resistance will be discussed, with particular reference to limitations in the ability of vector control programmes to conduct insecticide resistance management and monitoring on a small scale.

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A NOVEL VIDEO-TRACKING SYSTEM TO RECORD BEHAVIOR FOR NOCTURNAL MOSQUITOES ATTACKING HUMAN HOSTS

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The mosquitoes that transmit malaria and many other vectors spend most of their adult life within human homes. It is here that they bloodfeed and transmit infections and also where vector control, via residual insecticides, is most effective in preventing transmission. Knowledge of vector behavior in this environment is limited, particularly when describing how mosquitoes attack a human host and how insecticides impact on such behavior. This is partly because satisfactory technology for tracking these small nocturnal insects in traditional dwellings in disease endemic communities has never been available. As better knowledge of such behavior is a pre-requisite for rational design of novel interventions, we have developed a novel camera tracking system that allows the real-time observation of nocturnal mosquitoes and records their behavior when attacking human hosts, within or without a bednet. The system addresses the requirements for high resolution over a substantial volume (approximately 2.0 x 2.4 x 1.0m) under nocturnal conditions. We describe the data processing strategies required to extract individual mosquito trajectories and the algorithms to derive insights into mosquito behavior as they approach, search and interact with a bednet baited with a human host. The high resolution of the system has revealed different behavioral modes in mosquitoes attacking a human-occupied bednet, and enabled detailed quantification of flight activity, velocity and contact duration at precise locations. The complete tracking device has been deployed successfully at a remote location in rural Tanzania, enabling the behavior of wild mosquito populations to be investigated. The system has considerable potential for use in behavioral studies of mosquitoes and many other vector groups, and it may provide a new route for investigations ranging from fundamental questions on basic behavior to the evaluation of vector control tools.

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LABORATORY STUDIES FOR VECTOR COMPETENCE OF *CULEX ERRATICUS* FOR EASTERN EQUINE ENCEPHALITIS VIRUS

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Field studies of the ecology of Eastern Equine Encephalitis virus (EEEV) in the Southeastern USA have demonstrated that ***Culex erraticus*** is among the most common mosquitoes at many EEEV endemic sites. ***Cx. erraticus*** is often infected with EEEV, implicating it as a potential vector in this area. However, the competence of ***Cx. erraticus*** for EEEV has not been explored in detail, though early data have suggested that it might serve as a vector. To further explore the competence of this mosquito species for EEEV, ***Cx. erraticus*** females were collected from wetlands in Tampa, Florida and used in laboratory transmission studies. To confirm that females were not previously exposed to EEEV in nature, mosquitoes were first allowed to feed upon a droplet of honey that was then tested for presence of EEEV. Females were then starved for 48 hours before being allowed to feed upon chickens infected with EEEV (log10 PFU = 4.17-5.39 PFU/ml). Blood-engorged mosquitoes were then placed into individual containers and maintained for 14d. The rearing containers contained honey droplets to provide nutrition for the mosquitoes and to monitor for the presence of EEEV in mosquito saliva. The honey was changed out every other day. At 14d post feeding, mosquitoes were frozen, dissected and examined for the presence of EEEV by RT-PCR and IFA. Nucleic acids were also extracted from the honey samples and screened for the presence of EEEV RNA. EEEV RNA was detected in 89% of the bodies of the infected ***Cx. erraticus*** and in the legs of 64% of the individuals, providing evidence of a disseminated infection. Of the 19 individuals that survived for the entire study period, 16 (84%) of the mosquito heads were IFA positive for EEEV antigen; of these EEEV RNA was detected in the honey from 15 (79%), indicative of EEEV in the saliva. Together, these data suggest that ***Cx. erraticus*** is a competent vector of EEEV.

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EVIDENCE FOR *AEDES AEGYPTI* OVIPOSITION ON BOATS IN THE PERUVIAN AMAZON

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The dengue vector ***Aedes aegypti*** is invading peri-urban and rural areas throughout Latin America. Our previous research in the Peruvian Amazon has shown that river boats are heavily infested with immature and adult ***Ae. aegypti***, likely playing a major role in their long-distance dispersal and successful invasion. However, the presence of immature mosquitoes provides no information about the timing of oviposition, and whether it took place in the boats. Here, we used baited ovitraps deployed on river boats to test the hypothesis that ***Ae. aegypti*** oviposition occurs during boat travel. We deployed a total of 360 ovitraps on 60 different barges during August and October of 2013, and February of 2014 (with 20 barges sampled during each month). We found ***Ae. aegypti*** mosquitoes in 22 individual ovitraps from 15 out of 60 barges (Premise Index 25%) across all sampling dates. Further, the distribution of ***Ae. aegypti*** egg abundance was highly aggregated: 2.6% of traps (N=7) were responsible for 71.8% of eggs found, and 1.5% of traps (N=4) were responsible for all (100%) of the larvae found. Similarly, 5% of boats were responsible for 71.5% of eggs. To complement our findings that a few boats are responsible for most mosquito production and movement over long distances, we now add the observation that a few oviposition sites are responsible for much of the larval production. When oviposition data are considered together with transportation data, we found that 1) large barges are a significant source of propagule pressure (the frequency and intensity of new introductions) for ***Ae. aegypti*** mosquitoes at all life stages 2) potential mosquito introductions may occur more frequently at different times of the year, as human transit in the Amazon is seasonal (even though mosquito oviposition is not). Our results provide strong evidence that ***Ae. aegypti*** oviposition commonly occurs during boat travel, and that the whole life cycle may be completed during a large barge voyage. Baited ovitraps targeted to specific boats, sites within a boat, and times of year are a potential cost-effective means of monitoring and controlling mosquito populations on boats, and introduction to new sites.

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INTEGRATED ENTOMOLOGIC, PARASITOLOGIC AND IMMUNOLOGIC SURVEILLANCE FOLLOWING CESSATION FOR MDA IN SENTINEL COMMUNITIES IN SIKASSO, MALI

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After 7 years of annual albendazole/ivermectin mass drug administration (MDA) in 6 ***Wuchereria bancrofti*** (Wb) previously highly endemic villages in Mali when WHO stopping criteria were met in 2008, surveillance was instituted over the subsequent 5 years using a variety of methods that included; circulating filarial antigen (CFA) tests (both ICT and ELISA) and antibodies to the Wb-specific antigen (Wb123) in all 6-7 year old children. Entomological surveillance was also performed using dissection of ***Anopheles gambiae*** complex.vectors collected monthly (from July to December) by human landing catch (HLC) and an additional pyrethrum spray catch (PSC) mosquito collection that relied on screening pools of ***Anopheles gambiae*** complex.using reverse-transcriptase polymerase chain reaction (RT-PCR) for infection/infectivity. Data from the children demonstrated increases in CFA prevalences over time with 0% (0/289) in 2009, 2.7% (8/301) in 2011, 3.9% (11/285) in 2012 and 4.1% (13/318) in 2013(Trend Chi2= 10.19, p= 0.0014). In 2012 when Wb123 antibody was assessed concurrently, there was a reported prevalence of positive responses of 1.8% (5/285) that was similar to that of the CFA prevalence (5/285). For the entomologic assessments, over the various sampling times using HLC, only two Wb-infected ***Anopheles*** were observed despite 14,575 mosquitoes dissected. Using the PSC method and RT-PCR technique, no positive pools were observed.Although CFA positivity in children was used as the major surveillance tool, adults (8-65 years old) were also assessed. Unlike the children, adults showed a decrease of the CFA prevalence from 4.9% (39/800) to 3.5% (28/795) and 2.8% (50/1,812) respectively in 2009, 2011 and 2012 (Trend Chi2= 7.361, p=0.0067) though microfilaremic adults were found within ICT positive subjects 2.6% (1/39) in 2009 and 8.3% (3/36) in 2011. These data are consistent with a small but real degree of Wb transmission 5 years after cessation of MDA in a previously highly endemic area of Mali that met the stopping criteria. Most of this transmission appears to be driven by a relatively few microfilaria carriers among the adult population.

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DEVELOPMENT AND OPTIMIZATION OF THE SENTINEL MOSQUITO ARBOVIRUS COLLECTION KIT (SMACK)

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Sentinel animals have long been are used throughout the world to monitor arbovirus activity in remote areas. However, there are ethical and safety implications associated with using the animals, and cross-reactions

in serological assays make it difficult to distinguish closely related viruses. Mosquito traps are a viable alternative, but most require batteries and are often set overnight, thus making them unsuitable for remote locations. To overcome these challenges, our goal was to develop a passive sentinel mosquito arbovirus collection kit (SMACK) that allows for the detection of arboviruses on honey-baited nucleic acid preservation cards (Flinders Technology Associates; FTA) that can compete with standard battery-powered light traps. The efficacy of the SMACK as a mosquito trap was tested against the CDC light trap, EVS light trap, and CDC blacklight (UV) trap in a field trial conducted in tropical north Queensland, Australia. The SMACK caught comparable numbers to both CDC traps when CO_2 was supplied either by a gas cylinder (500ml/min) or dry ice (1kg), and captured significantly more mosquitoes (pooled data) (t_7=0.98, P=0.02) than the EVS trap. Daily survival of field-collected mosquitoes was significantly greater (t_{12}=2.68, P=0.01) in the SMACK (93.1±3.6%) compared to a standard passive box trap (PBT) (31.4±5.4%). This increased survivorship will maximize the number of sugar-feedings on honey-baited FTA cards as it was determined that 66.8±16% of field-collected mosquitoes sugar-feed at least twice over a period of 72hr. We are currently testing the ability of the SMACK to serve as a sentinel arbovirus surveillance platform at three locations in the remote Cape York area of northern Australia. Thus far, 7 FTA cards have tested positive for Kunjin virus (KUNV) and 2 have tested positive for Murray Valley Encephalitis (MVEV), with both MVEV and KUNV virus being detected from a single trap on one occasion. The simplicity and longevity of the SMACK, combined with its ability to detect arbovirus activity in remote locations, makes it a viable alternative to sentinel animal programs and a practical substitute for standard light traps.

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EXPERIMENTAL HUT ENTRANCE AND RESTING BEHAVIOR OF *ANOPHELES DARLINGI* FROM ZUNGAROCOCHA, LORETO, PERU

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Anopheles darlingi is the most important malaria vector in the Amazon region, therefore rigorous characterization of factors contributing to its vector competence is vital to its control and prevention of human-vector contact. Patterns of ***An. darlingi*** human landing rates (inside and outside of homes), house entry, and indoor resting were described using experimental huts located in Zungarococha village, a malaria-endemic area in Loreto, Peru. Landing rates (mosquitoes caught/person/hour) were evaluated for three days each month during a 12-month experimental period. Entry behavior (mosquitoes caught/trap/hour) was evaluated for six days each month during the same time period using interception traps placed on experimental huts windows and doors. Indoor resting behavior of wild-caught and colonized adult females was observed for six days each month during a 3-month period using a mark-release study design to record preferences on wall, ceiling, window, door, floor and other sites within huts. Results of these studies indicated a primarily peak in landing rates inside and outside of homes between 2100h to 2300h, and occasionally between 1800h-2000h outside homes; no defined peaks in hut entry were recorded. In fact, entry rates were very low compared with landing rates inside homes. The peak of indoor resting behavior for both wild-caught and colonized ***An. darlingi*** occurred between 1800h to 2100h with a preference for resting in the lower part of hut surfaces (door and wall) in both populations. Results from this study provide useful insights into the behavior of ***An. darlingi*** in malaria-endemic area in the Peruvian Amazon, which can help guiding vector control strategies in the region.

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BRUGIA MALAYI INFECTION INFLUENCES VECTOR LONGEVITY, FECUNDITY AND HOST-SEEKING BEHAVIOR

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Understanding vector-parasite interactions is increasingly important as we move towards the eradication of lymphatic filariasis. While mass drug administration is reducing the parasite burden within populations to low levels it is necessary to determine the effects of reduced microfilaria (mf) density on disease epidemiology and vector behavior. ***Aedes aegypti*** mosquitoes were exposed to the filarial parasite ***Brugia malayi*** at low (5,450 - 7,750 mf/ml), moderate (10,550 - 15, 400 mf/ml) and high (15,900 - 17,900 mf/ml) levels of microfilaremia, while control mosquitoes were fed uninfected blood. The effect of an infection on host-seeking behavior was examined using a short range host assay. The proportion of mosquitoes responsive to a human host at different developmental periods in the parasite life cycle was recorded and a subset dissected to determine parasite prevalence and mean intensity. Volume of ingested blood and fecundity were also compared between infected, exposed and control mosquitoes. Mosquito mortality was observed for 16 days post-exposure, and each mosquito dissected to record infection status. We observed a 5 fold increase in host receptivity when infective stage larvae (L3) were present as opposed to developing stage (L1 and L2) ($p<0.001$). In control mosquitoes, a reduction in receptivity was observed during the same period. Furthermore, receptivity was density dependent with non-receptive mosquitoes harboring a greater burden of L1 and L2 while receptive mosquitoes harbored a greater number of L3 ($p<0.001$). Density dependent decreases in mortality ($p<0.001$) and fecundity ($p<0.01$) were observed, but the ability of mosquitoes to feed to repletion remained unaltered.

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TESTING THE EFFECTS OF LATITUDE ON LIFE HISTORY OF ARBOVIRAL VECTORS *CULEX PIPIENS* AND *CULEX QUINQUEFASCIATUS*

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The mosquito species ***Culex pipiens*** and ***Cx. quinquefasciatus*** (Diptera: Culicidae) are important vectors of arboviruses worldwide, including West Nile Virus. Together, these sibling species span the entire United States and, where they overlap geographically, there is evidence for hybridization between the two. We conducted laboratory experiments to investigate the adaptability of mosquito populations to temperature, in order to examine population differentiation. ***Culex*** egg rafts were collected from 6 sites spanning three regions along the Eastern U.S. coast (north, central, south). These eggs were reared at two fixed temperatures (16°C and 26°C). Three life history traits were analyzed: larval growth rate (days to pupation), adult body size (correlated with pupal weight), and preadult developmental time (days to emergence). Mosquitoes from the three regions differed significantly in pupal weight, with southern mosquitoes smaller than the others ($p = 0.009$). Region effects on development time and growth rate were marginally significant, with generally faster development and higher growth rates for northern mosquitoes ($p = 0.099$). Both rearing temperature and sex were highly significant for all three traits ($p < 0.001$). Highly significant interactions between region and temperature for all three traits ($p < 0.001$) indicate a region-specific plastic response to temperature variation. In conclusion, these experiments highlight the plasticity of these two vector species. Their individual abilities to adapt to

different temperatures may help elucidate the impacts of climate change on the biological traits of these mosquitoes associated with vectorial capacity and arbovirus transmission.

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POPULATION ECOLOGY OF A RIFT VALLEY FEVER VIRUS VECTOR: A COMPREHENSIVE ANALYSIS OF ENVIRONMENTAL DRIVERS OF *AEDES MCINTOSHI* ABUNDANCE IN KENYA

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One disease of great economic and human health importance in East Africa is Rift Valley Fever virus (RVFV). Current mosquito forecasting models focus on climate and vegetation monitoring at broad temporal scales, but this approach assumes mosquito population dynamics are homogenous between species, and many questions remain regarding environmental drivers of individual species emergence. One example is the mosquito species ***Aedes mcintoshi***, identified as the primary vector of RVFV in East Africa. We hypothesized that environmental drivers at fine temporal scales contribute to ***Ae. mcintoshi*** counts at specific locations and that these drivers can be measured using existing remotely-sensed environmental data. We analyzed relationships between ***Ae. mcintoshi*** abundance counts from repeat sampling conducted by the United States Army Medical Research Unit - Kenya (USAMRU-K) at 28 georeferenced locations across Kenya and a suite of remotely sensed environmental data, using a generalized linear mixed modeling (GLMM) approach. Specifically, we analyzed the contributions of Moderate Resolution Imaging Spectroradiometer Land Surface Temperature data, Normalized Difference Vegetation Index data, daily Climate Hazards Group InfraRed Precipitation with Station data, and SPOT Vegetation Small Water Bodies Africa data to abundance. Preliminary results indicated that a relationship exists between ***Ae. mcintoshi*** counts and elevated precipitation approximately 2 weeks prior to the sampling date and elevated LST values up to 32 days prior to the sampling date. Relationships between total area of free water, humid vegetation, or dry land and ***Ae. mcintoshi*** counts was not detected surrounding sampling sites. Future directions include analyses of additional RVFV vectors and spatio-temporal interactions between species. Knowledge of these factors will contribute to a better understanding of the disease ecology of RVFV and complement current forecasting methods, contributing to more efficient vector control applications and distribution of public health resources.

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ENHANCEMENT OF *PLASMODIUM VIVAX* OOCYST PRODUCTION IN *ANOPHELES DARLINGI* EXPERIMENTAL INFECTIONS

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Because ***in vitro Plasmodium vivax*** gametocyte culture is not possible, obtaining infective ***P. vivax*** sporozoites for drug screening and other studies remains a challenge. The recently reported establishment of an ***Anopheles darlingi*** colony in Peru—freely mating and currently in its 24th generation—in proximity to ***P. vivax***-infected humans has the potential to provide stable sporozoite production. This study evaluated methods for standardizing and maximizing the production of ***P. vivax*** sporozoites in laboratory-reared ***An. darlingi***. With regard to enhancing ***P. vivax*** mosquito infected, we tested 1) the effect of anticoagulants EDTA, citrate, and heparin in donor blood samples; 2) the effect of adding penicillin/streptomycin to membrane feeds of mosquito in terms of infection and survival. Non-blood-fed adult female ***An. darlingi*** mosquitoes 3 to 7 days post-emergence were fed on ***P. vivax***-infected blood and maintained up to 15 days on 10% sugar at 27°C and 80% humidity in 12 hour light/dark cycles. Oocysts were enumerated by midgut light microscopy on day 8; whole body sporozoites were quantified in a Neubauer chamber on day 15. ***P. vivax*** midgut infection (***N***=150) assessed by oocyst counts were significantly correlated with donor gametocyte densities. Sporozoite production was highly variable (range 75-21,954 ± 331.6) and was not associated either with oocyst density or with donor sexual or asexual parasitemia. With 3 donors, mean oocyst per mosquito midgut was significantly higher with heparin (90.4) compared to EDTA (59.7) or citrate (42.56). Use of penicillin/streptomycin (20 µg/ml in blood mean; and in 10% sugar solution after blood feeding) (N=6440) did not significantly increase oocyst density but did increase female ***An. darlingi*** survival (54% to 76%) at day 14. The effect of these interventions on sporozoite production is being evaluated.

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MOSQUITO SALIVA PROMOTES A TH1 IMMUNE RESPONSE IN HUMANIZED NOD-SCID IL2RGAMMA-NULL MICE

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Aedes aegypti mosquito saliva increases dengue virus pathogenesis in humanized mice. Since mosquito saliva also acts as a vasodilator and conveys various advantages to mosquito blood feeding, we investigated whether ***Ae. aegypti*** mosquito bite stimulates an immune response in humanized mice. To answer this question, we used flow cytometry to monitor human innate immune responses in the skin, spleen, blood, and bone marrow of humanized mice post mosquito bite. We found that saliva alone increases activation of human dendritic cells in the bone marrow and natural killer cells in the peripheral blood 24 hours post bite. Also, we observed increases in both IL12 and IFN, which lead to a dominant T_h1 immune response. These results suggest that ***Ae. aegypti*** mosquito saliva preferentially promotes a T_h1 immune response in humans to avoid a T_h2 response. Because a T_h1 response promotes an antiviral state, whereas a T_h2 response promotes an allergenic state, viruses dependent on mosquitoes for transmission may have developed mechanisms to counteract the saliva-induced T_h1 immune response. In the future, we will use the same approaches to study these human immune responses in humanized mice bitten by mosquitoes infected with dengue virus.

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THE HOST FEEDING PATTERNS OF *ANOPHELES* MOSQUITOES FROM MALARIA ENDEMIC VILLAGES IN MADANG PROVINCE, PAPUA NEW GUINEA

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Vector-human contact during the blood-feeding event, where malaria parasites are exchanged between the two hosts, is an important factor that determines malaria transmission. However, in addition to humans, mosquitoes may feed on other vertebrates. The choice of a vector to feed on alternative hosts or to feed only on a subpopulation of humans can influence malaria transmission. Here, we investigated the host-feeding pattern of ***Anopheles*** mosquitoes from five different villages in Madang Province, Papua New Guinea. Mosquitoes were sampled using the barrier screen sampling method. DNA was extracted from blood-fed specimens

and host species were identified by sequencing the mitochondrial cytochrome B gene or a multiplex PCR using host-specific primers. Mosquitoes confirmed to have fed on humans were further evaluated by DNA profiling to determine molecular fingerprints of the individual people from whom they fed. The high human blood index (HBI) for ***An. koliensis*** (HBI = 0.91), ***An. punctulatus*** (HBI = 0.84), and ***An. farauti*** s.s (HBI = 0.63) was consistent with previous findings that showed them to be highly anthropophagic. ***An. longirostris*** (HBI = 0.76), which was considered zoophagic, appeared here to be anthropophagic. ***An. bancrofti*** (HBI = 0.23) appeared consistent with previous findings as a zoophagic species and ***An. farauti*** No. 4 (HBI = 0.58) is reported here for the first time as a generalist feeder. Despite strong preference for humans, these vectors are not strictly anthropophagic as pigs and dogs were also fed upon. Increasing the number of pigs and dogs as alternative hosts along with long-lasting insecticide-treated nets may help reduce malaria transmission in these villages. The DNA fingerprint data based on analysis of 212 human-fed ***An. farauti*** s.s from one village showed that these mosquitoes obtained their bloodmeals from a pool of 69 individual humans (81% males and 19% females). In addition, only 7/69 individuals (10%) contributed to 53% of the bloodmeals. These results showed evidence of aggregated feeding on a subpopulation of humans, a phenomenon that plays an important role in the sustenance and resurgence of malaria transmission.

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HOUSEHOLD-LEVEL ENVIRONMENTAL CHARACTERISTICS ASSOCIATED WITH *ANOPHELES* SPECIES ABUNDANCE IN MALARIA-ENDEMIC BLANTYRE, MALAWI

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Malaria is holo-endemic in southern Malawi, and many cases are reported among residents of Blantyre City. Virtually nothing is known about the ***Anopheles*** spp. present in that setting, including their space-time patterns of abundance and associated peridomestic environmental characteristics. We systematically sampled adult mosquitoes at 64 locations (5 households per location) along 8 transects radiating outward from the city center (~18 km distance) during the rainy and dry seasons of 2015. Each house (n=320) was aspirated and light-trapped, house characteristics (size, construction, screens, room use, animals) were recorded and people were questioned about demographics and anti-malaria efforts (ITNs, treatment). ***Anopheles*** funestus were most abundant; only a few An. aegypti s.l. were found. Light-traps captured ~58-fold more adults than did aspiration. Considerable variation in abundance among households was found, both within- and among the 64 locations. Sporozoite infection prevalence determined by PCR was low, and also varied among households. PCR-based analysis of blood meals, targeting the vertebrate cytochrome B gene, demonstrated that engorged females mostly fed on human blood. House construction was generally predictive of mosquito abundance, with closed eaves and bed net use being protective. Peri-domestic (<50m) habitats with any type of vegetation (vs. buildings or bare ground) were predictive of ***Anopheles*** abundance. These results suggest that fine-scale spatial heterogeneity in malaria risk is determined by complex relationships at the household level across a range of habitat characteristics traditionally considered to be urban.

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FILLING IN THE VECTOR MAP WITH A LARGE SCALE SURVEILLANCE FOR FIELD-COLLECTED MOSQUITOES IN LIBERIA

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A large-scale mosquito surveillance study in Liberia aimed at determining the prevalence of malaria and arboviruses from field-collected mosquitoes was initiated in 2010. Mosquitoes were collected in five counties of Liberia, for seven consecutive days and nights. Collection sites included coastal, forest, urban, and mountain / savannah terrains. At each site, three different traps were used: a CDC UV trap baited with CO2, a Gravid Trap baited with 50% hay infusion, and a Biogent-Sentinel (BGS) Trap baited with a lure. A comprehensive literature search of 37,000 specimens comprised of 70 species and 11 genera were collected and identified. An additional 70 species and three genera were gleaned from the literature, yielding a total of 140 species and 14 genera currently known to occur in Liberia. During this survey, 30 out of the 70 collected species are new records, with the majority of the new records belonging to the genera Culex and Coquillettidia. Due to the morphological indistinguishability of ***Anopheles gambiae*** sensu lato (Giles) complex, DNA was extracted from legs of each specimen with species identification determined by multiplex polymerase chain reaction using specific primers revealing 72.6, 25.5, and 1.9% of ***A. gambiae*** s.s specimens were S, M, and hybrid forms, respectively. Additionally insecticide resistance PCR assays demonstrated 92% RR, 6% SR, and 2% SS from the collected samples. Selected Anophelinae species were also screened for the presence of malaria causing sporozoites using enzyme-linked immuno assay, which showed 4% infection rate. Culicinae mosquitoes were analyzed by performing RNA extraction in the attempt to detect RNA corresponding to DENV 1-4, CHIKV, YFV, RVFV, WNV or families Flavivirus, Alphavirus, Buyavirus will be detected using protocols for realtime or conventional RT-PCR. However no arboviruses were detected in the collected mosquito specimens during this time period.

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DEVELOPMENT FOR A WEB SYSTEM THAT FACILITATES THE COLLECTION AND ANALYSIS FOR DENGUE RISK FACTORS USING OPEN-ACCESS GEOGRAPHIC INFORMATION SYSTEM

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In Colombia, the decentralized health system requires local authorities to take important decisions about the prevention and control of dengue. But actions are generally reactive, i.e. undertaken principally in response to disease outbreaks, limiting the opportunity for preventive actions. Additionally, methods of data collection and design of intervention strategies do not involve joint analysis of the biological, ecological, and social needs of each locality. Consequently, at the local level, there is no specific initial assessment which would allow proactive actions and adequate prevention in terms of intervention and control strategies, as well as a lack of continuity in personnel. We are developing a web system that facilitates the collection and analysis of entomological, epidemiological and social data using open-access geographic information systems, allowing spatial analysis of transmission. We will share the

identification of risk factors associated with dengue transmission locally from two intermediate cities in Colombia. We expect that the introduction of local analysis, integrating the various determinants of dengue transmission, and taking advantage of technological tools that facilitate entomological studies and disease surveillance, will allow appropriate prevention and control strategies; giving a greater chance of long-term sustainability. This system will empower dengue surveillance and control programs at the local, provincial and national levels.

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EMERGING OF CHIKUNGUNYA VIRUS CLADE WITHIN ASIAN GENOTYPE IN THE PHILIPPINES

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Chikungunya virus (CHIKV) is a mosquito-borne pathogen that can cause epidemic fever, rash, and arthritis. In the Philippines, sporadic outbreaks have been reported for several decades with increasing numbers during the 2012-2013. CHIKV has recently spread to the Americas during the 2013-2014. The Asian genotype has been identified in specimens collected during the outbreaks in the Americas and the Philippines. Obtaining more genetic information on Asian genotype will provide more insight into the genetic changes that may have led into the most recent CHIKV epidemic patterns. In this study, we analyzed the complete genomes of five CHIKV isolates from acute illness blood samples collected from a prospective longitudinal cohort study of febrile illnesses conducted in Cebu City, Philippines during 2012-2013. Phylogenetic analysis of the two concatenated open reading frames revealed two clades within the Asian genotype: an older clade (1958-1995) and a modern clade (2006-2014). The modern clade appeared in Malaysia in 2006 and has expanded since 2007 into two separate sub-clades. The five new sequences from the Philippines fell into the newer sub-clade of the modern clade, containing the CHIKV strains found recently on St. Martin, British Virgin Islands, and Mexico. The mutations in each clade of Asian genotype are further described in this study. Our findings increase our understanding of the molecular epidemiology of CHIKV strains responsible for recent geographic spread of chikungunya.

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IMPORTED CASES OF CHIKUNGUNYA INFECTION AMONG CANADIAN TRAVELERS IN 2014

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Since the spring of 2014, a large increase in travel-related Chikungunya cases have been diagnosed in Canada. As of April 1, 2015 over 400 confirmed Canadian cases of imported Chikungunya associated illness had been diagnosed in 2014 with the majority of Canadian provinces identifying at least one imported case. As well, approximately 100 probable and confirmed cases had already been diagnosed among Canadian travellers in early 2015 (as of April 1). Suspect case serum samples from provincial public health laboratories across Canada were screened using a CDC based IgM ELISA. Specimens positive for IgM were confirmed for presence of viral specific antibody using a plaque reduction neutralization test. Serum samples were also tested by real time and conventional RT-PCR for the presence of viral RNA. The confirmed numbers of imported Chikungunya infections identified in 2014 is a significant increase in documented cases as compared to past years when case rates ranged from 1-20 cases a year. Cases in which travel histories were available indicated that the majority of Canadian exposures resulted from travel to the Caribbean where the virus recently emerged, however, cases were also identified in travellers to the Asia – Pacific region where outbreaks are also occurring. Approximately 20 % of the cases were viremic indicating the possibility of local transmission if primary mosquito vectors become established in Canada. Given the continuing expansion of Chikungunya throughout the Americas and Asia a heightened awareness of Chikungunya among clinicians and the public in Canada and other countries is key. Ongoing communications regarding preventative measures when travelling to endemic areas is required to decrease risk of exposure to this emerging mosquito-borne pathogen.

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DEVELOPMENT OF A QUANTITATIVE, REAL-TIME REVERSE-TRANSCRIPTASE PCR FOR O'NYONG-NYONG VIRUS AND ABSENCE OF VIRUS DETECTION AMONG FEBRILE KENYAN CHILDREN

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O'nyong-nyong virus (ONNV) is a unique alphavirus as it is transmitted by ***Anopheles*** mosquitoes. ONNV has been detected in two large outbreaks centered in Uganda, and in a recent serological study, high rates of ONNV and chikungunya virus (CHIKV) transmission were detected in villages of coastal Kenya. However, little is known about intra-epidemic ONNV transmission or its contribution to acute febrile illnesses in this region. The purpose of this study was to develop a real-time reverse transcriptase PCR (rRT-PCR) for the detection and quantitation of ONNV and to use this assay to screen acute-phase serum samples from febrile Kenyan children. For assay design, we aligned the complete genomes of available ONNV stains, including one strain of Igbo Ora, in the NCBI Nucleotide database. Highly conserved regions of the non-structural protein 1 gene were selected for primer and probe design. Primer and probe concentrations were optimized using synthesized ssDNA containing the ONNV target, and performance was confirmed with extracted genomic RNA. The assay had a linear range from 8.0 to 2.0 log10 copies/µL with a lower limit of 95% detection of 22.4 copies/µL. Performance was similar when run with only ONNV primers and probe or as an ONNV-CHIKV multiplex. The ONNV assay showed no signal when tested using genomic RNA from dengue, chikungunya, or West Nile viruses. We then randomly selected serum samples from 200 children who were enrolled in an ongoing, acute febrile illness surveillance study in coastal and western Kenya. Patients presented within the first 3 days of fever. No patients had detectable ONNV or CHIKV RNA. Sixty-five patients (32.5%) had malaria by PCR, indicating exposure to ***Anopheles*** mosquitoes. Nucleic acid extraction and the absence of PCR inhibitors were confirmed for all samples in a separate reaction for RNase P. In conclusion, we developed a new rRT-PCR for the detection and quantitation of ONNV. Among 200 febrile Kenyan children, who presented during a non-outbreak period, no cases of acute ONNV infection were identified.

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ASIAN GENOTYPE DETECTED IN IMPORTED CHIKUNGUNYA CASES IN ARGENTINA, 2014-2015: A CHALLENGE FOR LABORATORY SURVEILLANCE

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Chikungunya virus (CHIKV) is a mosquito-borne pathogen that was endemic in Africa and south Asia until 2005 and 2006, when the virus spread into the Indian Ocean islands, Europa and Asia. In 2013, it emerged

in America as an important public health problem. All the pre-requisites for autochthonous activity of CHIKV are present in Argentina. The objective of this work is present the virological, serological and molecular studies for CHIKV detection in Argentina, during 2014 -2015. A total of 460 patients with signs and symptoms were notified. Acute sera samples were tested using qRT-PCR for CHIKV (nsp1 Asian genotype) and nRT-PCR (degenerate oligonucleotides, nsp4). Virus isolation in VERO C76 cells was attempted in a selection of samples. IgM antibodies were detected by ELISA according whit the algorithm recommended by PAHO. Serological confirmation and cross reactions with Venezuelan Equine Encephalitis virus, Eastern Equine Encephalitis virus, Western Equine Encephalitis virus, Mayaro virus and Una virus were performed by PRNT in VERO C76. CHIKV was detected in 62 patients, 97% (60/62) of them had recent travel to other countries (Dominican Republic, Venezuela, Colombia and Trinidad and Tobago). Cases were classified as confirmed (26, 42%) and probable (36, 58%). No cross-reactions were detected between the Alphaviruses evaluated by PRNT. Nucleotide sequencing of two fragments (155 bp nsp4 gene and 1200-bp E gene) in 3 viral strains isolated was performed and compared to other sequences available in Genebank. The sequences were aligned by the Clustal W method (BioEdit). Phylogenetic tree was performed by Neighbour Joining (MEGA 5) and Bayesian (Mr.Bayes) analysis. The topology of the tress were similar, the strains belong to Asian genotype currently circulating in other Latin American countries. Our results support the hypothesis that a single CHIKV strain introduction of Asian genotype on the Caribbean islands later spread to other American countries. The findings highlight the risk of introduction of this Alphaviruses in Argentina and the need for the health system prepared to give timely and efficient response to potential emergency.

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EVOLUTION FOR CHIKUNGUNYA VIRUS IN *WOLBACHIA*-INFECTED MOSQUITOES

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New approaches to preventing chikungunya virus (CHIKV) are needed because current methods are limited to controlling mosquito populations, and they have not prevented the invasion of this virus into new locales. A promising candidate for arbovirus control and prevention relies on the introduction of the intracellular bacterium Wolbachia into ***Aedes aegypti*** mosquitoes. Wolbachia biocontrol has advanced from laboratory experiments demonstrating that Wolbachia reduces virus replication in mosquitoes to deploying and evaluating this strategy in six countries. This primarily has been proposed as a tool to control dengue virus (DENV) transmission; however, Wolbachia infections confer protection for Ae. aegypti against CHIKV. Although this approach holds much promise for limiting virus transmission, at present our understanding of the evolutionary consequences of Wolbachia on any arbovirus is limited; therefore, anticipating evolutionary changes of CHIKV-Wolbachia-mosquito interactions is an important component involved in validating the safety and viability of this approach as a biocontrol mechanism. Herein, we present data assessing the ability of CHIKV to adapt to Wolbachia-mediated suppression in mosquitoes. The choice of CHIKV is timely due to the massive CHIKV epidemic occurring in the Americas. To assess this, we have developed a tractable, laboratory model to begin to understand the selective pressures associated with Wolbachia-mosquito-CHIKV co-adaptation. Wolbachia-infected Ae. aegypti have been established as part of a complementary Eliminate Dengue Program project in Colombia, and preliminary data suggest that the wMel strain of Wolbachia in these mosquitoes blocks CHIKV infection and also reduces the transmission potential of these mosquitoes. As such, we are evaluating the specific CHIKV phenotypes that arise during alternate passage between Wolbachia-infected Ae. aegypti and mice. The demonstration that Wolbachia is effective against CHIKV and that the strategy will not be undermined by virus evolution will represent a significant new development in the fight against Ae. aegypti transmitted arboviruses.

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EPIDEMIOLOGICAL AND VIROLOGICAL FEATURES FOR CHIKUNGUNYA VIRUS INFECTIONS AMONGST TRAVELERS RETURNING TO SPAIN, 2008-2014

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Chikungunya fever used to be only endemic in some parts of Africa, Southeast Asia and in the Indian subcontinent. In late 2013, the first documented autochthonous transmission of chikungunya virus was reported in the Caribbean island of Saint Martin and since then the infection has spread quickly in countries and territories in the Caribbean region, North, Central and South America. With the ongoing outbreak in the Caribbean, chikungunya virus is increasingly becoming a European public health threat. Transmission of chikungunya virus to humans occurs through bites of ***Aedes aegypti*** and ***Ae. albopictus*** mosquitoes. In Europe, ***Ae. albopictus*** is established primarily around the Mediterranean basin and has been demonstrated its competency for virus transmission. This resulted in locally-acquired infections in Italy, and in France, as rported previously. In this study, we analyzed six years of imported chikungunya infections in Spain. During the study period (2008-2014), a total of 1311 suspected chikungunya infections were studied and more than half were in 2014 alone. From 2008 to 2013, 30 laboratory-confirmed (PCR, IgM/IgG) chikungunya infections were imported whereas in 2014 there were 195 confirmed cases. Majority of chikungunya cases with known travel history in the period 2008-2013 reported travel to Asia whereas in 2014 the main travel destination were the Americas (Dominican Republic, Haiti and Venezuela). Virus sequencing revealed that all samples from Americas felt into Asian genotype (Caribbean Clade), although Indian ocean genotype was detected in cases imported from Asia and Africa .Most of the positive samples in 2014 clustered around May and October, the activity period for the vector present in Spain. Chikungunya virus is an emerging public health threat to Spain because the conditions for autochthonous transmission are met: presence of competent vector and a large number of travelers returning from affected areas like the Caribbean and northern South America.

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SCREENING TESTS DURING CHIKUNGUNYA OUTBREAK IN CARTAGENA DE INDIAS, COLOMBIA

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Chikungunya virus (CHIKV) and Dengue virus (DENV) are mosquito-borne viruses. Both have similar clinical manifestations including hemorrhagic fever except for the arthralgia more related to CHIKV. Clinical differentiation between dengue and chikungunya from other causes of febrile illness is difficult to achieve during early stages of the illness. In Colombia, access to laboratory tests is limited and diagnosis may rely solely on clinical symptomatology. The aim of this study was to describe clinical manifestations of acute febrile illness and apply a screening test for CHIKV and DENV during an outbreak of Chikungunya fever in Colombia. For this study patients were enrolled during a Chikungunya outbreak in Colombia that began in august of 2014 and persists nowadays. Patients who lived in Cartagena, had a history of fever ≤7 days were eligible. A commercial screening test for CHIKV IgG/IgM Ab and DENV IgA/IgG/IgM and NS1 antigen was performed for each patient at the beginning (acute phase)

and up to 30 days after the onset of the illness (convalescent phase). A total of 38 patients were enrolled, most patients were between 18 and 45 years old (57,9%) and females (55,3%). The duration of the fever during the episode was between 3-7 days (51,7%). Arthralgia (97,4%), chills (81,6%), malaise (81,6%), headache (76,3%), pruritus (60,5%), rash (60,5%) and myalgia (55,3) were the most frequent. Of the total, 53% of the patients had IgM antibodies for CHIKV; 63% had IgG antibodies for DENV; 31,6% had both CHIKV (IgM) and DENV (IgG) Ab and 21% of the samples had both tests negative. No one had NS1 Ag positive. Acute febrile syndrome in tropical areas is always related to arboviral diseases. We found, even when most patients fulfilled the criteria for CHIKV fever, not all of them had a positive result on the rapid test, and a high percentage had IgG Ab for DENV. We are aware that screening test are not approved as diagnostic tools for these diseases, this is why we suggest confirmatory tests. In patients who tested positive for IgG Ab against dengue we concluded that this could be a sign from a past episode not related to the current one, more when nobody one tested positive for NS1 Ag.

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MOSQUITO SALIVA ENHANCES EARLY ARBOVIRUS INFECTION WITH DIFFERENTIAL CLINICAL OUTCOMES

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Mosquito-borne viruses like those in the Togaviridae (Alphavirus genus) and Flaviviridae (Flavivirus genus) families are introduced into the vertebrate host along with saliva from the mosquito vector. Such viruses have evolved infection strategies that leverage salivary proteins to alter the early events in infection and may influence the progression of long-term disease. We have previously demonstrated in a murine model of dengue virus infection that ***Aedes aegypti*** salivary gland extract (SGE) results in an early enhancement of infection. We have now evaluated two additional viruses vectored by Ae. aegypti, yellow fever (YFV) and chikungunya (CHIKV) viruses, in order to understand the short- and long-term implications that SGE plays in pathogenesis and disease. Following infection in the presence of SGE, early YFV replication was as much as 40,000-fold greater in the draining lymph nodes and visceral tissues. In addition, ***in vivo*** imaging studies of CHIKV revealed an enhancement of infection at the site of subcutaneous inoculation and evidence of more rapid dissemination of virus distal to the site of inoculation. Enhanced replication of both viruses was sustained for at least four days following infection. Surprisingly, clinical disease in YFV-infected animals was similar regardless of SGE administration, although animals infected with YFV in the presence of SGE were statistically more likely to survive. Preliminary results of long-term CHIKV studies indicated that mice receiving SGE presented with a more severe musculoskeletal disease as measured by foot-pad swelling. Moreover, SGE may play a role in exacerbating chronic CHIKV disease. Our data suggest that diverse arboviruses may have independently evolved mechanisms to enhance and sustain early replication in the vertebrate host perhaps to increase the efficiency of infection or facilitate the acquisition of progeny by additional vectors. These studies emphasize the importance of developing animal models of mosquito-borne virus infection that consider the effect of the vector on pathogenesis.

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VENEZUELAN EQUINE ENCEPHALITIS VIRUS INFECTION INDUCES OXIDATIVE STRESS AND LEADS TO ALTERATIONS IN MITOCHONDRIAL DYNAMICS

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The multi-faceted organelles, mitochondria, have been shown to play roles in maintaining cellular metabolism, innate immunity and multiple signaling pathways. Composed of a population of organelles the mitochondria are actively and continuously dividing and elongating (mitochondrial dynamics) and undergoing controlled turnover. Recent studies have demonstrated that viruses have developed mechanisms to modulate the mitochondrial dynamics to facilitate viral replication. We have previously shown that Rift Valley Fever Virus induced oxidative stress activated transcription factors (p65 and p53), which in turn modulated cytokine secretion and apoptotic gene expression profiles leading to cell death. In this study we are investigating the new world alphavirus belonging to the Togaviridae family, Venezuelan Equine Encephalitis Virus (VEEV) where we hypothesize that VEEV infection induces oxidative stress which will interfere with mitochondrial dynamics thus impacting generic mitochondrial processes. Initial experiments using the MitoSOX red reagent indicated that VEEV induced the production of superoxide species in infected cells. Confocal microscopy analysis demonstrated that there is a differential "concentrating" mitochondrial phenotype observed in VEEV infected cells, which was not observed in uninfected cells. Ongoing studies entail defining a putative mechanism to the mitochondrial phenotype observed in VEEV infected cells. This work will aid in future investigations of the effect of mitochondrial dynamics in other alphaviruses and their role in pathogenesis, thus creating an avenue for therapeutic design as there are currently no approved vaccines or therapies to treat the disease.

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EILAT VIRUS INDUCES BOTH HOMOLOGOUS AND HETEROLOGOUS INTERFERENCE

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Most alphaviruses are mosquito-borne and exhibit a broad host range, infecting many different vertebrates including birds, rodents, equids, humans, and nonhuman primates. Occasionally alphaviruses can spill over into the human population and cause clinical disease characterized by debilitating arthralgia or fatal encephalitis. Recently, we described a unique alphavirus, Eilat virus (EILV), that readily infects mosquito but not vertebrate cell lines. Here, we investigated the ability of EILV to induce homologous and heterologous interference ***in vitro*** and ***in vivo***. Prior infection of C7/10 (Aedes albopictus) cells with EILV reduced virus replication of superinfecting homologous and heterologous alphaviruses. Virus titers of heterologous viruses were reduced by 10-10,000 fold and replication kinetics were delayed by 12-48 hrs. Similar to ***in vitro*** infection, prior ***in vivo*** EILV infection of ***Aedes aegypti*** mosquitoes prevented dissemination of chikungunya virus for 3 days. This is the first evidence of alphavirus-induced heterologous interference in mosquitoes, suggesting that EILV could be developed to reduce and/or prevent alphavirus transmission.

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EILAT VIRUS DISPLAYS A NARROW MOSQUITO VECTOR RANGE

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Most alphaviruses are arthropod-borne and utilize mosquitoes as vectors for transmission to susceptible vertebrate hosts. This ability to infect both mosquitoes and vertebrates is essential for maintenance of most alphaviruses in nature. A recently characterized alphavirus, Eilat virus (EILV), isolated from a pool of ***Anopheles coustani*** s.l. is unable to replicate in vertebrate cell lines. The EILV host range restriction occurs at both attachment/entry as well as genomic RNA replication levels. Here we investigated the mosquito vector range of EILV in species encompassing three genera that are responsible for maintenance of other alphaviruses in nature. Susceptibility studies were performed in four mosquito species: ***Aedes albopictus, A. aegypti, Anopheles gambiae,*** and ***Culex quinquefasciatus*** via intrathoracic and oral routes utilizing EILV and EILV expressing red fluorescent protein (-eRFP) clones. EILV-eRFP was injected at 10^7 PFU/mL to visualize replication in various mosquito organs at 7 days post-infection. Mosquitoes were also injected with EILV at 10^4-10^1 PFU/mosquito and virus replication was measured via plaque assays at day 7 post-infection. Lastly, mosquitoes were provided bloodmeals containing EILV-eRFP at doses of 10^9, 10^7, 10^5 PFU/mL, and infection and dissemination rates were determined at 14 days post-infection. All four species were susceptible via the intrathoracic route; however, replication was 10-100 fold less than typical for most alphaviruses, and infection was limited to midgut-associated muscle tissue and salivary glands. ***A. albopictus*** was refractory to oral infection, while ***A. gambiae*** and ***C. quinquefasciatus*** were susceptible only at 10^9 PFU/mL dose. In contrast, ***A. aegypti*** was susceptible at both 10^9 and 10^7 PFU/mL doses, with body infection rates of 78% and 63%, and dissemination rates of 26% and 8%, respectively. The exclusion of vertebrates in its maintenance cycle may have facilitated the adaptation of EILV to a single mosquito host. As a consequence, EILV displays a narrow vector range in mosquito species responsible for the maintenance of other alphaviruses in nature.

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THE SEROPREVALENCE FOR DENGUE FEVER IN SOLOMON ISLANDS IN 2014

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There was a large scale dengue outbreak in Solomon Islands in 2013. In order to fight against dengue fever, Taiwan government built up the first bio-safety level 2 (BSL-2) laboratory at National Referral Hospital in Honiara, Solomon Islands. This laboratory was designed by the Tropical Medicine Center of Kaohsiung Medical University Hospital. In 2014, we conducted the seroprevalence survey in Solomon Islands under the entrustment of Solomon Islands Government. Under IRB approval, we collected 1079 samples from the community in three different provinces (Guadalcanal Province, Western Province, Malaita Province) in Solomon Islands from September to November. We used dengue Capture IgG ELISA (Standard Diagnostics (SD), Korea) to detect the seroprevalence of dengue fever. The age of enrolled subjects ranged from 18~42 years old with median age of 38 years old. 72.4 % of them are female. The education level of them is the Elementary school (39.1%), Junior high school (26.3%), and Senior high school (22.5%). Only 3.4% of them recalled they ever got dengue fever. 62.2% of them had ever contracted malaria before. Different provinces had different seroprevalence rates of dengue fever. In Guadalcanal Province, we enrolled 306 subjects from different areas, and the mean seropositive rate is 46.4%. The distribution of the seropositive rate in Guadalcanal Province is as the following: Visale 62.5% (80/128), Burns Creek 34.6% (18/52), Betikama 30% (15/50), and Wanderer Bay 38.2% (29/76). In 2013, the dengue fever outbreak was chiefly located in Guadalcanal Province. The seroprevalence of dengue fever in Western Province is 34.1% (217/636) and 12.4% (17/137) in Malaita Province. In Western Province, two areas, Gizo (38.2%, 29/76) and Munda (38.7%, 184/476) are slightly higher than the other areas in seroprevalence. Malaita Province is the relatively lower seroprevalent in dengue fever and the residents might be more susceptible to the infection in the future. The dengue cases in Western Province might be under-estimated and should be more carefully diagnosed.

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THE EFFECT FOR LAND COVER ON THE ABUNDANCE AND DISTRIBUTION FOR SYLVATIC DENGUE VIRUS VECTORS IN MALAYSIAN BORNEO

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Mosquito-borne dengue virus has emerged from a sylvatic cycle, maintained in non-human primates and arboreal ***Aedes*** mosquitoes, to establish the human-***Aedes aegypti*** cycle that results in nearly 400 million infections annually. The sylvatic ancestors of human-endemic dengue virus continue to circulate in peninsular Malaysia and Borneo, with continual spillover into humans, sometimes causing severe disease. Systematic studies of sylvatic dengue virus in Southeast Asia, conducted in the 1960s-1970s, detected dengue virus in mosquitoes only once, in ***Aedes niveus sensu lato***. In recent decades, Malaysian Borneo has experienced intensive forest clearing, a potential risk factor for spillover of zoonotic viruses. How such clearing may affect sylvatic dengue spillover is unclear due to our incomplete understanding of dengue virus vectors in regard to identity and distribution. To bridge this knowledge gap, we conducted mosquito trapping in rural Sarawak, Borneo during two field seasons, 2013 and 2014, in three land cover types: homestead, agriculture and forest. Specifically, we tested the hypotheses that 1) ***Ae. niveus*** would be most abundant in forests compared to other land cover types, 2) In other land cover types, ***Ae. niveus*** would be most abundant at forest edges, and 3) compared to ***Ae. niveus***, ***Ae. albopictus***, a secondary vector of human-endemic dengue virus and a putative vector of sylvatic dengue virus, would be more evenly distributed throughout all land cover classes. Over 2,000 mosquitoes were sampled, identified morphologically and pooled by location, trap, day and species. Pooled samples were screened for arbovirus infection and used for molecular identification of mosquito species. Over 70% of the mosquitoes sampled in both field seasons were ***Aedes*** species; of these, 70% were ***Ae. albopictus*** in 2013 and 95% in 2014. Insect-specific viruses, including ***Euprosterna elaeasa*** virus, were detected in 7 ***Ae. albopictus*** pools, but sylvatic dengue virus was not detected in any pool. The complete analysis of the relative distributions of ***Aedes*** mosquitoes in this region will be presented.

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MOLECULAR EPIDEMIOLOGY FOR DENGUE VIRUS IN THE LATIN AMERICAN REGION: A SYSTEMATIC REVIEW

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Dengue is the predominant arthropod-borne viral disease affecting humans. The four serotypes of Dengue virus are antigenically and genetically distinct. The basis of DENV genetic diversity is still under research, but it seems that human and vector infections impose different selective pressures, moreover, epidemiological and enviromental conditions could affect the population structure of the DENV in a particular setting. This study was developed to describe the molecular epidemiological trends of Dengue virus and knowledge generated in specific molecular topics in Latin America and Caribbean islands from 2000 to November 2013. A systematic literature review of molecular epidemiology was conducted. 391 sources were identified, from those, 51 were included as relevant citations. Hyperendemicity arising from circulation of different serotypes in a region may be a factor in the association between dengue infection and severity of disease.Intra-serotype antigenic variation and the resulting differential generation of protective antibodies and immune responses are postulated as one of the reasons for high epidemiological impact of certain DENV serotypes. Distinct lineages with different dynamics were identified in each of the countries and coexistence, extinction and replacement of lineages occurred over the review period, and changes occurred have resulted in substantial genetic diversity with emergence of endemic and epidemic strains in different parts of the region. The extinction of earlier strains and appearance of new epidemic strains suggests genetic bottleneck as a cause of regional replacement. Reasons for the re-emergence of dengue in the Americas: spread of different DENV serotypes in bordering countries; permanent migration flow of viremic travelers, and increase in vector infestation due to inconsistent vector-control strategies. DENV seem to take advantage of diverse mechanisms to generate genetic diversity via genetic variability and host travel, as well as exploiting the increasing density of human hosts and urbanization.

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DENGUE VIRAL PERSISTENT INFECTION

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Dengue has been recognized to be one of the most important vector-borne human viral diseases in recent years globally. Dynamic clinical presentations in dengue virus (DENV) infected patients, ranging from asymptomatic, mild dengue fever, and dengue hemorrhagic fever associated with dengue shock syndrome, have been registered. Recently, it has been reported that infectious DENV can be recovered from healthy blood donors and that asymptomatic human beings could be accounted for the spread of dengue. This line of evidence implies that persistent DENV infection may exist in the natural setting. We utilized fresh HBM to establish humanized model to investigate the persistent infection of DENV. DENV was administered to 8 weeks old humanized mice by IV injection with 10^4 PFU. Peripheral blood was obtained at multiple time points, with two week intervals. With a FACS panel to assay the cells of human origin along with the identification of the infected cell, combined with plaque assay and immunohistochemistry to locate the virus in organs. We showed that the percentage of human cells in circulation was on average at 35% and that viral titers were seen starting from the second week of the infection and reached a mean peak titer at 10^3 PFU/ml, with each peak lasting for about two weeks. The viral titers were fluctuated after the peak, but maintained at a detectable level (above 500 PFU/ml) for 3 months until the mouse died, suggesting that it could be maintained if mice health conditions sustained. FACS analysis indicated that the phenotypes of cells harvesting the dengue viral antigen were of the megakaryocytic lineage, $CD45^+CD41a^+CD61^+$. Autopsy on the DENV infected deceased mice revealed that DENV was present in multiple organs, including liver, spleen, bone marrow, and brain by plaque assay. The highest viral titer was observed in the brain and especially in the region of hippocampus. Results indicated that DENV could reside in the brain of infected humanized mice. The results suggest that persistent infection may exist in the natural life cycle of DENV and that those of the megakaryocytic lineage cells in asymptomatic individuals may become a DENV carrier.

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SEQUENTIAL INFECTION WITH INFLUENZA AND DENGUE VIRUSES HINDERS IMMUNE CELL RECRUITMENT AND CAUSES LETHAL DISEASE

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Both influenza and dengue are major health problems worldwide. In 2009, Nicaragua experienced a massive and atypical dengue epidemic, even though the dominant dengue virus serotype (DENV3) was the same as in 2008 and 2010. The only identifiable epidemiological difference in 2009 was the influenza A-H1N1 pandemic, which was prolonged, compared to seasonal influenza, and overlapped with the dengue epidemic. We hypothesized that sequential or co-infection of influenza H1N1 and DENV contributed to the atypical and potentially more severe dengue disease. We established a mouse model of dual infection with a Nicaraguan pandemic H1N1 influenza virus isolate and the virulent DENV2 strain D220. DENV suppresses the interferon response, replicates, and causes disease in humans but not wild-type mice; therefore, we used mice lacking the interferon-α/β receptor (***Ifnar***$^{-/-}$) that are susceptible to both DENV2 and influenza virus infection. Sequential infection with influenza virus followed two days later by DENV2 caused 90% mortality in ***Ifnar***$^{-/-}$ mice at virus doses that induce mild disease during infection with either virus alone. The DENV viral load was significantly increased in the lung, liver, and spleen of sequentially infected mice compared to mice infected with only one virus, while influenza viral load was similar. We analyzed the host RNA response using nCounter technology and observed dramatic changes in chemokine and cytokine mRNA expression levels that suggested differences in immune cell recruitment in the lungs. Flow cytometry revealed impaired recruitment of monocytes, neutrophils, NK cells and T cells to the spleen and lung of sequentially infected mice compared to mice infected with only one virus. We are currently manipulating recruitment of different subsets of immune cells to determine the cause of immunopathology in sequentially infected mice. These studies will inform the development of potential treatment and vaccine strategies in endemic areas where dengue and influenza viruses co-circulate.

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VIRAL REPLICATION AND IMMUNOGENIC POTENTIAL DIFFERENCES BETWEEN TWO DENGUE 1 LINEAGES CO-CIRCULATING IN BRAZIL

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Dengue virus comprises four distinct serotypes (DENV-1-4). Within the serotype 1, previous genetic studies demonstrated the existence of

different lineages grouped into five genotypes. Phylogenetic analysis of the E gene showed that two lineages of DENV-1 (L1 and L6) co-circulated in São José do Rio Preto, São Paulo state, Brazil at least, between 2010 and 2012. This study has compared biological properties of these two lineages. Five cell lines (C6/36, Aag-2, Vero E6, LLC-MK2 and HepG2) have been infected with two DENV-1 isolates representatives of each lineage, at a MOI of 0.1 for growth curves. Additionally, one hundred forty adult female mosquitoes from two populations (PPCampos and Dom Pedro) and C57BL/6 mice have been experimentally infected with the same isolates. Later, qPCR was used to detect these viruses in supernatant of cells and in body and head samples of mosquitoes. FACS was used to analyze mice spleen cells. In silico epitope prediction was performed to evaluate the binding of B and T cell receptors to epitopes of the E gene using bioinformatics tools. Analysis of the growth curves showed that L1 replicated at higher level comparably to L6 in all cell lines tested as well as in Ae. aegypti mosquitoes. L1 presented a viral cDNA copies number significantly higher than L6 in both mosquitoes populations and also exhibited statistically significant higher rates of IR, VC and DIR. IR varied from 92.5% to 100% (L1) and 37.5% to 73.33% (L6). VC ranged from 85.01% to 100% (L1) and 30% to 43.34% (L6). DIR oscillated from 91.9% to 100% (L1) and 59.1% to 80% (L6). L6 had less antigenic potential to B cells while L1 had more antigenic potential to T cells in silico. L6 seemed to suppress B cells activation while L1 was a T cell activator ***in vivo***. These two lineages showed differences in their biological properties. Despite the better growth rate, L1 may elicit a better immune response while L6 with a lower growth rate might evade better the immune system, leading to an equilibrium and co-circulation of these lineages. Further analyses should be conducted to better understand how these characteristics correlate with epidemiological findings in subsequent years.

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ACCESSING HEALTH CARE IN VENEZUELA: A COMMUNITY BASED MIXED-METHOD SURVEY ON HEALTH CENTER ATTENDANCE FOR DENGUE AND FEVER

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Dengue is a major public health problem in Venezuela. Timely health centre (HC) attendance is crucial in reducing mortality and severity of dengue. The health care system in Venezuela comprises a public and a private sector. The public system includes the traditional primary/secondary (Ambulatorios), and tertiary level HCs (Hospitals) and is usually free of charge. To improve limited access to care for the poorer, a parallel public health care system ("Mission Barrio Adentro") was set up in 2003. We assessed the intended HC attendance in the case of fever and suspected dengue in an urban area of high dengue transmission. Between September 2013 and February 2014 a cross-sectional household survey was performed in Maracay, Venezuela. Intended HC attendance and the perceived barriers in the case of fever and dengue of adults and parents/guardians of children were assessed. Data was collected through structured questionnaires from 105 individuals. We show that people would visit several different HCs if needed, and that the health preferences differed throughout the community. The most frequent first choice of health centre was an Ambulatorio, in the case of fever (n=82; 78.8%) and dengue (n=84; 80.8%). Several economic, ethnic, logistic, and quality aspects influenced the preference to access the HCs. Individuals preferred to first attend traditional HCs as they trusted the care given at these institutions, but a barrier was the lack of treatment supplies. Although the lack of supplies was mentioned to a lesser extent in the case of the parallel HCs, people reported not to trust the medical staff, nor the diagnosis and treatment given in these HCs. Furthermore, the private care, which was considered best, was mainly accessible for those with a health insurance. A higher education (fever/dengue: p=0.001/p=0.001) and a non-manual occupation (fever/dengue: p=0.007/p=0.016) were associated with more intended private HC attendance. Access to care in Venezuela is currently a complex situation where individuals need to juggle between the different available public and private HCs in order to obtain proper/timely care and medical supplies.

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RELATIONSHIP BETWEEN MATRIX METALLOPROTEINASE EXPRESSION AND VIRULENCE FOR DENGUE VIRUS TYPE-2 INFECTED MOSQUITO AND MAMMALIAN CELLS

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Dengue virus infection is mostly asymptotic or produces a mild self-limiting acute febrile illness, dengue fever, or life threatening severe illness, dengue hemorrhagic fever, which is associated with increased vascular permeability in part as the result of elevated matrix metalloproteinases (MMPs). Herein, we characterized MMP-2 and MMP-9 production in mosquito and mammalian cells after infection with three strains of dengue virus type-2 ranging in virulence: the prototype New Guinea C (NGC), 16681, and PDK53 vaccine strain to test variations in viral properties in vaccine candidates and confirm the production of MMP as a possible marker for virulence. A zymogram gelatinolytic activity assay was employed to assess MMP-2 and MMP-9 production. We demonstrated that dengue infected mosquito and mammalian cell lines had unique MMP-2 and MMP-9 production patters depending on the virulence of the dengue strain and time after infection. MMP levels were highest after infection with the known virulent strain D2-16681, followed by the prototype NGC strain in both cell lines. The amounts of MMP appeared to correspond with the relative amount of infectious virions produced later in infection. These findings may contribute to understanding dengue pathogenesis and selecting markers to assist in the development of dengue vaccines.

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ANTIBIOTICS WITH UNEXPECTED TALENTS: EFFICACY AND MECHANISM FOR ACTION FOR FLUOROQUINOLONES AGAINST FLAVIVIRUSES

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Flaviviruses are positive-sense, single stranded RNA viruses, many are transmitted by arthropod vectors. Flaviviruses are responsible for some of the most significant vector-borne diseases worldwide, including dengue fever, yellow fever, West Nile encephalitis, and Japanese encephalitis. There are currently no antiviral drugs approved for the treatment of any flavivirus. Repurposing an FDA-approved drug as an antiflaviviral therapy would substantially reduce the time required to bring such a therapy to market. Several fluoroquinolones (FQ), which are broad-spectrum antibacterial drugs, are known to enhance RNA interference (RNAi), an intrinsic antiviral defense. We therefore hypothesized that these drugs might also show antiviral efficacy. To test this hypothesis, HEK-293 cells were inoculated with dengue virus serotype 4 (DENV-4) one virus per cell, after 2 hours the virus was removed and a FQ (enoxacin, ciprofloxacin, and difloxacin) was added using a serial dilution series. After 5 days, the cell supernatant was collected and titered on HEK-293 cells. The effective concentration 50 of these fluoroquinolones against DENV-4 in

HEK-293 cells was 7.6μM, 19.6μM, and 10.1μM, respectively, while the cytotoxic concentration 50 of these drugs was 545.2μM, 753.5μM, and 1513μM. To formally test whether inhibition of dengue virus by these fluoroquinolones is attributable to enhancement of RNAi, we investigated whether drug efficacy was lost when the RNAi pathway was knocked down. We first demonstrated, using Western blotting, that introduction of siRNAs targeting Argonaute 2 (Ago-2), a key effector in the RNAi pathway, depleted this protein in HEK-293 cells and persisted at least 6 days. We next demonstrated, using resazurin dye to assess cell viability, that Ago-2 siRNAs were not toxic to HEK-293 cells alone or in combination with enoxacin, ciprofloxacin, or difloxacin. Presently, we are testing the efficacy of each of the three fluoroquinolone drugs to inhibit DENV-4 replication in HEK-293 cells that have been pre-treated with either an anti-Ago-2 siRNA or a negative control siRNA; results of these experiments will be presented.

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DEVELOPMENT OF A PAN-DENGUE DIAGNOSIS PLATFORM USING DIRECT SERUM SAMPLES

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A single-tube reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay has been developed to detect all four serotypes of dengue virus (DENV) in serum. Transmitted by the Aedes mosquito vector, DENV is the causative agent of dengue fever with symptoms ranging from mild to severe, accompanied by debilitating and potentially fatal outcomes. There is no available vaccine or specific treatment for dengue, and early diagnosis is critical to providing appropriate supportive therapy to manage the disease. Pan-dengue LAMP primer mixtures and reaction compositions were optimized to detect viral RNA of all four dengue serotypes with similar efficiency. The analytical limit of detection (LOD) is 10^4 DENV RNA copies /mL in virus spiked serum. Serum samples from patients with acute dengue infection have about 10^3-10^8 RNA copies /mL, making the RT-LAMP LOD relevant to capturing a majority of clinical cases. With a blind test of 60 clinical serum samples, our assay reached sensitivities and specificities of 96.7% using conventional RT-PCR as the benchmark. The RT-LAMP reaction was performed on a companion portable prototype device with an 8-reaction capacity, thermal control, real-time fluorescence monitoring, and an interactive user interface. The entire assay can be completed in less than 60 minutes with the readout of dengue-positive or dengue-negative results available immediately at the conclusion of the test. The protocol requires minimal sample input (2 μL) and no RNA extraction step, which is another advantage to this method. Our assay represents a major advance in dengue diagnosis, as it combines the specificity and sensitivity of nucleic acid-based technology with the point-of-care diagnostic format.

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DENGUE-SPECIFIC ANTIBODY AND MEMORY B CELL RESPONSES FOLLOWING IMMUNIZATION WITH V180 TETRAVALENT RECOMBINANT SUBUNIT DENGUE VACCINE

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A Dengue vaccine that induces long-lasting protection against all four types of Dengue virus (DENV) is needed to protect against Dengue-induced disease. Following vaccination or infection, Dengue-specific humoral immunity is typically measured by IgG binding ELISA and antibody-mediated virus neutralization assay. Measurable antibody titers often wane over time and may become undetectable in the peripheral blood without a booster vaccination or virus exposure. Antibody levels are dependent on the number of memory B cells, and therefore assessment of memory B cells may be a better measure of long term immunity. For this purpose, we developed a sensitive memory B cell ELISPOT assay for each of the four DENV and we employed this assay to evaluate serotype-specific memory B cell levels following vaccination with V180 Tetravalent Recombinant Subunit Dengue Vaccine. In the V180 Phase I vaccine study, flavivirus-naïve subjects received three doses of recombinant envelope glycoprotein (DEN-80E) for all 4 DENV types at three dose levels, with or without adjuvant. Serum was collected at multiple times, including prior to and 28 days after each vaccination, and evaluated for DENV-specific antibody binding and virus neutralization. Prior to first vaccination and at the primary immunogenicity timepoint (28 days post dose 3, PD3), PBMC were collected for memory B cell analysis in a cohort of 18 study subjects in the high dose vaccine group. Preliminary data shows that V180 formulated with ISCOMATRIX™ adjuvant was highly immunogenic as measured in the serum antibody assays and the memory B cell assay, with an apparent balanced response across all 4 DENV types. We will present a comparison of these immunogenicity assays to (1) assess correlation among the assays, (2) analyze balance of immunogenicity in light of possible cross-reactivity between serotypes, and (3) analyze the time course and longevity of the immune response measured by each assay across vaccine groups. Our analysis will explore the relationship between DENV-specific memory B cell levels at 28 days PD3 and long term immunity as measured by antibody titers at 6 and 12 months PD3.

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HUMAN CD8 T CELL RESPONSES AGAINST THE FOUR DENV SEROTYPES ARE ASSOCIATED WITH DISTINCT PATTERNS FOR PROTEIN TARGETS

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All four dengue virus serotypes are now simultaneously circulating worldwide being responsible for 400 million human infections each year. Previous studies of CD8 T cell responses in HLA-transgenic mice and vaccinees demonstrated a different hierarchy in immunodominance of structural versus non-structural proteins as a function of the infecting serotype. This led to the hypothesis that there are intrinsic differences in the serotype-specific reactivity of CD8 T cell responses. We tested this hypothesis by analyzing serotype specific CD8 T cell reactivity in naturally infected human donors from Sri Lanka and Nicaragua using ***ex vivo*** IFNγ ELISPOT assays. Remarkably similar and clear serotype-specific patterns of immunodominance in both cohorts have been identified. Pooling of epitopes that accounted for 90% of the IFNγ response in both cohorts resulted in a global epitope pool. Its reactivity was confirmed in naturally infected donors from Brazil, demonstrating its global applicability. This study provides new insight in differential serotype-specific immunogenicity of DENV proteins. It further provides a potentially valuable tool for future investigations of CD8 T cell responses in the typically small sample volumes available from acute fever patients and/or children without requiring prior knowledge of either infecting DENV serotype or HLA type.

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ANTIVIRAL SMALL INTERFERENCE RNA PROFILES FROM LOW AND HIGH VECTOR COMPETENT MOSQUITO LINES FROM MEXICO UPON DENV-2 INFECTION

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The small interference RNA (siRNA) pathway is the major antiviral innate immune response in the DENV vector, ***Aedes aegypti***, and may control the outcome of transmission upon infection (i.e. vector competence). The aim of the work is to determine whether viral siRNA profiles from mosquitoes contribute to the vector competence phenotype. ***Aedes aegypti*** isofemale lines were derived from collections from the state of Veracruz, Mexico, and were assessed for vector competence by plaque assay and RT-qPCR. Three isofemale lines were chosen based on different vector competence phenotypes: isofemale line C/Z 9 does not have a midgut infection or midgut escape barrier (MIB and MEB, respectively) and has a high vector competence phenotype, C/Z 12 has a low vector competence phenotype due to a MEB, and C/Z 22 has a low vector competence phenotype due to a MIB. The isofemale lines were then orally infected with a low passaged Mexican DENV-2 strain, after 14 days post infection (dpi), midguts were dissected, and RNA from individual midguts were screened by RT-qPCR to determine infection status. 46, 48 and 17% of midguts were positive for DENV from the isofemale lines C/Z 9, C/Z 12, and C/Z 22, respectively. siRNA libraries were prepared from infected, exposed but uninfected, and unexposed midguts using the TruSeq small RNA library prep and were sequenced on the Illumina HiSeq platform. We expect to find similar antiviral siRNA profiles between the isofemale lines without a MIB (C/Z 9 and C/Z 12) and without a MEB (C/Z 9 and C/Z 22). This work will help us understand how the ***Aedes aegypti*** antiviral innate immune system contributes to the outcome of infection and DENV transmission.

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ENHANCING ANTI-DENGUE VIRUS ANTIBODIES TARGETING THE FUSION LOOP DELAY AND UPREGULATE PRO-INFLAMMATORY CYTOKINE PRODUCTION BY FCΓR-BEARING CELLS *IN VITRO*

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Secondary dengue infection with a heterologous serotype can lead to severe disease characterized by vascular leakage. Antibody-dependent enhancement (ADE) occurs when pre-existing, cross-reactive antibodies enhance viral infectivity. This phenomenon may be responsible for severe disease during secondary infections. The ADE hypothesis has been expanded to include the idea that FcγR-mediated viral entry into host cells can result in suppression of the cellular antiviral response. FcγRIIA-bearing K562 cells were infected with DENV that had been pre-incubated in the presence or absence hMAbs at neutralizing or enhancing concentrations. Infection kinetics were determined over a 120 hour period by plaque assay and qRT-PCR. Cytokine profiles were determined by cytometric bead array (CBA) assay. DENV-specific cytokine production was determined by subtracting the amount of cytokine (pg/ml) produced by mock-infected cell culture supernatants from the amount of cytokine (pg/ml) produced by infected cell culture supernatants. With the exception of cells infected with DENV plus a neutralizing concentration of HmAb 1.6D, all groups exhibited similar infection kinetics with viral titers peaking within 48 hours post-infection. Cells infected under enhancing conditions had viral titers approximately 100-fold higher than those infected without HmAbs present. In addition, cells infected under enhancing conditions significantly greater viral replication as measured by genomic copies than those cells infected under non-enhancing conditions. When compared to K562 cells infected with DENV alone, cells infected under enhancing conditions exhibited delayed pro-inflammatory cytokine production. However, the peak production of these cytokines was greater relative to cells infected with DENV alone. Enhancing hMAbs lead to increased viral replication after dengue infection of FcγRIIA-bearing cells. In addition, hMAbs targeting the fusion loop delay but increase the production of pro-inflammatory cytokines.

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DENGUE CHAT: AN INFORMATION AND COMMUNICATION TOOL TO PROMOTE COMMUNITY-BASED DENGUE VECTOR CONTROL

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Integrated vector control strategies that incorporate community-based interventions are currently considered a sustainable approach to try to curb the expansion of dengue. The best agents to eliminate potential and active mosquito breeding sites are the residents of communities affected by dengue. We report the development of Dengue Chat, an interactive cell phone and web platform that combines mobile technology, information, and evidence with game concepts to motivate community residents to report and eliminate mosquito breeding containers. Dengue Chat was developed with a capillary strategy of software production that involved young user-residents in Brazil, Mexico and Nicaragua in the researching, designing, and testing of the application. Dengue Chat crowd-sources the identification and mapping of breeding sites through photographic evidence, generating data that is returned as visual graphs and information to motivate the active management of dengue vector ***foci***. The elimination of the breeding containers is documented by a second photograph. The app and web interface are interactive, allowing residents to create their own profiles and exchange information regarding dengue in their neighborhoods. Dengue Chat also has an educational and social network component that relates to other relevant issues in the community. Players participate in teams and earn badges and points for their documented efforts in eliminating breeding sites. Dengue Chat is being piloted in 5 communities in Managua, Nicaragua, that are heavily affected by dengue and chikungunya. Baseline entomological surveys were carried out prior to implementation. The app is being deployed by teams of volunteer youth brigades under the supervision of a project facilitator and within the intrinsic community health leadership. Preliminary data demonstrates that Dengue Chat has high acceptability and is an effective communication and community engagement tool for dengue control. Results from the pilot studies are informing app re-design and providing direct feedback regarding the limitations and benefits of Dengue Chat, while potential scale-up is considered.

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SEROSURVEY OF DENGUE, ZIKA AND OTHER MOSQUITO-BORNE VIRUSES IN FRENCH POLYNESIA

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Since the 40s, dengue virus (DENV) has been the only mosquito-borne virus actually considered as major public health concern in the Pacific

region and more particularly in French Polynesia (FP). In 2013, Zika virus (ZIKV) was detected in FP for the first time and caused the largest outbreak ever reported. In order to anticipate the possible burden of arthropod-borne viruses (arboviruses), we conducted a serosurvey intended to identify the viruses the French Polynesian population is mostly immunologically naïve for or has already been exposed to. During the first semester of 2014, serum samples were collected from 196 participants aged 7-86 years (median 41 years) living in the most inhabited islands of the five archipelagoes of FP, and from 476 school children aged 6-16 years (median 11 years) living on the main island, Tahiti. Serum samples were tested for the presence of specific IgGs against the four different DENV serotypes, ZIKV, Chikungunya virus (CHIKV), Japanese encephalitis virus (JEV), West Nile virus (WNV) and Ross River virus (RRV). Seropositivity rates found among residents and school children were respectively 88% and 50% for DENV-1, 51% and 0% for DENV-2, 67% and 15% for DENV-3, 61% and 15% for DENV-4, 50% and 66% for ZIKV, 3% and 1% for CHIKV, 10% and 1% for JEV, 8% and 3% for WNV, and 35% and 1% for RRV. The level and distribution by age of the seropositivity for the different DENV serotypes is overall consistent with epidemiological data. Most notably, the lower level of seropositivity for DENV-2 compared to the other 3 serotypes may be associated with a higher risk of re-emergence of this serotype in FP. At least half of the residents and children have ZIKV IgGs, showing that most people were immunized during the 2013 epidemic. The results also suggest that RRV may have circulated in FP although it has never been reported. The low level of immunization (10% or less) against CHIKV, WNV and JEV may be associated with a high risk of emergence of these viruses in FP, as confirmed by the occurrence of a large CHIKV outbreak that started in FP in October 2014.

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LOW VECTOR COMPETENCE OF LOCAL *AEDES ALBOPICTUS* TOWARDS DENGUE VIRUS TYPE 1 STRAINS IN HAWAII

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Dengue is the most important arthropod-borne viral infection of humans, with an estimated 350 million cases reported each year in tropical countries. Dengue virus (DENV) is transmitted primarily by ***Aedes aegypti*** and to a lesser extent by ***Ae. albopictus***, which is associated with small and mild outbreaks. Two different DENV-1 genotypes, I and IV, were introduced in Hawaii in 1943 and 2001, respectively. ***Ae. albopictus*** is the most abundant Aedes species in the Hawaiian Islands. Given its abundance, increased global travel and spread of the virus, the vectorial capacity of ***Ae. albopictus*** towards dengue virus has major epidemiologic significance. However, there is no scientific data on how effective local ***Ae. albopictus*** are at transmitting DENV strains. In this study we investigated the competency of ***Ae. albopictus*** in transmitting DENV-1 strains isolated in Hawaii. Two ***Ae. albopictus*** mosquito populations trapped in Central Oahu (CO) and Southern Oahu (SO) were fed infectious blood meals of DENV-1 from Maui and Oahu isolated in 2001, and DENV-1 from Oahu isolated during the 1943 DENV outbreak. A subset of exposed mosquitoes was examined for virus levels by organ (body, legs/wings, saliva) at day 7 and day 14 after infection, and infection, dissemination and transmission rates were assessed. High levels of infection and dissemination were observed for the two 2001 viral strains from Maui and Oahu, whereas moderate level of infection was observed for the Oahu 1943 strain with low level of dissemination showing a differential response according to the genotype. Overall, low level of transmission was found for all viral strains suggesting the presence of a salivary gland barrier. These results can explain the limited size of the 2001 DENV outbreak in Hawaii. Phylogenetic evidence suggests introduction of DENV-1 from French Polynesia where a large outbreak driven by the vector ***Ae. aegypti*** was occurring at the same time. Interestingly no difference in the DENV genome between the Hawaii and the Polynesian strains were observed suggesting that the mild and small outbreak in Hawaii is directly linked to the low vector competence of the secondary vector ***Ae. albopictus***.

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COSTS OF DENGUE HOSPITALIZATION AND PUBLIC PREVENTION AND CONTROL ACTIVITIES IN URBAN SRI LANKA

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Dengue has become a major public health problem in Sri Lanka; however, the economic impact of the disease has not been studied in this setting. This study assessed the costs of dengue prevention and control activities and the direct medical costs of dengue hospitalizations in the Colombo District, the most affected district with the highest dengue caseloads in the country. The study was conducted in the epidemic year of 2012. Using information from the official databases of governmental agencies in charge of the dengue prevention and control activities in each administrative unit, we calculated the total financial costs of these activities and the average cost per capita. The direct medical costs of hospitalized dengue cases in the public health sector were derived using operational budgets and a sample of bed head tickets of adult and pediatric patients available from six secondary level hospitals. In 2012, the total financial cost of dengue prevention and control activities in the Colombo District was about $998,000, or $0.43 per capita. The mean direct medical costs to the public health care system per case of hospitalized dengue fever (DF) and dengue haemoraggic fever (DHF) were $221 and $316 for paediatric partients, respectively, and $203 and $272 for adult patients, respectively. These preliminary results highlight the high economic burden of dengue to the public health sector in the Colombo district in Sri Lanka during an epidemic year and contribute to the sparse literature on the economic burden of dengue in affected countries. This research was funded by "DengueTools" of the 7th Framework Programme of the European Community.

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DENGUE IN BANGLADESH: RESULTS FROM A NATIONWIDE SEROPREVALENCE STUDY

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Dengue is endemic in Bangladesh; however, seroprevalence studies in the capital city of Dhaka and one rural area suggest that there is spatial variation in risk. There is limited data on individual- and community-level risk factors in Bangladesh and the epidemiology is poorly understood. To address this gap we conducted a nationwide seroprevalence study during August to December 2014. We randomly selected 70 communities and visited up to 15 randomly selected households within each community. All household residents were asked to provide a blood sample and socio-demographic information. Indirect PanBio IgG ELISAs were used to identify

past dengue infections. ELISA optical densities separated individuals into two distinct groups of persons with and without past dengue exposure. We used bivariate analysis to identify individual risk factors associated with past dengue infection. In total, 2,911 individuals participated in the study with a median age of 26 (range 0 - 90) years. Prevalence of IgG against dengue was 11% in individuals aged ≤10 years, which increased to 18% in individuals aged 11-20 years (p-value=0.002), then further increased to 30% in individuals aged 21-50 years (p-value≤0.001), and remained at 30% in individuals ≥51 years of age. Males were 1.3 (95% confidence interval [CI] 1.1-1.5) times more likely to have been infected than females. Dengue seropositivity was higher in urban areas compared to rural areas (43 vs 19%, risk ratio = 2.2, 95% CI 1.9-2.5). Twenty-five percent of all communities had seroprevalence <10% and 10% had seroprevalence of >50%. Adults were more likely to be previously infected than children, but there were no meaningful differences after age 21, suggesting that endemic transmission may be relatively new to Bangladesh. Urban residents appear to be at much higher risk than rural residents with some rural areas having little evidence of previous dengue transmission. Additional studies to understand community-level differences in vector habitat and human movement could help predict which populations are at highest risk for future dengue outbreaks.

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PREDICTING POTENTIAL LINEAR B-CELL EPITOPES ON E GLYCOPROTEIN OF DENGUE VIRUS THROUGH IN-SILICO APPROACHES

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B-cell epitopes on the E protein of Dengue virus (DENV),the major target of neutralizing antibodies (NAb) and vaccine development for DENV were predicted by in-silico approaches. These bioinformatics analyses are attractive as a first line of screening, in identifying epitopes for therapeutic and diagnostic purposes, featuring for their low cost, convenience etc.Three tools,BepiPred, Ellipro and SVMTrip were used and the results obtained were compared with each other followed by a concise verification against available biochemical-assay positive data. E protein sequences of 50 strains from each DENV serotype with temporal differences (1963-2011) and geographical variation were analyzed. Predictions yielded 23 epitopes (length 6-62 AA). BepiPred and Ellipro predictions showed a higher similarity while predictions of SVMTriP were slightly different. Many of the predicted epitopes, at least partially, overlaps with regions that have been shown to generate Abs or be recognized by natural Abs in other studies. Some epitopes were further found on regions that are important in host cell attachment. The epitopes, EP5 (145-174) and EP6 (165-182) positioned on domain I (DI) showed a higher serotypic conservancy (%) with very low overall conservancy (%), demonstrating a potential target as serotype specific diagnostic markers. EP3 (62-123) and EP4 (65-85) are included in the highly conserved bc loop (73-79) region of DII, which is known to give rise to NAbs. EP11 (279-298) locates in the DI/DII hinge region, which is the principle target of long-lived serotype-specific NAbs that develop in humans after natural DENV3 and DENV4 infections. EP13 (311-316) and EP14 (310-329) on the DIII partially includes the highly conserved peptide sequence (309-320) on AB loop of the DIII and EP19 (371-402) includes highly conserved 393-401 region. EP20 (416-435) in the stem region showed an overall conservancy of 75% with 95% serotypic conservancy. The predicted epitopes by the 3 tools demonstrated good agreement in the results. As such this research concludes that in-silco approach is a recommendable initial step to screen potential linear epitopes in proteins.

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LONG HISTORY OF DENGUE TRANSMISSION, NEW GUINEA

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Dengue incidence has increased consistently in Southeast Asia and the Western Pacific in the past decades, and more than 70% of the global dengue burden is currently borne by people who live in this region. Despite presumed endemicity, little is known about the natural history of dengue in Papua New Guinea and there is no ongoing surveillance. We conducted seroprevalence assessments using serum samples collected from northern and southern coastal areas, offshore islands and the highland region of New Guinea between 1959-1963, and 2007-2010. DENV-specific neutralizing antibodies were demonstrated in sera from the northern coast of New Guinea and previous monotypic infection with all four serotypes was identified, however the majority of sera showed evidence of multiple previous DENV infections. No evidence of previous DENV infection was identified in the Asmat villages of the southern coast in sera collected in 1962; these same serum samples were previously found to be seronegative for measles IgG, supporting our finding that these isolated groups, in the absence of significant contact with outside forces, had not been exposed to DENV. DENV-3 was isolated from febrile patients in Madang in 2007-2008, and phylogenetic analysis of whole genome sequences showed these viruses clustered into a distinct group within genotype 1, a pattern suggestive of endemic DENV transmission. DENV-2 (Cosmopolitan genotype) was isolated from a febrile patient from Lihir Island in 2010. These findings confirm that multiple DENV serotypes have circulated along the northern coast of New Guinea for decades and support the notion that dengue has been a significant yet neglected endemic disease in PNG for more than 70 years. In spite of this apparent endemicity, severe dengue has not been reported in PNG. Dengue burden needs to be quantified and assessment of origin and distribution of DENV genotypes undertaken so as to develop an understanding of dengue transmission and virulence in PNG, particularly in the context of emerging dengue vaccines.

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THE DENGUE VACCINE INITIATIVE PROJECT: THE PASSIVE FACILITY-BASED SURVEILLANCE AND SERO-PREVALENCE SURVEY FOR DENGUE IN CHILDREN AND ADULTS OF NHA TRANG CITY, KHANH HOA PROVINCE, VIETNAM

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Dengue is one of the fastest spreading vector-borne viral diseases in the world and it is major health problem in Vietnam. In 2010, Vietnam experienced dengue outbreak with almost 130,000 cases and 110 deaths. In order to comprehensively determine true burden of dengue infection, Dengue Vaccine Initiative (DVI) conducts the passive facility-based fever surveillance, with the sero-prevalence survey in Nha Trang city, Khanh Hoa province in Vietnam. Since the launch of the surveillance in August 2014, we have collected acute and convalescent blood samples from febrile patients between 1-55 years of age to test for dengue fever using IgM and IgG ELISA, followed by RT-PCR. For the sero-prevalence survey, we identified 4400 randomly selected residents between 1-55 years of age to follow over rainy season and collected paired blood samples to estimate sero-conversion rate. To follow the changes in their immunity

status, those samples that show rise in the IgG ELISA undergo PRNT. As of March 2015, 541 patients have been enrolled in the fever surveillance. Laboratory test results are available from 176 subjects and among them, 67 were confirmed with dengue by ELISA IgM/IgG. The preliminary results show that the mean age was significantly higher among the dengue cases compared to non-dengue cases (23.9 vs. 13.0 years, P<0.001). Among dengue cases, only 7.5% (n=5) and 1.5% (n=1) of subjects were clinically diagnosed with DF and DHF, respectively. Rash, fatigue/weakness, and headache were found to be more commonly shown among the dengue cases compared to the non-dengue cases. As for sero-survey, the first bleeding was conducted in September 2014 and the second bleeding is planned in April 2015. After paired sera are collected, laboratory tests will be performed. More information such as circulating serotypes, the age-specific incidence rates and sero-conversion rates will be available for presentation at the conference. The study will provide valuation information on the true burden of dengue in Nha Trang city and will be used as evidence for decision-making for future dengue vaccine introduction in Vietnam.

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POTENTIAL GROUP AND SEROTYPE SPECIFIC B-CELL EPITOPES OF DENGUE NS1 PROTEIN AS IDENTIFIED BY A BIOINFORMATICS APPROACH

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Linear B-cell epitopes of NS1 protein from Dengue virus were predicted using a bioinformatics approach. The secreted form of Dengue NS1 in the bloodstream has been shown to stimulate a strong humoral response, therefore the utility of NS1 B-cell epitopes and antibodies against them as a diagnostic tool or for clinical management of the disease is of a current interest. Three B-cell epitope prediction tools, Ellipro, BepiPred and SVMTrip, were used to predict linear dengue NS1 epitopes and the predictions were evaluated by comparing the results among the three tools and with currently available data on immunogenic epitopes of NS1 as assayed by biochemical tests. Fifty sequences from each dengue serotype, representing a wide geographic area and a time span, were selected from NCBI gene bank. The sequences were aligned using MEGA6 software, and their conservation was evaluated using IEDB and Weblogo analysis tools. A total of 22 regions on the NS1 protein, ranging 6 to 21 amino acids in size, were predicted by the three prediction tools. The same regions were found to be predicted as epitopes by more than one tool, showing a good agreement in the results among the three tools. Further, many of the epitopes, at least partially, matched with regions, which have been previously identified to be immunogenic in other studies. The epitopes ^{72}NELNHILLENDMKFT^{86}, ^{102}MIRPQPMEYKY^{112} and ^{202}ESEKNET^{208} are highly conserved within each serotype but highly variable among the four serotypes. The results suggest a potential use of these epitopes as serotype specific diagnostic markers. Three epitopes, ^{155}EDYGFGIFT^{163}, ^{230}LWSNGVLESE^{239}, ^{261}TQTAGPWHLGKLELDFDLCE^{280} were more than 85% conserved among the four Dengue serotypes. Further, these epitopes were not conserved with other Flavivirus groups, West Nile Virus and Japanese Encephalitis Virus, demonstrating a potential role as group detection diagnostic markers for Dengue.

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ESTIMATING DENGUE TRANSMISSION INTENSITY FROM INCIDENCE DATA IN MULTIPLE COUNTRIES

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With an estimated 390 million annual infections worldwide, dengue is a global public health burden. Yet estimates of dengue transmission intensity remain ambiguous. Most primary dengue infections are asymptomatic, but secondary heterologous infection has been identified as a major risk factor for symptomatic and severe dengue. Therefore the majority of cases observed in hospitals or reported via surveillance systems are secondary infections. In many countries incidence data are the only type of data available. Although we hypothesise that seroprevalence data provide a more reliable estimate of the force of infection, in the absence of such data we propose a method of estimating dengue transmission intensity from age-stratified incidence data. We estimated the force of infection by fitting a catalytic model to data from 12 countries using a Metropolis-Hasting Markov Chain Monte Carlo algorithm. We also estimated the relative probability of detecting primary to quaternary infections and found that secondary infections consistently had the highest probability of detection. Basic reproduction numbers ranged between 1 and 6 which was consistent with results we obtained previously from seroprevalence surveys. We additionally estimated age-dependent reporting rates and the age threshold at which reporting rates changed. As expected this varied widely across surveys but reporting rates were consistently higher in the younger age-groups. The baseline reporting rates are indicative of the quality of the surveillance system. However there can be substantial bias in an epidemic year when not all suspected dengue cases will be laboratory confirmed and clinicians may diagnose any febrile illness as suspected dengue fever. Comparison of estimates derived from different data types will help to quantify the importance of silent transmission on dengue burden and identify areas where clinical dengue surveillance may require improvement.

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DENGUE SEROPREVALENCE IN TWELVE COUNTRIES: BASELINE SEROPOSITIVITY RATES FROM PREVIOUSLY CONDUCTED CLINICAL TRIALS IN ASIA AND LATIN AMERICA

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Dengue has been spreading rapidly around the globe and now considered endemic in most of the tropics and subtropics. The clinical manifestations of dengue disease are very wide, ranging from asymptomatic cases to fatal outcomes. The information on endemicity often comes from passive reporting and the true burden is relatively unknown. Age-specific seroprevalence data can, therefore, be useful to better understand dengue transmission and to address the extent of dengue burden; however, measuring seroprevalence against dengue is not a common practice in most countries and data are lacking. To obtain a better view of the magnitude of the problem in dengue endemic countries, we have revisited 10 Sanofi Pasteur CYD-TDV dengue vaccine clinical studies where blood was taken from the study subjects to assess the immunogenicity. Using their baseline seropositivity data, prior to receiving the candidate vaccine or placebo, with standardized age grouping (2-5 years, 6-11 years, 12-17 years, and 18-45 years), we evaluated the proportion of subjects being seropositive against dengue IgG or based on PRNT. A total of 3,701 subjects were included in the analysis from 12 dengue endemic countries in Asia and Latin America, where the bleeding had taken place between 2006 and 2011. In most countries where the studies were conducted, approximately 50% or more subjects were seropositive by the age of 5

years. Low seropositivity rate (approximately 20% seropositive by the age of 12 years) was observed from Singapore despite its location in the heart of South East Asia. On the other hand, high seropositivity rates were observed from Indonesia, Philippines, Colombia, and Honduras, where 90% or more subjects were seropositive by the age of 12 years. This highlights the extent of the burden of dengue in children and complements the passive reporting data in these countries. Knowledge of current age-specific seroprevalence rates is important in order to establish public health priorities and to adopt appropriate vaccination policies.

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COMPARISON OF CLINICAL DIAGNOSIS AND SYMPTOM CLUSTERS FOR DIFFERENTIATING DENGUE FROM OTHER ACUTE FEBRILE ILLNESSES IN IQUITOS, PERU

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Non-specific acute febrile illness (AFI) is a common clinical presentation of dengue virus (DENV) infection. This is a challenge for clinical management and timely identification of surges in disease incidence, especially in places where laboratory confirmation of DENV infection is either delayed or absent. We analyzed four years of hospital and clinic data from Iquitos, Peru, to quantify the utility of symptom clusters (such as might be used for syndromic surveillance) compared to clinical diagnosis for differentiating dengue from other AFI. Case data were restricted to patients ≥5 years old, in the first five days of fever, with PCR test results. PCR-confirmed cases were restricted to DENV-2. We used common factor analysis (CFA) to identify symptom clusters in DENV+ patients, and test their utility for differentiating DENV+ (n=1803) from DENV- (n=3939) illness. Presumptive clinical diagnosis was available for a subset of cases (95 DENV+ and 363 DENV-). Symptom cluster diagnosis and clinical diagnosis were evaluated using receiver operating characteristic area under the curve (AUC), where 1.0 is a perfect classification, 0 is the inverse of a perfect classification, and .50 is an arbitrary classification. Three distinct symptom clusters identified by CFA in DENV+ cases corresponded to 'body', 'respiratory', and 'gastrointestinal' symptoms. When these clusters were evaluated individually and in combination, the AUC ranged from 0.47 to 0.54, indicating that the discriminatory power was close to a random guess. Similarly, the AUC for clinical diagnosis was 0.46. Our results emphasize the difficulty of identifying dengue cases based on clinical presentation alone, and highlight the potential for reliable point-of-care diagnostics to improve clinical management, as well as the timeliness and accuracy of epidemiological data.

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DENGUE VACCINE INITIATIVE: PASSIVE FEVER SURVEILLANCE AND SEROLOGICAL SURVEY FOR DENGUE IN OUAGADOUGOU, BURKINA FASO

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Since 2000, there had been periodic notifications of dengue cases in Burkina Faso. Dengue presence has been confirmed and an outbreak was declared in 2013. However, there is limited information on dengue incidence and serotype distribution. To determine the true burden of dengue, a comprehensive epidemiologic study including a passive facility-based surveillance and a serological survey was launched in December, 2014, in Ouagadougou, Burkina Faso. For the fever surveillance, people between 1-55 years of age seeking health care in six health care centers (CSPS) of Ouagadougou, with current fever or history of fever (≤7 days) and without confirmed Malaria were evaluated for dengue infection. Two samples were taken from each patient, an acute sample during the first visit and a convalescent sample during the second visit, with an interval of 10-21 days. All subjects were tested using dengue rapid test (NS1/IGM/IgG) and IgM/IgG Capture ELISA. Those with positive results on any of thetests are further tested with RT-PCR. For the serosurvey, 3000 randomly selected residents of Ouagadougou between 1- 55 years of age were bled over an interval of six months and tested with IgG Indirect ELISA to calculate sero prevalence and subsequently the sero-conversion rate of the catchment population. Sero-converted samples will be tested using PRNT. So far, we have recruited 72 subjects from January to March 2015. The mean age is 23.12 years and 39 (68%) were female. From the 72 samples with lab testing completed, 23 (31.9%) were found to be dengue positive by ELISA, from which 95.7% (n=22) were secondary infections. There will be more data from the surveillance available at the conference. Also, from dengue-confirmed cases that sought care at the facility, direct and indirect cost-of-illness will be estimated and presented at the conference as well. The first serological survey is planned in May 2015 and the second bleeding would be taken around November. Additional data from the serological survey will also be available at the conference.

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THE EFFECT OF MOSQUITO LIFE STAGES ON THE INVASION DYNAMICS AND SPATIAL SPREAD OF *WOLBACHIA* IN *AEDES AEGYPTI*: IMPLICATIONS FOR DENGUE VECTOR CONTROL

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With approximately half the global population at risk of dengue infection, control of its primary mosquito vector ***Aedes aegypti*** is vital. One proposed control method is the release of mosquitoes infected with Wolbachia, which increases mosquito mortality, thus reducing the likelihood that dengue infected mosquitoes survive the extrinsic incubation period and transmit the virus. However, the success of this strategy depends on whether Wolbachia can spread spatially through the mosquito population from a few localised release sites. Previous theoretical work has examined how the threshold frequency of Wolbachia required for local fixation depends on the fitness costs associated with Wolbachia infection (i.e. increased mortality, reduced fecundity) and the levels of cytoplasmic incompatibility and maternal transmission. Using a stochastic patch model that incorporates egg, larval and adult stages, we find that for releases of adult mosquitoes, the threshold frequency of Wolbachia-infected adults required to achieve fixation in a single patch is always higher than previously derived values, and that this threshold depends on the oviposition rate, development rates for eggs and larvae, and mortality rates of all three stages as well as the fitness costs associated with Wolbachia. When considering the speed of Wolbachia spread through an array of patches, the wave front moves across the population more slowly that expected if only the fitness costs of Wolbachia are taken into account when predicting the wave speed; in some cases where previous theoretical work has predicted spatial spread would occur, the wave actually contracts and the Wolbachia-infected mosquitoes become extinct.

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TARGETED FULL GENOME AMPLIFICATION AND SEQUENCING OF DENGUE VIRUSES 1-4 IN SOUTH AMERICA

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Next-generation sequencing of dengue viruses (DENVs) is a powerful tool for dengue epidemiological surveillance around the world. Protocols using targeted approaches to selectively amplify complete DENV-1-4 genomes have been described. However, these published workflows

use primer sets that were specifically developed to detect DENV strains circulating in Asia. DENVs in South America differ from those in Asia. Thus, existing workflows for full genome amplification of DENVs are often inadequately suited to detect South American strains. There is one report of optimized work flows for DENV-2 in Brazil, but nothing for serotypes 1, 3, or 4, which also circulate in the area. Here, we report optimized workflows for full genome amplification of all four serotypes of DENVs in South America. Based on alignments of complete genome sequences available through GenBank, we have modified and expanded previously published sets of primers to generate complete genomes from DENVs 1-4. Following re-design of the primer sequences, cycling conditions were optimized using DENV-positive serum samples collected in Bolivia, Ecuador, Paraguay, Peru and Venezuela since the early 2000s. Full genome coverage in 5 overlapping segments was always possible from DENV-positive serum samples with CT values of ≤ 26 (as determined by McAvin RT-PCR). Samples with CT values higher than 26 required amplification of larger number of overlapping segments of smaller size in order to obtain full genome coverage. A handful of samples with CT values higher than 29 failed to generate complete genomes. The methods described here are sequencing platform-independent, hence, they can be used for next generation sequencing of DENVs circulating in South America using any next-generation sequencing approach. These optimized methods should facilitate both epidemiological surveillance and evolution studies of DENVs in Latin America.

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DENGUE VACCINE INITIATIVE PROJECT: THE SERO-PREVALENCE SURVEY AND THE PASSIVE SURVEILLANCE FOR DENGUE IN BANG PHAE, RATCHABURI, THAILAND

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Dengue fever is a major public health problem in Thailand. With so much awareness and documented high burden, Thailand is likely to be an early adopter of dengue vaccines. In preparation for the upcoming dengue vaccine introduction, the Dengue Vaccine Initiative (DVI) is conducting the passive facility-based fever surveillance and sero-prevalence survey to accurately document dengue disease burden in both adults and children in Bang Phae district of Ratchaburi province in Thailand. The data will provide essential evidence for decision-making for vaccine introduction. The population in the study area is approximately 50,000. In the fever surveillance, eligible febrile patients between 1-55 years-of-age were enrolled. We collected acute and convalescent blood samples to test for dengue infection using IgM/IgG ELISA NS-1 rapid test and RT-PCR. The sero-prevalence survey is conducted on a sample of 2000 randomly selected residents to determine the burden of inapparent dengue. With 6 months interval, the paired sera collected are tested with IgG ELISA and PRNT to assess age-specific sero-conversion rate. Starting October 2011, the surveillance was launched in Bang Phae Community Hospital (BPCH). Thus far, 759 patients were enrolled and 91 subjects were found to be dengue-positive. Most of the patients (n=82) were diagnosed with dengue fever and, among 88 samples that underwent PCR, 82% showed secondary infection (n=72). DENV-2 was the most common serotype. In sero-prevalence survey with 2012 subjects recruited in Bang Phae district, there were 1897 residents by the 4th bleeding among whom 76% of subjects were found to be IgG-positive. Over the study period, the sero-conversion rate was approximately 10% per year. PRNT has been performed for 70 subjects and the majority was from secondary infection (67%) with 57% of these showing homologous secondary infections. More PRNT data will be available for presentation at the conference. The data generated will provide essential evidence for policy-makers to make informed decision-making for dengue vaccine introduction in Thailand.

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A SOCIAL-EPIDEMIOLOGICAL STUDY TO UNDERSTAND DENGUE TRANSMISSION IN DHAKA, BANGLADESH

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Considering the complexities involved with dengue transmission, I argued that understanding transmission requires encapsulating different disciplinary knowledge as well as non-academic knowledge. I have applied an Ecohealth approach to investigate dengue transmission dynamics in Dhaka, Bangladesh.Using the Delphi method, all 90 Wards of Dhaka were classified into 'high', 'medium', and 'low' Socio-Economic Status (SES) zones. A total of 1,200 households were randomly selected which represented the SES zones. During 2011-2012, these sampled households were repeatedly inspected for Aedes mosquitoes and to collect blood samples from residing members. This transdisciplinary investigation focused on: i) the rates of human exposure to dengue virus (DENV) by identifying individuals with IgM and IgG antibodies in the serosurvey samples; ii) abundance of dengue vector mosquitoes in the same households; iii) risk perception, and Knowledge, Attitude and Practice (KAP) regarding dengue among community members and experts.KAP survey results indicated that 93.7% of the community members knew that mosquitoes act as the primary vector of its transmission; 87.3% were unaware that Aedes mosquitoes prefer to lay their eggs in water containers. The entomological survey results showed that 26.7% of all surveyed houses in the city were infested with ***Aedes aegypti*** mosquitoes. The ornamental functional categories of containers were most significant containers in producing maximum number of Aedes pupae; this was found to be a significant risk factor for seroprevalence and seroconversion. The examination of IgG seroprevalence revealed that seropositivity was strongly correlated with increased age and number of indoor potted plants. The serosurvey findings showed that seroprevalence was high (79.9%), revealing that most dwellers had been exposed DENV. However, there was no significant association between Aedes positive houses and houses with seroconverted persons. A persistently high rate of dengue infection in Dhaka is being influenced by the lack of knowledge and awareness of the eco-bio-social factors.

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GLOBAL ECONOMIC COST OF DENGUE ILLNESS

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To quantify the global cost of dengue illness, we systematically assembled and analyzed data on dengue burden across all 141 countries with active dengue transmission. We based numbers of cases on the Global Burden of Disease 2013. We used available empirical country-level data, and extrapolated to other countries based on GDP per capita or other indicators. For example, we derived the proportion of cases treated in other settings based on patterns for fever cases from Demographic and Health Surveys in dengue countries (n=71) and extrapolated to remaining countries based on region and share of births with skilled attendants. We estimated a global case-fatality rate from dengue deaths adjusted for reporting rates (available for 32 countries). We obtained direct and indirect costs from empirical studies in 14 countries and projected to remaining countries based on GDP per capita and treatment site using a log-log specification. We estimated that 58.4 million (95% certainty range 23.4-122.3) dengue cases occurred in 2013, with care distributed among the hospitalized (18%), ambulatory (42%), other (40%) sectors. The year's global direct and indirect cost of dengue illness in 2013 was $7.8 billion (range $3.5-$15.8b) in 2013 US dollars or $1.36 ($0.62-$2.77) per capita,

distributed among non-fatal hospitalized (42%), ambulatory (32%), other sectors (16%), and fatal cases (10%). Of these costs, $3.66b correspond to countries in Southeast Asia, East Asia and Oceania (n=33), $1.82b to Latin America and the Caribbean (n=43), $1.63b to South Asia (n=6), $0.33b to Sub-Saharan Africa (n=44), $0.09b to North Africa and the Middle East (n=6), $0.01b from Central and Eastern Europe and Central Asia (n=4), and $0.24b to high income countries (n=5). As we recognized care in other sectors and resource constraints in most health systems, our global estimate is substantially lower than the $39b of Selck et al (2014) estimated for 2010. Nevertheless, being more costly than rabies ($4.0b, Hampson, 2011), cholera ($3.1b, UPMC, 2010), and rotavirus gastroenteritis ($0.4b, Reingans, 2009), dengue imposes costs greater than several other major infectious diseases.

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ISOLATION OF A HIGHLY DIVERGENT HANTAVIRUS FROM THE EUROPEAN MOLE (*TALPA EUROPAEA*) IN POLAND

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Persistent uncertainties in hantavirus taxonomy result from the paucity of full-length genomes and the dearth of hantavirus isolates. Although referred to as novel viruses, nearly all of the more than 30 hantaviruses (family ***Bunyaviridae***, genus ***Hantavirus***) identified recently in shrews and moles (order Eulipotyphla) and insectivorous bats (order Chiroptera) exist only as viral sequences. Because Nova virus (NVAV), harbored by the European mole (***Talpa europaea***), represents a highly divergent hantavirus lineage which is widespread across Europe, its isolation has been a high-priority. Lung tissue homogenates, prepared from four NVAV-infected European moles captured in Huta Dłutowska in central Poland in 2013, were inoculated onto Vero E6 cell monolayers, then subcultured at two- to four-week intervals, at which time cells and culture media were analyzed for viral RNA by RT-PCR. After several failed attempts, NVAV RNA was detected in cells and culture media at 34 days after inoculation with tissues from one of four European moles. Subsequently, NVAV RNA was detected following inoculation of fresh Vero E6 cells with culture supernatant, indicating virus replication, and typical bunyavirus-like particles, measuring 80–120 nm in diameter, were found by transmission electron microscopy. Hantavirus genomic sequences of the isolate, designated NVAV Te34, were identical to that amplified from the original lung tissue, and phylogenetic analysis of the full-length L, M and S segments, using maximum-likelihood and Bayesian methods, showed identical topologies, with NVAV clustering with the highly divergent bat-borne hantaviruses. Studies to determine the pathogenicity of the NVAV isolate in infant mice are underway. The long-awaited isolation of NVAV, as the first mole-borne hantavirus, will accelerate the acquisition of new knowledge about its evolutionary origin, phylogeography and pathogenicity. Because European moles often reside near human habitation, individuals with known exposures, who develop febrile illnesses or unusual clinical syndromes, are being investigated for evidence of NVAV infection and disease.

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A PRACTICAL APPROACH FOR MOLECULAR EPIDEMIOLOGICAL STUDIES: ASSORTMENT OF VIRAL PATHOGENS IN SIMULATED ISOLATE ADMIXTURE

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Deep sequencing by Next Generation Sequencing (NGS) technology has been adopted as a valuable tool in molecular epidemiology to obtain comprehensive and unbiased genetic information from rapidly evolving pathogens. We developed a simulated isolate admixture, established NGS laboratory approaches, and tested four bioinformatics algorithm pipelines to identify and semi-quantify the viral pathogens in a simulated fifteen-virus admixture that can be used in public-health laboratories to develop analytical proficiency. The admixture was designed to simulate pooled respiratory samples collected from large epidemiological studies or specimens with multiple viral co-infections. Host genome depletion procedures consisted of mechanical host removal, enzymatic host removal, and/or virus concentration steps using conventional molecular laboratory equipments. The efficiency of host genome depletion procedures was critically evaluated according to individual pathogens that were successfully identified from the admixture and the quantity of the sequence recovered from the admixture. The percentage of viral pathogens identified from the admixture was consistently greater than 80% regardless of the analytical pipeline. For the sequence recovery, approximately 3.4 fold (95% CI 2.1- 4.7) and 13.3 fold (95% CI 8.4-18.2) viral contigs were identified by meta-IDBA, a read assembler for ***de novo*** metagenomic data, and Trinity, a ***de novo*** assembler of transcriptomes from RNA-seq data, respectively, prior to pathogen identification by blast on GENBANK database using sufficient host genome depletion procedures compared to untreated conditions (p=0.058 and p=0.046, respectively). In addition, host depletion procedures produced an average of 4 fold greater depth of coverage (DOC) of sequence reads by alignment analysis against identified viral pathogens when compared with no treatment. The host depletion steps along with all four analytical algorithm pipelines produced highly accurate viral identification from an admixture that can be applied to laboratory-based surveillance.

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IMMUNE RESPONSE TO IPV-OPV VACCINATION IN A BIRTH COHORT IN SOUTH AFRICA: THE MAL-ED STUDY

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In 2009, the South African National Vaccination Program introduced inactivated polio vaccine (IPV) in addition to oral polio vaccine (OPV) to ensure protection against polio. IPV is administered at 6, 10 and 14 weeks, and at 18 months, while OPV is given at birth and 6 weeks. Methods to study vaccine response have been described elsewhere (Hoest et.al 2014); briefly, the MAL-ED Study recruited a birth cohort in which vaccine history, monthly anthropometry, enteric infection, and breastfeeding practices were compared against polio antigens 1, 2, and 3 measured at 7 and 15 months. In South Africa, 250 children were enrolled, with 233 receiving

at least two OPV doses by their first blood draw (range 0-3 OPV doses) and 198 receiving at least three IPV doses (range 0-4 IPV doses). Analysis is restricted to children with at least 2 OPV doses and titers are converted to log2 scale. There is a significant decrease in titers from 7 to 15 months across all antigens: from 9.42 to 8.71 for antigen-1 (p=0.001); 9.84 to 9.38 for antigen-2 (p=0.006); and 9.80 to 9.07 for antigen-3 (p<0.0001). At 7M there is a significant positive trend between IPV doses and titers across all antigens with mean titers for 1, 2 or 3 IPV doses being 8.85, 9.18 and 9.56 for antigen-1; 9.21, 9.56 and 9.95 for antigen-2; and 9.18, 10.07 and 9.84 for antigen-3, respectively. At 15 months, this relationship is not significant and is negative for antigen-2: mean titers for 1, 2 or 3 IPV doses are 8.54, 8.50 and 8.73 for antigen-1; 9.58, 9.63and 9.32 for antigen-2; and 8.96, 9.38 and 9.00 for antigen-3, respectively. Compared with other MAL-ED sites, which only use OPV for immunization yet with varying schedules, South African children have lower average polio antigen-1 titers compared with Bangladesh, Brazil, Nepal, and Pakistan; higher antigen-2 titers vs. India, Pakistan and Tanzania; and significantly higher antigen-3 titers vs. all other sites. Variations by nutrition, enteric infection and breastfeeding practices will be discussed. Results provide insight into the merits of IPV use in conjunction with OPV for global polio immunization efforts.

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REPORT OF CIRCULATING GENOTYPE C STRAINS AND ONE UNKNOWN DIVERGENT STRAIN OF COXSACKIEVIRUS A16 IN PERU

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Coxsackievirus A16 (CVA16) is a major etiological agent of hand, foot, and mouth disease (HFMD), as well some neurological infections. In recent years, the number of studies investigating the etiology and molecular biology of this virus has increased. This work has focused primarily on CVA16 emergence from the Asian continent. However, information about CVA16 epidemiology in South America is limited. As part of our research investigating respiratory infections in Peru through passive, clinic-based surveillance methods, our laboratory reported the isolation of seven CVA16 isolates between 2005 and 2010 in children under six years of age. These isolates were collected from four ecological regions of Peru: coast, desert, rainforest, and highlands. Molecular epidemiological studies were carried out based on VP1 and/or VP4, and classified into three genotypes: A, B and C, and additionally subgenotypes B1-B2 and C1-C3. Using complete VP1 and VP4 gene sequences, we were able to molecularly characterize all seven of the CVA16 isolates. Six out of the seven isolates were genogroup C. To our knowledge, this study represents the first report of CVA16 molecular characterization in Peru, demonstrating that the primary circulating CVA16 strain in Peru is genotype C. However, we also found one divergent strain that did not cluster in any of the three genotypes reported so far. For future work we would like to complete a more complete phylogenetic analysis, including nucleotide analysis, with more strains from Peru and Latin America. This would allow for a more complete characterization of the possibility of an emergence of a new CVA16 genotype.

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EPIDEMIOLOGY OF ARBOVIRUSES IN THE NORTHEAST AMAZON BASIN OF PERU 2010-2014

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Arthropod-borne virus (arbovirus) infections continue to be a leading cause of morbidity and mortality around the world. Arbovirus infections are the primary cause of acute undifferentiated febrile illness (AUFI) in urban areas; dengue virus alone accounts for one hundred million cases each year, including more than 20,000 fatalities. Despite the public health relevance, the geographic range, relative impact, and epidemiologic characteristics associated with arbovirus infections are poorly described in many regions of the world. Here we describe the epidemiological data for arbovirus infection in patients with AUFI enrolled in a clinic-based surveillance study in Iquitos and Yurimaguas--the two largest cities of the northern Peruvian Amazon. Between December 2010 and October 2014, 2,328 samples screened positive for viral infection (PCR or viral isolation or ELISA IgM 4-fold increase between acute and convalescent samples); of these, 2,201 (94.5%) were positive for dengue virus infection while 127 (5.5%) were positive for other arboviral infections. These included Venezuelan equine encephalitis virus (VEEV), Mayaro virus (MAYV), group C viruses, and Guaroa virus (GROV). VEEV was the most common among the other arboviruses found. During 2011-2014, small but separate outbreaks of MAYV, VEEV, and GROV were detected. Seventy five percent (75%) of these non-dengue arboviruses were detected in residents from peri-urban and rural areas. Although 16-30 year olds were the most commonly affected age group for both dengue and other arbovirus infections, the cases of MAYV, VEEV, and GROV infections were higher among 31-45 years olds (X2=15, p=0.005). All arboviral infections appeared to impact males and females equally. In an effort to prevent futures outbreaks it remains important to continue existing febrile surveillance in the Peruvian Amazon Region where these arboviruses are present.

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A CRITIQUE OF EBOLA PREPAREDNESS AT A LEVEL ONE TRAUMA CENTER IN THE SOUTHEASTERN UNITED STATES

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The Ebola Virus was first discovered in Africa in 1976. Since that time, more than twenty outbreaks have been reported. We are currently in the middle of the worst Ebola epidemic on record. In the past year alone, a number of cases have been identified outside of Africa, specifically in Western Europe and North America. One of the most notable cases involved the death in Texas of an individual who reported a recent travel history to Liberia. Many Ebola patients will seek care at hospitals that may not be equipped properly for the high level of infection control that is required.To better prepare these facilities, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have published guidelines for caring for Ebola-infected cases. These guidelines are designed to maximize containment of the infection and the prevention of its spread. This presentation will examine the protocols for handling Ebola patients at a Level 1 trauma center located in a major metropolitan area in the Southeastern United States. More specifically, we will highlight protocols for Ebola treatment and containment and assess these guidelines against the WHO and the CDC recommendations.

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THE BURDEN OF JAUNDICE IN PREGNANCY-RELATED MORTALITY IN RURAL AND URBAN KENYA AND POSSIBLE ROLE OF HEPATITIS E, 2002-2014

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Maternal mortality is an area of severe health inequity, with 62% of global maternal deaths occurring in sub-Saharan Africa. Hepatitis E virus (HEV) infection also occurs disproportionately in impoverished settings and is the only cause of acute jaundice that increases maternal mortality. An outbreak in Dadaab refugee camp in 2012 indicated the presence of HEV in Kenya and infection has been consistently reported in nearby countries, but because of lack of surveillance, population-level mortality burden remains unknown. To estimate the proportion of pregnancy-related mortality associated with jaundice in Kenya we analyzed results of verbal autopsy (VA) interviews of family members, designed to determine the cause(s) of a recent death. We analyzed VA data from three sites: the Nairobi Urban Health and Demographic Surveillance System (HDSS) (data from 2003-2012) and a Population-Based Infectious Disease Surveillance site (2009-2014) in Nairobi's urban slums, and the rural Western Kenya HDSS (2002-2011). From all deaths among women of reproductive age, we identified those occurring during pregnancy or within 42 days of termination. We calculated the proportion associated with jaundice and pregnancy-related mortality ratios as the number of deaths per 100,000 live births. Of 5,601 verbal autopsies conducted for women of reproductive age we identified 400 pregnancy-related deaths. In the Western Kenya HDSS, 17% (45/263) were associated with jaundice and the pregnancy-related mortality ratio associated with jaundice was 93 per 100,000 live births. At both urban sites the proportion of pregnancy-related deaths associated with jaundice was 25% (16/65 and 2/8). The pregnancy-related mortality ratio associated with jaundice was 80 per 100,000 births in the Nairobi Urban HDSS. Therefore, jaundice is frequently associated with pregnancy-related deaths in both rural and urban Kenya, at similar proportions to those found in other HEV-endemic countries. HEV is preventable and an effective vaccine exists; laboratory-based studies to document the burden of HEV in Kenya and to verify use of VA data to estimate HEV mortality are warranted.

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UNGROUPED AUSTRALIAN ARBOVIRUSES

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In the early 1960s, the Rockefeller Foundation began support for a program of discovery of arboviruses in Australia which resulted in the identification of twenty-four viruses that were new to science and a number of arboviruses that had not been identified previously in Australia. When this program concluded with Dr Ralph Doherty's retirement as Director of the Queensland Institute of Medical Research, 50 agents recovered from mosquitoes, and several from a bird, which caused death or paralysis in new-born mice remained "unclassified". Viruses now have been propagated in cell culture from 23 of these. Nucleotide sequencing is in progress and has identified 8 strains of Sindbis (SINV), 6 of Murray Valley encephalitis (MVEV), 4 of Bovine viral diarrhoea (BVDV), 3 of Kunjin (KUNV) and one each of Kokobera (KOKV, multiple isolates from the tissues of a bird) and Ross River viruses (RRV). The BVDV isolates are believed to be contaminants from incompletely inactivated, locally collected, foetal calf serum rather than genuine isolates. There are no previous reports of the flavivirus KOKV being recovered from birds or of birds playing a role in its natural cycles of transmission. The nucleotide sequences of the isolates made from the various tissues of the bird were almost identical and were most closely related to a strain of KOKV recovered in Papua New Guinea in 1966 (MK 7979). Nucleotide sequencing of a further thirteen, previously unstudied, KOKV isolates from Australia, failed to identify KOKV similar to the bird isolates. The SINV isolates included a novel Australian lineage and the nsP3 genes of these viruses are being sequenced to identify lineage specific insertions, deletions and duplication events which have been described for almost all alphaviruses and which may distinguish Australian SINV lineages and/or be associated with lineage replacements. Phylogenetic analyses of the RRV and three KUNV are in progress. Nothing in the properties of the viruses, identified to date, explains why the serological methods of the day failed to identify them.

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POTENTIAL TRIGGERS OF DENGUE HEMORRHAGIC FEVER IN PATIENTS FROM BANGKOK, THAILAND

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Dengue fever (DF), a mosquito-borne disease, is among the most significant global health challenges. After dengue virus (DENV) infection, patients may progress to dengue hemorrhagic fever (DHF) within days. Evidence suggests that vascular endothelial cell dysfunction, coagulation disorders and increased plasma leakage occur rapidly after peak viremia and at defervescence, corresponding with elevated levels of circulating chemokines and cytokines. We seek to determine key triggers of DHF as part of an ongoing case-control study at the Queen Sirikit National Institute of Child Health in Bangkok Thailand. Our central hypothesis is that DHF cases demonstrate an elevated proportion of inflammatory (M1) compared to resting (M2) macrophages and demonstrate a unique cytokine expression profile early during infection that exacerbates endothelial cell permeability compared to DF controls. To date, we have enrolled eight hospitalized febrile patients, collecting both acute and convalescent blood samples. Aliquots of fresh blood were used to assess the frequency and vasoactive mediator expression profiles of CD14+ cells and subsets by flow cytometry. Preliminary results demonstrate an elevated M1 frequency and increased expression of TNF-alpha and VEGF in acute samples from DHF cases compared to controls. These data demonstrate that an early shift to M1 in DENV-infected patients may be critical to subsequently elevated vasoactive mediator levels and disease severity.

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MONKEYPOX VIRUS INFECTIONS IN HEALTHCARE WORKERS

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Human monkeypox (MPX) is caused by monkeypox virus (MPXV), a zoonotic ***Orthopoxvirus***. MPXV is endemic in western and central Africa; however, the overwhelming majority of human infections are reported from the Democratic Republic of the Congo (DRC). Healthcare workers (HCWs) are at risk of infection if they are providing consultation and care for patients. Infrastructure and resources for healthcare facilities are poor in DRC, and, therefore, there may be a heightened risk of infection if workers are unable to apply optimal infection control measures. Fourteen suspect monkeypox (MPX) cases in HCWs were identified in Tshuapa District, DRC between 2009 and August 2014. Diagnostic specimens were collected from 12 of the suspected cases; real-time polymerase chain reaction assays were conducted to assess MPXV and varicella zoster virus infection status. Exposure information was retrieved from the case report or collected retrospectively using an interview tool. Seven of the twelve suspect cases with laboratory testing results (58.3%) were classified as confirmed MPX. Five of the seven confirmed MPX cases (71.4%) had a smallpox vaccination scar. Considering all suspect MPX cases reported and investigated between January 2011 and August 2014, the overall proportion of HCW cases was 0.8% (range 0.6-1.8% by year). Among confirmed cases of MPX, the proportion represented by HCWs was 0.8% (range 0.3-3.1% by year). The estimated annual HCW incidence of MPX was 17.4/10,000. Three of the confirmed MPX cases (42.8%) reported exposure during the course of their duties at work, and four confirmed cases (57.1%) reported exposure while caring for an ill family member or friend. Ill HCWs put their families and close household contacts at risk, as well. Reinforcement of infection control practices should not only be emphasized for methods at a clinic, but also their work at home, should a family member become ill and need care. The use of vaccines to prevent occupationally-acquired infections should be considered as a first-line defense against infection for HCWs in DRC.

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FIRST EVIDENCE OF UNA VIRUS INFECTIONS IN INDIGENOUS AND NON-INDIGENOUS COMMUNITIES IN LORETO, PERU

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In the Amazon region, humans are exposed to sylvatic transmission by mosquitoes or small mammals, with arboviral diseases being most common illness transmitted in those areas. Arboviruses are classified according to antigenic relationships, and all cause febrile illness. This study aimed to understand possible arbovirus exposures in populations comprised of residents from two rural villages around Iquitos, Peru (non-indigenous), as well as riverside indigenous communities in Datem del Marañon province in Loreto, Peru. In collaboration with CDC rabies branch, Epidemiology Directorate of the Peruvian Ministry of Health (MoH), the Virology and Emerging Infectious Department of the U.S. Naval Medical Research Unit No. 6 in Lima conducted surveillance activities in these areas to identify antibodies to known arboviral pathogens, including alphaviruses, flaviviruses, and bunyaviruses. Informed consent was obtained from all study participants. All 364 samples were tested for ELISA IgG arboviral antibodies including the following groups: flavivirus (dengue virus (DV)), ilheus virus (IV), Saint Louis Encephalitis virus (SLEV), West Nile Virus (WNV), yellow fever virus (YFV)); alphaviruses (Venezuelan Equine Encephalitis Virus (VEEV), Mayaro Virus (MAYV), UNA Virus, Eastern Equine Encephalitis Virus (EEEV)); arenaviruses (Allpahuayo Virus (ALLV), and Tacaribe Virus (TCRV)), and orthobunyaviruses (bunyavirus, caraparu virus, maguari virus, murututu virus, oropouche virus), hantaviruses (Andes Virus (ANDV), and Rio Mamore Virus (RMV)). Samples were positive for alphavirus EEE = 2 (0.5%), UNA = 8 (2.2%). May = 6 (1.7%), and cross reactivity between VEE/MAY/WEE/UNA/EEE = 21 (5.8%). For hantavirus RMV = 67 (18.4%), for Arenavirus TAC = 4 (1%), ALL = 4 (1%), flaviviruses and bunyaviruses presented a high cross reactivity. We demonstrate the first evidence of UNA Virus circulating in humans, however, more tests are needed to confirm this serological result.

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SUSCEPTIBILITY OF POTENTIAL VERTEBRATE HOSTS TO HEARTLAND VIRUS INFECTION

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Heartland virus (HRTV; Bunyaviridae, Phlebovirus) has emerged as a causative agent of thrombocytopenia/ leukopenia in humans. Preliminary serological assessment of vertebrate sera obtained from areas adjacent to two index human cases in northwestern Missouri identified HRTV antibody positivity in raccoons (42.6%), horses (17.4%) and deer (14.3%). In order to further assess potential amplification hosts and potentially to develop an animal model for tick transmission studies, experimental inoculation of mice, rabbits, hamsters, chickens, goats and raccoons were performed. Inoculated rabbits, hamsters and chickens (104 PFU of HRTV s.c.) failed to demonstrate viremia from 1 to 7 dpi or neutralizing antibodies at 28 dpi. All species were boosted at 28 dpi with 104 PFU of HRTV. No detectable HRTV neutralizing antibodies were detected in chickens through 42 dpi after boost inoculation, but ELISA titers were evident (1:500-1500). PRNT80 10-20 titers were observed in boosted rabbits with ELISA titers up to 1:13,500. All boosted hamsters demonstrated neutralizing titers (1:10-80) and ELISA titers ranging from 1:13,500 to 1:≥ 45,000. Despite the high HRTV neutralizing antibody response identified in the field for raccoons, none of the six experimentally inoculated developed detectable viremias and only 2 had PRNT80 titers of 1:10, 20. Both goats experimentally inoculated with 104 PFU of HRTV failed to generate detectable viremias, but both generated low (1:20) neutralizing immune responses following the boost inoculation. All 3-week old C57BL/6 mice survived i.p. viral challenge with no demonstrable viremias and antibody responses were evident with up to 1:40 and 1:121,500 titers observed by PRNT and ELISA, respectively. In contrast, interferon receptor deficient AG129 mice demonstrated a dose-dependent mortality profile (LD50 of 9 PFU) and viremias of up to 108 PFU/mL sera were observed. While PRNT titers from sera taken from surviving mice were low, ELISA titers were as high as 1:40,500. Inoculated AG129 mice demonstrated gross hemorrhagic lesions with enlarged spleens associated with marked presence of HRTV antigen identified by IHC.

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SURVEILLANCE FOR NOROVIRUS AND OTHER ENTERIC VIRAL AND BACTERIAL PATHOGENS AS ETIOLOGIES OF ACUTE GASTROENTERITIS AT U.S. MILITARY RECRUIT TRAINING CENTERS AND ABOARD ACTIVE U.S. NAVY SHIPS (2011-2015)

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Acute gastroenteritis (AGE) is defined as the rapid onset of diarrhea with or without accompanying symptoms, such as nausea, vomiting, fever, or abdominal pain. An estimated 179 million cases of AGE occur each year in the United States. AGE is commonly reported within both training and deployed U.S. military populations; even outbreaks described as mild can have significant impacts on the health, readiness, and operational effectiveness of military personnel. Given the disruptive effect of AGE upon military training, readiness, and performance, it is crucial to establish a firm understanding of its causes among these "at-risk" populations so that we may better predict, prevent, and respond to future outbreaks. To this end, the Naval Health Research Center Enteric Disease Surveillance Program (EDSP) initiated clinic-based passive surveillance at five U.S. military training facilities for both sporadic and outbreak-associated AGE starting in April 2011. Recruits attending sick-call with the chief complaint of AGE/gastrointestinal (GI) symptoms were screened against the case definition, per protocol. Those meeting case definition who chose to volunteer signed an informed consent form, completed a case report form, and submitted a clinical stool specimen or rectal swab. Forms and specimens were transported to the EDSP laboratory, where standard culture-based and molecular assays were conducted to identify enteric pathogen etiology_the primary pathogen of interest being norovirus. From April 2011 through March 2015, a total of 1,391 cases were enrolled; median age was 19 years, 77% were male, and 64% were Caucasian. An enteric pathogen was identified in 450 cases, with norovirus accounting for 87%, followed by ***Salmonella*** spp. (6%). The overall norovirus-attack rate was 28% across all field sites. Ultimately, data derived from the EDSP will facilitate the development of more targeted and effective AGE prevention and/or intervention policies and programs that will help mitigate the impact and burden of infectious AGE/GI disease, not only in U.S. military recruits and operational forces but in the general population as well.

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CHARACTERISTICS ASSOCIATED WITH RUBELLA INFECTION AMONG CHILDREN IN THE DEMOCRATIC REPUBLIC OF CONGO

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Although the burden of rubella is unknown in sub-saharan Africa, there are approximately 110,000 congenital rubella cases occurring yearly worldwide. Rubella is vaccine preventable disease with the primary objective to prevent congenital rubella syndrome in infants. In the Democratic Republic of Congo (DRC), the rubella vaccine has not yet been introduced; therefore we assessed characteristics associated rubella infection among vaccine-naïve children 9-36 months of age in DRC. We used the Case-Based measles surveillance system with laboratory confirmation of disease to assess risk factors for rubella infection among children with suspected measles from January 1, 2010 to December 31, 2012. Children testing negative for measles were subsequently tested for rubella IgM antibodies. Rubella is present throughout the country with variable prevalence rates. We used Maniema as the reference for comparing odds of disease acquisition in the other 10 provinces. All of the other provinces had a greater chance for observing a case, Kinshasa (9.97 times), Bas-Congo (7.75 times), South- Kivu (4.94 times), Bandundu (4.27 times), Kasai-occidental (3.64 times), North-Kivu (3.62 times), Oriental Province (3.59 times), Equateur (3.51 times), Katanga (2.91 times) and Kasai-Oriental (2.44 times). Given that little else is known about the prevalence of Rubella in the DRC, surveillance data suggest that the virus is circulating in all 11 provinces in children. However, we continue to have no information about the prevalence of CRS. If DRC is to introduce a rubella vaccine, routine immunization should be strengthened for children, and CRS surveillance should be introduced. A main concern when introducing the rubella vaccination in just children is the coverage may decrease virus circulation, thus sufficiently shifting both the average exposure to rubella and susceptibility from children to older age groups including women of childbearing age, and therefore may increase the prevalence of CRS.

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERISTICS OF HEALTH CARE-ASSOCIATED RESPIRATORY SYNCYTIAL VIRUS INFECTIONS IN KENYA

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Human respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infection in infants and young children, with highest burden in low-income countries. RSV is an important cause of nosocomial respiratory infections; however data on hospital-acquired RSV in Africa are limited. We sought to describe the epidemiologic and molecular characteristics of health care-associated RSV infections in 3 urban hospitals in Kenya (2 in Nairobi and 1 in Kisumu). From September 2009 to September 2012 we collected nasopharyngeal and oropharyngeal samples from patients with hospital-onset fever (≥ 38°C) after≥ 3 days admission), plus cough or sore throat. RSV testing and subgrouping (RSV A or B) were done using real time polymerase chain reaction (RT-PCR). Where subgroup was identified, sequencing and phylogenetic analysis of G glycoprotein was performed. Among 41 RSV-positive cases, the median age was 11.4 months [IQR 7.2-24] and 63% were male. Thirty seven (90%) out of the total (41) had fever onset > 6 days after admission. Eight cases (19%) died. Among 16 (39%) samples successfully sequenced, 9 (56%) were RSV A and 7 (44%) were RSV B. RSV A strains diversified into two main clades, AN1 and ON1, with 8 isolates belonging to the NA1 genotype and 1 isolate belonging to ON1. All the sequences in group B belonged to the BA1 genotype. We observed 3 RSV A and 2 RSV B cases clustered by time (same admission date), places (same ward) and molecular characteristics suggesting possible case-to-case transmission. Others had same date of onset but were of different genotypes suggesting different sources of infection. Our data suggest that nosocomial transmission of RSV is occurring among urban hospitals in Kenya, posing a threat to hospitalized children. Further research is needed to identify sources of infection, which may include health care workers, visitors and other patients. Strengthening infection control measures could prevent nosocomial spread of RSV.

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MOBILE SUITCASE LABORATORY FOR RAPID DETECTION OF EBOLA VIRUS AT LOW RESOURCE SETTINGS

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The current outbreak of Ebola has killed over 10,000 people in Guinea, Sierra Leone and Liberia. Early identification and isolation of the infected Ebola cases are the most important control measures. Detection of the Ebola virus by using the rapid antigen lateral flow tests is an easy to be applied at the point-of-care. Nevertheless, the results must be confirmed by additional laboratory tests. Laboratory diagnosis mainly depends on Ebola RNA detection by reverse transcription real-time polymerase chain reaction (RT-PCR), which is available in central laboratories and has a turnaround time of more than three hours. For decentralized low resource settings, there is a need for a simple molecular point-of-need test. In this study, a mobile suitcase laboratory (62x49x30 cm) containing all reagents and equipment for the detection of Ebola RNA was developed. Moreover, it was operated by a solar power battery. All reagents were cold-chain independent in order to ease the use at poor resource settings. RNA extraction was performed by a magnetic bead based method, in which a simple fast lysis protocol was applied. In one reaction tube, the reverse transcription step as well as the DNA amplification and detection by the recombinase polymerase amplification (RPA) assay was achieved. Using spiked plasma samples, down to 15 Ebola RNA copies were detected in less than 30 minutes, while samples containing Crimean-Congo-Hemorrhagic-Fever, Yellow Fever, Lassa, Marburg, Rift Valley Fever, Dengue, Chikungunya and Zika viruses and ***Plasmodium falciparum*** were negative. In conclusion, the mobile suitcase laboratory is ideal for rapid sensitive and specific detection of Ebola virus especially at low resource settings. Currently, two mobile suitcase laboratories are being used in Guinea.

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CONTACT PATTERNS DRIVING EBOLA TRANSMISSION IN WEST AFRICA

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Over 22,000 confirmed, probable and suspected cases of Ebola have been reported in West Africa as of early 2015. Cases are asked if they have had exposure to sick individuals (reported contacts) in a funeral or non-funeral context prior to becoming ill. Exposures are reported by nearly a third of cases, here we analyse these data. The proportion of cases reporting a funeral exposure has decreased over time in some districts. We found a strong positive correlation between this proportion in a district for a given month and the within-district transmission intensity, quantified by the estimated reproduction number. We also found a strong positive correlation between within-district transmission intensity and the district's proportion of hospitalised cases admitted within <=3 days of onset of symptoms. There was no correlation between these two proportions, suggesting that both reduced funeral attendances and faster hospitalisation have independently influenced the intensity of local transmission. We were able to identify 16% of named contacts in the line-list of case report data collated by WHO. Linking cases to the contacts who potentially infected them provided partial information on the Ebola transmission network, which revealed a high to extreme degree of super-spreading. Multivariable regression models allowed us to identify predictors of being named as a non-funeral contact: severe symptoms, death, non-hospitalisation and older age; or a funeral contact: non-hospitalisation, older age and travelling prior to onset. Non-funeral exposures are strongly peaked around the death of the contact, with a high proportion of exposures occurring after hospitalisation. We found that non Ebola Treatment Units (ETUs) have been worse than ETUs at preventing exposure from hospitalised and deceased. Achieving elimination will require sustained safe funerals and fast hospitalisation. Continued real-time data capture, reporting and analysis is vital to track transmission patterns, inform resource deployment, and thus hasten elimination of the virus from the human population.

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A DESCRIPTIVE AND QUANTITATIVE ANALYSIS OF POTENTIAL UNDERESTIMATION OF HUMAN MONKEYPOX CASES IN THE PASSIVE SURVEILLANCE SYSTEM IN THE DEMOCRATIC REPUBLIC OF CONGO

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A major objective of disease surveillance systems is to provide critical information on burden of disease that will inform prevention and control policy and focus of public health efforts. In limited resource settings, such as the Democratic Republic of Congo (DRC), reported diseases might only represent a fraction of the total cases. We present a novel computational method using a scenario tree to model gaps in DRC's disease reporting system, using monkeypox (MPX) as an example. Case counts of MPX reported from January 1st, 2013 to December 31st, 2013 to the Direction of Disease Control in the Ministry of Health (MoH) were used. Parameters of health care utilization and disease reporting at all levels (from when a person becomes ill to when the information is received at the national level) were identified and quantified using health utilization surveys, personal interviews and expert opinion. In 2013, a total of 2,460 cases of suspected human MPX virus were reported to the Integrated Disease Surveillance and Response (IDSR) unit, in the simulated model, the actual number of cases could be 5.0 to 15.2 (mean 9.4) times higher than what was observed in the IDSR at the national level. According to our estimates the true number of suspected cases would have ranged from 12,380 to 37,600 (average=23,280) suggesting a far more severe picture of human MPX in DRC than currently thought. Our model found that most of the cases are lost during the health care ascertainment phase, in that 60% of the total cases may not seek any treatment. Additionally, many cases may be missed if they visited a private facility or were unable to pay for services at their public facility, and further cases were lost once reported to the health zone as there are no sanctions if weekly reports are not sent in. Improved involvement of community workers for disease surveillance, integration of private facilities into the reporting system, and identifying health zones which consistently do not send in reports could lead to a major increase in the numbers of cases reported to the national level and an improvement in surveillance accuracy.

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FIGHTING AGAINST ANTIMALARIAL RESISTANCE IN EARLY DRUG DISCOVERY

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Current antimalarial therapy is built upon only a few differentiated chemotypes and clinical resistance is emerging continuously threatening

the efficacy of antimalarial treatments. Antimalarial mode of action is intimately linked to the appearance of resistance in the field. Historically those antimalarials that inhibit defined molecular targets (e.g. antifolates or mitochondrial Cytb inhibitors) have selected for resistance much more rapidly than those drugs exhibiting complex modes of actions (e.g. endoperoxides or chloroquine).Recent studies have demonstrated that antimalarials with complex mode of actions display a fast killing profile when parasite viability is monitored after drug treatment. However compounds like atovaquone, pyrimethamine or DHODH inhibitors display a much slower rate of killing surely because they are affecting single targets inside of the parasite. As rate of killing profile is linked to the specific antimalarial mode of action it could be a relevant parameter to anticipate propensity to develop resistance by emerging antimalarials. Although robust technologies to determine killing rates exist in the field we have develop a novel medium-throughput assay that allows robust identification of fast acting compounds. This assay has been used to study a set of ca. 50 compounds that are being used as tool compounds to select for ***in vitro*** resistance in a target identification consortium supported by Bill & Melinda Gates Foundation. Preliminary results point out a much higher success for resistant selection with compounds displaying a slow or moderate killing rates vs those with a fast mode of action. These results suggest that antimalarial speed of action could be used as surrogate of the propensity to select for resistance.

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EVALUATING THE PREVALENCE OF DRUG RESISTANCE IN INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA DURING PREGNANCY

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Due to the poor patient compliance with prophylaxis and increasing resistance of parasite strains to chloroquine, administration of intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine/pyrimethamine is now recommended for all pregnant women living in areas with stable malaria transmission. However, resistance to sulfadoxine/pyrimethamine is on the increase which risks the drug being compromised. Thus, an urgent need exists to assess alternative drug regimens for IPTp. Numerous molecular epidemiologic studies showed that resistance to pyrimethamine is associated with the acquisition of mutations in ***Plasmodium*** spp. dihydrofolate reductase (dhfr) genes while resistance to sulfadoxine is associated with 3 mutations in dihydropteroate synthase (dhps gene). Each mutation leads to a decrease in sensitivity to pyrimethamine (dhfr gene) and sulfadoxine (dhps gene). On a systematic review, results indicated that 2 doses of IPTp with sulfadoxine/pyrimethamine retained activity to reduce placental malaria and low birthweight amongst pregnant that visited the clinic. About >72% of the pregnant women that visited the clinic benefited with 2 doses of IPTp in the proportional reduction of peripheral parasitaemia at delivery compared with that at enrolment while the rate of resistance was at <30%; and the proportion of placental infection was reduced by 75% compared with the efficacy of chloroquine prophylaxis administered the previous year. An alternative approach involves systematic detection of placental infection at delivery by using blood smear, rapid diagnostic test, or PCR with placental blood. Conversely, placental infection prevalence may change with time because of changes in sulfadoxine/pyrimethamine efficacy (likely to decrease) and quality of IPTp implementation (likely to increase). Such an approach would also provide baseline data to assess efficacy of all preventive measures against pregnancy-associated malaria, including IPTp and use of insecticide-impregnated bed nets, and will enable assessment of these effects in a specific population.

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INVESTIGATING CANDIDATE MOLECULAR MARKERS OF PIPERAQUINE RESISTANCE IN A NORTHERN CAMBODIAN COHORT WITH A HIGH RATE OF DIHYDROARTEMISININ-PIPERAQUINE TREATMENT FAILURE

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Dihydroartemsinin-piperaquine (DP), one of the last remaining effective drugs for multidrug-resistant ***Plasmodium falciparum,*** has been the first-line artemisinin combination therapy (ACT) in Cambodia since 2012. However, alarming rates of DP failure accompanied by a rise in piperaquine IC_{50}s have already been reported. We are investigating whether DP failures in the region are associated with sequence or copy number variation in candidate molecular markers of piperaquine resistance, including ***pfmdr1, pfcrt***, ***pfmrp2,*** and the X5r domain. 107 isolates from a DP trial with a 42-day PCR-corrected recrudescence rate of 54% are being analyzed. Preliminary analysis shows near fixation of the 184F mutant (98%) and wild type alleles only at codon 86 in ***pfmdr1***. Increased ***pfmdr1*** copy number (CN) is present in 15% of isolates and appears to be associated with greater ***in vitro*** susceptibility to piperaquine, though the association did not reach significance (median PPQ IC50 21.8nM vs. 30.7nM in samples with CN>1 and CN≤1, respectively, p=0.09). None of the nine patients with ***pfmdr1*** CN>2 developed recrudescence. A duplication at the X5r locus on chromosome 5 has been observed in drug-pressured parasites that acquired piperaquine resistance. Using digital droplet PCR, we found no increase in CN at four genes within the X5r domain. Analysis of codon 350 of ***pfcrt*** and a microindel in ***pfmrp2*** is ongoing. A clinically validated molecular marker of piperaquine resistance is urgently needed to determine the role of the partner drug in DP failures. Genome-wide association studies may be necessary to inform molecular surveillance worldwide.

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THE PROTECTIVE EFFICACY OF SEASONAL MALARIA CHEMOPREVENTION IN AN AREA OF EXTENDED SEASONAL TRANSMISSION IN THE ASHANTI REGION OF GHANA: AN INDIVIDUALLY RANDOMIZED TRIAL

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Seasonal malaria chemoprevention (SMC) is recommended by the World Health Organisation for the control of malaria in young children in areas of highly seasonal transmission in the Sahel and sub-Sahel. However, there is a large burden of malaria in areas with a longer transmission season that could potentially benefit from SMC with modified delivery schedules. We undertook an individually randomised, placebo-controlled trial of seasonal malaria chemoprevention in the Ashanti Region of Ghana, where the malaria transmission season peaks over a period of five to six months. Children under five years of age received SMC or placebo on five occasions during the rainy season. SMC was delivered from a central point in each community for the first three rounds, and from a central point in combination with community health workers for the last two rounds. Community health workers managed cases of malaria with artemisinin-based combination therapies, using rapid diagnostic tests to confirm infection prior to treatment. Relative to the placebo group, children who received SMC were less likely to carry malaria parasites after the rainy

season (19.9% vs. 12.4%, prevalence ratio 0.62 (95% CI: 0.49, 0.80); p<0.001). However, the distribution of haemoglobin and prevalence of anaemia was very similar between the two groups. During the rainy season, the incidence of malaria was reduced by around 40% in children in the SMC group, and by around 60% in children who received all five rounds of SMC. However, there was still an important burden of malaria before and after the period when SMC was administered. Our results suggest that SMC may be a valuable malaria control tool for areas with a longer transmission season, but further optimisation of SMC schedules may be needed to maximise impact. Achieving high coverage is crucial to the success of SMC programmes, and it appears that delivery through community health workers will be better able to achieve this aim.

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CONFIRMED *PLASMODIUM VIVAX* RESISTANCE TO CHLOROQUINE IN CENTRAL VIETNAM

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Plasmodium vivax resistance to chloroquine (PvCQR) is currently reported in almost all vivax endemic countries. In Vietnam, little evidence has been published so far on ***P. vivax*** susceptibility to CQ, and only one study in the early 2000s reported PvCQR in the Southern central region. Despite the paucity of published data, the national malaria control program is closely monitoring antimalarial drug resistance since 1995, and all 28-day ***in vivo*** studies carried out since 2003 on PvCQR in several sentinel sites reported late parasitological failures between 0-5.7%. Between May 2009 and December 2011, a 2-year cohort study was conducted in Central Vietnam to assess the recommended radical cure regimen based on a 10-day course Primaquine (0.5mg/kg/day) together with 3 days CQ (25mg/kg). We hereby report the results of the first 28-day follow-up estimating the cumulative risk of P.vivax recurrences together with the corresponding CQ blood concentrations among other endpoints. Out of 260 recruited P.vivax patients, 240 completed treatment and were followed up to day 28 according to the WHO guidelines. Eight patients (3.45%) had a ***P. vivax*** recurrent infection, at day 14 (n=2), day 21 (n=1) and day 28 (n=5). Chloroquine blood concentrations, available in 3/8 recurrent infections (day 14,21,28) were above the minimal inhibitory concentration (>100ng/ml whole blood) in all of them. Fever and parasitaemia (both sexual and asexual stages) were cleared by day 3. Anemia was common at day 0 (35.8%) especially in children below 10 (50%) and hemoglobin (Hb) recovery at day 28 was significant among anemic patients (median change d28-d0 =+1.7g/dl; IQR[+0.7; +3.2]). This report confirms for the first time ***P. vivax*** CQ resistance in Central Vietnam, and calls for further studies using standardized protocols for accurately monitoring the extend and evolution of PvCQR in Vietnam. These results, together with the mounting evidence of artemisinin resistance in Central Vietnam, further highlight the increasing threat of antimalarial drug resistance on malaria elimination in Vietnam.

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DIHYDROARTEMISININ-PIPERAQUINE VS ARTESUNATE-AMODIAQUINE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN GHANAIAN PREGNANT WOMEN: A NON-INFERIORITY, SAFETY AND EFFICACY TRIAL

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Dihydroartemisinin-piperaquine (DHA-PPQ) has potential for use as treatment for uncomplicated falciparum malaria in pregnancy. However, there is a paucity of safety data on the use of this drug combination in pregnancy and only two studies have reported its use in pregnancy so far. We assessed the safety and efficacy of DHA-PPQ in 417 second and third trimester pregnant women with uncomplicated malaria and compared these outcomes to those seen with artesunate -amodiaquine (ASAQ) in a non-inferiority trial using a margin of 5%. Pregnant women who tested positive for ***Plasmodium falciparum*** infection using both a rapid diagnostic test and microscopy were recruited, individually randomized to DHA-PPQ or ASAQ, and followed up actively on days 1, 2, 3, 7, 14, 28 and 42, at delivery and 6 weeks post-partum. During this period, assessment of adverse events, sampling of blood for haematological and parasitological assessments and data collection on neonatal morbidity and mortality were performed. Uncorrected parasitological efficacy by day 42 for DHA-PPQ was 89.0% (95%CI: 83.6, 93.0) and for ASAQ it was 86.5% (95%CI: 80.6, 91.2) in the per protocol population and 87.4% (95%CI: 81.9, 91.8) and 86.7% (95%CI: 80.8, 91.3) respectively in the modified intention-to-treat analysis. DHA-PPQ was non-inferior to ASAQ at both days 28 and 42 in both sets of analyses. There was no evidence of hepatic/ renal toxicity or white blood cell dyscrasia in either study arm. DHA-PPQ was better tolerated than ASAQ; vomiting (19.5% vs 29.4%; p=0.02), dizziness (14.5% vs 26.6%; p=0.003) and anorexia (12.0% vs 22.3%; p=0.007). No harms were associated with DHA-PPQ in the second and third trimesters and though the PCR-corrected estimates were not reported, the study is expected to contribute significantly to the evidence that will guide a policy decision on the use of DHA-PPQ use in pregnancy.

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UNDERSTANDING THE HISTORICAL SPREAD OF MOLECULAR MARKERS OF SULPHADOXINE-PYRIMETHAMINE RESISTANCE IN AFRICA

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The dispersal of ***Plasmodium falciparum*** strains resistant to sulphadoxine-pyrimethamine (SP) occurred at a time when molecular analysis techniques were sufficiently developed to map the spread of molecular markers of resistance for the first time. Understanding what influenced SP resistance in the past could help inform predictions on the possible spread of resistance to artemisinin and its partner drugs, and lead to better resistance containment strategies. We are developing a mathematical transmission model of the temporal spread of drug resistance to SP, incorporating rates of SP treatment, transmission intensity, asymptomatic infections, multiple clone infections, human immunity, and fitness costs of resistance. We fitted our model using Bayesian Markov Chain Monte Carlo methods to 274 prevalence data points of the dhps540E mutation in 35 locations in Africa which had multiple measures over time. We incorporated local data from 93 household surveys on use of SP and data on transmission intensity from the Malaria Atlas Project. The rate of increase in dhps540E frequency varied considerably between locations. SP intake was very high in some areas; we estimate that the number of SP treatments in under fives was more than 5-fold higher than

the number of clinical malaria episodes in parts of East Africa during the 2000's. There were no clear univariate statistical associations between the rate of increase in dhps540E frequency and SP use or transmission intensity. However, initial modelling results are able to reproduce the dhps540E prevalence patterns in most sites by taking into account multiple factors simultaneously, including SP use, transmission intensity, an estimated time of introduction of the mutation and pre-existing partial resistance to SP due to other mutations in dhfr and dhps. In a few locations, the reported SP use was too low to support the observed dhps540E prevalence, indicating there are further factors not currently captured by the model or the data, possibly including use of other sulpha drugs. Validating a mathematical model against historical data helps increase its applicability to modelling drug resistance in the field.

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LACK OF ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* IN UGANDA BASED ON PARASITOLOGICAL AND MOLECULAR ASSAYS

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Artemisinin-based combination therapies (ACTs) are standard treatment for falciparum malaria. The efficacies of these regimens are threatened by resistance to the artemisinin component, exemplified by delayed clearance of parasitemia after therapy, in southeast Asia. The resistance phenotype has recently been associated with two parasite features. First, in freshly cultured isolates, resistant parasites demonstrated increased parasitemias, compared to those in sensitive parasites, 66 h after a 6 h pulse with dihydroartemisinin (DHA). Second, polymorphisms in propeller domains of the recently described ***Plasmodium falciparum*** kelch (K13; PF3D7_1343700) gene were associated with resistance. However, delayed parasite clearance after therapy with ACTs has not been clearly documented in Africa. To better characterize resistance markers in Africa, we studied ***ex vivo*** susceptibility to DHA and examined K13 propeller polymorphisms in ***P. falciparum*** isolates collected in Kampala, Uganda from May-July, 2014. By standard ***in vitro*** assays all studied isolates were highly sensitive to DHA (IC_{50} 0.4-2.7 nM). Sensitivities to chloroquine varied widely (IC_{50} 9.8-1060 nM), with sensitivities consistent with genotypes at the ***pfcrt*** K76T allele. Using the ring-stage survival assay, after a 6 h, 700 nM DHA pulse, parasitemia was undetectable in 40/43 cultures at 72 h; most cultures followed beyond 72 h demonstrated parasites after 2-4 weeks. Two of 53 isolates had non-synonymous K13 propeller polymorphisms, but not the mutations associated with artemisinin resistance in Asia. In summary, consistent with clinical trial results from Africa, we did not observe parasitological or molecular evidence of artemisinin resistance in recent ***P. falciparum*** isolates from Uganda.

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SURVEILLANCE OF TRAVELERS: PREVALENCE OF ANTIMALARIAL DRUG RESISTANCE MARKERS AMONG IMPORTED *PLASMODIUM FALCIPARUM* CASES DIAGNOSED IN THE UNITED STATES, 2013

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CDC conducts surveillance to detect molecular markers associated with drug resistance among ***Plasmodium falciparum*** parasites imported into the US. These data may inform prevention and treatment guidelines for US travelers visiting malaria endemic countries. Blood samples from persons with malaria diagnosed in the US were submitted to CDC for molecular characterization. ***P. falciparum***-positive samples were genotyped for genetic polymorphisms associated with resistance to chloroquine (***pfcrt***), sulfadoxine (***pfdhps***), pyrimethamine (***pfdhfr***), mefloquine (***pfmdr1*** and copy number), atovaquone (***pfcyt b***) and artemisinin (***K13- propeller***) using Sanger sequencing and real-time PCR methods. The genotype data were connected with epidemiologic data. In 2013, CDC received a total of 136 samples from 29 states for malaria reference diagnosis and genetic characterization of resistance markers. CDC corrected/determined the species for 87 (64%) samples. ***P. falciparum*** was identified in 101 (74%) samples (including 2 mixed infections), ***P. vivax*** in 14 (10%), ***P. ovale*** in 14 (10%), and ***P. malariae*** in 6 (4%). Travel history was reported for 106 cases (78%); 96 (71%) traveled to Africa. Only 22 cases had reported some malaria chemoprophylaxis use. From 57 cases with information on treatment, 23 (40%) received atovaquone/proguanil, 20 (35%) doxycycline, and 5 (9%) chloroquine, including other combination therapies. Among the ***P. falciparum***-positive cases, 89 (88%) had genetic polymorphisms associated with resistance to pyrimethamine, 74 (73%) to sulfadoxine and 53 (52%) to chloroquine. No pre-treatment samples had ***pfcyt b***, ***K13-propeller*** mutations or multiple ***pfmdr1*** copies associated with resistance to atovaquone, artemisinin and mefloquine, respectively. These findings highlight the problem of antimalarial drug resistance and the importance of active surveillance for drug resistance among imported malaria cases in the US.

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IMPACT OF DRUG RESISTANCE MEDIATING *PLASMODIUM FALCIPARUM* POLYMORPHISMS ON CLINICAL PRESENTATIONS OF PARASITEMIC CHILDREN IN UGANDA

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Plasmodium falciparum genetic polymorphisms that mediate altered sensitivity to antimalarials may impact upon parasite virulence. In a recent cross-sectional study of parasitemic Ugandan children, isolates with mutant sequences at ***pfcrt*** K76T, ***pfmdr1*** N86Y, or ***pfmdr1*** D1246Y had one-fourth the odds of association with symptomatic malaria compared to wild type (WT) parasites. However, these results may have been confounded by selection of WT parasites by recent use of artemether/lumefantrine for prior episodes of malaria. To further explore these associations, we evaluated polymorphisms in samples from paired episodes of asymptomatic and symptomatic parasitemia in 114 cohort subjects aged 4-11 years from Tororo District, with the two episodes occurring within 3-12 months, and no prior treatment for malaria within

60 days. The N86Y mutant genotype was less commonly found with symptomatic (4/110, 3.6%) than asymptomatic (13/111, 11.7%; p = 0.028, Fisher's exact test) infection, compared to mixed or wild type genotypes; significant associations were not seen for the K76T or D1246Y alleles. Considering paired episodes in the same subject, thus controlling for host factors, the odds of symptomatic malaria were lower for mutant compared to WT or mixed sequence at N86Y (OR 0.23, 95% CI 0.04-0.84, p = 0.021, McNemar's exact test), but not the other alleles. However, symptomatic episodes (which have higher densities) were more likely than asymptomatic to be mixed (for N86Y OR 2.0, 95% CI 1.04-4.0, p = 0.036), likely explaining the higher prevalence of WT/mixed infections in symptomatic children. Excluding mixed infections, prevalences of WT and mutant alleles did not differ between symptomatic and asymptomatic episodes. Thus, associations seen between WT/mixed ***pfmdr1*** N86Y genotype and symptomatic disease were likely explained by confounding due to greater parasite densities in symptomatic children, arguing against an impact of ***pfmdr1*** and ***pfcrt*** genotypes on clinical outcomes, and highlighting the value of cohort studies for best assessing associations between parasite factors and clinical outcomes.

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RESPONSIVENESS OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* INFECTIONS IN ANEMIC AND NON-ANEMIC NIGERIAN CHILDREN TO ARTEMISININ-BASED COMBINATION TREATMENTS: EVIDENCE FOR HEMATOCRIT CONSERVATION IN ANEMIC CHILDREN

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The efficacy, hematocrit conservation and anemia recovery times were evaluated in 909 non-anemic and 437 anemic malarious Nigerian children <15 years old during a 7-year period of adoption of artesunate-amodiaquine and artemether-lumefantrine as first line treatments. Overall, fever and parasite clearance were significantly faster following treatment with AA (P = 0.002, P < 0.0001, respectively). Fever but not parasite clearance, and parasite reduction ratio 1 day after treatment began were significantly faster (P = 0.031) and higher (P < 0.001), respectively, in non-anemic compared to anemic children. Polymerase chain reaction-corrected parasitological cure rate on day 28 was similar in non-anemic and anemic children (96.1% (95%CI 91.2 - 101.1) versus 97.5% (95%CI 92.4 - 102.6)); P = 0.30) and were similar for both treatment groups. Fall in hematocrit/1000 asexual parasites cleared from peripheral blood were significantly lower in anemic compared to non-anemic children (P = 0.038) suggesting much hematocrit conservation in anemic children. Delayed anemia occurred in 2% of the children. Mean anemia recovery time was 15 days (95%CI 13.3 - 17.4) and it did not correlate with FIH/1000 asexual parasites cleared from peripheral blood (r = 0.004, P = 0.61). Kinetics of the deficit in hematocrit from 30% was estimated by a non-compartmental model. Declines in the deficit were monoexponential with a mean elimination half-time of 1.4 days (95% CI = 1.2-1.6). Anemia half-time correlated significantly positively with anemia recovery time in the same patients (r = 0.69, P < 0.0001). Bland-Altman analysis of 10 multiples of anemia half-time and anemia recovery time showed narrow limit of agreement with insignificant biases (P = 0.07). Artesunate-amodiaquine and artemether-lumefantrine are efficacious treatments of uncomplicated falciparum infections in anemic and non-anemic children and they may conserve hematocrit and hasten recovery from malaria-associated anemia.

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CROSS-SECTIONAL SURVEILLANCE FOR DRUG RESISTANCE-MEDIATING *PLASMODIUM FALCIPARUM* POLYMORPHISMS IN UGANDA

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Antimalarial drug resistance, mediated in part by known ***Plasmodium falciparum*** genetic polymorphisms, is of great concern in Uganda. Of interest is whether resistance determinants vary across the country and whether, with replacement of chloroquine/sulfadoxine-pyrimethamine (SP) by artemether/lumefantrine (AL) as the national treatment regimen, beginning in 2006, resistance determinants are changing. We analyzed samples from cross-sectional surveys in children 5-15 years of age in 3 districts with wide variation in malaria transmission (Tororo, Jinja, and Kanungu) in 2012 and 2013. The prevalence of transporter polymorphisms was similar at the 3 sites, and the prevalence of WT alleles increased at each site. Overall, WT/mixed sequences increased between 2012 and 2013 for ***pfcrt*** K76T (7.5% to 17.5%, p<0.0001), ***pfmdr1*** N86Y (75.5% to 89.1%, p<0.0001), and ***pfmdr1*** D1246Y (80.9% to 91.7%, p<0.0001). WT prevalences were much greater than those measured at multiple sites in Uganda in prior years. The changes are all consistent with the selective pressure of increasing use of AL in the country. For antifolates, in the partial results now available the prevalence of 5 mutations (***pfdhfr*** N51I, C59R, S108N; ***pfdhps*** A437G, K540E) that have been common since initial studies over a decade ago remained high. Only in Kanungu, two additional mutations that predict a greater level of resistance to SP, and which previously were rare in Uganda (mixed/mutant ***pfdhfr*** I164L 15%, ***pfdhps*** A581G 47%), were seen. Taken together, our results demonstrate significant changes in the prevalence of transporter polymorphisms with increasing use of AL to treat malaria, persistent prevalence of 5 common antifolate mutations despite decreased use of SP to treat malaria, and the presence of additional antifolate mutations that predict high level resistance in Kanungu. Another cross-sectional survey is now underway, and results for 2015 will be compared with those from prior years. Considering changing use of antimalarials and recent evidence for changing relative drug efficacies in Uganda, continued surveillance for drug resistance markers is an important priority.

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RISK OF CARDIOTOXICITY WITH REPEATED DOSING OF DIHYDROARTEMISININ-PIPERAQUINE FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN UGANDAN SCHOOLCHILDREN

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Intermittent preventive treatment (IPT) for malaria in schoolchildren offers a promising option for malaria control. Although studies suggest that repeated dosing of dihydroartemisinin-piperaquine (DP) for IPT is safe and effective, questions about the potential risk for cardiotoxicity remain, particularly when DP is administered with food. We conducted a cardiac monitoring and pharmacokinetic (PK) study alongside a cluster-randomized trial to evaluate monthly IPT of schoolchildren with DP in Jinja, Uganda. A sub-set of intervention participants was recruited using

convenience sampling. ECGs were performed at baseline (prior to DP), and on Day 1 (before DP), and Day 3 (4-5 hours after DP) for each IPT round. Information on food intake and recent medications was also collected. In reading ECGs, the duration of the QT interval was corrected using Fridericia's formula. In the final IPT round, piperaquine levels were measured on Days 1 (trough) and 3 (peak), from whole capillary blood samples collected by fingerprick into heparinized microtubes. In 2014, 197 children from 8 schools were screened, and 191 children were enrolled; 6 were excluded due to an abnormal baseline ECG (n=5), or lack of consent (n=1). The mean age of participants was 9.95 years (range 5 to 19 years), and 86 girls (45%) were included. A total of 1701 ECGs were performed. A QTcF interval of >450ms was detected in 31 (2%) ECGs, including 18 grade 1 (450-464ms), 8 grade 2 (465-479ms), and 5 grade 3 (>480ms) abnormalities; all were asymptomatic. Of those children with a QTcF >464ms, 12 (92%) were male, with a mean weight of 34.4 kg; 6 (46%) reported taking food within a 6-hour window around the DP dose, while 7 (54%) took DP while fasting. A total of 156 (82%) participants in the cardiac monitoring sub-study were enrolled into the PK study; blood samples were obtained from all participants, and analysis of the samples is ongoing. Full results, including the association between food intake, piperaquine drug levels, and QTcF prolongation, will be presented and their implications on the safety of DP for chemoprevention will be discussed.

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CAROTENOID BIOSYNTHESIS INHIBITION IN THE ASEXUAL INTRAERYTHROCYTIC STAGES OF *PLASMODIUM FALCIPARUM* BY FLURIDON

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Malaria remains a major health concern despite extensive efforts spanning over a century to combat the disease. ***Plasmodium falciparum***, like other Apicomplexans have retained a relict plastid known as the apicoplast and this organelle is only present in the parasite and not the human host. The isoprenoid biosynthetic pathway has been annotated to this organelle and the pathway results in the synthesis of isoprene units which can be channelled into carotenoid synthesis. This pathway could be a good target for the chemotherapeutic treatment of malaria. ***P. falciparum*** 3D7 clone were cultured as described by Trager and Jensen. The parasites were initially synchronised at the ring stages by treatment with 5% D-sorbitol and maintained in culture until they developed into trophozoites or schizonts. The various stages were then treated with different concentrations of fluridon (0, 120 and 150μM) and incubated for different time intervals (0, 12, 24, 48 and 60hrs). Parasite population was monitored by microscopy after the incubation and IC50 determined. The fluridon treated cultures had a decrease in parasite population as compared to the control and this was observed in all the asexual stages. The decrease in population was more evident as incubation time increased. The effective fluridon concentration was observed to be at 150μM. There was also a significant (95% CI, p=0.023) delay in the parasite life cycle from the rings through to the schizonts stage. The fluridon seem to inhibit the carotenoid biosynthetic pathway in these stages which suggests that the pathway is essential for parasite survival. The reduction in parasite population was observed in all the asexual intraerythrocytic stages of the parasite. This suggests a stage-specific dependence on carotenoid synthesis and this is the first time it has been demonstrated. The effect of the drug was profound on the ring stages suggesting that they require carotenoids for their development. The carotenoid biosynthesis pathway is inhibited by fluridon in all the asexual intraerythrocytic stages of ***P. falciparum***. This opens up new opportunities for developing chemotherapeutic agents for the treatment of malaria.

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EVALUATION OF *PLASMODIUM FALCIPARUM* ARTEMISININ RESISTANCE IN WESTERN THAILAND AS PART OF A DOD MULTI-CENTER TRIAL

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Artemisinin-resistant ***Plasmodium falciparum*** threatens the effectiveness of all artemisinin-based combination therapies. A multi-center artesunate-mefloquine (A+M) efficacy trial is on-going in three US DoD laboratories in Peru, Kenya, and Thailand, to compare parasite clearance rates at 72 hours after artesunate initiation. Participants receive 4 mg/kg artesunate at 0, 24, and 48 h, 15 mg/kg mefloquine at 72 h, and, at 84-96 h, 10 mg/kg mefloquine plus 0.5 mg/kg primaquine for transmission blocking. To calculate parasite clearance half-life ($PC_{1/2}$), parasite density is assessed by microscopy every 4h for the first 12h after first artesunate dose and eve-ry 6h for 72h or until two consecutive negative smears. Efficacy outcomes over 42 days are also assessed. Between Oct 31, 2013, and Jan 7, 2015, we enrolled 45 of 59 planned patients in Sangkhlaburi district near the Thai-Myanmar border. We found that AS-MQ combination ther-apy as administered was still highly efficacious in this region, with 100% adequate clinical and parasitological response (ACPR) at 42 days. However, the median $PC_{1/2}$ of 4.9 hours is approaching that used to define artemisinin resistance (5 hours). Further, 15% of patients had PC1/2 >5 hours, and 20% remained parasitemic at 72 hours (Day 3), thus meeting the WHO definition for suspected artemisinin resistance. Evidence of resistance based on ***in vitro*** drug-sensitivity testing and molecular resistance markers is also being evaluated. While our data suggest that clinical effectiveness has yet to be compromised, ongoing surveillance in this area is needed.

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A MULTI-STAGE PRECLINICAL CANDIDATE FOR THE POTENTIAL TREATMENT OF MALARIA

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Malaria is a devastating parasitic disease causing widespread mortality and morbidity across many parts of the developing world. Many medicines for the treatment of malaria are failing due to the increasing development of resistance and new therapies for both treatment and prevention of this deadly disease across all of its life cycle stages are urgently needed. In the search for new antimalarials, a collaborative project between the University of Dundee and Medicines For Malaria Venture (MMV) was initiated with the high throughput phenotypic screening (HTS) of an in-house library of protein kinase scaffolds. Initial screening identified multiple structurally diverse chemical series that blocked asexual blood stage parasite viability and served as a catalyst for a new drug discovery programme. Using a focussed medicinal chemistry approach involving drug design, chemical synthesis, biological testing and rigorous compound profiling, we identified a highly efficacious compound with potent activity against multiple life cycle stages. Efforts reported herein reveal a small molecule inhibitor of ***Plasmodium falciparum*** malaria with good pharmacokinetic properties and excellent possibilities for chemoprotection, single dose blood stage treatment and transmission blocking.

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THE EFFECTIVENESS OF CHLOROQUINE AND PRIMAQUINE FOR THE TREATMENT OF VIVAX MALARIA; A STUDY IN A TERTIARY CARE HOSPITAL IN THAILAND

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Malaria is an infectious disease that continues to be a major public health problem, and occurs throughout the tropical regions of the world. Chloroquine in combination with primaquine is the first-line treatment for ***Plasmodium vivax*** malaria in Thailand. In view of the declining efficacy of chloroquine in many ***P. vivax*** endemic areas, the possibility of emergence of chloroquine-resistant ***P. vivax*** in Thailand is a concern. The aim of this study was to assess the trends in therapeutic efficacy of chloroquine and primaquine for the treatment of uncomplicated ***P. vivax*** malaria. A retrospective review of medical records of patients admitted and treated for uncomplicated ***P. vivax*** malaria, during 2004 - 2013, was conducted at the Bangkok Hospital for Tropical Diseases (BHTD), Faculty of Tropical Medicine, Mahidol University, Thailand. A total of 335 uncomplicated ***P. vivax*** malaria patients were enrolled into the study, 362 patients achieved an adequate 28-day clinical and parasitological response (ACPR). 3 cases were classified as early treatment failure. The minimum-maximum of median parasite clearance time and median fever clearance time over the 10 year period was 46-59 hours and 14-28 hours respectively. There was no significant increasing trend of parasite clearance time over this time period. We concluded that treatment of ***P. vivax*** infection with a combination of chloroquine and primaquine has remained efficacious in Thailand. Increasing time-trends in parasite clearance times may be useful as an early warning mechanism for emergence of chloroquine-resistant ***P. vivax*** strains.

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SULFADOXINE-PYRIMETHAMINE AVAILABILITY AND (MIS) USE IN SUB-SAHARAN AFRICA: ANTIMALARIAL MARKET DATA FROM EIGHT COUNTRIES

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As evidence of reduced chloroquine efficacy against ***Plasmodium falciparum*** mounted in the 1990's, sulfadoxine-pyrimethamine (SP) became first-line malaria treatment in many endemic countries in sub-Saharan Africa (SSA). Between 2002-2005, countries in SSA adopted artemesinin combination therapies (ACT) as first-line treatments. SP is still recommended by the WHO and used across SSA for intermittent preventive treatment of malaria during pregnancy (IPTp). As such, availability and use of SP should currently be limited to distribution for IPTp at antenatal care (ANC) clinics. However, multi-country data collected by ACTwatch show widespread availability and use of SP outside of health facilities. Multiple malaria medicine outlet surveys were conducted between 2009-2014 in Benin, the DRC, Kenya, Madagascar, Nigeria, Tanzania, Uganda and Zambia. A census of outlets with potential to distribute antimalarials was conducted among a representative sample of administrative units. A drug audit documented product information, reported retail price and amount distributed to consumers in the past week for all antimalarials in stock. Across project countries, the vast majority of antimalarial-stocking private sector outlets including pharmacies, drug shops, and retailers were stocking SP. Conversely, SP availability was typically lower among public health facilities as compared with these private sector outlets. In some contexts, SP availability among public health facilities declined in recent years. SP is commonly distributed to patients by private sector outlets and accounts for one-quarter to one-half of antimalarials distributed. In some cases, SP market share has actually increased in recent years including in Madagascar, where SP distribution increased with the decrease in distribution of chloroquine. We examine trends over time and across 8 project countries regarding where SP is found, the origins of available SP (manufacturer and country of manufacture), and private sector price of SP compared with first-line ACT treatment. Implications for policies and strategies to ensure SP use is restricted to IPTp delivery will be discussed.

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PARASITE CLEARANCE AND EFFICACY OF ARTEMISININ-BASED COMBINATION FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN BOBO-DIOULASSO, BURKINA FASO

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Artemisinin-based combination treatments (ACTs) main feature is their ability to accelerate the clearance of ***Plasmodium*** ring stage infected erythrocytes. Recent emergence of Artemisinin-resistance in Cambodia may represent a major threat to the global effort to malaria control and elimination. Artemisinin-resistance polymorphisms have been associated with delayed parasite clearance following artemisinin combination therapy. We investigated the association between parasite clearance and ACTs efficacies in 200 uncomplicated malaria patients randomized. We sampled per-protocol 42 days follow-up data from patients of a Phase IIIb/IV clinical trial to assess the safety and efficacy of repeated administration of pyronaridine-artesunate, artemether-lumefantrine and dihydroartemisinin-piperaquine in patients with acute uncomplicated ***Plasmodium falciparum*** Malaria conducted in Bobo-Dioulasso. Parasitaemia at Hour 0, 12, 24, 36, 48, 60 and 72 counts were shared on WWARN Parasite Clearance Estimator online tool according to WWARN terms of use to generate parasite clearance half-life. Therapeutic efficacy has been evaluated according to the presence or not of asexual forms of malaria parasites on a blood smear during follow-up. The geometric mean of parasite clearance half-life is significantly lower in recovered patients group, 2.68 h (95% CI 2.28-3.08) versus 2.90 h (95% CI 2.45-3.35), p-value=0.035. Overall, in parasitological treatment failure group, there was a significant correlation between the failure day and the parasite clearance; the slope half-life decreases significantly with the timeout of treatment failure. Very Late treatment failure would be more due to recrudescent than new infections. Parasite clearance is a good tool for the evaluation of artemisinin-based combination efficacy for the treatment of uncomplicated ***P. falciparum*** malaria. The raising of the parasite clearance half-life, calculated with the three-day parasitemies after treatment, can determinate parasitological treatment failure during follow-up.

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SYSTEMATIC SAMPLING APPROACH REVEALS FEWER FALSIFIED FIRST LINE ANTIMALARIALS THAN PREVIOUSLY REPORTED

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Malaria is a curable disease provided patients have timely access to efficacious drugs, namely artemisinin based-combination therapies (ACTs), recommended as first line treatment by the World Health Organisation (WHO). The threat posed by falsified and substandard drugs is drawing increasing international attention, heightened by reports indicating that up to 35% (796 of 2,296) of antimalarial drugs purchased following the convenience approach from 21 Sub-Saharan African countries failed

content analysis. To investigate the threat we purchased over 10,000 ACTs using three sampling approaches (convenience, mystery client and overt) and updated sampling frames, to provide comprehensive surveillance of ACTs available in a given geographical region. The ACTs were collected in 6 countries - Cambodia, Ghana (Kintampo), Equatorial Guinea (Bioko Island), Nigeria (Enugu metropolis and Ilorin city), Rwanda and Tanzania (nationwide). Content analyses using mass spectrometry (qualitative) and high performance liquid chromatography with photo-diode array detection (quantitative) were used to measure the amount of active pharmaceutical ingredients (APIs) in 3 independent laboratories. Results were expressed as percentage of APIs stated on the packaging and used to categorise each sample as quality assured, substandard, degraded, or falsified. Our findings were reassuring in that out of the 10,092 samples (142 brands) we only found falsified formulations that did not contain the stated APIs in 2 countries: Nigeria (both Enugu state [1%] and Ilorin city [0.8%]) and Equatorial Guinea (Bioko Island [7.3%]). In contrast, although substandard drugs were found in all 6 countries, this did not exceed 7% of the samples analysed from Africa. The results were disseminated to the country-specific Ministry of Health, as well as the stated manufacturers and WHO. Data will be presented to illustrate that a representative sampling approach is essential, and that both mystery client and overt sampling approaches can be used, to accurately quantify and track the scale of ineffective drugs which jeopardise treatment of a life threatening disease.

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IN VIVO AND EX VIVO EFFICACY OF ARTEMETHER-LUMEFANTRINE ON *PLASMODIUM FALCIPARUM* ISOLATES IN MALI

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In Mali, previous studies showed high frequency of recurrent parasitemia after treatment with artemether-lumefantrine. We hypothesized that, recurrent parasites after treatment have a reduced sensitivity to artemether-lumefantrine. We conducted a longitudinal study on 641 volunteers recruited in Sotuba and Kolle, Mali. In-vivo sensitivity of ***Plasmodium falciparum*** to artemether-lumefantrine was measure as per WHO protocols. Polymerase chain reaction was used for molecular correction and to assess Pfcrt K76T and Pfmdr1 N86Y allele polymorphism. K13 propeller genotypes were assessed by sequencing. Ex-vivo field isolates sensitivities to artemether and lumefantrine were assessed using the hypoxanthine isotopic test. The 28-day uncorrected Adequate Clinical and Parasitological Response (ACPR) cure rates were statistically different between Sotuba and Kolle, respectively 96.31% (313 of 325 patients; 95% CI, 94.25 - 98.36) and 78.16% (247 of 316 patients; 95% CI, 73.59 - 82.73), $p < 0.001$. PCR corrected ACPR rates were comparable between the two study sites 98.77% (321 of 325 patients; 95 % CI, 97.4 - 99.9) and 97.47% (308 of 316 patients; 95 % CI, 95.73 - 99.2) in Sotuba and Kolle respectively ($p = 0.181$). The pfmdr1 86N allele was selected after treatment with artemether-lumefantrine (71% versus 91%, $p = 0.0012$) but not pfcrt 76T (76.8% versus 75.2%, $p = 0.09$). Fifty (50) ***P. falciparum*** isolates from 641 volunteers were successfully cultured ex-vivo. All of the isolates tested ex-vivo were sensitive to artemether and lumefantrine. Means values of IC_{50} for artemether were 1.8 nM in pre-treatment isolates versus 3.8 nM in post treatment isolates ($p = 0.001$) and 1.9 nM versus 6.9 nM ($p = 0.014$) for lumefantrine Field ***P. falciparum*** isolates were sensitive in-vivo and ex-vivo to artemether and lumefantrine but recurrent parasites had significantly higher IC_{50} for both components of AL.

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K13 MUTATIONS IN *PLASMODIUM FALCIPARUM* PERSISTING AFTER ACT TREATMENT OF KENYAN CHILDREN

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Studies in the greater Mekong sub-region reported a strong relationship between polymorphisms at the propeller domain of the Kelch 13 (K13) protein encoded by the pfk13 locus and delayed parasite clearance after artemisinin treatment. In Africa, ***Plasmodium falciparum*** remain susceptible and combination therapy regimens which include an artemisinin component display good efficacy. In 2009, we observed using parasite detection by qPCR that sub-microscopic persistence of ***P. falciparum*** occurred in about one third of children treated with artemisinin combination therapy (ACT) in western Kenya, and these children were much more likely to develop recrudescent patent parasitaemia 4 or 6 weeks after treatment. Mutation in the pfk13 locus was rare in the parasite population captured by this study, and there was no evidence variants of pfk13 contribute to the persistence phenotype we observed. A similar study was carried out in the same site in 2013 in order to monitor any change in parasite clearance time and in the emergence of any mutations at pfk13 and other key genes. The parasite clearance phenotype as well as the prevalence of mutations in pfk13, pfmdr1, pfcrt1 and pfap2μ will be presented. Any association between sub-microscopic parasites on day 3 after ACT treatment and mutations on those genes will be evaluated and results presented.

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DURATION OF ARTEMISININ COMBINATION THERAPY INFLUENCES PARASITOLOGICAL OUTCOME IN A MOUSE MODEL OF MALARIA

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The clinically active derivatives of artemisinin reduce the total parasite burden more rapidly than other antimalarials. The short plasma half-life of the artemisinin derivatives and the dosing duration (number of days of treatment) of artemisinin combination therapy (ACT) result in most of the artemisinin being cleared during a single 48 hour asexual cycle of ***Plasmodium falciparum***. Our laboratory hypothesizes that the ACT regimen duration plays a significant role in clinical outcome, keeping total dose of ACT constant. To better understand the role of treatment duration on clinical outcome, we have adapted a novel murine model of malaria, which utilizes a luciferase-reporter ***P. berghei*** at high parasitemia to study the rate of parasite killing ***in vivo***. Unlike other drug efficacy assays which assess cytostatic activity, our assay more accurately mimics high parasitemia infections and cytocidal drug activity in the field. Using this model, we have determined the effect of two different artesunate duration regimens on parasite clearance and survival. Unlike ***P. falciparum***, which has a 48-hour asexual cycle***, P. berghei*** has a 24-hour asexual cycle in the mouse. Three doses of 50 mg/kg artesunate were administered to BALB/cJ mice with 6-10% parasitemia at two different duration regimens: 3 doses at 0, 8, and 20 hours, and 3 doses at 0, 24, and 48 hours. We have found that extending single-drug artesunate treatment from 1 asexual life cycle to over 3 asexual life cycles significantly prolongs time of absent parasitemia and results in a slower parasite multiplication rate for recrudescent parasites. In combination with quinoline partner drugs, the longer dose duration of artesunate effects cure measured at 30 days, whereas recrudescence is observed in combination with the shorter dose duration. Also, pyronaridine was shown to have a single dose cure with one tenth of the human equivalent dose. Lengthening dose duration to span three lifecycles makes existing artemisinin treatment more effective.

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IMPACT OF INTEGRATING THE DELIVERY OF SEASONAL MALARIA CHEMOPREVENTION WITH NUTRITION SUPPLEMENTATION IN NORTHERN NIGERIA ON HEALTH OUTCOMES: A PRAGMATIC INTERVENTION TRIAL

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In 2012 WHO recommended Seasonal Malaria Chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine (SP-AQ) to prevent childhood malaria in the Sahel sub-region. As malnourished children are twice as likely to die from malaria, there is potential for improving malaria and nutrition outcomes by combining the delivery of SP-AQ with Lipid-based Nutritional Supplements (LNS). From August to November 2014, SP-AQ was delivered monthly door-to-door to children under five years old by community volunteers in seven wards of Madobi, Kano State, northern Nigeria. In three of the wards, children 6-24 months old were also provided LNS. About half of the 4000 children 6-24 months old who received SMC also received LNS. Cross-sectional baseline and endline household surveys conducted in August and November 2014 collected data on demographics, anthropometric measurements, coverage, recent fever history, and adherence to interventions among children aged 6-24 months at baseline. We measured the impact of adding LNS on coverage estimates, recent fever incidence, and nutrition outcomes using multivariable logistic regression models. SMC coverage was 88% (95% CI 87-89%, n=1594) with no observed difference between the two arms; LNS coverage was estimated at 83% (95% CI 81-84%). Reported adherence to the 3-day course of SP-AQ was 82% across both arms, but adherence to LNS measured by consumption the day before was 57%. At baseline 78% of 1700 children were stunted, 54% were underweight and 15% were wasted. Adjusting for demographic factors and baseline, children who received four rounds of SMC and LNS had similar nutrition outcomes to children who received SMC only in preliminary analyses. Adding LNS to SMC resulted in a significant drop in fever incidence between August and November compared with SMC alone (p=0.02). Co-packaging SMC and nutritional supplements is operationally efficient, but no benefit in terms of coverage or nutrition outcomes was observed. Further study should explore subgroups and whether four rounds of LNS with observed adherence is sufficient to address the high prevalence of malnutrition observed in this population.

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EMERGING OF MUTATIONS IN K13 PROPELLER GENE OF *PLASMODIUM FALCIPARUM* ISOLATES FROM DAKAR, SENEGAL IN 2013-2014

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Malaria resistance to most antimalarial drugs has developed in Southeast Asia and has spread to Africa. Since 2005, the World Health Organization recommended artemisin-based combination therapy (ACT) as first-line treatment in malaria. In Senegal since 2006, ACT is recommended by the Senegalese National Malaria Control Programme as first-line treatment for uncomplicated malaria. The emergence of ***Plasmodium falciparum*** resistance to artemisinin and its derivatives, manifested as delayed parasite clearance following the treatment with artesunate monotherapy or ACT, has recently developed in Southeast Asia. Recently, mutations in the propeller domain of the Kelch 13 (K13) gene (PF3D71343700) were associated with ***in vivo*** and ***in vitro*** resistance to artemisinin in Southeast Asia. Four mutations, Y493H, R539T, I543T and C580Y, were recently correlated to ***in vivo*** and ***in vitro*** artemisinin resistance in Southeast Asia. While no mutation was found in ***Plasmodium falciparum*** isolates from Dakar, in 2012-2013, three mutations, N554H, Q613H and V637I, were identified among 92 isolates (12%) in 2013-2014. Five polymorphisms were identified in the ***Plasmodium***/Apicomplexa-specific domain (K123R, N137S, N142NN/NNN, T149S and K189T/N). The mutations associated with ***in vitro*** resistance in Southeast Asia such as Y493H, R539T, I543T and C580Y were not observed, nor than the M476I mutation obtained ***in vitro*** on a Tanzanian strain. Further studies are needed to better characterize the role of these mutations in artemisinin resistance.

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EVALUATION OF K13-PROPELLER POLYMORPHISMS IN *PLASMODIUM FALCIPARUM* PARASITES FROM NIGERIA

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Drug resistance is indeed a raging 'monster' in the battle against malaria. As a result, artemisinin combination therapies (ACTs) have been globally adopted for antimalarial chemotherapy. ACT is known to be very potent but recent reports have observed in the form of slow parasite clearance in Southeast Asia that these drugs may not be as effective as in the recent past. As part of the measures to curb antimalarial drug resistance, the World Health Organisation (WHO) recommended continuous molecular surveillance of resistance markers to these drugs in order to detect early warning signs. In Nigeria, artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) have been adopted as preferred ACT options. Until recently, there was no candidate marker for artemisinin resistance rather, candidate markers for their combination partners were used to evaluate efficacy. ***Plasmodium falciparum*** chloroquine resistance transporter (pfcrt) and ***P. falciparum*** multidrug resistance gene 1 (pfmdr1) are associated with decreased sensitivity to amodiaquine and lumefantrine, but effects of these polymorphisms on therapeutic responses to artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) have not been clearly defined. Recently, the ***P. falciparum*** K13-propeller was identified as a key determinant of delayed parasitological clearance after artemisinin treatment, and single nucleotide polymorphisms (SNPs) in K13 were associated with ***in vitro*** and ***in vivo*** resistance in Asia. As part of molecular surveillance of resistance markers to ACTs, we investigated by direct sequencing, the mutations in K13-propeller region in 150 ***P. falciparum*** positive blood spots from Nigerian children treated with ACTs in a clinical trial between 2007-2008. The results will serve as preliminary data on the interplay between recurrent parasitaemia and polymorphism in the ***P. falciparum*** K13-propeller gene in Nigeria.

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EVALUATION OF THE CAPACITY OF SENEGAL'S MALARIA DIAGNOSTIC ALGORITHM TO IDENTIFY PATIENTS WITH MALARIA PARASITEMIA

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Appropriate case management of malaria depends on access to an accurate diagnostic test. Malaria rapid diagnostic tests (RDTs) enable point-of-care testing nearly as sensitive and specific as reference microscopy and permit accurate diagnosis at every healthcare level. The Senegal National Malaria Control Program introduced RDTs in 2007, along with a diagnostic algorithm stipulating that if a febrile patient of any age has symptoms indicative of a febrile illness other than malaria (e.g., cough or rash), they would not initially be tested for malaria, but treated for the apparent illness and receive an RDT if they return in 48 hours without improvement. We conducted a year-long study in 16 epidemiologically representative health posts to determine the algorithm's capacity to identify cases of confirmed malaria. Health post personnel enrolled patients of all ages with fever (>37.5 C) or history of fever in the past 2 days. After clinical assessment, the nurse determined whether the patient would have received an RDT according to the diagnostic algorithm, but performed an RDT for all enrolled patients. Over one year, 6039 patients were enrolled; 47% were under 5 years, and 58% required an RDT according to the algorithm. Overall, 23% had a positive RDT, 34% during rainy season and 9% during dry season. The algorithm identified 78% of patients with a positive RDT, but this varied according to transmission season (rainy 80%, dry 70%), epidemiologic strata (high transmission 75%, low transmission 95%), and age group (under five years 68%, five years and older 84%). In all but the lowest transmission zone, use of the algorithm results in an unacceptably large proportion of patients with malaria who do not receive timely diagnosis and treatment. While the algorithm was adopted within a context of malaria control, with the goal of treating patients with symptomatic malaria, Senegal has now adopted a goal of malaria elimination. In the context of malaria elimination, the paradigm of case management needs to shift toward the identification and treatment of all patients with malaria parasitemia, and case management policy has been changed to reflect this.

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EVIDENCE OF NON-FALCIPARUM MALARIA IN KEDOUGOU, SENEGAL

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Expanded efforts to control malaria in Senegal have resulted in increased used of rapid diagnostic tests to identify the primary disease-causing ***Plasmodium*** species, ***P. falciparum***. However, these tests do not detect other malaria-causing species such as ***P. ovale*** and ***P. malariae*** that have previously been reported in Senegal. Current studies on species circulating in Senegal are lacking; and, assessing the population prevalence of malaria using appropriate diagnostic methods is essential for assessing the need for health care services. Based on this, we developed real-time polymerase chain reaction speciation assays and analyzed 475 HRP2-based rapid diagnostic tests collected between 2013 and 2014, from a single site in southern Senegal, Kedougou, a hyper endemic region with malaria prevalence greater than 10%,. We observed ***P. malariae*** (n=3) and ***P. ovale wallikeri*** (n=2) as co-infections with ***P. falciparum*** among patients with positive rapid diagnostic tests (n=187 positive tests), including one patient positive for all three species. Among 288 negative tests, both ***P. ovale curtisi*** (n=3) and ***P. ovale wallikeri*** (n=1) were identified, as well as ***P. malariae*** (n=1). Furthermore, approximately 10% of the negative samples had detectable levels of ***P. falciparum*** by real-time PCR. These findings suggest that non-falciparum malaria is of concern in this country, particularly in light of reduced incidence of ***P. falciparum*** nationwide. Use of a specific rapid diagnostic test does not necessarily provide an accurate view of malaria transmission in Kedougou, Senegal; and, more sensitive and specific methods are required.

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DIFFERENTIAL RESPONSE OF TOTAL IGG LEVELS IN SERUM FROM *PLASMODIUM FALCIPARUM* INFECTED INDIVIDUALS IN THE AMAZON REGION AGAINST PROTEINS EXPRESSED IN A BACULOVIRUS EXPRESSION SYSTEM

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Erythrocyte invasion by the merozoite is a multiple steps process that involves specific interactions between erythrocyte receptors and parasites ligands. Therefore proteins related to invasion are important vaccine candidates and/or targets for diagnostic tool development. A recent study carried out in Peru assessed the protective immune response in plasma of Asymptomatic vs. Symptomatic ***Plasmodium falciparum*** infected individuals, using Protein microarrays against 850 asexual recombinant proteins derived from ***P. falciparum*** strain 3D7. The results of this study indicated a higher immune responses for asymptomatic individuals in comparison with the symptomatic ones against 52 proteins, including invasion proteins EBA-175 and RH2b; MSP-10 protein was immunoreactive for both groups.The aim of this study was to evaluate the total IgG levels in 30 serum samples from ***P. falciparum*** infected individuals (symptomatic and/or asymptomatic) against proteins PfRH2b, EBA-175 and MSP-10 by ELISA. The proteins were expressed in a baculovirus-recombinant protein system using Sf9 insect cells. All serum samples confronted against proteins PfRH2B, EBA-175 and MSP-10, showed a differential total IgG response against these recombinant proteins individually. These preliminary results demonstrated that recombinant ***P. falciparum*** invasion proteins synthesized by the baculovirus expression system were well recognized by patient serums, suggesting that this methodology could be a useful tool for the synthesis of ***P. falciparum*** proteins to be used as vaccine candidate proteins and/or as new targets for the development of Rapid Diagnostic Test.

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EXPRESSION OF *PLASMODIUM FALCIPARUM* - RECOMBINANT PROTEINS (RH2B, EBA-175 AND MSP-10) USING BACULOVIRUS SYSTEM IN SF9 INSECT CELLS

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Malaria caused by ***Plasmodium falciparum*** kills over 1 million people a year. A recent study carried out in Peru indicated a higher immune responses for asymptomatic individuals in comparison with the symptomatic ones against 52 proteins by Protein Arrays, including invasion proteins EBA-175 and RH2b; MSP-10 protein was immunoreactive for both groups. The aim of the present study was to standardize a baculovirus-recombinant protein system expression in Sf9 insect cell. We designed specific primers for the pfrh2b, pfeba175 and pfmsp10 genes. DNA products were cloned in order to be expressed using the Bac-to-Bac

system (Life TechnologiesTM). The expressed proteins were his-tag purified using iMAC system (BioRad). In order to evaluate the immunoreactivity of these proteins ten positive sera from symptomatic patients positive to ***P. falciparum*** by microscopy were used. Eight out of ten were positive for PfRH2B, four out of ten were positive for PfEBA-175 and all ten were positive for PfMSP10 recombinant protein. These preliminary results demonstrated that the recombinant proteins obtained using baculovirus system were recognized by patient serum, however more serological evaluation are necessary for further validation. This protein expression system is a useful tool to produce recombinant proteins that can be used as candidates to develop rapid diagnostic test to detect ***P. falciparum***, since the current rapid diagnostic test, mainly base on pfHRP2 are no longer useful for the Amazon region where the parasites circulating in this area do not have this gene.

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ANTIGEN DISCOVERY OF NOVEL SEROLOGICAL MARKERS OF RECENT *PLASMODIUM VIVAX* INFECTION

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As countries near elimination, the identification of sporadic pockets of ongoing malaria transmission becomes a challenge in the growing number of low prevalence areas, especially for ***Plasmodium vivax***. In addition, ***P. vivax*** elimination will be difficult without being able to identify and target asymptomatic carriers of ***P. vivax*** hypnozoites. Current tools focus either on the diagnosis of ***P. vivax*** bloodstage infections, limited in duration and number, or on the measure of past cumulative exposure, using long-lasting serological markers, none of which are suited to the identification of transmission pockets or hypnozoite carriers at risk of relapse. To close this gap, we have conducted a large-scale antigen discovery screen to identify new serological markers able to specifically detect recent (0 to 9 months old) ***P. vivax*** infection. Two large libraries of ***P. vivax*** antigens, collectively representing 1,600 individual proteins and more than 2,600 protein fragments, were deployed for high throughput screening of serum samples using an AlphaScreen™ liquid array system (307 wheat germ cell-free protein fragments) or custom-made protein microarrays (2,320 E. coli cell-free protein fragments). The immunoreactivity profiles of 31 children (5 to 10 years old) from Papua New Guinea and 60 Thai and Brazilian patients >7yrs. of age following radical cure of PCR-confirmed ***P. vivax*** infection were investigated for candidate antigens displaying an ideal profile for serological markers: either significant seroreversions or >4-fold drops in antibody concentration from 0 to 9 months after radical cure. This study proves the principle of serological markers of recent exposure to ***P. vivax*** infections. The resulting panels of antigens will be investigated for their performance in detecting concurrent and recent infections using plasma samples from longitudinal studies in 4 different ***P. vivax*** endemic settings.

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DEVELOPMENT AND OPTIMIZATION OF A NEW QUANTITATIVE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY ASSAY FOR THE SAFE TREATMENT OF *PLASMODIUM VIVAX* HYPNOZOITES

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A major challenge to achieving successful global elimination of malaria is the treatment of the ***Plasmodium vivax*** hypnozoite reservoir. Primaquine, an 8-aminoquinoline compound, has been available for many years and has proven efficacy in killing hypnozoites. Its use however, can induce haemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. This X-linked disease is the most common human enzymatic deficiency; prevalence of up to 30% has been observed in some malaria endemic areas. Fears of G6PD deficiency and drug-induced haemolysis have greatly decreased the uptake of primaquine therapy in ***P. vivax*** endemic countries, resulting in high burden ***P. vivax*** as a consequence. This risk of haemolysis can be dramatically reduced with G6PD testing prior to treatment or identifying homogeneous populations with low risk of G6PD deficiency. Each of the current G6PD testing methods have significant limitations either in their ability to be technically implemented or in their affordability in resource-poor settings. Consequently, there is a need for an accurate, robust, and inexpensive G6PD test that can be deployed in resource-poor settings. We describe the optimisation and validation of a new quantitative G6PD test with an integrated quantitative haemoglobin (Hb) test. This new combined test showed strong agreement with the current gold-standard tests but can be performed with much less sophisticated equipment at a fraction of the cost. Field evaluations of this new G6PD test are currently being conducted.

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EVALUATION OF GENEDIA® MALARIA P.F/PAN AG RAPID TEST RELATIVE TO MICROSCOPY IN A MALARIA ENDEMIC AREA ETHIOPIA

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Early and accurate diagnosis of malaria followed by prompt treatment reduces morbidity and mortality in endemic regions. Presumptive treatment of malaria is widely practiced where microscopy or rapid diagnostic tests are not readily available. Introduction of rapid diagnostic tests (RDTs) for the treatment of malaria in many low-resource settings need evaluation of their performance. This study evaluated the performance of GENEDIA® Malaria P.f/pan Ag Rapid Test in malaria endemic area Ethiopia. This study ws undertaken to evaluate the diagnostic performance of GENEDIA® Malaria P.f/pan Ag Rapid Test relative to microscopy for the diagnosis of ***Plasmodium falciparum*** and ***P. vivax*** malaria in Ethiopia. In this cross-sectional study from November to December 2013, patients who had malaria symptoms and visited malaria control center in Adama, Oromia Region were recruited. Thin and thick blood smears were prepared from finger prick and stained by 10% Giemsa. Microscopic examination was done under 100x magnifications for ***Plasmodium*** species identification and determination of parasitaemia. The RDT was performed as per the manufacturers instructions. A total of 417 febrile patients were diagnosed, of which 149 were microscopy positive for Pf (n=47) , Pv (n=93) and P.f and P.v or mixed(n=9) The sensitivity, specificity, positive and negative predictive value of GENEDIA® Malaria P.f/pan Ag rapid test was 95.3%, 96.6%, `94.0%, 97.4 % respectively. In conclusion, the diagnostic performance of GENEDIA® Malaria P.f/pan Ag

rapid test has sensitivity, specificity, positive and negative predictive value of 95.3%, 96.6%, `94.0%, 97.4% respectively with respect to malaria microscopy in this study.

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A SYSTEMATIZED REPRESENTATIVE SELECTION QUALITY CONTROL PROTOCOL FOR THE QUANTITATIVE ASSESSMENT AND IMPROVEMENT OF MALARIA MICROSCOPY IN TANZANIAN MILITARY HEALTH FACILITIES

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Malaria microscopy is the gold standard methodology for confirmation of malaria parasites in human blood. It enables accurate detection, quantification and identification of species to support treatment and patient management, as well as research efforts to understand malaria prevalence. The accuracy and reliability of results are critically dependent upon the quality of blood film preparation, staining procedure, microscopist's expertise and reading time. Studies have demonstrated poor malaria microscopy performance in Africa, including Tanzania. The Walter Reed Army Institute of Research (WRAIR) and Tanzania Peoples Defence Forces (TPDF) have established a Quality Monitoring and Improvement Program of malaria diagnostics at select TPDF camps. A key objective is to perform crosschecking of malaria slides prepared in current and future research sites. Malaria microscopy QC programs generally focus exclusively on reading accuracy. In contrast, the WRAIR/TPDF protocol includes quantitative assessment of preparation, staining and reading. All blood films prepared for routine testing are stored and monthly QC samples are selected in situ from the laboratory registers and crosschecked by the Walter Reed Malaria Program on a quarterly basis. 10 negative and all positive slides for each month are selected using a random representative sampling protocol. Representative sampling ensures that the QC slides are selected (in a random manner) from the start, middle and end of the working day, and from various days distributed across the month. The quality of blood films are quantitatively assessed macroscopically for blood film preparation quality, and microscopically for staining quality and reading accuracy. Over a 4 month period, the mean smear and staining quality were 74.3% (53.6-86.7) and 72.2% (50-86, n=251) respectively, mean sensitivity was 38.7% (33-45, n=160), and specificity was 82% (70-100, n=25).Results shows that the improvement or drop in quality of performance over months can be depicted and monitored using a systematized random sampling protocol with an objective selection of smears prepared in different times.

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QPCR DETECTION OF SUB-MICROSCOPIC *PLASMODIUM FALCIPARUM* PARASITEMIA AFTER ARTEMISININ COMBINATION THERAPY IN MALI

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Parasite clearance time (PCT) after artemisinin-based combination therapy (ACT) is increasing in Asian settings. The association between parasite clearance following ACT with increased transmission to mosquitoes and parasite recurrence was demonstrated. The prevalence of sub-microscopic ***Plasmodium falciparum*** parasitemia in Malian people after ACT is less documented. Since October 2011 we have conducted prospective studies of four artemisinin combination therapies in Kollé and Bougoula, Mali. We determined parasite clearance dynamics by duplex quantitative polymerase chain reaction (qPCR) for patients for whom a complete set of day 0, 1, 2, and 3 dried blood spots were available. We obtained 208 and 186 patients for episodes 1 and 2, respectively. Residual parasitemia on day 3 after initiation of treatment was observed during episode 1 in 22% and 27% at Kollé and Bougoula respectively. For episode 2, residual parasitaemia was detected in 10% and 30% of patients respectively for Kollé and Bougoula. Our results indicate that sub-microscopic parasitaemia persists in 10 - 30% of patients at day 3 post-ACT treatment in Mali. The implications of these observations for ACT efficacy will be discussed.

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ULTRASENSITIVE MOLECULAR SCREENING FOR *PLASMODIUM VIVAX* INFECTION IN PREDOMINANTLY DUFFY NEGATIVE POPULATION IN MALI, WEST AFRICA

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The preponderance of Duffy-blood-group negative populations in the Sahel has led to the assumption of very low prevalence or no ***Plasmodium vivax*** malaria infection in this region based on the resistance of Duffy negative people to ***P. vivax*** infection. This reasoning has long been widely accepted, and may have resulted in a diagnostic bias and mis-diagnosis of ***P. vivax*** in West Africa by microscopy. Molecular tools enable us to detect species-specific infection including submicroscopic low-density parasitemia of ***P. vivax***. Molecular screening may offer an efficient tool for investigating ***P. vivax*** infection to inform malaria elimination policies in Africa. Blood samples were collected from 400 children aged 0-14 years during quarterly scheduled visits in prospective cohort studies from 2009 to 2012 in Bandiagara, Mali. An ultrasensitive Pf_Pv-multiplex qPCR using Roche LightCycler 480 was carried out using DNA extracted from filter paper samples. ***P. vivax*** positive results were repeated three times for confirmation. Our preliminary data have detected four cases of vivax infection in 2009. One patient among the four positives was chronically infected by ***P. vivax*** during consecutive quarterly visits. These results suggest that ***P. vivax*** may be more common than previously believed in this setting. Sensitive molecular tools have the potential to provide important information for planning malaria elimination strategies in the Sahel.

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EVALUATION OF NEXT GENERATION INFECTION DETECTION TESTS FOR MALARIA ELIMINATION AND ERADICATION

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Over the last decade, increased funding for malaria control has resulted in the adoption and scale-up of interventions, leading to a worldwide decrease in mortality cases. However, it has recently become evident that in both low and high transmission settings, there is a large proportion of individuals who harbor low density infections that are not detected by microscopy or rapid diagnostic tests (RDTs). Other diagnostic tools such as PCR, ELISA, and RT-PCR lack affordability, ease of use, rapid time to results, and portability, all of which are essential for large scale use in resource-limited settings. As a result, infected individuals that are undetectable with current tools remain a reservoir of infection and contribute to continued malaria transmission within a population. Clearly, there is a need for more accurate tests. The Infection Detection Test (IDT) Development Initiative seeks to enable access to the most appropriate diagnostic tools that will support regional elimination and global eradication of ***Plasmodium falciparum*** malaria. The IDT Initiative is focused on the development and commercialization of a high quality, low cost, easy to use, and highly sensitive ***P. falciparum*** diagnostic test by 2017. Similar to existing RDTs, the IDT will implement a lateral flow format, and will utilize the HRP2 biomarker because HRP2 persistence in peripheral blood suggests recent infection. Currently, commercial RDTs have a limit of detection (LOD) of 800 pg HRP2/mL. The goal of the IDT Initiative is to produce a new diagnostic tool that has an LOD of 80 pg HRP2/mL, and this work has led to the development of five promising prototypes. Each prototype includes developments or improvements to one or more of the following: alternative reporters, workflow innovations, advanced capture agents, sample preparation, and non-invasive sampling. Using native and recombinant HRP2 proteins, as well as clinical samples, we evaluated the performance of these prototypes. Here, we present the preliminary results of the IDT prototype evaluation and identify the relative advantages of several design approaches.

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ASSESSMENT OF NON-ADHERENCE TO ANTIMALARIAL TEST RESULTS IN PUBLIC AND PRIVATE HEALTH FACILITIES IN NIGERIA

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Current global guidance requires that all suspected malaria cases should be confirmed by microscopy or rapid diagnostic test (RDT) before treatment. The Nigerian National Malaria Elimination Programme is implementing strategies to promote availability of quality assured microscopy and mRDTs in public and private health facilities across the country but there are concerns that the failure of health workers and prescribers to adhere to results of these confirmatory tests undermines the benefits of these interventions. This study was performed to assess the practice and reasons for non-adherence to malaria test results. Non-adherence rate was defined as percentage of cases that tested negative by microscopy or mRDT who received antimalaria treatment despite the test result. Record of microscopy or mRDT test results, and treatments given to the most recent cases of suspected malaria seen in 61 health facilities sampled from all the six geo-political zones of Nigeria were analyzed. The included facilities received regular supplies of quality-assured mRDTs from the National Malaria Elimination Programme or through the implementer of the grant-supported initiative to improve malaria diagnosis and treatment in the private sector. Confirmatory tests (mostly mRDT) were performed in 431 of 610 eligible cases; 169 had no test. Results showed 285 (66.1%) had malaria parasitaemia and 146 (33.9%) had no parasitaemia. Thirty one patients who had negative test results were treated with antimalarial drug (ACT) giving a non adherence rate of 21.2%. Top three reasons given by prescribers for non-adherence were (i) low confidence in accuracy of mRDT, (ii) disregarding test results when illness symptoms are "too obvious"; and (iii) insistence by some patients to be treated despite their negative test results. These findings would be helpful in developing interventions to address non-adherence and draw attention to the need for more in-depth studies of this practice.

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A STUDY OF DIFFERENT METHODS FOR ESTIMATING OF BLOOD MALARIA PARASITE DENSITY

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A retrospective study of malaria parasitaemia estimation by using actual and different assuming number of WBC and RBC counts was conducted at the Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Thailand. Clinical data from 512 patients, who infected either falciparum or vivax malaria and diagnosed of malaria by using thick and thin blood smears, were analyzed. The application of a WHO recommended 8,000 WBCs/μL and assuming RBC number for estimating malaria parasitemia led to overestimation and resulted in low reliability when compared to the actual counts of WBC and RBC. The difference in the geometric mean parasitemia calculated by using actual WBC and RBC counts and compared to parasite densities with assuming number of WBC and RBC counts were significantly lower and higher except for assuming WBC 5900/μL in thick blood film; assuming RBC 4,800,000/μL in thin blood smears in male patients and assuming RBC of 4,300,000/μL in female patients.

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NEGATIVE MALARIA RAPID DIAGNOSTIC TEST (MRDT) CASE MANAGEMENT IN THE PRIVATE RETAIL OUTLETS: RESULTS FROM MYSTERY PATIENT SURVEYS IN NIGERIA AND UGANDA

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Malaria remains endemic in Uganda and Nigeria, with over 80% of the population at risk of disease in both countries. The two countries have adopted the WHO recommendation of universal parasitological confirmation before treatment. The recommendation includes the private sector where 40-60% of febrile patients seek care. However, adherence to case management guidelines following a negative test result remains unclear especially in the private retail outlets offering malaria testing services. Mystery patient surveys, an effective method for assessing provider's behavior, were conducted to understand providers' case management following a negative mRDT result. The surveys were conducted in November 2014 and December 2013 in Nigeria and Uganda respectively, as part of a broader multi-country private sector mRDT project. In both countries, mystery patients were confirmed malaria negative, healthy adults consenting to at most 3 malaria tests over a one week period. They were trained to present at randomly selected private retail outlets known to offer malaria diagnostic services and act as patients, presenting with symptoms likely to indicate suspected malaria thus leading the provider to recommend a malaria test. Their experiences were later recorded through an interviewer administered questionnaire. A total of 131 visits from 63 mystery patients were made in Nigeria and 193 visits from 65 mystery patients in Uganda. In Both countries, the

outlets were mainly drug shops and clinics. In Nigeria, all visits received a test whereas 176/193 (91%) of visits in Uganda received a test. Despite a negative confirmatory result prior to the visit, 53/131(40%) and 62/176 (35%) of cases tested were given a positive test result in Nigeria and Uganda respectively. Among cases testing negative, 26/78(33%) in Nigeria and 27/114(24%) in Uganda were given an anti-malarial, predominantly ACTs. Adherence to non-malaria case management guidelines is still sub optimal. Evidence suggests alteration of negative results. There is need for to further understand the factors driving anti-malarial prescription despite negative results

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BUILDING A SYSTEM OF QUALITY ASSURED MALARIA DIAGNOSTICS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Using a parasitological test for suspected cases of malaria is increasingly becoming the norm in the Democratic Republic of the Congo. However, assuring that tests - both microscopy and malaria rapid diagnostic tests (RDTs) - are done properly and the test results are used appropriately, remains a difficult challenge in the country. To address this, the President's Malaria Initiative (PMI) through the PATH-led MalariaCare partnership has supported the National Malaria Control Program (NMCP) to develop and implement a system of quality- assured malaria diagnostics. This system is designed on continuous improvement principles and includes update training, certification of trainers, on-site supportive supervision linked with regular review and feedback by Quality Assurance (QA) supervisors. MalariaCare has worked with the NMCP to train a cadre of diagnostics experts in microscopy skills and in quality RDT performance. Best performers - from both central and provincial reference laboratories - are then trained as on-site supervisors. They then work with local laboratory and clinical staff at health facility level to perform on-site outreach training and supportive supervision (OTSS) in health facilities in six of the DRC's eleven provinces. The OTSS visits focus on skills observation and on the spot problem solving. The primary goals have been to improve preparation and accuracy of malaria slide reading, assurance of appropriate RDT results, and adherence by clinicians to the test results. Recently a cadre of clinician supervisors has also been trained and joined the laboratory experts in providing regular on-site supervision visits. To assure steady quality improvement in the program the supervisors meet to review outcomes and plan improvements during annual lessons learned workshops. While the largest portion of the facilities are new, a subset with two years of representative data have shown steady improvements in the quality of diagnostic and clinical indicators. Consequently, the QA system is being expanded to include on-site proficiency testing and linkage of community health worker networks to QA program health facilities.

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FEASIBILITY AND ACCEPTABILITY OF INTRODUCING MALARIA RAPID DIAGNOSTIC TESTS (MRDTS) AND PRE-REFERRAL RECTAL ARTESUNATE (RA) INTO COMMUNITY CASE MANAGEMENT IN MCHINJI, MALAWI

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WHO recommends the use of malaria Rapid Diagnostic Tests (mRDTs) for malaria diagnosis at all levels of the health system and use of rectal artesunate (RA) at community level as a pre-referral treatment for severe malaria. We conducted this mixed method study to assess the feasibility, acceptability and impact of integrating mRDTs and RA into community case management on quality of care and utilization of CCM services. During the study, mRDTs/ RA were available in 97% of the village clinics and 96% of mRDTs were correctly performed by community health workers. Cross sectional surveys showed that care seeking from community health workers increased from 36.8% before the intervention to 89% after the intervention whilst the proportion of caregivers that sought care from informal sources of care declined from 16.5% to 1.9%. Prompt access to care (within 24 hours) increased from 42% to 93%. For children that received RA, 79% complied with referral when assessed within 24 hours to 7 days of referral advice. Non compliance to referral increased with distance from the health centre. Both mRDTs and RA were highly accepted by caregivers and community health workers, and reasons given included the provision of diagnostic services and timeliness of treatment at community level. This study has shown that integration of mRDTs and pre-referral rectal artesunate into community case management is both acceptable and feasible. The intervention improves utilization of child health services, malaria diagnosis, treatment and compliance to referral advice among children less than five years of age in hard to reach areas of Malawi.

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ASSESSING ADHERENCE TO THE REVISED CASE MANAGEMENT GUIDELINES FOR THE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESSES IN RURAL GHANA

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The World Health Organization has released revised Integrated Management of Childhood Illnesses(IMCI) case management algorithm that incorporate the use of malaria rapid diagnostic test. Adherence to the revised guidelines is critical to proper diagnosis and management of malaria and non-malaria fevers, including the rational use of antibiotics. As part of an implementation research in the middle-belt of Ghana, we conducted an observation study to assess the extent of adherence to the revised guidelines. Between April and July 2013, we conducted structured observation of clinical practices in the management of under-five children reporting with fever to eighteen (18) health centers in the Brong Ahafo Region of Ghana. Data was collected using a structured checklist and the data was matched against the standard set in the revised WHO guidelines. A total of 4111 observations were carried out among children 3-59 months. Among children diagnosed with malaria and prescribed ACT, only 26% had RDT performed. Only 28% had their ability to eat, drink or breastfeed assessed. History of convulsion or convulsing at presentation was checked for approximately 22%. Just about 18% were assessed for ear problems while only 14.7% got assessed for malnutrition and anemia. 16% of the observed children received better than average IMCI assessment (7 or more of the 11 IMCI task). Adherence to the revised IMCI guidelines is sub-optimal in this area in Ghana. Innovative approaches are needed to increase clinician adherence and ensure appropriate management of under-five febrile illnesses in the era of test-based management of malaria.

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THE USE OF FIONET™ TECHNOLOGY AS A TOOL IN IDENTIFYING THE PROBLEMS WITH RDT QUALITY IN MILITARY HEALTH FACILITIES OF TANZANIA

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Over 93% of the Tanzanian population is at risk for malaria infection. In the past, malaria diagnosis in health facilities below hospital level relied on clinical diagnosis and rarely microscopy. The World Health Organization advocates the use of Rapid Diagnostic Tests (RDTs) where microscopy is not feasible. Despite the promising use of RDTs, quality control (QC) is challenging. While cross-checking malaria slides is a common external QC method, cross-checking RDTs by off-site personnel can be unreliable because RDT result lines begin degrading within hours. Fionet™ technology is a web-based workflow guidance system that addresses RDT quality control issues. The system uses the Deki Reader™ (DR) and standard mobile devices to provide step-by-step guidance for performing RDTs, and to capture and transmit digital records of each test. Web-based oversight of RDT performance is enabled through Fionet™ to provide real time reporting and case management. RDT QC through Fionet™ was implemented at selected Tanzanian military facilities in collaboration with the US Army. Images of RDTs performed at the sites were viewed through the web portal for preparation problems and discordant results between device and human interpretations. Concerns were communicated remotely with health workers at the point of service for improvement. In 2014, 2.9% (960/32655) and 0.1% (26/32655) of all uploaded images were identified to have quality problems related to interpretation of results and RDT preparations respectively. Of the false results, 56% (539/960) were false negative and 44% (421/960) were false positive. False positive interpretations were commonly found in mixed species infections (64.5%; 247/383); false negative interpretations were found more in single species infections (54.5%; 294/539). The main factors leading to errors in RDT interpretations were assumption of presence of imaginary positive line (44.5%; 150/337) and missing weak positive test lines (35.9%; 121/337).

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HIDDEN *PLASMODIUM* AND THE RISK OF TRANSFUSION TRANSMITTED MALARIA IN NON-ENDEMIC AREAS

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Malaria in Brazil is endemic in the Amazon, although autochthonous cases, mostly asymptomatic, have been reported in the Atlantic Forest near the coastal area. Epidemiological investigations in this region revealed carriers that represent a challenge for blood banks since the proportion of submicroscopic infections in such low transmission areas (prevalence by microscopy ≤ 10%) can reach 70 - 80%. Moreover, the pre-donation questionnaire may fail to detect individuals harboring ***Plasmodium*** due to its low parasitemia and absence of symptoms. In order to improve transfusion safety we evaluated molecular and serological tests on a population of risky blood donors. We tested samples from 91 candidates for blood donation from non-endemic areas where asymptomatic infections have been reported. Thick blood smear, qPCR, nested PCR, ELISA with both recombinant ***P. vivax*** MSP119 (ELISA-Pv) and total ***P. falciparum*** extract (ELISA-Pf) and indirect fluorescent assay with P. malariae (IFA-Pm) were used. Two samples (2.2%) (0.6-7.6) were positive by thick blood smear with very low parasitemia. Genus qPCR exhibited amplification in 3.3% (1.1-9.2) and species-specific nested PCR in 2.2% (0.6-7.6%) indicating the presence of P. malariae. ELISA detected 42.8% (33.2- 53.1) of samples reagent for ***P. vivax*** and 6.6% (3.1-13.6) for ***P. falciparum***. IFA-Pm was reagent for P. malariae in 15.4% (9.4-24.2). Among the 40 positive samples by serology, 97.5% were detected by ELISA-Pv, 35.0% by IFA-Pm and 15.0% by ELISA-Pf (Chi^2, p<0.001). Considering parasitological and molecular methods, 4/91 blood donors (4.4%) were probably infectious. Based on serology, 44.0% were exposed to ***Plasmodium***. The high prevalence of antigens and antibodies in this population points out to the risk of transfusional malaria in this area, as asymptomatic donors are currently missed by the clinical-epidemiological screening. We are currently evaluating the power of a few specific questions on donor's proximity to the Atlantic Forest in addition to laboratorial methods to disclose ***Plasmodium*** carriers, aiming to reduce the risk of transfusional malaria in this area.

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COMPARISON OF DIFFERENT DIAGNOSTIC TOOLS FOR THE DETECTION OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS ACROSS GEOGRAPHICALLY DIVERSE AREAS

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Global malaria control has been drastically improved in the recent past, resulting in an almost halved mortality rate and a 26% decrease in infections worldwide since the year 2000. The significant epidemiological shifts that this has led to need to be fully understood to progress the elimination agenda. Foremost is the increasing fraction of malaria transmission due to asymptomatic infections in low endemicity settings, a large part of which is characterized by submicroscopic parasitaemia. Such individuals are extremely difficult to identify for treatment. Indeed, recent data suggest that up to 50% of human-to-mosquito transmission could be caused by such infections in very low endemicity areas. To evaluate the extent and detectability of asymptomatic ***Plasmodium falciparum*** infections, we recently collected large sets of venous whole blood samples from asymptomatic adult volunteers in several areas of low malaria endemicity in Cambodia, Peru and Senegal. Sample sets were constructed to include one fifth asymptomatic ***P. falciparum*** positive specimens and four fifths negative specimens as determined by locally performed molecular detection tests (PCR or loop-mediated isothermal amplification). The ability of reverse transcription quantitative PCR, rapid diagnostic tests (RDTs), and microscopy to correctly identify ***P. falciparum*** infections in our sample sets was measured in reference laboratories and compared to conventional nested PCR. In addition, the ***histidine-rich protein 2*** gene (***pfhrp2***), a key biomarker for parasite detection by RDTs, was sequenced and the whole blood concentration of HRP2 determined for positive specimens by ELISA. By correlating the presence of ***P. falciparum*** infection in asymptomatic individuals with parasitaemia, detectability by multiple techniques of varying sensitivity, and HRP2 expression level,

this study provides unique insights into this significant parasite reservoir. Analysis of this comprehensive data will inform the optimization of strategies to identify asymptomatic but infective carriers of ***P. falciparum***.

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A NEW, ROBUST AND FASTER SINGLE-STEP PCR FOR *PLASMODIUM* SPOROZOITE DETECTION IN MOSQUITOES

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Malaria is the most important vector-borne disease. Countries as well as organizations all over the world are working towards its control and/or elimination. Knowledge on infective ***Anopheles species*** (mosquitoes carrying ***Plasmodium*** spp sporozoites in their salivary glands and bucal parts) in the field, is fundamental to this goal. The detection of infective mosquitoes is required for vector incrimination, the determination of entomological inoculation rate (EIR), and for interventions monitoring toward the reduction of malaria transmission. Currently sporozoite detection relies on PCR methods, where the 18s-rRNA nested PCR assay is the most extensively used. This standard technique is not amenable for high throughput analyses, due to being laborious and time-consuming. In addition, this assay is affected by debris and carry-over from host cells as well as traces of reagents/template used during DNA extraction and reactions in this multi-step process. In this study, over 3000 mosquitoes collected in the Western Province, Solomon Islands, were evaluated for sporozoite infection using a newly developed assay based on the cytochrome oxidase I gene (COX-1). This assay, is faster, simpler, amenable for high-throughput studies, and has a higher detection limit when compared to the standard 18s-rRNA.

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CARDIAC SAFETY OF ARTEMISININ-BASED COMBINATION THERAPY AMONG ADULTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS AND STABILIZED ON ANTIRETROVIRAL THERAPY IN MALAWI

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Human Immunodeficiency Virus (HIV) and ***Plasmodium falciparum*** malaria co-infections frequently occur in sub-Saharan Africa but there are limited data on the safety of artemisinin-based combination therapy (ACT) in HIV-infected individuals taking antiretroviral (ARV) drugs. ACT components (piperaquine, amodiaquine and lumefantrine) have been associated with cardiac abnormalities in a concentration dependent manner, and have triggered a call for more safety data from various high risk sub-populations. ARV drugs such as protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) that affect activity of cytochrome P-450 enzymes may elevate concentrations of ACTs and have raised concerns about increased risk of cardiac toxicity. As part of a pharmacokinetic trial assessing safety of ACTs among non-malaria HIV infected individuals stabilised on ART, we compared the incidence and severity of QTc prolongation and cardiac related adverse events in ARV-naïve individuals (n=41) and those taking NNRTIs (n=71) and PIs (n=42) in combination with a standard three-day regimen of dihydroartemisinin-piperaquine, artesunate-amodiaquine or artemether-lumefantrine. We will present the QTc interval prolongation and its correlation with ACT plasma levels and treatment emergent cardiac adverse events. Effect of predictors such as age, gender, electrolyte levels and intake of cotrimoxazole prophylaxis on QTc prolongation across study arms are assessed using logistic regression. Robust safety profiles of ACTs in malaria-HIV co-infected populations will help inform treatment guidelines and support national malaria control programs which consider large ACT mass drug administration campaigns

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EXPOSURE TO ARTEMISININS IN PREGNANCY AND THE RISK OF ADVERSE PREGNANCY OUTCOMES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Malaria in pregnancy is responsible for up to 100,000 neonatal deaths and 10,000 maternal deaths annually. Severe malaria may lead to severe maternal anemia miscarriage, stillbirth, prematurity, and low birth weight. In animal models, artemisinin derivatives may be embryotoxic and teratogenic if administered at specific times of pregnancy. No increased risk of adverse pregnancy outcomes has been observed in humans but the studies were limited by too small sample sizes to detect rare but clinically significant differences in adverse pregnancy outcomes. The purpose of this meta-analysis is to examine the association between exposure to artemisinins during pregnancy and the risk of adverse pregnancy outcomes including miscarriage, stillbirth, and congenital anomalies. We conducted a comprehensive search of MEDLINE, EMBASE, and the Malaria in Pregnancy Consortium (MiPc) Library to identify prospective cohort and randomized controlled trials of pregnant women exposed to artemisinins that followed the women through pregnancy and presented pregnancy outcomes. In addition, we reviewed the citations list of key publications and reached out to authors for additional, unpublished data. Our search identified 22 published studies that met our inclusion criteria. Additional outcome data has been requested from six studies, and five studies offered to-date to provide individual level data for analysis. The 22 studies enrolled 26,430 women, of whom 3,772 were exposed to an artemisinin during pregnancy, 554 exposed during the first trimester. The proportion of women experiencing a stillbirth or congenital anomaly after exposure to an artemisinin in the 2nd and 3rd trimester of pregnancy ranged from 0% to 8.5% and 0% to 5.9% respectively in the study populations. Pooled analyses of the data are ongoing and will be presented to the World Health Organization as part of an Evidence Review Group on the safety of artemisinins in pregnancy. The results of the systematic review highlight the need for additional data on artemisinins exposure, especially during early pregnancy.

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FOSMIDOMYCIN AS AN ANTIMALARIAL DRUG: A META-ANALYSIS OF CLINICAL TRIALS

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With first indications of resistance against artemisinin compounds, the development of novel alternative antimalarials remains an urgent need. One candidate is fosmidomycin (Fos), a phosphonic acid derivative. This PRISMA guideline-adhering and PROSPERO registered systematic review and meta-analysis provides an overview of the state-of-the-art of the clinical development of Fos as an antimalarial. Pooling six clinical trials of Fos against uncomplicated malaria in African children yielded an overall day 28 cure rate of 85% [95% CI: 71-98%]; a parasite clearance time of 39 hours; and a fever clearance time of 30 hours. In four adult cohorts, the corresponding values were 70% [95% CI: 40-100%], 49 hours

and 42 hours, respectively. Data suggest that, besides the partner drug, formulation determines efficacy. We advocate further clinical development Fos-combinations. PROSPERO registration number: CRD42014013688

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ARTEMISININ-BASED COMBINATION THERAPY EFFICACY IN KISUMU, WESTERN KENYA: ESTABLISHMENT AND ANALYSIS OF PARASITES USING THE RING-STAGE ASSAY

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Artemisinin resistance leads to reduced parasite clearance rate, marked by higher survival rate of exposed ring-stage parasites during ring stage survival assay (RSA). Although adequate clinical and parasitological response to artemisinin-based therapies (ACTs) remain high in Africa including Kenya, the efficacy of these compounds must be constantly monitored. This study reports the establishment of RSA to monitor the efficacy of ACTs in western Kenya. Clinical isolates collected from field sites as part of routine surveillance and those from an efficacy clinical trial were used in the study. Patients with uncomplicated malaria were recruited into the study where blood samples were collected and analyzed using immediate ***ex vivo*** RSA. The assay was performed as previously described (Witkowski et al, 2013). Artemisinin sensitive (F32-TEM) and resistant (F32-ART) clonal lines were used as controls. A total of 102 clinical isolates were analyzed, 28 from the efficacy study. For the clinical isolates, the RSA median (interquartile range) was 0.78% (0 - 4.62). Twenty eight samples had RSA of 0% whereas 16 had RSA above 10%. The highest RSA values recorded were 58% and 60%; these were from the surveillance study samples. The highest RSA from the efficacy clinical study samples were 20% and 31%. For the controls, F32-TEM had RSA of 0 (n = 6) and F32-ART had RSA of 11% (n = 4). Genetic analyses of these samples including population structure and drug resistance markers are underway. Compared to the controls and previous reports, our data show an underlying concern for efficacy of ACTs in western Kenya. This is in line with our recent study which showed that there has been significant change in parasite genotype in western Kenya in recent years. Genetic analyses of the samples in this study should yield critical data. There is need for continued close monitoring of parasite genotype, phenotype and clinical dynamics in response to continued use of ACTs in western Kenya.

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OBSERVED QTC PROLONGATION AND PHARMACOKINETIC PROFILE OF A PROGRAMMATIC REGIMEN WITH AN INCREASED DOSE OF DIHYDROARTEMISININ-PIPERAQUINE IN YOUNG CHILDREN 5-24 KG IN MALAWI

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Dihydroartemisinin-piperaquine (DHA-PPQ) is highly efficacious against uncomplicated falciparum malaria. Due to its long half-life and associated chemoprophylactic effect, DHA-PPQ is also considered a promising candidate for mass drug administration in transmission reduction settings. However, PPQ has a relatively narrow, poorly defined therapeutic dose range which poses dosing challenges. Concerns particularly remain whether observed PPQ concentration-dependent cardiac QTc prolongation could vary between (and pose a clinical risk in) specific vulnerable population subgroups. While recent pooled efficacy and pharmacokinetic (PK) analyses suggest that young children require a higher weight-adjusted dose due to increased PPQ clearance, no paediatric cardiac safety data exists at these higher doses. This hampers DHA-PPQ's safe and effective introduction into national control programmes. We conducted an open-label dose optimization study to describe the population PK profile of PPQ and cardiac safety of a regimen with a higher average dose of DHA-PPQ in children of 5–24 kg with uncomplicated falciparum malaria [PACTR201303000506302]. Children (n = 100) received a dosing regimen with supervised doses of 1.7–3.8 mg/kg DHA and 13.6–30.0 mg/kg PPQ given once daily over three days, using whole or half-tablets (20/160 mg and 40/320 mg DHA/PPQ). Follow-up was 63 days. QTc was measured before and 4–6 h after the last dose and compared to baseline and Day 28 using digital 12-lead electrocardiograms. No Fridericia corrected QT interval (QTcF) >500 ms or clinical cardiac safety signals were observed with this regimen. However, a substantial QTcF prolongation was measured 4–6 h after the last DHA-PPQ dose compared with baseline: Mean 54 ms, <30 ms in 13%, 30–60 ms in 49% and >60 ms in 37% of children. We will present the results from the population pharmacokinetic/pharmacodynamics (PK/PD) analysis and describe the association between drug exposure and cardiac response. Targeted, small PK/PD safety studies can provide key data on subgroups and inform safe and effective DHA-PPQ dosing regimens for control programmes.

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A QUANTITATIVE TOOL FOR ASSESSING IDEAL DRUG COMBINATIONS FOR MALARIA THERAPY

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Drug combinations are recommended for effective malaria therapy and to prevent emergence of resistance by the WHO and MMV. Yet we are not aware of quantitative criteria for assessing potential antimalarial combinations for their compatibility, efficacy, and safety. We developed a quantitative score to assess drug combinations covering marketed antimalarials, as well as compounds in clinical development and preclinical evaluation. The score includes 13 values, representing different criteria that are important in drug combinations, and each value can be variably prioritized to provide a quantitative score for the combination. These values are: 1) anticipated efficacy in planned use (e.g. Single Exposure Radical Cure and Prophylaxis [SERCaP]); 2) safety of the drugs alone and in combination (worse if both have the same adverse effects); 3) developmental stage of the drugs (later in development is better); 4) pharmacokinetic/dynamic compatibility (matched time above minimum paracidal concentration is best); 5) total dose (in mg, less is better); 6) anticipated co-formulation issues; 7) prophylactic activity against liver stages; 8) probability of emergence of resistance; 9) food effect with administration; 10) drug-drug interaction between the partners; 11) drug-drug interaction of the combinations with other drugs; 12) differing mechanisms of actions; and, 13) intellectual property flexibility. Applying these criteria, and weighting SERCaP success ten-fold, showed OZ-439 to be an optimal partner with: KAF-156, DSM-265, and Ferroquine. This scoring procedure will help to clarify potential antimalarial partners to prioritize for further pre-clinical and clinical testing as effective malaria combination therapy.

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SAFETY AND EFFICACY OF REPEATED OF REPEATED ADMINISTRATION OF PYRONARIDINE-ARTESUNATE OR DIHYDROARTEMISININ-PIPERAQUINE VS ARTESUNATE-AMODIAQUINE IN CHILDREN AND ADULT PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM* SP MALARIA OVER OF TWO YEARS PERIOD AT BANFORA/NIANGOLOKO SITES IN BURKINA FASO

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The safety and efficacy of repeated administration of three ACTs [(pyronaridine-artesunate (PYR) or dihydroartemisinin-piperaquine (DHA-PQ) vs artesunate-amodiaquine (ASAQ)] are in evaluation in three West African Countries, part of the West African Network of Clinical trials for AntiMalarial drugs (WANECAM). The current study presents the partial data of safety and efficacy of repeated administration of PYR or DHA-PQ vs ASAQ over a period of 2 year in children and adults with uncomplicated ***Plasmodium*** sp malaria at Banfora/Niangoloko sites in Burkina Faso. This study is a comparative, randomized, open label longitudinal clinical trial involving children and adults with uncomplicated ***Plasmodium*** sp. malaria. Following the screening, 763 participants were enrolled in ASAQ (315), DHA-PQ (224) and PYR (224) arm, from July 2012 to December 2013. Each of the participants received during their subsequent episodes the same drug and went through the same trial procedures as for the initial episode. The partial results at the end of the enrolment in December 2013 showed that 185 of 315 (58.7%) patients, 129 of 224 (57.6%); and 138 of 224 (61.6%) experienced at least 2 malaria episodes and 10.8%, 7.3% , 9.4 % experienced 5 malaria episodes in the ASAQ, DHA-PQ and PYR arms respectively. The average time between the first and the second malaria episode was statistically longer ($p<0.05$) in DHA (157 days) compared to ASAQ (135 days) and PYR (117 days) arms. Adequate clinical and parasitological response (ACPR) by day 28 was 93.0 %, 97.8% and 98.2% in ASAQ, DHA and PYR arm respectively. The 42 day cure rate (not adjusted by PCR) was 80.3 %, 93.8% and 78.2% in ASAQ, DHA-PQ and PYR arms respectively. Our preliminary results confirmed the two news drugs (DHA-PQ and PYR) are safe and their efficacy comparable to the ASAQ in uncomplicated malaria treatment in high malaria transmission region. The study is still ongoing to complete the follow of the all the patients over a period of two years.

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MORTALITY, MORBIDITY AND DEVELOPMENTAL OUTCOMES IN CHILDREN BORN TO WOMEN RECEIVING EITHER MEFLOQUINE OR SULPHADOXINE-PYRIMETHAMINE AS INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY: A RANDOMIZED CONTROLLED TRIAL

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Malaria infection during pregnancy confers substantial risks for the woman, her fetus and the newborn child. Mefloquine (MQ) has showed to be an effective antimalarial in sub-Saharan Africa, though controversy about its safety in pregnancy has been subject of debate. A recent trial showed that African pregnant women receiving MQ for intermittent preventive treatment of malaria in pregnancy (IPTp) had less clinical malaria than those receiving sulphadoxine-pyrimethamine (SP) while the safety profile was similar. Furthermore, MQ is recommended for prophylaxis in non-immune pregnant women travelling to endemic areas and is a potential partner drug in artemisinin-based combination treatment (ACT) recommended in pregnancy or for women of childbearing age. However, safety of MQ in pregnancy has been poorly assessed in children over the first month of life and little is known about its long-term impact on infant's morbidity, mortality and development. In the context of a multicenter randomized controlled trial to evaluate the safety and efficacy of IPTp with MQ compared to SP in pregnant women from Mozambique, Benin, Gabon and Tanzania, 4247 newborns were followed up until 12 months of age. Nutritional status and psychomotor development were assessed at months 1, 9 and 12 and incidence of malaria, anemia, hospital admissions, outpatient visits and mortality calculated for their first year of life. No differences in the proportion of stunting, underweight, wasting and severe acute malnutrition at month 1, 9 and 12 of age between infants born to women who had IPTp with MQ or SP were found. Higher risk of being unable to stand without help, walk without support and bring solid food to the mouth were observed in the MQ group at month 9 (RR 1.07 [95%CI 1.00-1.14] p= 0.040, RR 1.10 [95%CI 1.01-1.21] p= 0.039 and RR 1.32 [95%CI 1.03-1.70] p= 0.031) though no significant differences were found in the other psychomotor development milestones assessed. Incidence of malaria, anemia, hospital admissions, outpatient visits and mortality were similar in both groups.

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A TRIAL OF THE SAFETY OF SINGLE LOW DOSE PRIMAQUINE IN ADDITION TO ACTS COMMONLY USED IN SENEGAL: PRELIMINARY RESULTS

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WHO recommends the addition of a single dose of primaquine (0.25 mg base/kg) to artemisinin combination treatments (ACTs) as a component of pre-elimination or elimination programs. However, primaquine has been little used in Africa and there are concerns about its safety, as the drug can cause acute haemolytic anaemia in individuals with G6PD deficiency. This open randomised controlled trial was conducted to assess the safety of adding low-dose primaquine to the normal ACT regimen in adult patients in Senegal. Patients with ***P. falciparum*** malaria (parasitaemia{1,000-100,000} trophozoites/µL) were randomized to receive treatment with ACT or ACT plus low-dose primaquine. Haemoglobin concentration was measured at enrolment, and on day 3 and day 7 after the start of treatment. G6PD status was determined for each patient using a qualitative field test (CareStart™). The primary outcome was the change in haemoglobin concentration from day 0 to day 7, which was compared between trial arms using analysis of covariance. Secondary endpoints included haemoglobin concentration on day 3 and gametocyte carriage on day 7. One hundred and thirteen patients (56 in the ACT arm and 57 in ACT plus primaquine arm) were randomized. At enrolment, gender, mean weight, parasitaemia, haemoglobin, and prevalence of G6PD deficiency were similar in the two arms. Mean haemoglobin concentration on day 7, was similar in primaquine and control groups (11.9 and 12.1 g/dL respectively). The difference in Hb concentration on day 7 in the primaquine group compared to controls after adjusting for Hb at baseline, was -0.029 (95%CI -0.51,0.45) g/dL. Haemoglobin change at day 7 was significantly associated with haemoglobin at enrolment, weight and gender. There was no evidence of an association with treatment drug, G6PD status, and parasiteamia at enrolment. This study provided preliminary evidence that the administration of single low dose primaquine (0.25 mg/kg) in addition to ACT treatment, to adult patients with acute ***P. falciparum*** malaria, is safe and does not induced significant drop in haemoglobin level both for G6PD normal and deficient individuals.

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IMPACT OF SCREENING FAILURE ON RECRUITMENT PROCESS DURING A CLINICAL TRIAL OF THE WEST AFRICAN NETWORK OF CLINICAL TRIALS OF ANTIMALARIAL DRUG (WANECAM), BOUGOULA-HAMEAU, MALI

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The conduct of antimalarial's drug or vaccine clinical trials required a rigorous selection of the study volunteers for their participation to the clinical study. The inclusion and exclusion criteria would have an impact on the enrollment rate in a specific study site although the prevalence of the disease indicates an appropriate enrollment rate at a predefined time. The objective of this study was to assess the impact of the screening failure on the recruitment of study volunteers planned for a defined period. During a phase IIIb/IV multicenter clinical trial study of WANECAM, we consented patients with positive blood smears, performed a clinical examination including an Electrocardiogram (ECG) and blood draw for hematology and liver function tests (LFT). Patients with QTc > 450ms and/or LFTs (ALT/AST)> 2x ULN were not eligible as well as those with Hemoglobin less than 7.0 g/dL and females of 12 years or older with a positive urine pregnancy test etc. From December 17, 2012 to January 15, 2015, a total of 1380 infants and adult male and female with positive blood smear freely consented/ assented for the study and underwent the screening procedures. From them we enrolled 1173 patients in the WANECAM study. The rate of screening failure was 15.0%. The proportion of prolonged QTc (Bazet) among screening failure (n =207) was 58.7% followed by low Hemoglobin rate (18.4%) and Liver Function Tests (ASAT/ALAT) > 2xULN (10%). Details on the other exclusion criteria will be provided. When calculating the sample size and study duration for drug or vaccine trial, previous knowledge of possible causes and proportions of screening failures is necessary in addition to the usual parameters such as prevalence of the disease and loss of follow up.

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A PHASE IIA PROOF-OF-CONCEPT STUDY TO ASSESS THE EFFICACY, SAFETY, TOLERABILITY AND PHARMACOKINETICS OF SINGLE DOSES OF DSM265 IN ADULT PATIENTS WITH ACUTE, UNCOMPLICATED *PLASMODIUM FALCIPARUM* OR *VIVAX* MALARIA MONO-INFECTION OVER A 28-DAY-EXTENDED OBSERVATION PERIOD IN IQUITOS, PERU

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DSM265 is from a new class of antimalarial chemotherapy that inhibits the pyrimidine biosynthetic enzyme, dihyroorotate dehydrogenase (DHODH). The DHODH enzyme is essential to ***Plasmodium*** parasites, as they lack pyrimidine salvage pathways and require de novo synthesis to supply the pyrimidine source. DSM265 entered clinical development in 2013 and could potentially be a novel component of either a new combination single-dose treatment or chemoprophylaxis of uncomplicated malaria. Here, we discuss interim results on efficacy, safety, tolerability and pharmacokinetics of DSM265 when administered as single dose to adult patients with acute, uncomplicated ***Plasmodium falciparum*** or ***vivax*** malaria mono-infection in Iquitos, Peru. Beyond the primary objective of determining the efficacy of DSM265 as monotherapy in uncomplicated malaria on Day 14, we followed patients over a 28-Day-extended observation period for parasite recrudescence/reinfection and safety, tolerability and pharmacokinetics of DSM265. We report data on post-treatment prophylaxis over the extended observation period. In a third part, we show how the results from the previously reported induced blood-stage malaria (IBSM) study using ***P. falciparum*** infected red-blood cells together with a refined PK/PD model and ***ex vivo*** experiments from field isolates guided optimal dose selection of the starting dose for this POC study. The independent two arm study design was adaptive sequential. This design allowed flexibility in dose selection of subsequent cohorts and thereby enabled us to calculate the PRR and MIC of DSM265 for both ***Plasmodium*** species.

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RANDOMIZED CLINICAL TRIAL OF AQ-13 (AN INVESTIGATIONAL ANTIMALARIAL) IN COMPARISON TO COARTEM FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Our previous studies have shown that AQ-13 and other 4-aminoquinolines with modified side chains are active against chloroquine (CQ)-, mefloquine- and multi-resistant ***Plasmodium falciparum in vitro***. Because previous studies have also shown that AQ-13 is as safe as CQ in human subjects, we performed a randomized clinical trial to test whether AQ-13 was as effective as artemether + lumefantrine (AL, Coartem) for the treatment of uncomplicated ***P. falciparum*** malaria in human subjects. Subjects enrolled in this study were Malian males ≥ 18 years of age with uncomplicated ***P. falciparum*** malaria and asexual parasite counts between 2,000 and 199,999 parasites per µl (NTC Clinical Trial Number 01614964). After oral treatment with either AQ-13 (1,750 mg in doses of 700, 700 and 350 mg on days 1, 2 and 3) or AL (4 tablets twice daily x 3 days=480 mg artemether and 2880 mg lumefantrine), subjects were followed as inpatients on a Clinical Research Center for days 1-7 and twice weekly as outpatients from days 8-42. Although the second group of 33 subjects is still being enrolled, there have been no differences between AQ-13 and Coartem in terms of efficacy (0 early parasitologic or clinical treatment failures on or before day 3 in either group, no subjects in either group has failed to clear all asexual parasites on or before day 7), safety and adverse events including ocular side effects (0 adverse events, Grade 3, Grade 4, serious adverse events, arrhythmias or ocular side effects in either group). These results suggest that both AQ-13 and other AQs with similarly modified side chains (with 2-3 or 10-12 carbons vs. the 5 carbon [isopentyl] side chain of CQ) may be effective for the treatment of human subjects with uncomplicated malaria due to CQ-resistant ***P. falciparum***.

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HIGH-RESOLUTION MELT ANALYSIS TO COMPARE THE EFFICACY OF AN INVESTIGATIONAL ANTIMALARIAL (AQ-13) WITH COARTEM (ARTEMETHER-LUMEFANTRINE) FOR TREATMENT OF UNCOMPLICATED MALARIA DUE TO CHLOROQUINE-RESISTANT VS. CHLOROQUINE-SUSCEPTIBLE *PLASMODIUM FALCIPARUM*

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The use of High Resolution Melt (HRM) analysis to distinguish between specific parasite genotypes during the evaluation of a candidate antimalarial can provide additional information about its efficacy against parasites that do (or do not) have the point mutation (resistance) of interest. To test this hypothesis, we are using HRM to identify (and distinguish between) chloroquine-resistant (CQ-R) and chloroquine-susceptible (CQ-S) parasites containing the T76 and K76 point mutations, respectively. Because the T76 point mutation (SNP) within the ***Plasmodium falciparum*** chloroquine transport gene (PfCRT) is necessary for CQ-R, the frequency of the T76 genotype among the parasites causing the initial infections is being compared for subjects randomized to receive the investigational antimalarial (AQ-13) vs. the control treatment (Coartem) in a Phase 2 clinical trial of efficacy for the treatment of uncomplicated ***P. falciparum*** malaria. Although the mutant (T76) and wild-type (K76) alleles can be distinguished by gel electrophoresis after restriction endonuclease digestion with ApoI, that method can yield false-negative results for genotypes present as minority variants within a polyclonal infection because of the limited sensitivity of gel electrophoresis. In contrast, HRM can detect alleles comprising less than 10% of the parasites present in polyclonal infections. For this reason, we are using HRM genotyping to make this distinction and this assay has now been established and standardized at the University of Bamako within the West African International Center of Excellence in Malaria Research (ICEMR). The results obtained thus far have shown no differences between the two treatment groups. After the second group of 33 subjects has been enrolled and studied this summer, the codes for treatment groups will be broken during September so the uncoded results can be presented at the ASTMH Meeting.

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DEVELOPMENT OF A CONTROLLED HUMAN MALARIA INFECTION MODEL FOR THE ASSESSMENT OF TRANSMISSION BLOCKING INTERVENTIONS

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With the declining burden of malaria and the increasing focus on elimination the development of tools to evaluate interventions that interrupt transmission is assuming increasing priority. Rapid and cost-effective evaluation of transmission-blocking drugs and vaccines requires a model that accurately predicts efficacy in humans. Although controlled human malaria infection (CHMI) studies have been used successfully to assess pre-erythrocytic and blood stage interventions, these current models do not assess the ability of interventions to interrupt ***Plasmodium falciparum*** transmission from humans to mosquitoes. The reason for this is that during CHMI, treatment must be initiated promptly once a threshold of asexual parasitemia is reached, thus preventing the normal development of gametocytes, which usually only appear after an extended period of asexual stage infection. In an ongoing study we are exploiting our observation that piperaquine treatment of blood stage ***P. falciparum*** infection clears asexual parasitemia and is followed by the appearance of gametocytes in peripheral circulation ~2 weeks post treatment. The aims of this clinical trial are i) determine if these gametocytes can be transmitted to Anopheles mosquitoes and complete the sexual stage of development, and ii) assess the transmission-blocking activity of the experimental synthetic ozonide, OZ439. The study is being undertaken in 3 cohorts of 6 volunteers, with 2 subjects in each cohort receiving no gametocytocidal intervention (-ve control), 2 subjects receiving primaquine (+ve control) and 2 receiving OZ439. Parasitemia and gametocytemia will be monitored by qPCR and following gametocyte detection, transmission studies will be performed using both direct feeds on volunteers and direct membrane feeding assays on venepuncture blood. Successful mosquito infection will be determined by oocyst/sporozoite visualisation and confirmed by qPCR. This study aims to develop and optimise a reproducible model that can assess both gametocytocidal and transmission-blocking activity for the rapid selection of effective drugs and vaccines. Results of this trial will be presented.

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EXPLORATION OF 5-AMINOPYRAZOLE-4-CARBOXAMIDE AS POTENTIAL MALARIA TRANSMISSION BLOCKING THERAPY

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Currently available drugs to treat malignant malaria, caused by ***Plasmodium falciparum***, kill the asexual blood stage parasites and treat the symptoms, but do not kill the gametocytes, and do not stop transmission of malaria to mosquitoes. Thus, the vicious cycle of malaria continues, leading to an estimated 584,000 deaths a year. Effective control and eradication of malaria will require new tools to prevent transmission of ***P. falciparum***. We previously established the efficacy of a pyrazolopyrimidine (PP) scaffold of bumped kinase inhibitors (BKIs) in malaria transmission blocking. BKIs block transmission of malaria to mosquitoes by inhibition of ***P. falciparum*** calcium dependent protein kinase 4 (PfCDPK4), which blocks exflagellation of ***P. falciparum*** male gametocytes. We explored alternative inhibitor scaffolds, and report here the design of malaria transmission blocking inhibitors with a 5-aminopyrazole-4-carboxamide (AC) scaffold that specifically exploits the PfCDPK4 atypical gatekeeper pocket. We characterized the AC BKIs for inhibition of PfCDPK4 and ***P. falciparum*** gametocyte exflagellation. To date, 28 compounds have been found to inhibit PfCDPK4 with an IC50 of <100 nM and a further 7 with IC50 of <50 nM. In addition, 26 compounds reduced parasite exflagellation by >50% at 1 μM, Most of these compounds lacked evident toxicity issues, in that at >10uM, there was no inhibition of the mammalian kinase Src, hERG activity, or proliferation two mammalian cell lines, CRL8155 and HepG2. The AC BKIs will be further optimized, through iterative study using ***in vitro*** and ***in vivo*** PK and toxicity models, to optimize clinical safety and efficacy, with long-lasting transmission blocking exposure. The AC and PP BKI scaffolds have yielded valuable compounds to block transmission and show promise to help malaria control and eradication programs.

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DEFINING EFFECTIVE, APPROPRIATE, IMPLEMENTABLE STRATEGIES FOR MALARIA ELIMINATION IN MILITARY FORCES IN CAMBODIA AS A MODEL FOR MOBILE POPULATIONS

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Multidrug resistant malaria remains a critical public health problem in Southeast Asia despite intensive containment efforts. This has led to refocused efforts on malaria elimination, and Cambodia has declared an ambitious goal of achieving elimination by 2025. However, important gaps remain in case management, service delivery, prevention, and vector control, particularly in hard-to-reach mobile populations including military personnel, an under-recognized malaria transmission reservoir. The operational elements of a comprehensive malaria elimination initiative in mobile and migrant populations in Southeast Asia have yet to be rigorously tested in a clinical trial design. We will conduct a two-arm, controlled, cluster-randomized, open-label pilot study to determine the effectiveness of monthly malaria prophylaxis (MMP). Monthly dihydroartemisinin-piperaquine and weekly low dose primaquine for 3 months will be compared to a focused screen and treat (FSAT) strategy following recently updated Cambodian national treatment guidelines. Military encampments will be randomized to the MMP or FSAT intervention in order to treat a total of up to 1200 soldiers. In addition, each site will be randomized to a single-blind treatment of duty uniforms with permethrin to guard against outdoor biting mosquitoes, or sham treatment with water. Conventional malaria microscopy with be compared with RT-PCR and rapid diagnostic testing. All G6PD deficiency screening will be done via two commercially available rapid diagnostic tests, qualitative and quantitative testing to allow for comparison. By including all currently available screening, preventive, and diagnostic modalities, the study aims to identify whether elimination is currently possible and to determine the most cost-effective and efficient approaches in the military as a model mobile population in Cambodia.

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OPTIMIZING ALLOCATION OF COMMUNITY HEALTH WORKERS USING A NOVEL GEOSPATIAL TOOL IN NAMIBIA

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Namibia's malaria burden continues to decrease from 439,760 cases in 2002 to 4,745 in 2013. However, the country is challenged by having one of the lowest population densities in Africa, with people distributed unevenly across enormous distances. It is reported that over 60% of the rural population need to walk to the nearest health facility, the majority of which are 3-4 hours away. Increasing access to healthcare at the community level was supported by the rollout of community health workers (CHWs) in 2014. The initial placement of CHWs was evenly distributed and the National Vector-borne Diseases Control Programme (NVDCP) sought advice on how to best re-allocate CHWs to ensure maximized coverage of communities in need. For the efficient allocation of resources, CHWs should be placed in highly populated communities where there is limited access to healthcare and a high malaria transmission potential. Allocation was prioritized in communities with the greatest population density (>5 people per square km), highest predicted risk of malaria (incidence >5 cases per 1000), and with location furthest from health facilities, based on expert recommendations from the NVDCP. The ideal CHW allocation resulting from this analysis suggested targeting 625 priority villages out of the 6988 endemic villages (8.9%) and comparison of the recommended deployment with current CHW locations demonstrates substantial gaps in recent CHW placement. This methodology was translated into a simple, web-based dashboard, which does not require any advanced geospatial analysis skills and could be accessed by the NVDCP and regional partners. In an elimination setting, malaria burden is dynamic, and CHW placement should be iteratively reassessed and updated as the epidemiology of malaria evolves. The findings for the allocation of CHW in Namibia will be used to plan for the next malaria season in 2016, while this methodology could be beneficial for resource allocation in other elimination settings.

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MASS SCREENING AND TREATMENT DOES NOT IMPACT MALARIA INCIDENCE IN WEST TIMOR, INDONESIA

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Mass screening and treatment (MST) is an intervention strategy used in Indonesia towards the goal of eliminating malaria. The aim of this study was to identify the impact of two regimens of MST on malaria incidence and prevalence in a region of moderate malaria transmission. Inhabitants (n=1,868) of 3 villages (Lamea, Seserai and Weoe) and 1 hamlet (Metanamasi) in Belu Regency, West Timor, were randomly allocated into 3 arms, i.e. one that received MST twice (arm-1, n=649) in 3 months, an arm that received MST only once (arm-2, n=422) and a control arm that did not receive MST (arm 3=777) during the same time span. Dihydroartemisinin-piperaquine plus primaquine was used to treat positive cases based on microscopic blood slide results. The malaria status of a cohort of primary school children from these 3 arms were also examined monthly for 6 months Results of the study will be presented.

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SERIAL MOLECULAR IDENTIFICATION TO CONFIRM THE PRESENCE OF *PLASMODIUM KNOWLESI* IN INDONESIA

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In order to achieve malaria elimination, intervention efforts must target all ***Plasmodium*** species, including zoonotic ones. The use of sensitive and specific molecular detection of malaria parasites can address this challenge. We introduced the use of molecular detection for reactive case detection of malaria in Aceh Besar District, Aceh Province, Indonesia, an area in the pre-elimination stage with known endemicity of ***P. vivax*** (Pv) and ***P. falciparum*** (Pf), but not ***P. knowlesi*** (Pk). ***Plasmodium***-specific loop-mediated isothermal amplification (LAMP) was performed on dried blood spots collected from microscopy confirmed cases identified at 5 sentinel health facilities as well as family members and neighbors residing within the reactive case detection radius. From June 2014 - March 2015, passive and active case detection (testing 778 household members and neighbors of passively detected cases) using microscopy detected 20 cases (7 Pf, 11 Pv, 1 P. malariae, 1 suspected Pk). LAMP detection confirmed microscopy findings and identified 3 additional submicroscopic infections in the community. ***Plasmodium*** speciation by nested PCR targeting the ***cytochrome b*** (***cytb***) gene followed by restriction digests were done and confirmed some but not all of the microscopy results (4 Pf, 1 Pm and 1 suspected Pk cases were identified as Pv). Further PCR and sequencing of ***P. knowlesi***-specific small subunit ribosomal RNA gene identified that 7 of 21 cases identified as Pv by ***cytb***-PCR were actually Pk, including one found in active case detection. We report the first cases of Pk identified in Indonesia outside of Borneo island, through passive and active case detection. Pk detection by microscopy is difficult because the morphology of different stages of Pk life-cycle is very much similar to other malaria species. Moreover, commonly used molecular methods mis-identify Pk as Pv. These findings pose challenges to areas with ***P. vivax*** and possible ***P. knowlesi*** endemicity, particularly those aiming to eliminate malaria. Simple molecular methods to detect all ***Plasmodium*** species are needed to support malaria control and elimination efforts.

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HOUSING QUALITY AS A POTENTIAL RISK FACTOR FOR LOCALLY ACQUIRED MALARIA INFECTION IN SWAZILAND

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Poor housing quality may contribute to mosquito exposure and increase risk of malaria infection, but evidence in elimination settings is lacking. Here, surveillance data was used to study the association between housing quality and locally-acquired infection in Swaziland. Subjects were malaria index cases detected through passive surveillance and their household and community contacts screened in reactive case detection from August 2012 to January 2015. Cases were defined as Loop-mediated isothermal amplification (LAMP) positives with no travel history and controls as LAMP negatives with no travel history. Housing quality was defined using wall, roof and window type variables, which were then combined to develop a composite housing quality index. Bivariate and multivariable logistic regressions, adjusted for household-level clustering, were used to analyze relationships between infection, housing quality and other risk factors. Of the 993 index cases, 840 (85%) received a LAMP test and 102 (12%) were locally-acquired cases. Of the 11,464 people screened, 11,079 (97%) received a LAMP test and 138 (1%) were locally-acquired cases. In bivariate model with separate housing quality predictors, higher odds of infection were associated with poor quality external wall (OR 2.1 95%CI 1.4-3.1), poor quality internal wall (OR 1.8 95%CI 1.2-2.7) and poor quality roof (OR 2.1 95%CI 1.4-3.2). Higher infection odds were also associated with farming and manual labour occupations (OR 2.5 95%CI 1.6-3.9). In the multivariable model, only poor quality external wall was associated with higher infection odds (OR 2.5 95%CI 1.3-4.9). Overall, poor housing was associated with higher infection odds in bivariate (OR 1.7 95%CI 1.2-2.5) and multivariable models (OR 1.8 95%CI 1.2-2.8). Insecticide-treated bed net use and sleeping under a sprayed structure were associated with non-significant reductions in infection odds. There were no associations with age, gender, region and nationality. Housing quality was an important determinant of locally-acquired infection in Swaziland, suggesting improved housing as a potential elimination strategy.

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LEVERAGING COMMUNITIES TO PURSUE MALARIA ELIMINATION: A COMPARISON OF NATIONAL-LEVEL CERTIFICATION TO VILLAGE-LEVEL CERTIFICATION AND SUBSEQUENT EFFECTS ON COMMUNITY ENGAGEMENT

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The World Health Organization certifies malaria elimination exclusively at the national scale and has no mechanism to certify smaller geo-political units as malaria free. This certification process fails to recognize that malaria epidemiology is highly heterogeneous and smaller geo-political units may become malaria free long before an entire country. The WASH sector, in contrast, certifies open defecation free (ODF) status at village-level. The use of smaller geo-political units in the WASH sector has two advantages: 1) it drives the construction and use of latrines and hand washing stations and 2) it engenders community engagement. In Zambia, real-time feedback of WASH data to villages regarding their success or failure in attaining ODF status has resulted in great gains in the number of ODF villages. ODF status has been achieved at district-level in Chiengi (the first district in sub-Saharan Africa to achieve this certification) and other districts are close. The direct connection between those responsible for action at village-level and the certification of ODF status has fostered the community-driven progress in Zambia's WASH sector. Related to malaria elimination, several districts in Southern Zambia have made great progress towards eliminating malaria transmission, including the capital city, Lusaka. With certification for "malaria free" only applying at national level, however, a country like Zambia which possesses high heterogeneity of malaria transmission is far from country-wide attainment. We propose that sub-national certifications of malaria elimination or even a muted "malaria safe" designation at the facility or village level would better engage and empower communities to achieve and sustain malaria-prevention behavioral change and eventually malaria elimination. Additionally, we suggest a sub-national malaria elimination framework to catalog and communicate decentralized malaria elimination status and to better motivate communities and provide evidence that efforts are continuing to shrink the malaria map.

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MAPPING OF POPULATION MOVEMENT PATTERNS AND ESTIMATING OF IMPORTED MALARIA IN MALARIA-FREE JAVA/BALI IN INDONESIA

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Controlling and eliminating malaria in Indonesia is a challenging endeavor. About 60% of Indonesian lives the islands of Java and Bali where malaria-free and low risk of infection zones occur. However, sustaining this achievement and preventing the re-introduction of malaria will be very difficult. These movements incur substantial risk of importing and re-establishing malaria transmission on islands that have eliminated malaria. This project is designed to quantifying population movements, along with understanding the geographic and demographic dimensions of such movements, as the key to combating threats to sustaining elimination. Knowing the importation risks, high risk visit periods and the most important paths of malaria importation will allow malaria control authorities to design interventions that can reduce transmission in all regions that are primary sources of infected travelers. Techniques to estimate specific patterns and numbers of human movements using mobile phone usage data among the islands will be explained.

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TOWARDS MALARIA ELIMINATION: ETHNOGRAPHIC AND MALARIA EPIDEMIOLOGICAL RESEARCH IN EASTERN INDONESIA

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Malaria continues to be a severe public health problem in Indonesia with approximately 130 million Indonesians living at risk. In the absence of effective prevention, appropriate treatment-seeking behavior and accessibility of adequate health services are essential for decreasing the risk of severe complications, deaths and transmission of the disease. This cross-sectional study aimed to investigate factors affecting treatment-seeking in 3 malaria-endemic communities in Alor district in eastern Indonesia. Mixed (qualitative/quantitative) methods used, include (1) a GPS-aided household census to enumerate the study population (n=3077); (2) ethnographic research, including observations and semi-structured interviews with community members and health care providers (n=44); (3) a structured community-based survey (n=350) and semi-structured interviews with health care providers (n=6), and a check of diagnostic facilities and malaria treatment records at the field sites. Guided by a socio-ecological framework, thematic content analysis identifies different spheres of influence on malaria-treatment seeking behavior, including local (mis-)understandings about the disease and its transmission, as well as competing socio-economic concerns at the individual and social levels, and the lack of adequate diagnostic and treatment facilities at the structural level. Multiple regression analyses explore associations of personal and socio-economic correlates with appropriate treatment-seeking behavior. This study highlights that in order to address the needs of populations in malaria-endemic areas in eastern Indonesia, we must understand the complexity of multiple interacting factors, avoiding simplified assumptions about obstacles to appropriate treatment-seeking behaviors.

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CHARACTERIZATION AND ANTIBIORESISTANCE OF CULTURABLE MICROFLORA BACTERIA IN THE MIDGUT OF ADULT *ANOPHELES GAMBIAE* AND *AN. COLUZZII*

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The bacterial microbiota colonizing the mosquito midgut plays an important role in interactions between mosquitoes and parasites, thus modulating the level of malaria transmission. Characterization of the bacterial flora living in the mosquito vector of malaria may inform new control strategies aiming to reduce malaria transmission. In this study, we isolated and identified the bacteria flora present in the gut of adults female ***Anopheles*** by conventional ***in vitro*** culture techniques and sequencing. To verify the stability of paratransgenesis bacteria candidate and refractoriness bacteria pathogens for human, we evaluated the susceptibility of bacteria flora isolated in the midgut of ***An. coluzzii*** and ***An. gambiae***. We showed that the composition of the midgut bacteria in field-collected mosquitoes exhibits a great variability in contrast to laboratory-reared mosquitoes, which could be explain by the bacterial richness of the larval habitats. Among the isolated bacteria, Enterobacteriaceae and Staphyloccocacea families were most prevalent. A species-specifics association, ***An. coluzzii*** and ***An. gambiae*** has founded. We also observed interest bacteria as Asaia sp and Pantoea sp for

paratransgenesis and potentially pathogenic bacteria for Human such as ***Escherichia coli, Serratia ficaria*** or Klebsiella pneumonia. The antibiotic susceptibility test showed that the ideal candidate of paratransgenesis were sensitive an different antibiotics using abusively in Burkina Faso.

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FOUL WIND, SPIRITS AND WITCHCRAFT: ILLNESS CONCEPTIONS AND HEALTH-SEEKING BEHAVIOR FOR MALARIA IN THE GAMBIA

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As the disease burden in the Gambia has reduced considerably over the last decade, heterogeneity in malaria transmission has become more marked, with infected but asymptomatic individuals maintaining the reservoir. The identification, timely diagnosis and treatment of malaria-infected individuals are crucial to further reduce or eliminate the human parasite reservoir. This ethnographic study focused on the relationship between local beliefs of the cause of malaria and treatment itineraries of suspected cases. An ethnographic qualitative study was conducted in twelve rural communities in the Upper River Region and the Central River Region in the Gambia. The data collection methods included in-depth interviews, participant observation, informal conversations, and focus group discussions. While at first glance, the majority of people seek biomedical treatment for 'malaria', there are several constraints to seeking treatment at health centres. Certain folk illnesses, such as Jontinooje and Kajeje, translated and interpreted as 'malaria' by healthcare professionals, are often not considered to be malaria by local populations but rather as self-limiting febrile illnesses - consequently not leading to seeking care in the biomedical sector. Furthermore, respondents reported delaying treatment at a health centre while seeking financial resources, and consequently relying on herbal treatments. In addition, when malaria cases present symptoms such as convulsions, hallucinations and/or loss of consciousness, the illness is often interpreted as having a supernatural aetiology, leading to diagnosis and treatment by traditional healers. In conclusion, although malaria diagnostics and treatment seeking in the biomedical sector has been reported to be relatively high in the Gambia, local symptom interpretation and illness conceptions can delay or stop people from seeking timely biomedical treatment, which may contribute to maintaining a parasite reservoir of undiagnosed and untreated malaria patients.

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INTENSIFYING SURVEILLANCE OF ANTIMALARIAL MULTI-DRUG RESISTANCE IN THE CAMBODIAN NATIONAL CONTROL PROGRAM BY INTEGRATING GENETIC EPIDEMIOLOGY INTO ROUTINE ACTIVITIES OF VILLAGE MALARIA WORKERS

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Drug resistant ***Plasmodium falciparum*** parasites are the most serious threat to malaria control in Cambodia and to the Greater Mekong System (GMS). Monitoring of genetic markers of drug resistance and parasite diversity provides an important tool for mapping the geographical distribution of resistance and for identifying subpopulations of parasites. This can be used to respond to local outbreaks of malaria by distinguishing clonal expansion of multidrug resistant parasites from other causes of increased transmission. Furthermore, through the application of population genetics and mathematical modelling, geographical corridors of spread of resistance can be identified, informing intervention policies. We have established a genetic epidemiological surveillance system to guide malaria elimination in Cambodia, in particular targeting multidrug resistant falciparum malaria. The system leverages on an existing malaria information system (MIS) which records slide-confirmed malaria cases data notified by village health workers (VMWs) or health facility staff in 2539 villages in endemic areas of Cambodia. VMWs and other health facility staff provide a first point of care by diagnosing and treating malaria cases, and collect basic epidemiological data on age, sex, location, pregnancy status, and likely location of infection. To support genetic surveillance, the collection of dried blood spot (DBS) samples on filter paper for all confirmed cases of falciparum and mixed species infection has been integrated in the routine diagnosis and treatment workflow. Samples are then routed for processing and genotyping using existing sample referral mechanisms; the resulting data is used to create maps of markers, population analyses and mathematical modelling. Here, we describe the initial phases of implementation of this new approach to surveillance and its findings. The National malaria control program of Cambodia aims to intensify elimination efforts by further integrating genetic epidemiological approaches into village-based surveillance programs.

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IMPLEMENTING ENHANCED HIGH-RESOLUTION SURVEILLANCE USING SPATIAL DECISION SUPPORT SYSTEMS (SDSS) TO GUIDE TARGETED RAPID RESPONSE IN MULTI-DRUG RESISTANT AREAS OF VIETNAM

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Emerging untreatable malaria in the Greater Mekong Subregion (GMS) could result in a public health disaster when it reaches Africa. A project was established to research, develop and implement enhanced surveillance

and targeted appropriate intervention measures to stop the spread of multi-drug resistant malaria through elimination of the disease in the region. The aims of the project are to implement a spatial decision support system (SDSS) approach to conduct high-resolution surveillance to guide swift and targeted responses. Sites have been established in Phu Yen and Quang Tri Provinces of Vietnam. Publically available topographic geographic information have been uploaded as baseline information. In Phu Yen province, 4683 households with 17653 people have been georeferenced. Seventy five current and recent malaria cases have linked to the geolocated households. Only 5-10% acquired malaria at the household level. Most patients could specifically identify the likely transmission location where they were 8-16 days (liver incubation period) earlier. Remote area geographic reconnaissance data is currently being systematically collected from the largely forested transmission locations. Data with GPS coordinates are being collected with handheld computers and inexpensive smart phones using Open Data Kit (ODK). All new cases are being reported within 24 hours on smart phones. Current and historical case and entomology data are being captured to automatically classify transmission ***foci*** and generate response areas-of-interest (AOI). Supporting data (e.g. population, indoor residual spraying status, bed need distribution/use, location and number of sleeping locations) will be used to automatically produce output for village health workers and district level units to mobilize appropriate responses. The current project, ending in August 2015, will allow the full SDSS system will be presented. This new approach utilizes novel geo-spatial tools to support targeted, appropriate and aggressive response measures to support malaria elimination in areas of global significance.

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MALARIA CASE INVESTIGATION IN SENTINEL SITES IN VIETNAM

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The National Malaria Control Program in Vietnam established seven sentinel sites in areas of different malaria endemnicity in Vietnam in 2014 in preparation for malaria elimination by 2030. One component of this project involved case interviewers to better understand the factors leading to on-going malaria transmission. Between July 2014 and March 2015, 322 confirmed malaria cases were interviewed in 4 provinces as follows: Binh Phuoc 217, Phu Yen 72, Gai Lai 31 and Lai Chau 1. Sixty-one percent of cases were reported as ***Plasmodium falciparum***, 34% ***P. vivax*** and 4% both species. 97.5% of cases had a diagnosis by microscopy, 80% both microscopy and RDT and the remainder only RDT. The concordance rate between the diagnostic tests was very high. Of the 214 cases with available data, 86% reported a diagnosis made within two days or fewer from the onset of symptoms. ***P. falciparum*** cases were treated with dihydroartemisinin-piperaquine and ***P. vivax with*** chloroquine. For both species, 98% were given primaquine according to national guidelines. Follow-up on day 3 was conducted in 134 people; only one person had not cleared parastiemia. Overall, 16% of patients were women, ranging from 8% in Phu Yen to 39% in Gai Lai ($p < 0.001$), while 11% of cases were in children < 16 years. Sixty percent of cases were reported from 9 different ethnic minority groups. The probable transmission locations were described as follows: at home 6%, in the forest or on a forest-fringe farm 47%, and while traveling 26%. For 102 cases (32%), the transmission site was suspected to be across borders, of which Cambodia was specifically mentioned in 42. Of the cases from Cambodia, 79% were Pf, all were in men and 83% occurred in forest workers. Of the 163 individuals asked, 30% reported knowing of family or neighbors who had malaria recently. Information presented here reveals that the quality of malaria diagnosis and treatment is good in the public health sector in Vietnam. This questionnaire is now being rolled out all malaria-endemic areas in Vietnam. In Phu Yen province, additional information are now being captured to more specifically identify transmission locations in preparation for pilot implementation of reactive case detection.

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AN EXTENDED MOLECULAR BARCODE FOR TRACKING *PLASMODIUM FALCIPARUM* PARASITE POPULATIONS

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Major epidemiological shifts associated with the transition toward malaria-elimination are mirrored by changes in the genetic diversity of malaria parasite populations. These genetic signatures can be monitored using population genetic-based tools to determine efficacy of malaria intervention efforts prior to measurable changes in disease prevalence. We sought to develop a genotyping tool that would detect key changes in parasite population structure as transmission declines that would be cost-effective and applied to large-scale population analysis. We also wanted a tool that would facilitate our understanding of basic biological processes of parasite transmission. Thus, we strategically increased the number of informative loci to develop a readily deployable and robust panel of single nucleotide polymorphism (SNP) assays comprising an extended molecular barcode. The extended barcode assays, designed using Agena's (Sequenom) mass spectrometry-based technology include SNPs exhibiting high minor allele frequency and low divergence (e.g. FST), filtered from screening over 500 whole genome sequenced samples. We found 99% concordance across a set of previously sequenced monogenomic samples, identified the limit of detection (50 pg/ul), and found the dynamic range of the minor allele could be accurately resolved when present > 10-15% of the sample. The increased number of loci and allele frequency information improve our ability to predict the number of distinct parasite genomes in samples with higher complexity of infection. In silico barcodes, derived from whole genome sequencing data, demonstrate the extended barcodes utility for detecting ancestry among parasites on a more cost-effective basis compared to whole genome sequencing. Agena assay concordance across clinical samples using DNA extracted from monogenomic samples collected on filter paper from Senegal was 98% concordant with Taqman genotyping. We are now applying this extended barcode approach to follow changing population genetic signals in Africa as campaign interventions such as mass drug administration are being applied toward malaria elimination.

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USE OF SURVEILLANCE AND MOBILE PHONE DATA TO PREDICT SPATIAL PATTERNS OF MALARIA TRANSMISSION AND INFORM TARGETED INTERVENTION STRATEGIES IN HAITI

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To achieve malaria elimination goals with limited resources, national programs will need to design and target interventions strategically. Spatial targeting of interventions can be guided by understanding heterogeneity of risk and human movement between areas of differential risk. We assessed risk and exportation of malaria via human movement across Haiti, where the national program aims to interrupt local transmission by

2020. Malaria Test Positivity Rates (TPR) among symptomatic individuals, reported to the national sentinel site surveillance system (2011-2013), were combined with demographic and ecological covariates using logistic regression to predict spatial patterns of transmission. The resulting risk map was overlaid with human mobility patterns predicted using anonymized mobile phone call data records from September to November 2010. Geographic communities with highly connected infected individuals were identified and within communities, administrative units likely to export infection were highlighted. We estimated that 15-30% of Haiti's population lived in areas of medium to high transmission risk (TPR >15%). Combining risk with mobility data, Haiti was divided into six communities, between which travel is relatively low but, within which there is likely substantial movement of infected individuals. Malaria exportation rates and the number of cases exported by administrative unit were estimated. While any combination of these results can be used for geographic targeting of interventions, we highlighted three administrative units in each community with the highest exportation rates. For optimal efficiency, malaria interventions may be targeted within a community simultaneously, reserving those most aggressive for high risk ***foci*** that export infection. At the same time, strong passive surveillance should be maintained across the country. Although use of TPR to estimate risk is limited by health seeking behavior and diagnostic practices, evidence generated by the combination of surveillance and mobility data can help programs design targeted interventions to interrupt local malaria transmission.

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TEMPORAL INSTABILITY OF SPATIAL CLUSTERS OF *PLASMODIUM FALCIPARUM* INFECTION IN AN AREA OF LOW MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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The spatio-temporal dynamics of malaria transmission are not well characterized in areas transitioning from endemic transmission to pre-elimination and elimination. Data from a population-based, serial cross-sectional cohort study were used to detect spatial clusters of malaria in a low transmission setting of southern Zambia. The prevalence of malaria was determined using an algorithm that included those positive by polymerase chain reaction (PCR) and those positive by rapid diagnostic test but negative by PCR who reported taking anti-malarials in the prior two weeks. The malaria prevalence decreased from 9% in 2008 to <1% in 2013. Spatial only cluster detection analysis (SaTScan) was preformed to identify clusters for each year from 2008-2013. Space-time cluster detection analysis was performed to identify spatial clusters over the years 2008 to 2013 accounting for temporal trends. Statistically significant spatial clusters were detected with the spatial only analysis for 2008, 2009, 2010, and 2012; however, these clusters did not overlap. The space-time cluster detection analysis detected two statistically significant space-time clusters that were stable over time after accounting for temporal trends. These two temporally stable clusters overlapped spatially with clusters detected in the spatial only model from 2008, 2009, and 2010. The spatio-temporal stability of clusters between 2008 and 2010 suggests a persistent ecological reservoir of malaria transmission. However, as transmission further decreased after 2010, the absence of spatio-temporal clusters suggests the disappearance of a stable reservoir, with malaria likely to be imported rather than locally acquired. As areas transition from endemic and pre-elimination to elimination, targeting control measures to eliminate the remaining few cases is essential. When there is evidence of spatial clusters and a persistent malaria transmission reservoir, interventions such as reactive drug administration can be effective. But when this reservoir is eliminated, passive detection of imported cases and measures to reduce re-establishment of a reservoir are warranted.

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PILOT STUDIES OF *GRAMMOMYS SURDASTER (G. DOLICHURUS)*, THE NATURAL HOST FOR *PLASMODIUM BERGHEI* PARASITES, AS A MODEL TO STUDY WHOLE ORGANISM VACCINES AGAINST MALARIA

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A highly efficacious vaccine to prevent infection is needed for the goal of malaria eradication. Inbred mice are commonly used to test candidate malaria vaccines, yet are unreliable to predict efficacy in humans. To establish a more rigorous animal model, we acquired African woodland thicket rats (TR) of the genus ***Grammomys***, the natural hosts for *Plasmodium berghei* (Pb). TR were transferred from a colony at INRB in Kinshasa to establish a breeding colony at NIAID in Rockville, MD, and were identified as ***G. dolichurus (surdaster***) by skull and teeth measurements and mitochondrial DNA genotyping. We established that TR are highly susceptible to infection by all rodent malaria strains tested (Pb ANKA, Pb NK65, ***P. chabaudi chabaudi*** (Pcc) AS, Pcc CB and ***P. yoelii*** 17XNL): for all parasite species, 1-2 infected mosquito bites or 25-100 sporozoites (SPZ) administered intravenously consistently resulted in patent parasitemia. We then assessed efficacy of whole organism vaccines (WOV) to induce sterile immunity. Using Pb radiation attenuated SPZ (RAS), C57BL/6 mice were 100% protected after immunization with a total of 30K RAS PbSPZ administered in 3 doses (10K RAS PbSPZ per dose) at 3-4 weeks interval, against challenge with 200 PbSPZ or 1K PbSPZ. In contrast, this regimen protected only 40% of TR against challenge with 200 PbSPZ, and 0% against challenge with 1K PbSPZ. A higher immunizing dose of 200K RAS PbSPZ administered in 3 doses at 3-4 weeks interval (100K +50K +50K) protected 100 % of TR from challenge with 200 PbSPZ, but only 20% from challenge with 1K PbSPZ. Using a chemoprophylaxis vaccination approach, we again observed differences between laboratory mice and TR. After 3 doses of 3K PySPZ under Pyrimethamine drug (PYR) cover at 3-4 weeks intervals, 75% of BALB/c mice but 0% TR were protected from challenge with 200 ***P. yoelii*** 17XNL SPZ (PySPZ). After 3 doses of 10K PySPZ under PYR treatment at 3-4 weeks interval, 50% of TR were protected from challenge with 200 PySPZ. The results show that TR are a more stringent rodent model for evaluating WOV for malaria, and also confirm that WOV efficacy in a stringent model can be enhanced by increasing SPZ dose.

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EFFICIENCY OF REACTIVE CASE DETECTION WITH FOCAL DRUG ADMINISTRATION FOR MALARIA ELIMINATION: SIMULATION STUDY BASED ON CROSS-SECTIONAL SURVEYS IN RURAL SOUTHERN ZAMBIA

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As malaria elimination is pursued, identifying and treating all remaining infected individuals will be critical. Passive or reactive case detection (RACD) with rapid diagnostic tests (RDTs) may not identify individuals with sub-patent or asymptomatic infection who serve as persistent reservoirs. Focal mass drug administration is a strategy in which individuals residing within a specified radius of an index case are presumptively treated for malaria without testing. RACD and focal mass drug administration are potential strategies to eliminate parasite reservoirs in low transmission

settings. Household malaria surveys were conducted within Choma District, Zambia between 2009 - 2013. Questionnaires were administered and a blood sample was collected to detect ***Plasmodium falciparum*** by RDT and polymerase chain reaction (PCR). Household structures within the study area were enumerated using satellite imagery. Simulations were performed to extrapolate data from surveyed to non-surveyed households. Residents who were RDT-positive, reported seeking care at health facilities, experienced fever or headache as symptoms were considered index cases detected through passive surveillance. Radii of increasing size around each index household were examined to determine the proportion of households with an infected individual detected through RACD or treated through focal drug administration. Based on simulated data, cross-K function analysis identified spatial clustering of missed asymptomatic and sub-patent infection in residents of households around index houses. RACD and treatment of residents of households within 500 meters of the index case would have identified only 56% of all households with an RDT-positive asymptomatic resident and only 54% of individuals with sub-patent infection. RACD will identify asymptomatic individuals who do not seek care but will not be sufficient to eliminate malaria in this setting.

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IVERMECTIN FOR MALARIA CONTROL: MODEL VALIDATION TO EXISTING DATA AND DESIGNING TRIALS TO DETECT AN IMPACT USING CLINICAL AND ENTOMOLOGICAL METRICS

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Ivermectin has been identified as a potential new addition to the arsenal of interventions to control and eliminate malaria. The mosquitocidal impact of ivermectin is well documented and well understood, however it remains unclear how mass treatment using ivermectin in malaria endemic populations would impact transmission. Nearly all the studies investigating the effect of ivermectin on vector mortality use laboratory reared mosquitoes, which live considerably longer than wild mosquitoes. Cluster randomised trials are needed to see how the mosquitocidal effect translates to a population level impact, on both mosquitoes and humans. In this study we use a previously published mathematical model of the impact of ivermectin on malaria transmission to estimate the optimal timing and frequency of mass ivermectin treatment to maximise the impact on clinical incidence and entomological metrics (parity rate, survival rate and sporozoite rate). We perform sample size calculations to estimate the number of mosquitoes that would need to be caught and the number of clinical cases to be detected each day to significantly detect an impact on transmission. The optimal time to start a mass ivermectin intervention is during the peak of the rainy season, as this is when mosquito density is greatest. The total maximum reduction in clinical incidence is achieved when ivermectin is distributed monthly; however this reduction is over a longer period of time, and fluctuates more; meaning detecting a significant impact requires more monitoring and larger sample sizes. Treating every 2-3 week's results in a more sustained, albeit shorter, reduction in incidence, which would be easier to detect in a trial. To eliminate malaria, ivermectin alone is very unlikely to be the answer; however it could be a useful adjunct to other malaria interventions. In particular, adding ivermectin to mass treatment with an ACT could result in the same overall impact on transmission, but at a lower coverage level. Here, we argue that well designed, adequately powered cluster randomised trials are an essential step to determine the potential impact of ivermectin as a malaria control tool.

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EVALUATING THE COSTS OF IMPLEMENTING A PACKAGE OF INTERVENTIONS AND SURVEILLANCE SYSTEMS TO SUPPORT MALARIA ELIMINATION IN SOUTHERN PROVINCE, ZAMBIA: A MICRO-COSTING ANALYSIS

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The Zambian Ministry of Health and partners are implementing several interventions and surveillance systems in Southern Province, Zambia, in support of the national malaria control and elimination agenda. These include: (1) long-lasting insecticide-treated net distribution; (2) indoor residual spraying; (3) rapid reporting of malaria surveillance indicators; (4) systematic case investigation with reactive focal testing and treatment using rapid diagnostic tests and artemether-lumefantrine; and (5) population-wide mass drug administration and household-level focal drug administration with dihydroartemisinin-piperaquine. Accurate and comparable information on the costs of implementing these interventions and surveillance systems is necessary to inform operational and resourcing decisions for malaria elimination. We are estimating the costs of implementing these interventions and surveillance systems in 173 health facility catchment areas in ten districts in Southern Province in 2014-2015. Costs are being estimated from the providers' perspective using an ingredients-based approach. Data on resource utilization (labor, equipment, drugs, diagnostics, and other supplies), unit prices, and intervention and surveillance system outputs are being collected from programmatic records, interviews with program managers and staff, and other sources to estimate implementation costs and cost drivers for each intervention and surveillance system. This information will be useful for budgeting and planning; identifying opportunities to improve program efficiency; and informing additional analyses on the cost-effectiveness and budget impact of different approaches to malaria elimination.

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EXCLUSIVE BREASTFEEDING AND CLINICAL MALARIA RISK IN INFANTS IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO

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Malaria remains a significant health burden in the developing world, and particularly, in Sub-Saharan Africa. Children under the age of five years bear the greatest burden, suffering the highest rates of malaria-related mortality and morbidity. The World Health Organization recommends exclusive breastfeeding (EBF) for the first six months of life because it offers protection against various infectious diseases. However, the effects of EBF on the risk of malaria infection among infants are unclear. In the present study, 495 infants were evaluated for parasitemia using qualitative PCR on dried blood spots collected from the infants during their 24-week follow-up appointment. During the visit, information on infant feeding

practices, fevers in the past month, medication use, and bed net use was also collected. The main exposure of interest was EBF. The outcomes of interest were parasitemia, defined by a positive PCR, and clinical malaria, defined as parasitemia with fever. Of the 495 infants, 137 (27.7%) were EBF at 24-weeks. Malaria parasitemia was confirmed by PCR in 52 (10.5%) of the infants. Of the infants with parasitemia, 21 (4.2%) were reported to be febrile at the 6-month visit or anytime in the month prior to the visit, and were classified as clinical malaria cases. EBF was not associated with a reduced risk of parasitemia OR = 0.82 (95% CI 0.39, 1.62) but was associated with a reduced risk of clinical malaria OR = 0.13 (95% CI 0.00, 0.80). Of the 21 infants with clinical malaria, only one case was from an EBF infant. This study suggests that EBF is protective against clinical malaria and should be supported in malaria-holoendemic areas. However, to better understand the relationship between EBF, parasitemia risk, and malaria immunity, further investigation is needed.

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AN APPLICATION OF BOOSTED MODELS FOR PROPENSITY SCORE ESTIMATION WITH A MULTIFACTOR EXPOSURE IN AN INTERMITTENT PREVENTIVE THERAPY IN PREGNANCY FOR MALARIA OBSERVATIONAL STUDY

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The World Health Organization recommends the use of intermittent preventive therapy in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) in sub-Saharan Africa to prevent the adverse consequences of malaria in pregnancy. Increasing parasite resistance to SP has called into question the effectiveness of IPTp with SP. The effectiveness of IPTp-SP on multiple birth outcomes was assessed in eight sites in six countries with varying degrees of parasite resistance. In an attempt to minimize the effect of potential confounders, we fit propensity scores models. SP dose is best treated as a categorical variable (0, 1, 2, or 3+ doses) and standard, two-group propensity score models are suboptimal. Hence, we implemented generalized boosted models allowing the exposure to have a multinomial distribution. Boosted models are a machine learning technique that utilizes non-parametric, tree-based regression models to calculate propensity scores and derive weights designed to estimate the average treatment effect. Outcome models incorporated weights for each individual as sampling weights and included site as a cluster-level term. We focus on the application of this approach to -IPTp-SP observational data, explain the modelling process and the outcome model inferences, and discuss the impact on estimating SP treatment efficacy.

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SPATIO-TEMPORAL ANALYSIS OF MALARIA FEVERS IN A LARGE OPEN COHORT IN WESTERN KENYA; IMPLICATIONS FOR TARGETING 'HOTSPOTS'

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Geographic variation in malaria burden or risk of infection between villages and households within a village has been documented. As malaria transmission declines, these variations become more pronounced, giving rise to 'hotspots'. A hotspot of malaria transmission is defined as 'a geographical area within an endemic focus of malaria transmission where transmission intensity exceeds the average level'. Controlling hotspots may be strategic in moving towards malaria elimination if hotspots 'fuel' continual transmission within a larger area. Targeting control measures towards these hotspots is predicted to be more effective than spreading equivalent resources over larger areas or populations. Most of the information available about febrile malaria hotspots comes from data collected over small geographic areas and/or short time intervals. What is unclear from previous analyses is whether such hotspots are stable across multiple seasons and years. Here we present a spatio-temporal analysis of 3 years of morbidity data collected from a population census of 75,000 people. Every 6 months, individual morbidity events were recorded in a 'round' of data collection. We explore the stability in space and time of ***foci*** of self-reported malaria fevers. On average, residents reported 1050 malaria morbidity events in each 6-month period, 35% in children under-5 years. There were significant differences in incidence between rounds. Six-monthly rounds had between 2 and 11 significant clusters. Clusters in children under 5 did not coincide geographically with those in the 6 + years group. Clusters moved consistently from South to North in subsequent rounds, with the trend most apparent in young children. Cluster locations are not consistent across time; only 14.2% of the household falls into a cluster in more than one round. We will present further analyses on the relationship of locally moving clusters to dynamic factors such as local environment measured via satellite imagery, and population factors such as migration and births. Malaria fever hotspots that move in space over time present challenges to targeting control measures.

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A SCHOOL-BASED SEROLOGY STUDY TO VALIDATE USE OF ROUTINE DATA FOR TARGETING MALARIA INTERVENTIONS IN THE CENTRAL HIGHLANDS OF MADAGASCAR

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The Central Highlands in Madagascar are an area of unstable malaria transmission and remain epidemic-prone. Donors funded district-wide indoor residual spraying there from 2009-2011, but transitioned to targeted spraying in 2012, using health facility (HF) data to identify communes with the highest malaria incidence. To assess the validity of HF surveillance data to identify high-transmission ***foci***, we conducted a school-based serology study in 7 districts. Using serology as a gold standard for detecting ***foci*** in low-transmission settings, we assessed how well HF surveillance data identified high-transmission communes. From May-July 2014, teams visited 93 accessible communes (of 106) in the 7 districts, and two primary schools per commune. At each school, 30 children and their caregivers had capillary blood taken for a malaria rapid diagnostic test (RDT) and immunological analysis, and a brief questionnaire administered. Teams also collected school absenteeism data from registers and visited health facilities for information on data quality. Seropositivity was defined using mixture models for several antigens, including PfMSP1 and PfAMA1. School-based seroprevalence for each commune was compared to malaria incidence (HF RDT-positive cases per population). In total 12,771 children and parents at 186 schools had complete data. RDT positivity was very low at 0.53%, ranging from 0% (66/93 (71%) communes) to 13.3% by commune. Seroprevalence of MSP1 was 18.2% (range: 3.0% to 63.0% by commune) and for AMA1 was 26.5% (range: 4.4% to 70.4%). Correlation between seroprevalence and malaria incidence at commune level was 0.46 for MSP1 and 0.45 for AMA1, $p<0.001$ for each. HF data identified 21 of 30 communes with the highest transmission according to MSP1 and AMA1 (sensitivity=70% (95%CI: 50-85%)). Thus, routine HF surveillance data was relatively reliable for

identifying areas of higher transmission but missed 30% of malaria ***foci***. Additional analyses will be presented, including catalytic models of seroconversion rates and exploration of HF data quality and school absenteeism in predicting malaria ***foci*** to aid targeting of interventions.

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ASYMPTOMATIC AND UNCOMPLICATED MALARIA HOTSPOTS ACT AS YEAR-ROUND RESERVOIRS IN THE CHITTAGONG HILL DISTRICTS

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Malaria is endemic in 13 of 64 districts in Bangladesh, with the highest rates in the Chittagong Hill Districts, where ***Plasmodium falciparum*** is the predominant species. This study describes the epidemiology of asymptomatic and uncomplicated malaria infections in two unions of Bandarban, an area with a population of 23,000. Active surveillance for malaria infection was conducted by testing a random sample of 3,999 people from October 2010 to October 2012, without regard to symptoms. Of this sample, 53 tested positive for ***P. falciparum*** malaria through RDT and/or microscopy and 1 tested positive for ***P. vivax*** infection via microscopy, equating to a ***P. falciparum*** prevalence estimate of 13.4 (10.2-17.6) per 1,000 population. There was no difference in prevalence of these actively detected infections between the high and low seasons (p-value 0.78). These infections clustered in similar areas as symptomatic malaria cases. 72% of those with actively detected ***P. falciparum*** malaria reported at least one symptom commonly associated with malaria (fever, muscle aches, fatigue and headache) in the prior two weeks compared to only 21% of those not infected with ***P. falciparum*** malaria (OR (95% CI) = 9.3(5.0-18.3). We found a higher prevalence of these infections among those who were older, from tribal populations, did not use bed nets, and whose homes had animals, were closer to the forest, and were located less than 100 meters from an open drain or further than 100 meters from a river or stream. A subset of 1,275 people were selected from the active surveillance study to be followed every three months for 9 months to estimate malaria incidence. ***P. falciparum*** incidence was estimated to be 23.4 (15.2-36.1) per 1,000 person-years in this population. We conclude that hypoendemic asymptomatic and uncomplicated ***P. falciparum*** malaria continues in the Chittagong Hill Districts; the infections clustered in hot spots similar to the areas of symptomatic malaria and were associated with a number of household and demographic factors. Unlike symptomatic malaria which is highly seasonal, these actively detected infections were present year round and act as reservoirs for infection.

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ACUTE FEBRILE SURVEILLANCE IN CAMBODIA: MALARIA TRENDS AND CORRELATION WITH CLINICAL DIAGNOSIS

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Malaria is one of many potentially life-threatening infectious causes of fever in Cambodia. However, antimalarials are often given empirically, and confirmatory testing is not always available. With the rise of resistance in the SE Asia region, it is of increasing importance to ensure that medications are used judiciously on a population scale. For the benefit of the individual patients, it is important to ensure that other treatable causes of febrile illness are identified, if present. Data were reviewed from a subset of the Surveillance and Etiology of Undifferentiated Febrile Illness Study in Cambodia collected by Naval Medical Research Unit-2 Phnom Penh. Retrospective analysis of the passive surveillance data collected from 2010 through 2014 was conducted to assess for prevalence and the strength of association between clinical and laboratory-confirmed diagnoses, as well as the presence of other causes of fever. Overall, 13,618 febrile patient records were reviewed. Blood specimens were obtained for microscopy and rapid diagnostic testing. Of these, 4820 patients had been assigned a clinical diagnosis of malaria. 1477 patients (30.6%) were laboratory-confirmed for malaria by at least one test, giving an overall incidence of 10.8%. 78% of these patients were adults, and mean age was 25.1 years. Although a clinical diagnosis of malaria suggested a high odds of having malaria (OR 23.9, CI 20.0-28.6, p< 0.0001) and was highly sensitive (90.6%, CI 89.0-92.0%), the specificity was poor (71.3%, CI 70.5-72.1%) and positive predictive value was only 27.8% (CI 26.5-29.1%). Additionally, 73 of the laboratory-confirmed malaria patients were also confirmed to have either viral or rickettsial co-infection. Among febrile patients in whom alternate diagnoses were identified, influenza, dengue, chikungunya, and rickettsial illnesses were represented. It is therefore concluded that in an endemic region, it is prudent to maintain a high index of suspicion for malaria, including the presence of subacute malaria co-infection, but not to discount the possibility that other explanations for fever exist.

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SPATIAL AND TEMPORAL DYNAMICS OF *PLASMODIUM FALCIPARUM* INFECTION AND VECTOR DISTRIBUTION IN A SETTING OF HIGH COVERAGE OF CONTROL INTERVENTIONS

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The reduction in the malaria burden previously reported in The Gambia is largely due to the successful scaling up of control interventions. Understanding the current dynamics of malaria transmission in a context of high coverage of control interventions is critical to inform pre-elimination efforts. A prospective cohort study was conducted in twelve villages across the country. During the transmission season, all residents aged over 6 months old had a blood sample collected monthly for later molecular analysis and clinical malaria cases were captured by passive detection at local health facilities. Mosquito abundance and species distribution were determined by collections with CDC light traps, human landing catches and larval surveys. From June to December 2013, 4235 participants were followed up. The median age was 13 years (IQR 5, 28) and bed net coverage was 71.6% (2774/3876). Overall incidence rate of ***Plasmodium falciparum*** infections was 0.33 per person per year. Incidence was markedly heterogeneous, with lower values in the west (0.4 to 0.5 infections per person year) and higher in the east (1.4 to 2.8 per person year). Overall, 68 % of the infections were asymptomatic. The highest incidence of infection was in November (0.81 per person year) while the highest mosquito densities were found in September. ***Anopheles (An.) gambiae s.l.*** was the predominant species (80.4%) in all regions except the central region where ***An. funestus*** was predominant. Children aged 5-15 years (HR=1.4, 95% CI; 1.2-1.9) and adults (HR=1.2, 95% CI, 1.0 - 1.7) had a higher risk of asymptomatic infection. Individuals with severe anaemia had a significantly higher risk of being infected (HR=2.3, 95% CI; 1.5 - 3.6). Bed net ownership and female gender were associated with a reduced risk of infection. In The Gambia,

despite high coverage of control interventions, there is still significant ongoing malaria transmission. Additional interventions targeting residual transmission are urgently needed.

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THE BURDEN OF MALARIA INFECTION AMONG YOUNG AFRICAN INFANTS IN DIFFERENT MALARIA TRANSMISSION SETTINGS

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Infants are thought to be protected against malaria during the first 6 months of life largely due to transfer of maternal antibodies and presence of foetal haemoglobin. However, the true burden of malaria in infants is not well characterized and may be underestimated. A better understanding of malaria risk in early infancy is therefore critical for drug development and policy setting. A cross sectional survey was conducted in three West African countries: The Gambia, Benin and Guinea Conakry, representing areas of low, moderate and high malaria transmission respectively. Infants aged 0-6 months were enrolled and two children aged 1-9 years and 10-15 years in the same household were included to estimate the difference in risk of infection between infants and older children. Malaria diagnosis was by RDT, microscopy and molecular methods. Prevalence of antibodies against MSP119 was determined by indirect ELISA. A total of 6,761 children were enrolled; 2,270 from The Gambia, 2,276 from Benin and 2,215 from Guinea Conakry. Prevalence of malaria infection using PCR in infants up to 6 months old was 11.8%, with a significantly higher prevalence (21.7%) in Guinea Conakry compared to The Gambia (3.7%) and Benin (10.2%). Seroprevalence ranged from 5.7% in The Gambia to 41.6% in Guinea Conakry. Mean parasite densities were significantly lower in infants than in children 1-9 years in The Gambia and Benin. Malaria infection in infants was significantly associated with fever or history of fever and anaemia. Infants <5 kg had a significantly higher odds of malaria infection. There was a lower odds of malaria infection in infants aged 0-3 months with subsequently increasing odds of infection from 3 to 6 months of age. Malaria in young infants is therefore not rare, can be symptomatic, and has major health consequences, most notably anaemia. Our findings provide evidence that the period of perinatal protection may be shorter than 6 months and that 0-6 month is not a homogenous age group. The sizeable burden of malaria among young infants in endemic countries should be addressed by targeted preventive interventions, adequate drug formulations and treatment guidelines.

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ROLE OF ANTE-NATAL CLINICS (ANCS) ON THE COMPLIANCE OF USE OF LLINS AMONG PREGNANT WOMEN IN PORT HARCOURT RIVERS STATE

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A study to evaluate the role of Antenatal Clinics (ANCs) on the compliance of Long Lasting Insecticide Nets (LLINs) among some pregnant women in Rivers State was carried out. A total of 300 pregnant women (150 from government and 150 from private ANCs) were randomly selected and examined in this study. Information on LLINs usage and personal data were gotten through well-structured questionnaires. This study showed that 90% (135 out of 150) of the pregnant women attending ANCs in private healthcare centres used LLINs while 80% (120 out of 150) of the pregnant women attending ANCs in a government healthcare centre used LLINs. High LLINs compliance rates were recorded in both private and government healthcare centres and maybe due to increased malaria awareness and health lectures from ANCs health workers. LLINs have been shown to play a vital role in reducing malaria infection among pregnant women and should be encouraged.

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MALARIA SURVEILLANCE IN SELECT TANZANIAN MILITARY HEALTH FACILITIES THROUGH PASSIVE CASE DETECTION

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Malaria surveillance is the foundation for control and elimination programs, resource allocation, clinical research, and is essential for measuring the success of any intervention. Quality malaria surveillance is hindered by the challenges of performing accurate malaria diagnostics and the need to efficiently gather and analyze incidence data from multiple sources in a timely manner. Researchers at the Walter Reed Army Institute of Research (WRAIR) have partnered with the Tanzanian Peoples Defence Forces (TPDF) to implement a passive malaria surveillance strategy based on the use of malaria rapid diagnostic tests (RDTs) in 8 Tanzanian military camps. The strategy managed RDT quality and efficient data aggregation through the use of Deki Readers (***in vitro*** diagnostic devices used for reading and quality controlling commercially available RDTs). The readers were used with RDT testing when possible, which then transmitted de-identified patient data, diagnostic results and RDT digital images to Fionet (mobile software with a secured web-based portal). The data was available on-line for approved users for external QC, real-time monitoring, permanent data storage, and report generation. Quarterly assessments were conducted to collect data from RDT tests not performed with the readers. The malaria surveillance data from 2014 from the regions of Pwani, Morogoro, Tanga, Mara, Tabora, Kigoma, and Kagera will be presented. The data trends articulate the dynamic seasonality (based on the rainy seasons) or persistently high prevalence of malaria per camp, and variations in the timing of malaria seasons between sites. The data also highlighted which camps suffered the highest malaria incidence and were in most need of resources. However, device failures and lapses in power supply, RDT stock out, and poor telecommunication are known factors which have compromised the data integrity. The logistical challenges of implementation and general evaluations/observations of the strategy will therefore also be discussed. Despite the challenges, the Fionet system has been useful to efficiently collect quality passive malaria surveillance data.

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ASSESSMENT OF *PLASMODIUM FALCIPARUM* CASE-BASED SURVEILLANCE AT THE TWO MAJOR UNIVERSITY TEACHING HOSPITAL SOUTHWESTERN NIGERIA: A COMPARATIVE STUDY

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Guidelines focusing on National core indicators tools and methodology for monitoring and evaluating of Roll Back Malaria in Nigeria, in conformity

with other countries in Africa region has been developed. This study gives an insight on screened patient blood presented with ***Plasmodium falciparum*** focusing on diverse tools of surveillance ranging from age differences, gender status, education, level of awareness and individual therapeutic approach. Blood samples were collected from the fingers pricked of 200 patients (100 from each Hospital) who visited either of the two university teaching hospital (Obafemi Awolowo University (OAUTHC) and university college hospital (UCH), Nigeria) between August-December 2010 for Malaria parasite test. Rapid Test Kit Global device was used in detection of the presence of ***P. falciparum***. All data generated was presented with Chi-square description statistical analysis using Statistical Package for Social Sciences (SPSS) version 15.0 for windows. Difference were shown to be statistically significant where p<0.05. In the OAUTH, higher infection rate observed among age group <20 years (71.4%), followed by 20-30 years (18.2%) and less than 40 years (16.7%). The prevalence rate increased among male which constituted 23 (49.2%), compared with female subjects 15(31.3%), Un-educated 29(62.1%), Educated 20(10%), Semi-educated 43(37.2%) , Non -experience 8(25%), Non-drug compliance individuals 7(57.1%) Patients on malaria medication 70(38.6), Local herb users 8(25%). Also, 84(38.1%) showed higher level of awareness to ***P. falciparum*** while Non-awareness 7(42.9%), Indifference, 9(33.3%). Frequently infected individuals 22(40.9%), Non-frequent infected 30(36.7%). Rarely infected 36(36.17%), Non- response 12(41.7%). Patients utilized other means of protection, 22(31.8%), Insecticides, 36(55.6%), Mosquito net 40(22.5%). At the UCH, 12 positive results were recorded out of 100 patients tested. Higher infection rate was observed among age group <20(19%), 20-30(13%), >40(4.0%), The prevalence rate increased among female which contributed 9(12.2%), compared with male subjects which constituted 3(11.5%). Semi-educated 36(25%), Uneducated 21(4.8%), Educated 43(4.7%). Patients on malaria medication showed 65(13.8%), Non-drug compliance individuals 27(11.1%), Local-drugs/herbs users showed no attack, insecticides means of protection constituted 39(15.4%), Mosquito net 18(11.1%), Patients utilized other means of protection 43(9.3%), Level of awareness 84(38.1%), Non-awareness 7(42.9%), Indifference 9(33.3%). Frequent infected individuals showed 10(20%), Non-frequent 54(14.8%) and Rarely infected constituted 36(5.6%). Development of adequate information on ***P. falciparum*** infection risks is highly needed in the studied tertiary hospitals. Novel technologies to prevent, monitor, diagnose and efficient treatment of malaria mostly among lower age group must be adopted through Local and National Malaria Control Programme.

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BURDEN OF ASYMPTOMATIC AND SYMPTOMATIC FALCIPARUM AND VIVAX MALARIA ON CHINA-MYANMAR BORDER

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China launched a national malaria elimination program to end local malaria transmission by 2015, excluding the Yunnan province bordering Myanmar, Vietnam and Laos. Both ***Plasmodium falciparum*** and ***P. vivax*** are endemic in Yunnan presenting as they are elsewhere in the greater Mekong subregion (GMS). Malaria infection may be present in the human host with or without symptoms of illness, and both symptomatic and asymptomatic infections must be eradicated for successful malaria elimination. Asymptomatic infection poses a unique challenge to elimination strategies, since it typically harbors low density parasitemia, and the ability to detect it is limited by the sensitivity of detection methods as well as the volume of blood examined. The prevalence of asymptomatic malaria infection is higher when measured using sensitive molecular methods than when using standard diagnostic methods such as microscopy or rapid diagnostic tests (RDTs). We conducted a prospective cross-sectional field study in Mong Pawk and Laiza, two remote communities residing in the China-Myanmar border in July 2014 (rainy season) to estimate the prevalence of malaria, using multiplexed real-time PCR (RT-PCR), nested PCR, and RDT. Blood samples were collected on filter paper (50µL) from 765 participants (731 asymptomatic and 34 symptomatic). Overall the prevalence of ***P. falciparum*** and ***P. vivax***, detected by the multiplexed RT-PCR, was 4.71% (36/765) and 7.58 %(58/765), respectively. Important risk factors for asymptomatic infection, assessed by a logistic regression analysis, appeared to be the site and age. We found that 88% and 54% of asymptomatic infection were missed by RDT and nested PCR, respectively, whereas all symptomatic infections were identified uniformly by all three methods. We conclude that while RDT or nested PCR can sufficiently detect symptomatic infections in this setting, more sensitive detection methods, such as multiplexed RT-PCR, are required to accurately identify asymptomatic malaria and accurately measure the true prevalence of malaria infection to inform elimination strategies along the China-Myanmar border.

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THE ECOLOGY AND EPIDEMIOLOGY OF *PLASMODIUM* PARASITES CIRCULATING AMONG WILD CHIMPANZEE RESERVOIRS LIVING IN EAST AFRICA

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Molecular epidemiological analyses have traced the origins of human ***Plasmodium falciparum*** and ***P. vivax*** to ***Plasmodium*** parasites circulating among African great apes. These sylvatic hosts harbor a diverse assemblage of ***Plasmodium*** species capable of reaching notable prevalence (***Laverania*** relatives of ***P. falciparum***: up to 48%; ape ***P. vivax***: up to 8%). Although there is currently no evidence that humans are susceptible to ape ***Laverania*** parasites on contemporary timescales, surveys have revealed occasional cross-species transmission of ***P. vivax*** from apes to humans. Evaluation of the zoonotic potential of these reservoirs first necessitates elucidation of the ecological factors that underlie spatiotemporal variation in parasite prevalence. We exploited the infrastructure afforded by a long-term longitudinal study of wild chimpanzees in western Uganda to quantify the interaction between chimpanzee ecology and ***Plasmodium*** parasite infection. In agreement with previous analyses, chimpanzee fecal samples tested positive for mtDNA from three ***Laverania*** species—***P. gaboni*** (~13% of fecal samples), ***P. reichenowi*** (~6%), ***P. billcollinsi*** (~2%)—and ape ***P. vivax*** (~1%). Our analyses also confirm that the probability of infection declines with age, suggesting that analogous immunological processes may mediate ***Plasmodium*** infection of humans and chimpanzees. Other ecological factors, such as temporal variation in dietary quality, also influence the probability of infection. The nature of these relationships varies among representatives of the ***Laverania***, highlighting a source of epidemiological variation that has previously been overlooked. Finally, data from multiple chimpanzee communities highlight spatial variation in parasite prevalence, including the absence of infection within a Tanzanian population despite intensive sampling. Integration of these results with analyses of vector population dynamics and erythrocyte invasion assays will facilitate more rigorous evaluation of the co-evolutionary dynamics and zoonotic potential of ***Plasmodium*** parasites circulating among great apes in sub-Saharan Africa.

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THE USE OF SEROLOGICAL DATA TO PREDICT *PLASMODIUM FALCIPARUM* TRANSMISSION IN THE LOW-ENDEMIC NATION OF HAITI

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As ***Plasmodium falciparum*** (Pf) incidence in a region declines, strategies for the comprehensive detection of active infections become increasingly laborious and expensive. In addition, current technologies perform poorly for detecting very low density infections, and a paucity of cases creates difficulties in identifying ***foci*** of transmission. The human immune system generates a robust humoral response to many Pf antigens, and IgG production can persist for years following exposure, allowing for a greater historical perspective in assessing transmission. Here, we present comparisons of different analytical methods used for the interpretation of serological data from Haiti, which is thought to be an area of low, but persistent, Pf endemicity. Samples were collected in December 2012 from 5,479 individuals across 62 cluster-based community survey sites. Antibodies against the Pf antigens LSA-1, MSP-1, and AMA-1 were assayed through a bead-based multiplex assay, and data utilized for nationwide spatial analysis of serological intensity. Though the seroresponses of MSP-1 and AMA-1 were found to be independent, each antigen provided similar spatial estimates, showing approximately 20% of the study participants as being seropositive. Estimates for the prevalence of liver-stage antigen LSA-1 antibodies were much lower, with 1% of individuals seropositive. For each of the 62 sites, seroresponses were heterogeneous throughout the nation with a range of 0.3% to 46% persons seropositive within a site. Higher concentrations of seropositive individuals were found within the Central Plateau and along the southern coast of the Ouest Department. When analysis was restricted to persons under 10 years of age, these areas were also shown to contain higher percentages of seropositive children, suggesting stable and persistent transmission. This study gives further rationale for the use of serology to define areas of malaria transmission when parasite rates become low. Future serosurveys in Haiti will look to validate this approach, and provide better resolution in this nation of high Pf geographical heterogeneity.

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THE SUCCESSION OF MALARIA VECTOR SPECIES IN WESTERN KENYA

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The mass distribution of insecticide-treated nets (ITN) together with other interventions during the last decade has led to a decline in malaria and its vector populations. Due to behavioral variation and different insecticide resistance levels among vector species and larval habitat changes, major changes in vector species composition has been reported. However, the extent of species shift and vector community succession is largely unknown. Using data collected from 2001 to 2015, shifts in vector density and species composition were examined in western Kenya. Results showed there was a major population decrease in all malaria vectors after the 2006 mass ITN campaign, and the decline was more pronounced in ***Anopheles funestus***. However, decline in vector population were insignificant after the second round mass ITN distribution in 2011. In western Kenya highlands, species composition changed from 19 : 81 : 0% for ***An. funestus*** : ***An. gambiae*** s.s. : ***An. arabienesis*** in early 2000s, to 10 : 77 : 13% between 2006 and 2008, and then 70 : 27 : 3% in 2014. In the lowland, ***An. funestus*** accounted for 70% of total ***Anopheles*** in early 2000s and reduced to about 50% from 2006 to 2008, and it rebounded to 65% in the last 3 years. ***An. arabiensis*** accounted for about 1% of ***An. gambiae*** s.l, and increased to 40% in 2008 and this proportion remained stable. Surveys in 2014 indicated that more ***Anopheles*** rested in cowshed than in bedrooms and cowshed sampling captured almost equal number of ***An. gambiae*** s.l. and ***An. funestus*** in all sampling sites. Blood meal sources, species PCR identification, surveys for outdoor biting mosquitoes and larval habitats are currently being conducted. Overall, both proportion and density of indoor resting ***An. funestus*** rebounded significantly after 5 yearly of ITN scaling up. This study highlights the limitations of conventional indoor spray catch as a transmission surveillance tool as in the era of intensive use of ITN. Vector control strategies should target refugia of vectors and outdoor biting mosquitoes.

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DECREASE IN PRESUMPTIVE TREATMENT FOR MALARIA AFTER SCALE-UP OF RAPID DIAGNOSTIC TESTS: RESULTS FROM A NATIONALLY REPRESENTATIVE HEALTH FACILITY SURVEY

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Malaria transmission in Haiti is low and heterogeneous. The national malaria guidelines recommend diagnosis of febrile patients with an approved test, and treatment with chloroquine (CQ) plus primaquine (PQ) to cure illness and reduce ***Plasmodium falciparum*** transmission. Two years after conducting a baseline survey and scale-up of training and rapid diagnostic tests (RDTs), we conducted a national cross-sectional cluster sample survey of health facilities (HF). The sampling frame of all 907 HFs nationwide included data on geolocation and all-cause utilization. Sixty-four HFs were probability sampled, with 32 each from areas with relatively low or high risk, based on a 2011 malaria risk map; HFs with low utilization were under sampled. Survey teams conducted two-day visits from Nov 2014 - Jan 2015; outpatients present on survey days were systematically screened for fever. Post-consultation information was collected from patients and providers on illness history, HF-based test results, and prescribed medications. Gold-standard blood samples were analyzed by Haiti's reference laboratory. Of 64 sampled HFs, 56 were operational. Of 2,048 outpatients screened, 577 (28%) were febrile and attended for a first consultation, of these 451 (78%) consented and completed all survey procedures. Providers ordered a malaria test for 341 (76%) patients; and of these 209 (61%) patients had test results available by the end of the survey day (179 by RDT, 30 by microscopy). The HF-based tests confirmed malaria in four patients (all by RDT) and two of these were treated with CQ and PQ. Altogether, providers prescribed CQ to 26 (6%) patients, 13 were also prescribed PQ. Among those without test results available (n=242) 18 (7%) were prescribed an antimalarial. This is an apparent decrease in presumptive treatment compared to the 2012 results (31%). Malaria is an uncommon cause of fever among Haitian outpatients. Availability of RDTs at HFs has improved since 2012, and presumptive treatment has decreased. Additional effort could promote full implementation of the treatment guidelines.

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IMPACT OF THE SCALE-UP OF MALARIA CONTROL INTERVENTIONS IN MALI 2001-2012

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Enabled by significant increases in funding for malaria control, substantial scale-up of malaria control interventions has occurred over the past decade (2001-2012) in Mali. To assess the impact of this scale-up, an evaluation was conducted using the approach endorsed by the Roll Back Malaria Monitoring and Evaluation Reference Group (RBM MERG). Trends in all-cause childhood mortality (ACCM) were analyzed against trends in coverage of malaria control interventions as well as trends in other factors likely to affect child survival. The evaluation used data from nationally-representative household surveys to estimate intervention coverage and morbidity and mortality for surveys years (2001, 2006, 2010 and 2012) from the highly endemic areas of Mali (excluding Gao, Tombouctou, Kidal, and parts of Mopti). Household ownership of at least one insecticide treated net (ITN) increased from 49% in 2006 to 84% in 2012. Use of ITNs increased between 2006 and 2012 among the general population (21% to 61%), pregnant women (28% to 73%) and children less than five years of age (26% to 69%). The proportion of recently pregnant women receiving at least two doses of sulfadoxine-pyrimethamine for prevention of malaria during pregnancy increased from 4% in 2006 to 20% in 2012. ACCM declined from 225 to 95 deaths per 1,000 live births between 2001 and 2012. The declines of ACCM were greater in 2006-2012 (192 to 95 deaths per 1,000 live births) compared to 2001-2006 (225 to 192 deaths per 1,000 live births) corresponding to the period of rapid roll-out of malaria interventions. Changes in contextual factors such as vitamin A supplementation, diarrhea prevalence, and other socio-economic factors were also measured to develop a complete picture of mortality declines. Existing evidence from this analysis suggests that investments in malaria control interventions have contributed to observed declines in all-cause childhood mortality in Mali between 2001 and 2012.

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PREVALENCE OF *PLASMODIUM* SPP. AND ANEMIA AMONG SCHOOL CHILDREN IN TWO ECOLOGICAL ZONES IN GHANA

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Malaria remains a public health problem in Ghana. The prevalence of parasitaemia is usually determined by hospital-based surveys. However, a true representation of prevalence of parasitaemia in the population would be among healthy carriers of the malaria parasite in the population. We compare the prevalence of ***Plasmodium*** spp. and anemia among school children in the forest and the coastal zones of Ghana. Cross sectional surveys were conducted in a coastal savanna town (Cape Coast) and a town located in the Forest zone (Begoro) between October and November 2013, representing the end of the peak malaria transmission season. A total of 1037 pupils aged between 6 and 14 years were screened from three randomly chosen basic schools. The overall parasite prevalence was 21% (95% CI: 17%, 24%) and 20% (95% CI: 17%, 24%) in coastal and forest zones, respectively. In the coastal zone three species of ***Plasmodium*** were identified: ***P. falciparum*** accounted for 79% (95% CI: 73%, 86%) of infections whilst ***P. malariae*** accounted for 14% (95% CI: 9%, 20%) and P. ovale accounted for 0.7% (95% CI: -0.7%, 2.0%) of the infections. In the forest zone, two species of ***Plasmodium*** were identified: ***P. falciparum*** and ***P. malariae*** accounted for 75% (95% CI: 68%, 83%) and 22% (95% CI: 15%, 29%) of the infections, respectively. A few mixed infections (3% and 5.5%) were observed in the forest and coastal sites, respectively. Prevalence of asymptomatic parasitaemia was higher in male children than female children (56%; 95% CI: 49%, 63% vs 44%; 95% CI: 37%, 51%; P= 0.004), and was associated with Anemia in children (Odds Ratio = 0.42, P <0.0001) adjusting for study site. There was no evidence of a difference in the mean parasite parasitaemia in all ages groups (F= 1.69, P =0.187) and between the study sites (T= -0.0082, P = 0.9934). We conclude that though malaria parasite prevalence and mean parasitaemia, at the end of the peak transmission season, are same among school children in the coastal and forest zones of Ghana, asymptomatic parasitaemia is associated with gender and anaemia. Malaria interventions in basic schools should focus more on male children.

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ESTIMATING THE MALARIA ATTACK RATE IN A TANZANIAN MILITARY CAMP. A COMPARISON BETWEEN SUBJECTS FROM IMMUNE AND NON-IMMUNE POPULATIONS FOR FUTURE MALARIA PROPHYLAXIS, VACCINE AND TREATMENT STUDIES

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In Tanzania, malaria is the main cause of both in/out-patient attendance and deaths. Malaria is a major threat to non-immune travellers from malaria-free areas and peace keepers who are deployed to malaria endemic countries. This study is aimed at determining the malaria attack rate in groups from low and high transmission areas at malaria endemic national service (JKT) camps. This study was conducted at the Mgambo JKT camp in the Tanga region of Tanzania and involved military recruits originating from areas of low and high transmission. Consented recruits were screened negative for malaria, enrolled and followed-up biweekly for six months (a total of 12 visits). Blood smears were collected for malaria diagnosis by microscopy and subjects with positive malaria slides (study end point) were treated and censored. Each participant was screened for G6PD deficiency using a fluorescent spot test (FST). Of recruits enrolled (n=508), 67.1% came from low malaria transmission areas and 11.7% had G6PD deficiency. 94.1% completed the 12 follow-up visits or met the study end point. The malaria positivity rate was 43.5 % and there was a trend towards a higher positivity rate among recruits from low compared to those from high malaria transmission areas (46.0% ***vs*** 38.3%, p=0.099). The malaria attack rate was 72 cases/100 with higher rates among recruits from low compared to those from high malaria transmission areas (79 cases/100 ***vs*** 61 cases/100, p=0.08) but the difference was not statistically significant. After adjusting for G6PD deficiency, endemicity and use of bed-nets, the risk of getting a malaria attack was significantly higher among recruits from low transmission areas (OR=1.50, 95% CI =1.04 - 2.17, p=0.029). Recruits from low malaria transmission areas were at a higher risk of malaria due to lower malaria immunity. However, similar parasite positivity and malaria attack rates in the two groups were possibly due to the high malaria transmission in Mgambo and the surrounding area from heavy rains during the study period.

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AUTOCHTHONOUS MALARIA IN THE ATLANTIC FOREST OF THE RIO DE JANEIRO STATE, BRAZIL

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Malaria transmission was considered eliminated from Rio de Janeiro (RJ) in 1968, but some autochthonous sporadic cases are still described. From 2006 to 2014, 14 cases acquired in the state were diagnosed and treated at the National Institute for Infectious Diseases of Fiocruz, in tourists coming from areas covered by the Atlantic Forest. All were vivax malaria cases, with atypical parasites (morphologically different parasites with less numerous merozoites within the schizonts) as compared with the classical ***Plasmodium vivax***. Antibodies (Ab) to all ***P. vivax*** CSP (circumsporozoite protein) variants, with predominance of VK210, were observed in patients. Thirty-six percent of patients were positive for the presence of Ab to the P.malariae/brasilianum CSP and 73 % had positive results for anti ***P. falciparum*** CSP Ab. We estimated the prevalence of ***Plasmodium*** infection by serological and molecular tests in the 220 case-contacting neighbors in an area within a perimeter of five kilometers around the index case. Positive serology for MSP1-19 (Elisa test) ***P. vivax*** in the neighboring contacting-population was around 58%, with titles ranging from 1:100 to 1:3200. All individuals presented, however, as negative in the molecular tests. In all areas where serological and molecular tests were performed we have captured and identified vectors circulating in the locality. Most of them were ***Anopheles*** (***Kerteszia) cruzii*** (Dyar and Knab, 1908) and none was found carrying parasites. Complementarily, we found a death free-living ***Alouatta*** from the Atlantic Forest with positive PCR (***P. vivax***/***P. simium***). These data point to the circulation of ***Plasmodium*** in the areas and, although they can support the hypothesis of autochthonous malaria as a zoonosis in the region, they also raise some questions on the transmission mechanisms: ie; the apparently high number of subjects carrying antibodies contrasting to the reduced number of malaria cases as well as the presence of almost only tourists among patients with symptomatic infection.

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A PROSPECTIVE COHORT STUDY REVEALS NO ASSOCIATION BETWEEN ABO BLOOD GROUPS AND THE RISK OF UNCOMPLICATED MALARIA

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Although several studies have demonstrated an association between blood group O and reduced risk of severe ***Plasmodium falciparum*** malaria, the relationship between ABO polymorphisms and the risk of uncomplicated malaria remains unclear. To address this knowledge gap, we conducted a prospective cohort study of 510 children aged 3 months to 12 years in Mali during an intense 6-month malaria season. During the study period, ***P. falciparum*** infections and cases of febrile malaria were detected through bi-weekly PCR and weekly active clinical surveillance, respectively. The prevalence of ABO blood groups in this cohort was 25.5% A, 29.4% B, 6.9% AB, and 38.2% O. There was no association between ABO blood groups and the risk of ***P. falciparum*** infection. Multivariate analyses adjusting for confounders of malaria risk revealed no association between ABO blood groups and the time to febrile malaria, the risk of experiencing febrile malaria, or the incidence of febrile malaria. Finally, parasite densities during acute malaria were similar across ABO blood groups. Therefore, unlike sickle cell trait, which has clearly been linked to a reduced risk of both severe and uncomplicated febrile malaria, this study indicates that the protective effects of blood group O are restricted to severe malaria.

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THE USE OF GPS DATA LOGGERS TO DESCRIBE SPATIO-TEMPORAL MOVEMENT PATTERNS AND CORRELATIONS WITH MALARIA RISK IN AN AREA OF HYPERENDEMIC MALARIA IN NORTHERN ZAMBIA

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Human movement has been identified as a potential driver for malaria transmission; however, the importance of this factor in different epidemiologic settings has not been fully examined. Nchelenge District, Luapula Province in northern Zambia has hyperendemic malaria, with a parasite prevalence greater than 50% by rapid diagnostic test (RDT). Participants enrolled in a longitudinal cohort as part of the Southern Africa International Centers of Excellence for Malaria Research (ICEMR) were invited to participate in a population movement study using GPS data loggers. During each bimonthly longitudinal household visit, 12-15 participants aged 13 years and older were asked to carry a logger for one month. Data will be collected for a total of one year starting in August 2014, resulting in at least 80 participants over six data collection periods. GPS devices are motion activated and record GPS coordinates every 2.5 minutes while the participant moves. Following the month of data collection, the loggers were returned and participants answered a questionnaire and provided a finger prick blood sample for malaria RDT, microscopy, and PCR to detect ***Plasmodium falciparum*** DNA. Data were uploaded into ArcGIS and R statistical programs to produce movement tracks and density maps. Analyses were conducted to correlate cases of incident malaria with time spent near the home, use of bed nets, history of indoor residual spraying, and time spent in areas identified to be at high risk of malaria based on risk maps. The relative importance of malaria risk at the household compared with malaria risk in areas of daily movement are compared. To date, 54 participants have contributed over 1,500 days of data comprising 115,000 GPS points. Half of the participants had incident malaria infections after their month of participation, with incidence slightly higher in men (57%) than women (47%). Data collection will be completed in June 2015.

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PROGRESSIVE AND SUSTAINED DECLINE OF MALARIA BURDEN FOR NINE YEARS AT THE COMMUNITY LEVEL IN NORTHEASTERN TANZANIA

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Passive Case Detection involving early diagnosis and prompt treatment of malaria was introduced in 2006 in four villages (two in each of the lowland and highland strata) of Korogwe district, North-eastern Tanzania. A significant reduction in the incidence of malaria and parasite positivity rates was previously reported. We present further updates on the burden of malaria in this area with changing malaria epidemiology. In 2006, individuals with history of fever within 24 hours or fever at presentation (axillary temperature ≥37.5°C) were presumptively treated by Community Owned Resource Persons (CORPs) using sulphadoxine/pyrimethamine. From February 2007, individuals aged ≥5years with positive rapid

diagnostic test for malaria (mRDTs) were treated with artemether/ Lumefantrine (AL) while under-fives were treated irrespective of mRDT results. Logistic and Poisson regression were used to assess the changes in parasite positivity and incidence rates. Between January 2006 and December 2014, 21934 cases aged 0 - 98 years were attended whereby 24.6% were under-fives and majority (68.9%) was from lowlands. Overall, 30.9% of the cases had fever at presentation and the positivity rate was 15.5%. Parasite positivity rate was significantly higher in the lowlands compared to highlands (16.6% Vs 13.4%; p<0.001). After adjusting for the effect of year, age and altitude strata, the positivity rate decreased by 78% between 2006 and 2014 (OR =0.22, 95% CI = 0.16 - 0.31) and the incidence rate decreased by 75% (IRR =0.25, 95 % CI =0.18 - 0.34) with a significant decline in lowland compared to highland villages (p<0.001). However, the proportion of cases with fever remained unchanged (>30%). Despite a continued decline in the incidence of malaria and parasite positivity rates, fever cases remained unchanged. Future studies should address causes of persistently high level of febrile illnesses.

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HIDDEN COST OF INFECTION: MALARIA ACCELERATES TELOMERE DEGRADATION

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Approximately one half of the global population lives in malaria endemic areas where majority of malaria infections occur in the form of chronic asymptomatic infections. The potential for any long-term effect of such chronic/repeated infections, where individuals either completely recovered or carry asymptomatic infections is poorly understood. We have recently shown accelerated ageing and reduced lifespan mediated through faster telomere degradation in birds with chronic asymptomatic malaria. Great reed warblers that pick up various species of malaria parasites while wintering in the tropics and become asymptomatically infected for life after an initial acute malaria. Although these birds are chronically infected with malaria parasites, we found that these asymptomatic infections reduce lifespan, as well as lifetime number and quality of offspring. Furthermore, these delayed fitness effects were mediated through faster degradation of telomeres, a result supported by additional controlled infection experiments on birds in captivity. In human accelerated telomere degradation has been found in chronic and inflammatory diseases. Whether malaria infections also have effect on telomeres in humans, we have analyzed travelers with single treated ***Plasmodium falciparum*** malaria infections that were repeatedly followed over a year at Karolinska University Hospital in Sweden. We found that malaria infections accelerate telomere degradation in humans even after successful treatment. Furthermore, telomere length restored in these patients one year post infection. Further understanding of dynamics of telomere attrition and restoration in relation to intensity of malaria exposure and chronicity of infections is needed.

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MALARIA EPIDEMIOLOGICAL STRATIFICATION IN VIETNAM, 2014

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Malaria stratification is a classification of areas according to the risk of malaria and its level of endemicity. The malaria epidemiological stratification over the last five year period was implemented in all provinces (58) and cities (5) of Vietnam in 2014. The objective of this re-stratification was to identify different levels of malaria endemicity in order to develop more effective interventions for malaria control and elimination, based on the current malaria situation and national plan. Determination of indigenous malaria cases is a prerequisite for classifying each malaria zone. The seven indicators for the zone classification used were 1.) Average number of confirmed cases/1,000 population over the last 5 years, 2.) Presence of at least one of the three major malaria vectors, 3.) Socioeconomic disadvantaged or border commune, 4.) Poor health system, 5.) Drug-resistant parasites, 6.) Chemically-resistant mosquitoes, and 7.) Migratory populations. Each indicator was scored and the sum of the scores was used to define the level of endemicity and priority for interventions. This score was used to characterize each commune (county equivalent) into one of five zones (no malaria transmission, area at risk for reintroduction of malaria, low (>0-1/1,000), medium (1-5/1,000), or high (>5/1,000)). The areas free of malaria included 5,840 communes (52% of communes, 64,541,280 people), while the area at risk of malaria resurgence included 3,448 communes (21,445,395 people). The low malaria endemic areas include 1,095 communes (7,710,946 people); the medium malaria endemic area consists of 529 communes (2,813,221 people) ; the highly malaria endemic areas consisted of 240 communes (1,167,628 people). Based on these numbers 48% communes and 34% of the population are in endemic areas, with only 7% communes and 4% of the population in moderate to high transmission areas. This new stratification will be used to set priorities and target malaria control and elimination efforts to the areas where they are most needed. It will highlight areas where the malaria program needs extra effort and helps to make the best use of limited resources.

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IMPACT OF ONE VS. TWO ROUNDS OF ANNUAL INDOOR RESIDUAL SPRAYING (IRS) ON MALARIA PARASITAEMIA IN CHILDREN IN NORTHERN GHANA

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The frequency of indoor residual spraying (IRS) is crucial for the effectiveness of IRS and the optimal allocation of malaria control resources. This study was conducted between November 2010 and April 2013 to compare the impact of annual vs biannual pyrethroid IRS in Bunkpurugu Yunyoo District (BYD), in the Northern Region of Ghana, which has high malaria transmission. BYD has a peak malaria season in September-November, at the end of its single rainy season, and a low malaria season in March-April at the end of its dry season. For this study, half of BYD (Area A) was sprayed once a year prior to the rainy season and the other half (Area B) was sprayed prior to both the rainy and dry seasons, using a pyrethroid, alphacypermethrin (Fendona 5 WP, 25mg/m2 application rate) in both areas. IRS impact was measured by peak and low season parasitemia surveys among children under 5 years, starting with the pre-IRS 2010 peak and 2011 low seasons as baselines. Probability proportional to size estimates (PPSE) gave a minimum sample size of 828 children per survey in each IRS area. All selected children were tested for parasitaemia by microscopy, and a questionnaire was used to collect demographic and health information. 826 to 1,022 children in each area were surveyed per round. For peak transmission season, parasitemia prevalence at baseline was marginally lower in Area A compared with B, 49.6% (95% CI: 46.2, 53.1) vs 54.9% (95% CI: 51.9, 57.9), respectively. End-of-study peak season prevalence significantly declined to 44.4% (95% CI: 41.3, 47.6) in Area A whilst peak season prevalence in Area B remained unchanged at 50.7% (95% CI: 47.6, 53.7). For low season, the baseline prevalence in Area A, 31.7 % (95% CI 28.8, 34.8), was significantly lower than in Area B, 39.1% (95%CI 35.6, 42.6). End-of- study low season prevalence

significantly declined in both sites to 24.1% (95% CI: 21.5, 26.9) in Area A and 26.3% (95% CI: 23.6, 28.9) in Area B. There is no consistent evidence that IRS with pyrethroids applied twice a year was more effective than once a year in this district, where there is a short uni-modal transmission season.

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HIGH PREVALENCE OF MALARIA DESPITE PREVENTION AMONG PREGNANT WOMEN ATTENDING A DISTRICT HOSPITAL IN DOUALA, CAMEROON

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Gestational malaria is still a major health problem in Cameroon despite free delivery of IPT nationwide. Epidemiological data are needed to monitor the effectiveness of the preventive measures undertaken so far. This study aimed at determining an update of the prevalence and determinants of malaria in a District Hospital in Douala; the biggest town of Cameroon. A cross-sectional study was conducted at the District Hospital of Cité des Palmiers in Douala for a three-month period (May - July 2013). Pregnant women were met within the hospital and a questionnaire was administered to collect socio-demographical, midwifery and malaria-related data. In addition Giemsa stained thick film was performed for malaria diagnosis at their admission. A total of 121 pregnant women aged 17 to 42 were included in the study. The overall prevalence of malaria infection was high (81/121; 66.94%) among the women. However, the majority of infections were asymptomatic (67/121; 55.37%) despite the high coverage of IPT (92.6%). IPT was not timely related to routine diagnosis. The rate of ITN ownership was 63.6% and malaria infection varied significantly with respect to parity ($9\chi 2$ = 6.23; p = 0.016). Women living in wooden houses had more mosquito nets and were less infected than those living in block houses. Negative correlations, however not significant were observed between malaria infection and IPT (z = - 1.38; p = 0.16) and also between malaria infection and antenatal consultation (z = -1.41; p = 0.16), More than two-third of pregnant women were infected with malaria despite multiple preventive actions undertaken by the Cameroonian government. The high rate of asymptomatic infection suggests that active case detection could be helpful as well as synchronization with prevention tools like sanitation and the use of contact repellents.

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MALARIA CHARACTERISTICS AND TRENDS, UNIVERSITY HOSPITAL, TEGUCIGALPA, HONDURAS, 2000-2014

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The University Hospital is the main public health hospital in Honduras. Parasitology Service, Clinical Laboratory Department, performs malaria diagnosis using Giemsa stained thick/thin smears/slide (Mon-Fri, 6 am – 1 pm). Patient registration forms were reviewed with the aim to describe malaria characteristics and trends for the period 2000-2014. A database was prepared from daily/monthly registration reports. Microscopic diagnosis is described as number and average examined blood smears and positive smears by parasite species/year. For the period 2007-2014, epidemiologic and clinical characteristics of positive patients are described, including age, sex, origin of infection, pregnancy status, and therapeutic response. During the 15-year period, 723 annual average requests were analyzed (range 333-1043), including 677 average new cases (301-962), and 30 average post-treatment controls (9-59). A total of 665 malaria cases were diagnosed (44 annual average, range 15-84), 87.5% (582) ***Plasmodium vixax***, 11.4% (76) ***P. falciparum***, and 1.0% (7) mixed infections; 64.1% (426) subjects >15 years old, 20.0% (133) 5-14 age group, 6.6% (44) 1-4 age group and 3.8% (25) <1 year old; 5.6% (37) no age recorded. In 2007-2014, there were 27 cases in pregnant women, average age 21.4 years (14 – 42), 92.6% (25) ***P. vivax***. In 2009-2014, there were 228 malaria cases from which the therapeutic response was evaluated in 127 (55.7%), 118 (51.8%) ***P. vivax***, 8 (3.5%) ***P. falciparum***, 1 (0.4%) mixed infections. The origin of infection was traced to Francisco Morazán 38.6%, other departments 48.7%; Congo, Africa 0.4%; blood transfusion 0.9%, and no information 11.4%. All cases showed good clinical and parasitological response to chloroquine 25 mg/Kg in 48 hours, except one case that required a second cycle. In the last three years, annual cases are below the 15-year period annual average number (44) and are decreasing (40, 26, 15). Because its characteristics and performance in clinical care, academic and research activities, the University Hospital plays an important surveillance and response role, contributing to the activities for malaria elimination in Honduras.

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THE IMPACT OF FIONET™ TECHNOLOGY ON PASSIVE MALARIA SURVEILLANCE IN TANZANIAN MILITARY HEALTH SYSTEM

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Malaria is a major cause of death and illness in Tanzania. In the military health system, malaria surveillance relied on microscopy data; the reliability of malaria microscopy is highly dependent on technician expertise. Recently, malaria rapid diagnostic tests (RDTs) and Fionet™ were deployed to support malaria diagnostics in selected military health facilities. Fionet™ is a digital health system that improves field performance and oversight of rapid diagnostic testing. Fionet™ is a web-based workflow guidance designed for use with standard mobile devices and the Deki Reader™ (DR). It provides step-by-step guidance for performing RDTs. The DR captures a digital record of each RDT performed and allows web-based oversight of RDTs is enabled to provide real time case management. Fionet™ was deployed in eight military health facilities throughout Tanzania. 85% (33648/39585) of RDTs were analyzed and uploaded through Fionet. Fionet enabled real-time data tracking and reporting. 33% and 80% of all data from the sites were uploaded in <1 hour and 24 hours respectively. Prior to Fionet™ implementation, malaria surveillance data typically took 30-40 days to reach TPDF headquarters and did not affect clinical decision-making. Also, remote monitoring enabled managers to instantly correct site personnel, thus reducing errors in preparation and interpretation of test results. Due to the availability of real time data, malaria incidence was determined at each site and the needed supply chain support was more efficient by reducing waste. In addition, collected surveillance data demonstrated which TPDF camps suffered the highest malaria incidence, which supported the selection of study sites for further epidemiological studies through active malaria case detection. Fionet™ has improved diagnostic capacity, real-time malaria data tracking and reporting. It has affected informed decision making positively.

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IMPACT OF INTERMITTENT MASS SCREENING AND TREATMENT (IMSAT) ON COMMUNITY MALARIA PARASITEMIA PREVALENCE IN AN AREA OF HIGH TRANSMISSION - KENYA 2013-2014

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The asymptomatic malaria infected population contributes significantly to the infectious reservoir. Strategies targeting this population may help to reduce malaria transmission. We implemented a multi-year, community-based, two-arm cluster randomized controlled trial to evaluate the impact of intermittent mass screening and treatment (iMSaT) on malaria parasitemia prevalence in an area with high long-lasting insecticide-treated bednet (LLIN) coverage in Siaya County, Kenya. Villages within 3km of each of 10 health facilities were grouped into two clusters. One cluster was randomly assigned to intervention- the other to control. All residents ≥1 month of age in intervention communities (n>27,000 persons) received three rounds of mass screening with malaria rapid diagnostic tests (RDTs) and all positives were treated. Coverage (percent of intervention population screened) was 73.0% in September 2013, 79.5% in January 2014, and 75.0% in April 2014. Cross-sectional surveys (XSS) were conducted during peak malaria transmission in July 2013 (baseline) and 2014 (post-intervention). In each XSS, twenty compounds from each of twenty clusters were randomly selected from a complete list of compounds. Finger-prick blood samples were tested for malaria by light microscopy. Prevalence ratios (PR) were analyzed using generalized estimating equations. Study population demographics and LLIN coverage and use were similar between arms and surveys. Baseline prevalence (n=1893 residents) in the control and intervention arms were 37.6% (95% confidence interval [CI], 33.3-42.4) and 34.3% (95% CI, 29.3-39.5), respectively. After three rounds of iMSaT, malaria prevalence (n=1934 residents) in the control and intervention arms were 38.7% (95% CI, 34.4-43.6) and 32.0% (95% CI, 26.6-38.4), respectively. After one year of three rounds of iMSaT, the prevalence ratio for the relative change in malaria parasitemia prevalence between the intervention and control communities was 0.91 (95% CI: 0.78-1.06), a relative decrease of 9% (p=0.21). The second year of iMSaT is underway and data from the third XSS will be available by August 2015.

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ASSESSING THE FEASIBILITY OF MALARIA ELIMINATION EFFORTS AMONG WORKERS EMPLOYED ON PRIVATE PLANTATIONS IN CAMBODIA

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Resistance of ***Plasmodium falciparum*** to artemisinin is a serious global health challenge, and has been increasing in South-East Asia. As malaria prevalence declines in this region, new approaches to target asymptomatic carriers must be adopted to eliminate ***P. falciparum***. Mobile and migrant populations, especially those working in forests and plantations, are considered to be at high risk and pose considerable risk of spreading artemisinin resistant malaria, both within and across borders. We carried out a plantation worker survey in Northeast Cambodia to understand better how to target these groups. Forty plantations were visited in June (dry season) and October (wet season). 4,227 workers were interviewed and had blood samples taken. Plantation workers were classified as temporary or permanent, and individual and plantation level risk factors for malaria infection were analysed. Malaria prevalence was very low: 0.9% in the dry season and 1.1% in the wet season (p=0.11) by polymerase chain reaction. The strongest risk factor was plantation size, with the odds of infection by P.falciparum decreasing as the plantation size increased in the dry season (odds ratio=0.084, p=0.008), and the wet season (OR=0.81, p=0.009). High levels of parasitemia was clustered around certain plantations, with three having prevalence of over 10% in the wet season and one with a 30% prevalence. Migrant workers were no more likely than permanent workers to have malaria in the dry season (0.83% and 0.85%, p=0.904) and more likely to be parasitemic in the wet season, although not significant (1.9% and 0.9%, p=0.109). Forest exposure and previous travel are shown to affect infection in the wet season. Low malaria prevalence on plantations suggests that mass drug administration on plantations is not likely to be the most effective way to target this population. Seasonal targeting of certain workers and on certain plantations may be the best approach.

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PLASMODIUM VIVAX TRANSMISSION IN AFRICA

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Malaria in sub-Saharan Africa has historically been almost exclusively attributed to ***Plasmodium falciparum***. The high prevalence of Duffy negativity provided a rationale for excluding the possibility of ***P. vivax*** (***Pv***) transmission. Current diagnostic and surveillance systems are not designed to identify or report ***Pv*** accurately, resulting in a void of routine epidemiological data about its significance in sub-Saharan Africa. Review of varied evidence sources, including traveller infections, community prevalence surveys, local clinical case reports, entomological and serological studies, however, contradicts dogma. Here, these data reports are weighted in a unified framework to reflect the strength of evidence of indigenous transmission they provide in terms of diagnostic specificity, magnitude of individual reports and corroboration between evidence sources. Strong evidence of transmission was available from 12 of the 47 countries evaluated (26%), distributed across West, Central, and Eastern Africa, while four had no evidence of transmission. Approximately 86 million Duffy positive hosts were at risk of infection in Africa in 2015. Analysis of the mechanisms sustaining ***Pv*** transmission across this continent of low frequency of susceptible hosts, found that ***Pv*** prevalence was consistent with transmission among exclusively Duffy positive sub-populations. Finally, reports of apparent Duffy-independent transmission are discussed. While ***Pv*** is evidently not a major malaria parasite across most of sub-Saharan Africa, the evidence-base presented here highlights its widespread low-level endemicity across the continent. An increased awareness of ***Pv*** as a potential malaria parasite, coupled with policy shifts towards the use of combination rapid diagnostic tests and species-specific reporting, will allow a robust assessment of the true public health significance of ***Pv*** in Africa, a parasite currently invisible to most public health authorities, but which can cause severe clinical illness and requires specific control interventions.

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URBAN MALARIA INCIDENCE AND GEOGRAPHIC CLUSTERING ACROSS AN URBAN-TO-RURAL CONTINUUM: RESULTS FROM A CASE-CONTROL STUDY OF CHILDREN IN BLANTYRE, MALAWI

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Urban areas pose challenges to malaria prevention because the location of transmission is often unknown, household-level environmental conditions are highly diverse, and the definition of "urban" is debatable. As part of a case-control study of malaria among under-fives in and around Blantyre City, Malawi, we analyzed disease patterns and risk factors associated with environmental, demographic and infrastructural characteristics. Blantyre (~1.1M pop.) has highly diverse land use/land cover (LU/LC), house density, facilities and services inside its ~225 sq km city limits. Active surveillance at 6 health facilities (HFs) inside and peripheral to the city was undertaken from April 2012 - October 2014. Children with malaria symptoms who were PCR-positive for ***Plasmodium*** infection (cases) were age- and location-matched with one or two PCR-negative children (controls) from the same HF. A total of 202 cases and 353 controls met the eligibility, consent, and matching criteria. Follow-up household visits determined each house location, construction traits, and peri-domestic LU/LC. A standardized questionnaire addressed demographics, malaria prevention, and travel-associated risk. GIS analyses of household location, geographical features, and satellite-image derived LU/LC compared cases and controls, controlling for demographic and behavioral factors. The urban-rural status of each household was classified by measures e.g. census population, house density, peri-domestic agriculture, proximity to infrastructure, and a PCA-derived "urbanicity score." Multivariate statistics demonstrated no simple associations between case-control status and standard definitions of "urban." However, household-level LU/LC and geographic features were highly predictive of malaria risk. Spatial statistical analyses suggested clustering unrelated to governmental urban designations, whereas derived classifications involving LU/LC and other measures were more predictive. Our findings have important implications for household- and community-level malaria risk, and for where malaria control efforts might be most effective.

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USE OF LONG-READ DEEP-SEQUENCING TO CHARACTERIZE GENETIC DIVERSITY AND PATHOGENIC VARIANTS OF VAR2CSA IN WOMEN WITH PLACENTAL MALARIA

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Placental malaria causes maternal anemia, severe malaria, infant death, preterm birth and low birth weight (LBW), largely owing to ***Plasmodium falciparum*** sequestration in the placenta. The sequestration results from the binding of infected erythrocytes (IEs) to placental chondroitin sulfate A (CSA); this interaction is mediated by ***P. falciparum*** protein VAR2CSA expressed on the surface of IEs. Within VAR2CSA, the ID1-DBL2x-ID2 region both binds CSA with similar avidity as the entire protein as well as elicits cross-reactive immune responses ***in vitro***. Therefore, the ID1-DBL2x-ID2 region is a promising candidate for vaccines against placental malaria. Such a vaccine may be enhanced by the identification of pathogenic variants of VAR2CSA, owing to the great sequence diversity of var2csa genes. However, the extent of genetic diversity of the ID1-DBL2x-ID2 region is incompletely understood, and no pathogenic var2csa genotypes have been described. Using placental and peripheral blood samples from 150 ***P. falciparum*** infected pregnant women in Benin and Malawi, we are conducting a molecular epidemiologic study to use next-generation sequencing technology in order to characterize the genetic diversity of the 1.5kb ID1-DBL2x-ID2 region and identify pathogenic variants that are associated with adverse birth outcomes. Alpha (within group) and beta (between groups) diversity will be compared between pregnant women of differing gravidities and birth weight. Additionally, genetic diversity will be compared between paired peripheral and placental samples from women with malaria at delivery to better understand the role of var2csa in cytoadherence of IE to the CSA and the subsequent sequestration in the placenta. Initial testing of 7 clinical samples from Malawi yielded 15 unique haplotypes occurring at a wide range of frequencies (80-1%), indicating that there exists a large reservoir of quantifiable var2csa variants. By exploiting this diversity using new sequencing technologies and analytic approaches, the results from the study will help elucidate the pathogenesis of malaria in pregnancy and directly inform on-going vaccine development efforts.

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A DEEP SEQUENCING APPROACH TO ESTIMATE MALARIA COMPLEXITY OF INFECTION IN THE DEMOCRATIC REPUBLIC OF CONGO

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In areas of stable malaria transmission, asymptomatic malaria infections can consist of multiple genetically distinct ***Plasmodium falciparum*** strains. These multi-strain ***P. falciparum*** infections can impact malaria transmission dynamics, reduce treatment efficacy, influence clinical outcomes, and facilitate the emergence of novel malaria parasites with increased virulence and drug resistance mechanisms. The number of genetically distinct ***P. falciparum*** strains within a host is defined as the Complexity of Infection (COI). In this study, we utilized a deep sequencing approach designed to detect minor frequency alleles and estimate ***P. falciparum*** COI. We targeted a subset (n=81) of human DNA samples collected as part of the 2007 Demographic and Health Survey (DHS) in the Democratic Republic of Congo (DRC). Additional samples (n=965) were grouped based on geographic locations and pooled together to generate 88 population clusters distributed across the DRC. DNA extracted from each blood sample was utilized as template for PCR amplification of the polymorphic single-copy ***P. falciparum*** apical membrane antigen 1 (***pfama1***) gene. Resulting PCR products were deep sequenced on the Ion Torrent Platform and ***pfama1*** haplotypes were determined via a custom bioinformatics pipeline. Potential confounders, such as age, sex, geographic location, and HIV status were analyzed to determine the impact on the ***pfama1*** haplotypes and COI within a single individual.

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IN VIVO EFFICACY AND SAFETY OF ARTEMETHER/ LUMEFANTRINE VS DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED MALARIA AND ASSESSMENT OF PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE

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Following changes of malaria treatment guidelines in Tanzania in 2006, Artemether/Lumefantrine (AL) was the only ACT which was introduced for treatment of uncomplicated falciparum malaria. However, alternative drugs such as dihydroartemisinin-piperaquine (DP) are urgently required to ensure effective case management.To assess the efficacy and safety of DP and AL for treatment of uncomplicated falciparum malaria and the role of parasite genetic/genomic factors on the treatment outcome among patients treated with these ACTs.This was an open-label, randomized, non inferiority trial that recruited children aged 6 months to 10 years with uncomplicated malaria at two sites of Muheza District Hospital and Ujiji Health Centre in Tanga and Kigoma regions, respectively. Enrolled children were treated with either AL or DP and followed-up for 28 (extended to 42) and 42 (63) days for AL and DP, respectively. Parasite and fever clearance were monitored in the first 72 hours post treatment. The primary outcome was parasitological cure on days 28 and 42 for ALu, and day 42 and 63 for DP. Of the 1094 patients screened, 492 were enrolled with more patients enrolled at Ujiji (n=317) compared to Muheza (n=192). There was no early treatment failure and the crude cure rates on day 28 were 72.2% and 72.0% in patients treated with AL at Muheza and Ujiji, respectively. For the patients treated with DP, the cure rate on day 42 was 77.5.0% at Muheza and 76.5% at Ujiji. With extended follow-up to day 42 for ALu and day 63 for DP, the cure rates were lower in the two groups and at both sites (Muheza, 58.6.0% and 61.6%; Ujiji, 50.0% and 47.1%, for ALu ad DP, respectively). Over 50.0% of patients from Muheza had not cleared parasites after 40hrs post treatment (compared to <10.0% at Ujiji) and only one patient treated with DP at Muheza (1.1%) still had parasites after 72hrs. High parasite clearance for both drugs indicates that there is no tolerance/resistance to artemisinins. However, high rate of treatment failure among patients treated with AL and DP could be due to re-infections which will be corrected after PCR analysis.

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GENETIC SIGNATURES OF MALARIA SELECTION VALIDATED BY ASSOCIATION WITH CURRENT AND HISTORIC MEASURES OF MALARIA TRANSMISSION INTENSITY

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Malaria exerts strong selection pressure on the human genome as evidenced by the congruence of the population prevalence of malaria parasites with sickle-cell trait and other haemoglobinopathies. Proxies of malaria transmission intensity, such as altitude, have also been used to demonstrate genetic associations with malaria. In this study we examined the variation of human genetic polymorphisms as function of three measures of malaria transmission with differing longevity: village altitude as measure of long term malaria transmission, anti-malarial antibody seroconversion rate as a medium term transmission measure and village level parasite prevalence as a measure of current transmission. Samples were collected from 8138 individuals aged 1-45 years resident in 24 villages in north east Tanzania; village altitude ranged from 165 to 1788 above sea level, seroconversion rates from 0.019 to 0.941 person per year and ***Plasmodium falciparum*** parasite prevalence from 17.3 to 96.5%. Genotyping was conducted using Sequenome Massarray for 275 single nucleotide polymorphisms (SNPs) previously associated with protection from malaria; 175 passed a very strict quality check. By analysing the summary data of the villages we showed that alpha-thalassaemia, sickle-cell gene, a specific mutation in CD36 and several SNPs on the G6PD locus were strongly associated with long-term malaria exposure. We also found moderate associations between recent-to-current malaria exposure and several polymorphisms on immune response genes, including IL3, IL13 and TNF. The performed analysis highlights the strength of integrating large and detailed epidemiological studies with high-throughput genotyping technologies, thus providing robust evidence for the long-term genetic selection of sickle cell and alpha-thalassaemia traits and a mutation in CD36 and more recent genetic selection of several immune-related genes.

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EVOLUTIONARY RATES OF *PLASMODIUM* SPP. GAMETOCYTE EXPRESSED GENES AND THEIR PUTATIVE ROLE AS UNIVERSAL TRANSMISSION BLOCKING VACCINE CANDIDATES

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Although efforts to reduce malaria transmission have been highly successful, the development of resistance to antimalarial drugs by the parasite and to insecticides by the vector may hamper their sustainability. In addition, marked differences between transmission patterns and life cycles of the major causal agents (***Plasmodium vivax*** and ***P. falciparum***) increase the complexities that elimination programs are facing worldwide. Among the alternatives considered to overcome these obstacles, transmission blocking vaccines (TBV) have gained interest due to their ability to disrupt transmission and, in some cases, generate lasting immune responses. Given that many proteins are orthologous across the genus, some focus has been given to finding a universal malaria vaccine. In this study, the long term evolutionary trends of 312 genes with gametocyte biased expression were evaluated across 7 ***Plasmodium*** species. Also, 10 known TBVs candidates currently under different stages of development were evaluated. We first estimated the rates of synonymous (Ds) and non-synonymous (Dn) substitutions correcting by time. The difference on the variation of Ds ($F(0.7579)=0.3847$, $p>.05$) and Dn ($F(0.3903)=0.532633$, $p>0.05$) rates was not statistically significant between gametocyte sexes. However, variation of the Dn rate between membrane vs. non-membrane expressed genes regardless of sex was significant, $F(9.1960)=0.002646$, $p>0.05$. Episodic selection was detected on the ***P. vivax*** or ***P. falciparum*** branches in 52 genes. However, a more stringent analysis adding 4 ***Plasmodium*** species detected evidence for episodic selection only on 18 of those 52. Although many genes expressed in the gametocytes are highly conserved, still a few of them are less conserved or even under episodic selection in ***P. vivax*** or ***P. falciparum***. Considering this long-term term evolutionary trends is important for those actively exploring TBV candidates that could elicit cross-reactive immunity between both parasites.

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NEXT GENERATION SEQUENCING METHODS FOR THE ANALYSIS OF COMPLEXITY OF INFECTION IN MALARIA PATIENTS IN INDIA

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Genetically complex malaria infections comprising two or more distinct clones of the parasite ***Plasmodium*** within the host can influence disease phenotypes and outcome, vector transmission, and resistance to antimalarial drugs. Deep-sequencing methods provide high resolution, scalability and sensitivity, and are thus especially suited for the examination of intra-host diversity of malaria infections. We developed an amplicon sequencing protocol for the Ion Torrent sequencing platform in order to evaluate complexity of infection in ***P. vivax*** isolates. Based on SNP incidence and frequency obtained from whole genome sequencing of 200 global field isolates of ***P. vivax***, we selected a panel of five loci in highly polymorphic genes, which includes members of the MSP and SERA multigene families. We carried out a pilot amplicon sequencing run using mixtures of ***P. vivax*** reference strains to assess whether SNP allele frequencies at the selected loci can be used to determine the relative proportion of individual strains in these mixtures. Subsequently we will use this amplicon panel to evaluate the within host diversity of ***P. vivax*** isolates from three epidemiologically different sites in India, namely, Chennai (Tamil Nadu), Nadiad (Gujarat), and Raurkela (Odisha), as part of the Center for the Study of Complex Malaria in India.

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POLYMORPHISM OF MALARIA VACCINE CANDIDATE MEROZOITE SURFACE PROTEIN 5 (MSP5) IN MALIAN *PLASMODIUM FALCIPARUM* USING PACIFIC BIOSCIENCES AND SANGER SEQUENCING PLATFORMS

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The highly polymorphic nature of most candidate vaccine antigens may hamper an initially efficacious vaccine's short or long term success in the field. Haplotype prevalence and genetic diversity within vaccine candidate merozoite surface protein 5 (MSP5) were estimated from specimens collected from asymptomatic infections (n=42) and clinical malaria episodes (n=42) in children from Bandiagara, Mali, by Sanger sequencing and Pacific Biosciences (PacBio) single molecule real-time sequencing methods. PCR primers were designed to amplify a 3800 base pair product that encompasses the genomic segment encoding MSP5. Equimolar amounts of each sample amplicon were pooled for multiplex PacBio sequencing. Amplicons were individually assembled for each sample after barcode-based demultiplexing. Using Sanger sequencing methods we identified 92 unique sequences based on chromatogram peak heights. We generated 123 MSP5 sequences by PacBio sequencing with an average, minimum and maximum of 1.4, 1 and 5 sequences per sample, respectively. The average gene coverage by PacBio platform was 97X (range: 9-500) while an average predicted accuracy of 95% (range: 0.33-1) was observed. Overall, sequencing using the Sanger platform cost $82 dollars per sample while PacBio-generated sequence cost $62 per sample. The results from this study show that PacBio sequencing is a cost-effective alternative to Sanger sequencing, and that this third-generation sequencing platform can detect more diversity than that identified using Sanger sequencing.

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EUPATHDB: AN INTEGRATED GENOMIC DATA RESOURCE FOR EUKARYOTIC PATHOGENS

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The Eukaryotic Pathogen Database Resource (http://eupathdb.org) is a free online omics resource that provides a large number of search and analysis tools to the research community. These include: comparative genome analysis, population genetics tools, functional enrichment analysis, data visualization and advanced search capability across a large number of genome sequences and functional datasets. EuPathDB integrates diverse data types (genome sequence/annotation, proteomics, RNA-Seq, metabolomics, population re-sequencing, SNPs, etc.) and applies a standard analysis pipeline to all sequences that generates data

such as domain predictions, orthology profiles and GO term associations. Functional genomics datasets are also analyzed with standard workflows, providing a base for comparing, visualizing and mining data within or across different datasets with only a Web browser. EuPathDB specifically supports eukaryotic pathogens for taxon-specific data mining (AmoebaDB.org, CryptoDB.org, FungiDB.org, GiardiaDB.org, MicrosporidiaDB.org, PiroplasmaDB.org, PlasmoDB.org, ToxoDB.org, TrichDB.org, TriTrypDB.org) as well as cross-taxon inquiries (EuPathDB.org) and orthology inferences across representative organisms from the three kingdoms of life (OrthoMCL.org). Over the next few years EuPathDB will be developing new tools and functionalities that include a private user workspace for primary data analysis, functional analysis tools for result summarization, genome browser and query improvements. The power of EuPathDB lies in the large number of genomes (>224) and integrated functional datasets (>180); sophisticated data mining optimized to support hypothesis driven research; an intuitive graphic web-interface; fast transition between data mining and visualization; and the capacity to mine the details of a single genome-wide dataset or mine across several datasets. EuPathDB provides support via email (help@eupathdb.org), Web demonstrations and help desks, encouraging suggestions for pertinent datasets and new features. Please visit us in the exhibitor hall for a demonstration.

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GENETIC DIVERSITY OF VAR2CSA FROM FIELD ISOLATES, BASED ON A COMBINATION OF LONG-RANGE PCR AND SINGLE-MOLECULE SEQUENCING

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Pregnancy-associated malaria, PAM, a leading cause of maternal anemia and low birth weight, is characterized by the sequestration of ***Plasmodium falciparum***-infected erythrocytes in the placental microvasculature via binding of VAR2CSA, a parasite-encoded protein, expressed on the surface of infected cells. Sero-epidemiological studies suggest that VAR2CSA is a target of naturally acquired immunity, supporting the possibility that VAR2CSA could be a target for a PAM vaccine. However, based on limited data, there appears to be extensive genetic diversity within the var2csa gene that must be taken into account in the design of an effective vaccine. Sequencing the full-length gene from clinical isolates has been challenging due to a low complexity associated with var2csa. We have developed a novel strategy to characterize the extent of var2csa genetic diversity using Single Molecule Real Time (SMRT®) sequencing (Pacific Biosciences). Forty three samples collected from Malawi along with two laboratory strains were used to amplify the full gene in two overlapping fragments of ~5kb each. We successfully sequenced the first fragment spanning the first four domains (DBLpam1-DBLpam3) of the protein. In total 143,603 reads with a median length 2,728 (N50=4566) were generated from one SMRT® cell. After filtering, clustering reads, and removal of chimeric sequences, 98 sequences with an average coverage of 163x and accuracy equal or greater than 99%, including 21 full-length-amplicon sequences of fragment 1, were obtained. Our results showed an extensive polymorphism of N-terminal of var2csa with an average nucleotide diversity of 12%. Of the 4 domains, DBLpam3 was the least variable. Compared with published sequences, Malawian var2csa sequences clustered with 3D7 and FCR3 laboratory reference strains. We are using this approach to sequence the membrane proximal C-terminal region (fragment 2) and characterize the genetic variation of var2csa from parasite from different geographic regions. The findings will provide a framework for strain-transcendent VAR2CSA-based vaccine development.

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GENOMIC CHARACTERIZATION OF NF54, THE *PLASMODIUM FALCIPARUM* STRAIN IN THE WHOLE-ORGANISM MALARIA PFSPZ VACCINE

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NF54 (Nijmegen Falciparum #54) is the ***Plasmodium falciparum*** (Pf) isolate used in the whole-organism malaria vaccine called Sanaria® PfSPZ Vaccine. This vaccine, composed of radiation attenuated, aseptic, purified, cryopreserved sporozoites, provided 100% protection from homologous controlled human malaria infection (CHMI) in a small safety and efficacy trial using intravenous administration. Clinical trials are underway to replicate these results and to determine efficacy against heterologous CHMI. The annotated genome of NF54 is not yet available, but will be an essential tool to interpret the result of efficacy studies, including the comparison of outcomes between homologous and heterologous CHMI, as well as protection against natural infections. The NF54 genome is likely very similar to that of the reference 3D7 Pf isolate, a clone from NF54. Analyses of publicly available whole-genome sequencing data for NF54, in the form of paired-end 100 bp Illumina reads, reveals the presence of 171 SNPs, of which 36 are non-synonymous, confirming both the close relationship between the two genomes and the presence of a limited number of differences. However, the highly biased genome composition of Pf, together with its considerable repetitiveness, prevents the exhaustive characterization of those differences by read mapping, also an inadequate approach to identify potential genomic regions unique to NF54. In order to extensively characterize its genome, we have cultured NF54 and generated an 18,800 bp insert Pacific Biosciences (PacBio) library. This long-insert library was sequenced in four SMRT cells, with an average of 139,499 reads per cell, and an average mean and maximum read length of 7,433 and 48,513, respectively, for a total of over four gigabases of PacBio data. The genome is currently being assembled; a data set generated in a similar manner for another Pf isolate resulted in a nearly closed 24Mb genome assembly of 40 contigs. The newly generated NF54 assembly will be extensively characterized and compared with clinically relevant strains, including 3D7,as well as the 7G8 clone, currently being used in heterologous CHMI.

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NATURALLY ACQUIRED ANTIBODIES TO DUFFY BINDING PROTEIN OF *PLASMODIUM VIVAX* (PVDBP) AND PROTECTION FROM CLINICAL MALARIA IN RURAL AMAZONIA

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Plasmodium vivax merozoites recognize specific receptors on the host cell surface to invade their target cells, young erythrocytes known as reticulocytes. One of the major parasite's ligands is the Duffy binding protein (PvDBP), expressed in micronemes, which binds specifically to an erythrocyte membrane glycoprotein known as Duffy blood group antigen/

receptor for chemokines (DARC). Interaction between the cysteine-rich domain II of PvDBP and DARC is crucial for red blood cell invasion; DARC-negative individuals are thus usually refractory to blood-stage infection. Here, we tested whether naturally antibodies to PvDBP conferred protection against clinical vivax malaria among ***P. vivax***-exposed rural Amazonians. We measured IgG antibodies in 1126 plasma samples from 466 individuals (1-7 samples per subject) using a multiplex assay with microspheres conjugated with five different variants of PvDBP (Sal-I, AH, P, O and C). A high proportion of samples tested, ranging from 64% to 85% according to the variant, had IgG antibodies to PvDBP. We found a clear but short-lived boosting by comparing antibody levels in samples collected before and after laboratory-confirmed episodes of ***P. vivax*** malaria. However, survival analysis using multilevel Cox models found no association between high-level antibody response to PvDBP and time to the next laboratory-confirmed ***P. vivax*** malaria episode (hazard ratio = 0.97; $P = 0.86$). We further tested whether the presence of antibodies that are able to block binding of the Sal-I variant of PvDBP to Duffy was associated with clinical immunity and found that 12% of the 564 samples analyzed displayed high blocking activity (≥ 80%) ***in vitro***. Compared with subjects with antibodies displaying low-level (≤ 40%) or no inhibitory activity, those with highly blocking antibodies had a longer time to the next clinical ***P. vivax*** episode, but the difference did not reach statistical significance (hazard ratio = 0.66; $P = 0.32$). We are currently exploring the variant-specificity of blocking antibodies by using additional PvDBP variants in functional ***in vitro*** assays.

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GENOME-WIDE COMPARATIVE TRANSCRIPTOME ANALYSES OF VIRULENT AND AVIRULENT RODENT MALARIA PARASITES AND HOST RESPONSES IN THE BLOOD AND SPLEEN PROVIDE MOLECULAR INSIGHTS INTO AN INTIMATE HOST-PARASITE INTERACTION

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Using fluorescent and bioluminescent parasites, we demonstrate that severe pathology in mice infected with virulent ***Plasmodium chabaudi*** CB strain is associated with greater reticulocyte preference and stronger cytoadherence compared with avirulent AS strain. The reticulocyte preference results in more severe anemia as parasites erode replenishment of erythrocytes, and a more pronounced anemia signature in the host blood transcriptome. Comparative RNA sequencing analysis reveals an association with higher levels of reticulocyte-binding protein transcripts in the CB parasite. Furthermore, genome-wide transcription profiling of mouse blood and spleen during acute blood-stage infection shows an earlier and stronger apoptosis signature with virulent CB infection, whilst a heightened and sustained T cell response signature with avirulent AS infection. Together, these comparative transcriptome analyses elucidate key aspects of host-parasite interactions that shape the outcome of a malarial infection.

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PD1 MODULATES NEONATAL VΔ2 T CELL RESPONSES TO MICROBIAL ANTIGENS

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In utero exposure to microbial antigens primes the fetal immune system, often with adverse consequences for infant immune responses. Vδ2 cells, a subset of γδ T cells, play important roles in antimicrobial immunity and in the response to BCG vaccination. Their repertoire and responses are altered in neonates prenatally exposed to ***Plasmodium falciparum*** (***Pf***) and these alterations may affect responsiveness to childhood vaccines and susceptibility to later malaria exposure. Our studies focus on the mechanisms for attenuation of Vδ2 function due to ***in utero Pf*** exposure. We hypothesize that PD1, a negative regulator of T and B cell responses critical for maintenance of peripheral and feto-maternal tolerance, is key for neonatal Vδ2 dysfunction. We found that neonatal Vδ2 cells up-regulate PD1 upon activation ***in vitro*** and, unlike their adult counterparts, maintain its expression for up to 28 days. When PD1 is engaged by its ligand PDL1 during T cell receptor stimulation, Vδ2 cell effector functions are decreased. These effects depend on the dose of PDL1 and are positively correlated with the fraction of Vδ2 cells that are PD1+. PD1 expression by neonatal Vδ2 cells is inversely associated with DNA methylation in the PD1 promoter. PD1 mRNA levels are higher in neonatal compared to adult Vδ2 cells at both 14 and 28 days post-stimulation, while mRNA levels of Blimp1, a transcriptional repressor of PD1, are lower in neonatal Vδ2 cells. These differences seem part of a functional program specific for the fetal/neonatal stage of development. Fetal Vδ2 cell function may be regulated by PD1 to prevent excessive inflammatory responses before and shortly after birth. Prenatal stimulation of Vδ2 cells caused by maternal infection may induce prolonged PD1 expression and hinder functional Vδ2 development after birth, thus leading to impaired early immune responses to pathogens and some live vaccines. Our ongoing studies on Vδ2 cell phenotype and function in Malawian newborns with documented prenatal exposure to ***Pf*** will advance our understanding of neonatal immune regulation and help to develop interventions to improve protection of infants against a wide range of pathogens.

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ALLELIC FREQUENCY OF INTRONIC SNPS IN HUMAN FC RECEPTOR (FCΓR) GENE DIFFERS AMONG DOGON AND FULANI CHILDREN WITH UNCOMPLICATED MALARIA IN MALI

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Interethnic differences in malaria susceptibility may is influenced by multiple factors including host immunogenetics. Single nucleotide polymorphisms (SNPs) in immunological important molecules, such as Fc receptors (FcγRs) for IgG have been previously studied among Fulani and non-Fulani sympatric tribes to determine if they contribute to

protection. The complex nature of the gene cluster encoding FcγRs and recent reports of genotype-phenotype mismatch in this cluster suggests that other polymorphisms, probably intronic ones, may also influence malaria susceptibility in these populations. The aims of this study were to investigate two intronic SNPs (rs3933769 and rs396991) and a functional SNP (rs1050519) located in the FcγRIIC, FcγRIIA and FcγRIIB genes among Fulani and Dogon tribes living in sympatry in Mali. The study further tested the association of these SNPs with various clinical and immunological indices in 242 Fulani and Dogon children with uncomplicated malaria. All SNPs were genotyped with predesigned TaqMan® SNP Genotyping Assays. The study confirmed known malariometric and immunologic differences between sympatric Fulani and non Fulani tribes. We found the mutant alleles of both the rs3933769 and the rs396991 alleles were less frequent in the Fulani compared to the Dogon ($p < 0.0001$ and $p = 0.0043$). The distribution of the mutant rs1050519 allele was however similar in the two tribes ($p = 0.064$). All SNPs studied were not associated with levels of antibody responses with the exception of rs36991 which was associated with IgG1 ($p = 0.023$) in the Fulani. Carriers of the rs3933769 mutant allele harboured less than half of the parasite burden than those without the mutant allele ($p < 0.0001$). Differences in allelic frequencies between rs3933769 and rs396991 among Fulani and Dogon indirectly suggest that these SNPs could influence malaria susceptibility in the study population. Association of rs3933769 with parasite density is particularly noteworthy.

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ALTERED PLATELET COUNTS INFLUENCED LEVELS OF PRO-INFLAMMATORY CYTOKINES OF *PLASMODIUM FALCIPARUM* INFECTED PATIENTS IN A SEMI-URBAN AREA OF NIGERIA

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Malaria is still a leading global health concern with 3.4 billion people at risk and 198 million clinical cases, sub-Saharan African countries having the highest burden. Our preliminary studies revealed that Pro-inflammatory cytokines are increased in malaria ,however, some hematological parameters were significantly implicated.We therefore hypothesized that some blood components play protective roles in malaria. In 2014,volunteers(613) with a history of fever and forty (40) Uninfected controls were screened microscopically, according to WHO standard, for ***Plasmodium falciparum*** in a cross-sectional study at IJEDE General Hospital. Full Blood Counts (FBC) of participants was determined using Finray hematology Auto-analyzer.Serum levels of TNF-α, Interlukin-1β (IL-1β) and Interlukin-12(IL-12) were determined by capture ELISA while statistical analysis was done using SPSS Version 20. Study protocol was approved by NIMR IRB.A total of 128 patients comprising of 47% males and 53% females with a median age of 10 years were recruited. Malaria prevalence was 20.85% while Haemoglobin, Mean Cell Hemoglobin and Mean Cell Volume were significantly ($P<0.05$) lower in the control group. Thrombocytopenia (Platelets counts < 150 X 109 / L) was prominent in infected participants and was negatively correlated ($r=0.003$) with IL-12. Mean levels of TNF-α, IL-1β and IL-12 were significantly ($P< 0.02$) higher in the test participants than the controls. Increase in Parasitaemia resulted in insignificant increase in TNF-α. Results from this study suggests that hematological indices are key in the immunity against malaria and may modulate disease outcomes and pattern of progression as parasite killing ability of Platelets is related to pro-inflammatory response.

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MEDIATORS OF MONOCYTE OPSONIC PHAGOCYTOSIS OF *PLASMODIUM FALCIPARUM*

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Antibodies (Abs) mediate naturally acquired immunity (NAI) to blood stage malaria, in part by opsonizing parasites and infected red blood cells for phagocytosis. Thus, immunity to blood-stage malaria requires proper and efficient activation of phagocytosis. Using the THP-1 monocytic cell line, we have recently reported that high levels of merozoite opsonizing antibodies were associated with protection from clinical malaria. Phagocytosis is regulated by both stimulatory and inhibitory surface Fc receptors (FcγRs) on circulating monocytes, and several polymorphisms in FcγRs have been associated with altered malaria disease outcomes. Expression of FcγRs can be regulated by cytokines, and IFNg and IL-4 reciprocally regulate expression of stimulatory and inhibitory receptors, respectively. The influence of monocyte expression levels of various FcγRs receptors on antibody-mediated phagocytosis in malaria is poorly understood. To examine this question, we have tested phagocytosis by the THP-1 cell line of isolated merozoites, and separately, phagocytosis of beads coated with malaria blood-stage antigens that have been opsonized with antibodies from Papua New Guinean individuals. We are currently investigating the impact of knockdown of the stimulatory and inhibitory FcγRs and regulation by IFNγ or IL-4 on phagocytosis. Currently our results are preliminary, however by the time of the meeting we expect to have sufficient results to address this question.

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PROBING IMMUNE MECHANISMS OF SELECTION OF PARASITES WITH COMMON GENETIC SIGNATURES OVER FOUR TRANSMISSION SEASONS IN SENEGAL

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As malaria transmission declines, ***Plasmodium falciparum*** parasite populations display decreased genetic diversity due to emergence of parasites with common genetic signatures (CGS). Previously, we characterized immune responses to one cluster of these highly related parasites and their variant surface antigens (VSA), and observed CGS parasites express similar ***var*** genes, more than expected by chance, and year-to-year variation in immune recognition of VSAs on CGS-infected red cells. Here, we extend these studies to seven different clusters of CGS parasites with different dynamics of emergence, growth, and decline to better understand how their immune reactivity could influence parasite frequencies from year to year over four transmission seasons (2011-2014). We characterized parasites in these seven clusters regarding similarities in their genotypes and virulence gene expression (invasion ligands and ***var*** genes), and addressed whether genetic variation and gene expression are similar within clusters compared to other clusters and controls. We explored the relatedness of parasites within barcode-identified clusters by sequencing the highly polymorphic loci CSP, TRAP, and SERA-2 and observed that parasites within clusters are identical at these loci, but that

each distinct cluster contains unique haplotypes for these loci. As before, we found CGS parasites within clusters express the same ***var*** Ups classes compared to controls, implying that gene expression is also similar within clusters. To probe potential mechanisms of immune selection influencing the dynamics of growth or decline of these clusters, we are currently characterizing the functional humoral immune responses against these parasites by ***ex vivo*** rosetting assays and ***in vitro*** VSA reactivity assays. These experiments should shed light on mechanisms of genotype-specific immune selection over time within the population. These samples provide a unique opportunity to explore common immune responses in patients infected with identical parasites.

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VAR2CSA: THE SPECIFICITY OF NATURALLY ACQUIRED ANTIBODIES THAT BIND TO THE SURFACE OF CS2 INFECTED ERYTHROCYTES

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Placental malaria caused by ***Plasmodium falciparum*** is a major health problem affecting both the mother and fetus. Infected erythrocytes (IE) expressing VAR2CSA on their surface sequester in the placenta by binding to Chondrotin Sulfate A. Previous studies showed that high anti-VAR2CSA IgG levels improve pregnancy outcome, e.g., increase birth weight, reduce anemia, lower placental parasitemia, and prevent drug treatment failures. VAR2CSA has 6 DBL domains. Pregnant women produce antibodies (Ab) that bind to the surface of CSA binding IE. It is not clear if these Ab bind to one or multiple DBL domains. The goal of this study was to determine the specificity of naturally acquired Ab that bind to CS2 IE. In our study, clinical information and archival plasma samples collected between 1996 -2001 at delivery from 1,377 pregnant Cameroonian women living in Yaoundé (low transmission) were used. Since the samples were collected prior to implementation of intermittent preventive treatment and insecticide-treated bed nets, natural immunity determined the presence or absence of placental malaria (PM). Plasma from women who were PM- and PM+ of different gravidities were screened for Ab to full length VAR2CSA (FV2) and other DBL domains using a bead-based multiplex assay. The results identified which DBL domains each woman recognized. Then, a subset of samples was tested for Ab binding to the surface of CS2 IE (express VAR2CSA) and to 3D7 and FVO IE (non CSA-binding phenotypes). IE were incubated with plasma, then anti-human IgG FITC, and the amount of fluorescence per cell surface area was determined by FACS. In the initial study using 25 samples with different levels of Ab to FV2, a significant correlation between Ab to FV2 and cell surface staining intensity was obtained ($r = 0.62$; $p=0.001$: Spearman Correlation). Additional samples are being screened in the cell surface staining assay and levels of reactivity are being compared with the DBL domains recognized by each sample.

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HIGH AVIDITY ANTIBODIES TO VAR2CSA: ARE THEY ASSOCIATED WITH PROTECTION FROM PLACENTAL MALARIA IN A LOW TRANSMISSION SETTING?

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Pregnancy-associated malaria is characterized by the sequestration of ***Plasmodium falciparum*** infected erythrocytes in the intervillous space of the placenta, that is mediated by the binding of parasite membrane protein VAR2CSA with Chondriotin Sulfate A on the surface of placental cells. The presence of ≥ 35% high avidity antibodies (Ab) to VAR2CSA early in pregnancy correlates with the absence of placental malaria at delivery in a high transmission setting. However, very little is known about high avidity Ab to VAR2CSA in low transmission areas, and if one or multiple pregnancies are required to produce such Ab. We therefore looked at a panel of blood samples obtained at delivery between 1995 and 2001 in Yaoundé, Cameroon (low transmission setting). The samples were collected prior to use of chemoprophylaxis (IPT) and bed nets, so natural immunity determined presence/absence of placental malaria. These samples were screened for IgG Ab to Full Length VAR2CSA (FV2) using a bead-based immunoassay and 708 samples were identified for further use in the avidity assay. The percentage of strong binding (high avidity) IgG equals the amount of Ab that remain bound to FV2 after 30 minutes of incubation with 3M NH4SCN. Results showed that ~26% of the 708 women had ≥35% high avidity Ab at delivery. Very few primigravidae had high avidity Ab; and Ab avidity increased significantly between the 1st and 2nd as well as the 2nd and 3rd pregnancies; however, it remained constant thereafter (3rd through 7+ pregnancies). In the dataset, the prevalence of placental malaria (PM) decreased 2-fold with gravidity. After adjusting for age and malaria status, an increase in avidity with gravidity ($P=0.0028$) was found. Avidity increased gradually, with only a 0.9% increase per pregnancy. A higher mean avidity was found in PM-negative compared to PM-positive secundigravidae ($P=0.0319$), but no difference was seen between PM+ and PM- in the other gravidity groups. In summary, even though avidity to FV2 increases with gravidity and PM decreases with gravidity, very few women in this low transmission setting have high avidity Ab.

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ACUTE MALARIA PREFERENTIALLY ACTIVATES CIRCULATING TFH SUBSET THAT LACKS THE CAPACITY TO INDUCE NAÏVE B CELLS TO PRODUCE IMMUNOGLOBULINS

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Malaria-specific antibody responses in children are relatively short-lived, leaving them susceptible to repeated bouts of febrile malaria. The cellular and molecular mechanisms underlying this apparent immune deficiency are poorly understood. Recently, T follicular helper (Tfh) cells have been shown to play a critical role in generating long-lived antibody responses. We show that Malian children have resting PD-1+CXCR5+CD4+ Tfh cells in circulation that resemble germinal center Tfh cells phenotypically and functionally. Within this population the PD-1+CXCR5+CXCR3- Tfh cell subset is Th2-polarized and superior to the Th1-polarized PD-1+CXCR5+CXCR3+ subset in providing B cell help. Longitudinally, we observed that acute malaria drives Th1 cytokine responses, and accordingly, the less functional Th1-polarized Tfh subset was preferentially activated and its activation did not correlate with antibody responses. These data provide important insights into the cellular basis of suboptimal antibody responses to malaria in children and establish a link between the quality of Tfh cell responses and the outcome of B cell germinal center responses during a natural infection. These findings also suggest that vaccine strategies that promote PD-1+CXCR5+CXCR3- Tfh cell responses may improve malaria vaccine efficacy in children.

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PLASMA LEVELS OF INTERLEUKIN-12 IN PATIENTS WITH *PLASMODIUM FALCIPARUM* MALARIA IN IKORODU, LAGOS NIGERIA

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Cytokines are stimulated during malaria infection or the disease itself. Interleukin (IL) 12 is a pro-inflammatory cytokine produced from monocytes/ macrophages and other cell types that is needed in the early defence against malaria. IL-12 plays a central role in regulating innate and adaptive immune responses, and interacts with several other cytokines for increased immunoregulatory activities. The objectives of this study were to determine plasma concentrations of IL-12 in malaria patients and its association with the parasitemia, age, clinical symptoms and anemia in patients with ***Plasmodium falciparum*** malaria in Ikorodu, Lagos Nigeria. Malaria microscopy and blood parameters were carried out using standard protocols and the level of IL-12 in plasma was measured using the Enzyme Linked Immunosorbent Assay (MABTECH AB, Sweden). The concentration of IL-12 in malaria patients ranged from 23.45pg/ml to 99996.98pg/ml. A negative correlation (r = -0.143) was found between the mean levels of IL-12 (7521.69pg/ml) in 144 participants who were microscopy positive and mean parasitaemia (41519.20 p/μL) ($p \leq 0.05$). The levels of IL-12 were high (16352.05 ± 1434.17pg/ml) in patients who presented with symptoms such as fever compared with patients which did not present with fever symptoms. Interleukin (IL) 12 levels were higher in patients who were above 14 years but there was no statistical significant relationship between IL-12 levels and the ages of patients. IL-12 correlated with Hemoglobin levels (r=0.004) but it was not statistically significant. IL-12 was low (2173.66±3225.04pg/ml) in patients with severe anaemia (<8g/dl) and was high (11759.71±21566.09pg/ml) in patient with mild anaemia (8-9.9g/dl). In conclusion, IL-12 in this investigation has been demonstrated to be involved in the immunoregulation of malaria infections and this immunoregulatory activity is improved with aging. This study also confirmed that the tendency for a patient to secrete IL-12 is associated with protection against parasitemia, clinical malaria and anaemia.

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DELAYED ACQUISITION OF *PLASMODIUM FALCIPARUM* ANTIGEN-SPECIFIC CD4+ T CELLS IN HIV-EXPOSED UNINFECTED MALAWIAN CHILDREN RECEIVING DAILY COTRIMOXAZOLE PROPHYLAXIS

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Cotrimoxazole (CTX) prophylaxis, currently recommended in HIV-exposed uninfected (HEU) children as protection against HIV-related opportunistic infections, also has some anti-malarial efficacy.We have recently shown that HEU children have reduced magnitude and breadth of IgG antibody responses to distinct ***Plasmodium falciparum*** merozoite antigens. In this study, we determined the effects of CTX prophylaxis-induced reduction in ***P. falciparum*** exposure on the magnitude and quality of ***P. falciparum*** antigen specific CD4+ T cells in HEU children. Peripheral blood was collected from HEU and HIV-unexposed uninfected (HUU) children at 6, 12 and 18 months of age. Proportion of CD4+ T cells subsets were determined by immunophenotyping. ***P. falciparum*** specific CD4+ T cells responses were measured by intracellular cytokine staining assay. There were no differences in the proportions of CD4+ T cell subsets between HEU and HUU children at all ages. HEU children had lower frequenciesof ***P. falciparum***-specific CD4+ T cells producing IFN-γ ($p = 0.035$) and IFN-γ/ TNF (double producers) ($p = 0.007$) at 12 months after being on CTX prophylaxis for a year compared to HUU children. The study showed that cotrimoxazole prophylaxis-induced reduction in ***P. falciparum*** exposure results in delayed acquisition ***P. falciparum***-specific CD4+ T cell responses in HEU children. This delayed acquisition of ***P. falciparum***-specific CD4+ T cell responses might increase susceptibility to clinical malaria after cessation of prophylaxis.

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MAPPING EPITOPES WITHIN INTRINSICALLY DISORDERED PROTEINS OF *PLASMODIUM FALCIPARUM* USING COMPUTATIONAL APPROACHES: IMPLICATIONS FOR IMMUNITY AND VACCINES

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Malaria remains a significant global health burden, with ***Plasmodium falciparum*** responsible for the majority of malaria related deaths. The proteomes of ***Plasmodium spp.*** are predicted to contain a large number of intrinsically disordered proteins, but little is known about their subcellular localisation or immunogenic properties, or their potential as vaccine candidates. We employed several computational algorithms to investigate predicted protein disorder and the impact on adaptive immunity. The proportion of disordered regions was similar for the proteomes of five human and murine ***Plasmodium*** spp. Disordered proteins were shown to be particularly enriched within apical proteins, exported proteins and proteins localised to the parasitophorous vacuole. Tandem repeat regions in proteins have been suggested to be immunodominant, and we found that tandem repeat regions occur predominantly within regions of disorder. Presentation of peptides via MHC class I and II molecules plays an important role in the generation of adaptive immune responses, and we have found that disordered protein regions are predicted to contain relatively few MHC class I and II binding peptides owing to inherent differences in amino acid composition as compared to structured domains. In contrast to MHC binding peptides, linear B-cell epitopes were predicted to be enriched in disordered regions of the malaria proteome, suggesting that recognition of disordered regions by acquired and vaccine-induced antibodies is entirely likely. Several disordered proteins or regions are current vaccine candidates, while a number of others appear to have strong potential for inclusion in multi-antigen vaccine constructs. We will present a newly developed online tool (PlasmoSIP) for the visualisation of protein structural features, predicted immunological features, and known polymorphisms for proteins from ***P. falciparum***. Further study is required to experimentally verify these predicted structural and immunological features and to determine the importance of disordered epitopes as targets of protective immunity.

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ANTIBODY RESPONSE AFTER CHALLENGE WITH *PLASMODIUM FALCIPARUM* SPOROZOITES BY NEEDLE AND SYRINGE WITH DIFFERENT DOSES AND ROUTES IN MALARIA-NAÏVE AND SEMI-IMMUNE INDIVIDUALS

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Controlled human malaria infection (CHMI) trials represent an important tool to study the immune response of the human host against ***Plasmodium falciparum*** parasites. We studied the antibody (Ab) response (IgM, IgG, IgG1, IgG2, IgG3 and IgG4) against 21 pre-erythrocytic and erythrocytic ***P. falciparum*** antigens in volunteers from 4 CHMI trials performed with aseptic, purified, cryopreserved ***P. falciparum*** sporozoites (PfSPZ Challenge) in Europe (malaria-naïve individuals) and Africa: 1. Barcelona, Spain (36 volunteers received 2,500 (n=18), 25,000 (n=6) or 75,000 PfSPZ (n=6) by intramuscular injection, and 3,200 PfSPZ (n=6) by direct venous inoculation [DVI]); 2. Tübingen, Germany (24 volunteers received 50 (n=3), 200 (n=3), 800 (n=9) and 3,200 (n=9) PfSPZ by DVI); 3. Lambaréné, Gabon (5 malaria-naïve volunteers, 10 semi-immune volunteers, and 10 semi-immune volunteers with sickle cell trait, received 3,200 PfSPZ by DVI); and 4. Tübingen, Germany (40 volunteers received 3,200 PfSPZ by DVI 10 weeks after being administered different doses of PfSPZ (3,200 (n=9); 21,000 (n=9); 51,000 (n=9)) or placebo (n = 13) under chloroquine prophylaxis, also via DVI. We measured the Ab response at different time points before administration of PfSPZ, at the time of first parasitemia, and at days 35 and 90 after PfSPZ injection. To our knowledge this is the first study to systematically assess the Ab response against a defined set of ***P. falciparum*** antigens in the context of CHMI, as well as the first characterization of the immunoglobulin isotype and subclass pattern. Our results showed that IgM Ab levels peaked at day 35 after PfSPZ CHMI, showing lower levels at day 90, and observed against the majority of ***P. falciparum*** antigens. IgG Ab response on the other hand, peaked at day 90 and it was significant only against a small set of ***P. falciparum*** antigens. Additionally, we observed a PfSPZ Challenge dose-antibody response and different Ab profiles depending on previous malaria exposure, and a consistent Ab response against a defined set of antigens in previously exposed individuals, both naïve and semi-immune, that could be used as markers of ***P. falciparum*** exposure.

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INCREASING THE POTENCY OF ATTENUATED SPOROZOITE VACCINES WITH A GLYCOLIPID ADJUVANT

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The world needs a highly effective malaria vaccine and robust immunity can be induced in humans with ***Plasmodium falciparum*** (Pf) sporozoites (PfSPZ), that invade hepatocytes, but arrest during liver stage development. In recent trials Sanaria® PfSPZ-based vaccines, composed of aseptic, purified, cryopreserved, radiation attenuated PfSPZ Vaccine, or non-attenuated PfSPZ with chloroquine (PfSPZ-CVac) were safe and highly protective. PfSPZ Vaccine protected 100% and 92% of non-immune individuals after 5 doses and 87% after 3 doses in 2 independent trials and provided durable and heterologous (75%) strain protection. PfSPZ-CVac was 100% efficacious in non-immunes. Even under natural exposure settings in Africa, PfSPZ Vaccine provided ~50% protection of semi immune individuals over 6 months. Adjuvants are potent vaccine additives to reduce the number of doses, interval between doses, immunogen quantity/dose, and to prolong efficacy. However, adjuvants are generally not used for live vaccines, nor been shown to improve CD8+ T cell mediated immunity in humans, required to protect against SPZ. In our screens to identify such an adjuvant, a novel glycolipid, 7DW8-5 that binds CD1d, and stimulates iNKT cells, was the only one to demonstrate significant enhancement in a mouse malaria model involving irradiated (irr) ***P. yoelii*** (Py) sporozoites (irrPySPZ). 7DW8-5 enabled reduction in number of doses for 75% protection with irrPySPZ from 3 to just 1 dose administered IV (30/40 protected with, 4/30 without adjuvant), from 4 to 2 doses (70%-100% protection) and even 1 dose (60% protection) given intradermally, with protection lasting for at least 14 weeks. Most importantly, 4 IV doses over 7 days of $2x10^3$ cryopreserved irrPySPZ with and without 7DW8-5 protected 96% (15/16) and 44% (7/16) of mice respectively (p=0.006). In NHPs, 7DW8-5 with PfSPZ Vaccine induced enhanced splenic CD8+ and CD4+ T cells responses. Together, these findings provide strong rationale to combine 7DW8-5 with PfSPZ vaccines, in an accelerated immunization regimen that will vastly improve commercial, clinical, and public health benefits of PfSPZ vaccines.

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ISOLATION AND CHARACTERIZATION OF FUNCTIONAL HUMAN MONOCLONAL ANTIBODIES TO *PLASMODIUM VIVAX* DUFFY BINDING PROTEINS

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Plasmodium vivax (Pv) invasion of reticulocytes requires binding of Duffy Binding Protein (PvDBP) to the Duffy Antigen Receptor for Chemokines (DARC) on erythrocytes for most individuals. About 3.7-8.6% of individuals residing in ***P. vivax***-endemic areas acquire high-binding inhibitory antibodies (BIAbs) (defined as >80% binding inhibition relative to malaria-naïve donor antibodies) to region II of PvDBP (PvDBPII), which correlates with partial protection against ***P. vivax*** malaria. Consequently, PvDBPII is a leading vaccine candidate. However, PvDBPII is polymorphic and whether naturally-acquired or vaccine-induced BIAbs to PvDBPII are strain-transcending is unknown. In initial DARC-binding ELISA studies, we found that naturally-acquired BIAbs from Brazilian and Cambodian individuals show the presence of both strain-specific and strain-transcending BIAbs. To identify which PvDBPII epitopes mediate strain-specific versus strain-transcending BIAbs, we obtained PBMCs from Cambodians with high levels of BIAbs, and sorted single PvDBPII-specific IgG+ memory B cells, PCR amplified their IgG heavy and light chain variable regions, and cloned them into a human IgG expression vector to generate a panel of human monoclonal antibodies (mAbs). From one individual, 13/15 mAbs recognized PvDBPII and 8/10 mAbs blocked binding of PvDBPII to DARC. These 15 mAbs fell into 9 clonal groups. We are now characterizing these mAbs in terms of their ***P. vivax*** strain-specificity, the PvDBPII epitopes they recognize, their affinity for PvDBPII, and their ability to block ***P. vivax*** invasion of reticulocytes ***in vitro***.

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DYNAMICS OF CIRCULATING T FOLLICULAR HELPER CELLS AND PLASMABLASTS DURING CHEMOPROPHYLAXIS VACCINATION WITH PYRIMETHAMINE AND *PLASMODIUM KNOWLESI* IN RHESUS MACAQUES

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Whole organism ***Plasmodium*** vaccines can induce long-lasting sterile protective immunity. Understanding which components of innate and acquired immunity play a role in conferring sterile immunity will aid in vaccine development. Recent studies with the whole ***P. falciparum*** (Pf) organism product PfSPZ Vaccine showed a correlation between vaccine-induced antibody levels and protection in humans. T follicular helper cells (Tfh) play an integral part in the development of high affinity effector and memory B cells, and studies in influenza vaccine have shown that circulating Tfh cells can predict the development of functional antibodies. The dynamics of Tfh during whole organism vaccination are unknown. We examined this during a Chemoprophylaxis Vaccination (CVac) study in monkeys. Rhesus macaques were vaccinated by direct venous inoculation (DVI) of ***P. knowlesi*** sporozoites (PkSPZ) followed by oral pyrimethamine on days 1, 2 (n=4) or days 2, 3 (n=7) post DVI. Blood samples for Tfh and plasmablasts analysis were obtained on days 0 (day of vaccination), 2, 5, 7, 14 and 27 post DVI. Preliminary results show that levels of circulating plasmablasts and Tfh cells remain at baseline after first vaccination. However after second vaccination, levels of both plasmablasts (p=0.016) and Tfh cells (p=0.016) increase significantly by day 2 post DVI, then return to baseline by day 7. Circulating Tfh undergo a second and sustained rise at day 14 of second vaccination. The initial circulating Tfh rise is observed in the ongoing third and final vaccination. At completion of the study, we will determine if levels and kinetics of Tfh and plasmablasts correlate with antibody levels and can predict development of lasting protective immunity. Since it is now established that the antibody response after immunization with PfSPZ Vaccine is dramatically different in non-immune US and semi-immune African populations, we believe that this approach to studying the development of immunity will be useful for understanding how to optimize immune responses to PfSPZ in all populations.

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CLODRONATE LIPOSOMES INDUCE PARTIAL DEPLETION OF MONOCYTES AND INCREASE PARASITEMIA IN *PLASMODIUM FALCIPARUM*-INFECTED *SAIMIRI SCIUREUS* MONKEYS

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Non-human primates of the genera ***Saimiri*** and ***Aotus*** are recommended models for experimental malaria studies, being susceptible to infection by ***Plasmodium falciparum*** and ***P. vivax***. However, splenectomy is usually required to achieve high and reproducible parasitemias. The spleen is a key organ in the immune response to malaria infections and therefore splenectomy is an undesired condition. We asked whether administration of clodronate liposomes (CL), which cause partial depletion of monocytes/macrophages in several organs, including the spleen, would allow the establishment of sizeable and reproducible parasitemias in ***P. falciparum***-infected ***Saimiri sciureus*** monkeys, preventing the need for splenectomy. CL caused marked death of ***Saimiri*** leukocytes ***in vitro***. Groups of 6-9 S. sciureus monkeys inoculated with $1x10^6$ ***P. falciparum***-infected erythrocytes, as well as control monkeys inoculated with saline, received either 0.5 or 1.0mL of CL, intravenously, twice a week starting at the day of ***P. falciparum*** inoculation, with a total of 6-8 injections. While saline-treated monkeys kept parasitemia under control (average 8.1% lasting for over 18 days without need for antimalarial treatment), half of the monkeys receiving 0.5mL LC and 2/3 of monkeys receiving 1.0 mL CL developed high parasitemias (>20%) requiring treatment by day 13. Animals tolerated well CL administration but some showed hepatocyte vacuolization. Although peripheral CD14+ cell counts were not significantly affected by CL administration, ***P. falciparum***-infected, CL-treated animals showed milder splenomegaly compared to infected saline-treated monkeys. Splenic monocyte populations were only partially depleted and were still able to retain hemozoin, showing that the spleens were functional. Partial depletion of Kupfer cells was also observed, with lower levels of ferrous iron, but hemozoin-containing Kupfer cells were still observed in CL-treated animals. These data indicate that CL administration is a viable and effective alternative to surgical splenectomy for malaria studies using ***Saimiri*** monkeys, leading to higher parasitemias with preserved splenic function.

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HUMORAL IMMUNITY CONTRIBUTES TO CLEARANCE OF ARTEMISININ-RESISTANT PARASITES IN SOUTHEAST ASIA

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We have proposed that the ability to clear drug resistant parasites is a phenotype for protective malaria immunity and have found that parasite genetic factors account for only about half of the variation in parasite clearance half-life after artesunate treatment. To assess the contribution of humoral immunity to clearance of artemisinin resistant parasites after artesunate treatment of falciparum malaria, sera from 100 Cambodian, 78 Myanmar, 111 Thailand, and 120 Vietnamese participants in the Tracking Resistance to Artemisinin Collaboration (TRAC) study of artesunate efficacy were probed against two protein microarrays: a customized diversity-reflecting protein microarray and a microarray of 500 reactive ***Plasmodium falciparum*** and ***P. vivax*** antigen fragments. The customized protein microarray included 268 diverse apical membrane 1 (AMA1) fragments, 20 merozoite surface protein 1 (MSP1) fragments, and 30 Rh5 fragments, all based on sequences derived from field samples, as well as 176 fragments of PfEMP1s based on the 3D7 reference genome. Preliminary analyses show that stronger and broader seroreactivity to ***P. falciparum*** antigens was associated with rapid parasite clearance for samples with K13 mutations, particularly with respect to field-derived AMA1 variants. This approach provides a potential way to control for the effects of immunity in assessments of antimalarial drug efficacy.

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MODELLING MASS DRUG ADMINISTRATION IN MALARIA-ENDEMIC COUNTRIES IN THE PRESENCE OF IMPORTED INFECTIONS

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Mass Drug Administration (MDA) has received renewed interest as a potential tool for the control and elimination of malaria. MDA, the administration of drug therapy to a large segment of the population, has the ability to not only reduce current prevalence, but to decrease onward transmission of the malaria parasite. As the evidence base for MDA is not large, mathematical modelling may be used to simulate the intervention under a variety of epidemiological and logistical conditions to assess the impact of MDA on prevalence and transmission. This paper contributes to the literature by simulating MDA in an open population i.e. in the presence of imported infections. By modelling MDA in high and low transmission settings, with varying levels of imported infections, this paper shows that the impact of MDA in an open population is dampened, particularly in low transmission settings where imported infections originate from areas with higher transmission. In such situations it was found that MDA in the source of imported infections has a larger impact than MDA in the local area of interest. MDA is not a lasting intervention with prevalence reverting to previous levels after 3 years. The presence of vector control may assist with extending this impact. As countries change focus from control to elimination of malaria, the management of imported infections increases in importance. As such the impact of MDA may be lower than expected if imported infections are ignored.

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GETTING THE LUMEFANTRINE DOSE RIGHT FOR THE TREATMENT OF UNCOMPLICATED MALARIA; A POOLED PHARMACOMETRIC APPROACH

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Artemether-lumefantrine (ARM-LF) is the most widely used antimalarial treatment in the world. Paediatric dosages are available and it is safe for women in the second or third pregnancy. Low cure rates have been reported in pregnant women and under-exposure to LF in these vulnerable populations might increase the risk of resistance development. Objective: To evaluate the pharmacokinetic-pharmacodynamic (PK-PD) properties of LF and conduct in-silico dose optimisations, using a population-based model approach and pooled data from paediatric, pregnant and adult patients from Africa, South East Asia and Oceania. Population PK properties of LF, and demographic and disease-related covariates were evaluated in 1,347 densely sampled patients. Sparsely and densely sampled venous blood, capillary plasma, capillary blood and venous plasma concentration-time data from 595, 190, 840 and 966 patients, respectively, were used for external model validation. Recurrent malaria data in all patients were used for a PK-PD time-to event model and this model was subsequently used for in-silico dose optimisation. Pregnancy affected LF absorption and bodyweight distribution and clearance resulting in lower day 7 concentrations compared to non-pregnant adult patients. Admission parasite biomass correlated with relative bioavailability resulting in lower exposure to LF in more severe patients. LF dosage correlated with relative bioavailability resulting in dose-limited absorption. LF concentration affected the treatment outcome significantly. In-silico simulations were conducted to evaluate the tentative treatment success after a prolonged treatment, intensified treatment and increased dosage for paediatric and pregnant patients. In conclusion, LF concentrations correlated with the

time to recurrent malaria. The in-silico dose optimisations suggest that an intensified treatment is effective and most practical when implemented for paediatric and pregnant patients. Confirming studies evaluating the optimised dose regimens are required to ensure a high efficacy in pregnant patients and young children.

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OPTIMIZED *PLASMODIUM FALCIPARUM* HEPATOCYTE INFECTION MODEL FACILITATES DRUG AND VACCINE DEVELOPMENT

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Malaria is a life-threatening disease that mainly affects children in developing countries, infecting 250 million people and causing over 700,000 deaths per year. ***Plasmodium falciparum*** is the parasite credited with much of the morbidity and mortality. Development of disease in human hosts requires the parasite to invade the liver and replicate in hepatocytes before releasing to the blood stream to cause clinical disease. This liver stage of the infection has proven difficult to study due to the lack of an ***in vivo*** model for ***P. falciparum*** and the challenges of ***in vitro*** studies. ***P. falciparum*** infects primary human hepatocytes, but these cells do not propagate in culture, are in short supply, prohibitively expensive, and result in highly variable infection rates. The human hepatocarcinoma cell line HC-04 is susceptible to ***P. falciparum*** and ***P. vivax*** sporozoite infection but also suffers from poor infection efficiency. Here, we report on the development of a HC-04 monolayer culture platform that results in a 20-fold increase in infection over previous studies. It is well-known that ***in vitro*** culture systems are different from ***in vivo*** systems in many ways, such as nutrient composition, oxygen levels, the presence of signaling molecules, and barometric pressure. We hypothesized that by mimicking the ***in vivo*** conditions as closely as possible in our ***in vitro*** system, we would be able to optimize invasion efficiency of the parasite in the HC-04 cells. We systematically examined variables in our culture model while monitoring ***P. falciparum*** invasion by microscopy in order to determine the optimal culture media and conditions. Additionally, we found that the HC-04 cell line was quite heterogeneous, so we performed dilution sub-cloning in an attempt to isolate a clone that facilitated improved invasion. We successfully identified HC-04.J7, which allowed more efficient invasion by the parasite than did the heterogeneous HC-04 population. Establishment of this method for optimized ***in vitro*** hepatocyte invasion can facilitate future studies of the parasite's mechanism of invasion, as well as allow for the assessment of drug and vaccine candidates.

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MALARIA TEST POSITIVITY RATES AMONG PATIENTS AT FIVE OUTPATIENT HEALTH CENTERS LOCATED IN AN ENDEMIC REGION IN UGANDA: IRS VERSUS NON-IRS DISTRICTS

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In March 2014, the Uganda Malaria Surveillance Project expanded to include five level IV outpatient health centres (HCs) in the Lango sub-region of northern Uganda to monitor the impact of indoor residual spraying of insecticide (IRS). Two of the sites were in districts that received IRS with bendiocarb twice a year since 2010: Apac (Aduku HC) and Kole (Aboke HC) districts. The other three sites were in districts without IRS: Dokolo (Dokolo HC), Amolator (Amolator HC), and Otuke (Orum HC). From March to December 2014, 82,310 patient visits were recorded at the HCs, ranging from 12,960 in Aduku to 22,056in Dokolo. The proportion of patients with suspected malaria ranged from 25-35% in the two IRS districts and 45-58% in the three non-IRS districts. Overall, 87% of patients with suspected malaria underwent laboratory testing, ranging from75% in Amolator to 99% in Aduku and Aboke. Overall, 67% of testing was done using an RDT (as opposed to microscopy), ranging from 22% in Aduku to 94% in Dokolo. The outcome of interest was the test positivity rate (TPR): the proportion of malaria tests positive by RDT or microscopy. TPRs were considerably higher in non-IRS districts (Amolator=57%, Dokolo=63% and Orum=67%) compared to IRS districts (Aduku=19% and Aboke=25%). Prior to bendiocarb spraying in Apac, TPR at Aduku was as high as 58% in 2009, results comparable to current TPRs in non-IRS districts. Upon multivariate analysis using log-binomial regression, patients from non-IRS districts were nearly threetimes more likely to test positive for malaria than those in the IRS districts (RR=2.71, 95% CI 2.59-2.82, p<0.001) after controlling for age and the type of diagnostic test performed. Other than distribution of insecticide treated nets in June of 2014 across all the districts (94% ownership), IRS was the only intervention directly targeting malaria transmission in the region. The higher TPRs in the non-IRS districts compared to the IRS districts in a region of Uganda with historically high transmission highlights the importance of IRS for malaria control in endemic areas.

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PROJECTED PREVALENCE OF MALARIA IN ALTA VERAPAZ, GUATEMALA FOR THE YEARS 2020 AND 2050 USING GLOBAL CIRCULATION MODELS AND ECOLOGICAL NICHES

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We analyzed a total of 257 confirmed malaria cases (***Plasmodium vivax*** and ***P. falciparum***) for the year 2006 georeferenced at village level in Alta Verapaz, Guatemala. We used a Pearson correlation to validate precipitation (both number of days and daily accumulated rain), mean temperature and maximum/minimum temperatures collected from local weather stations for the same year to used them as potential predictors. After this first approach, we used historical climate data obtained from WorldClim (1950-2000, 30 arc-seconds resolution) and Maximum

entropy (MAXENT®) to generate a baseline map of probabilities of distribution using the georeferenced cases and 19 bioclimatic variables derived from monthly temperature and rainfall. A Jackknife analysis was ran to estimate the standard error associated with each of the variables and a contribution/permutation table was generated leading to the final arrange of variables with high predictive values: annual precipitation, precipitation of the driest month and precipitation of the warmest quarter (AUC = 0.95). We validated our baseline map of probabilities by comparing the variables generated by MAXENT with those from the local weather stations with a statistically significant correlation (α= 0.05) and by comparing the probability outcome per county generated by the baseline map with the actual percentage of cases for the same counties. Using the baseline as a validated framework, we used the predictors to generate a map of probabilities of occurrence of ecological niches at the county level for the IPCC climate change scenarios A1b, A2b, B1 and B2 for the years 2020 and 2050.

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ESTIMATION OF AMOUNT OF ARTEMETHER AND LUMEFANTRINE EXCRETED THROUGH BREAST MILK

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Artemisinin based combination therapies are widely used and recommended by WHO as first-line therapy for uncomplicated ***Plasmodium falciparum*** malaria in nursing mothers. However, artemether-lumefantrine (AL, Coartem®) is not recommended during lactation (no breast feeding at least up to 28 days post last dose) as the excretion of AL in breast milk has not been studied. Clinical data on dihydroartemisinin (DHA) suggest clinically insignificant amount of DHA is excreted in breast milk (peak concentration: 35 ng/mL) after 200 mg oral artesunate. In the absence of clinical data, amount of AL excreted into breast milk was estimated based on the milk-to-plasma drug concentration ratio (M/P ratio) obtained from preclinical studies. In a pre-postnatal preclinical study there were no developmental changes in rat pups fed exclusively on milk of mothers who received 50 mg/kg/day of AL (7.1 mg/kg artemether, 42.9 mg/kg lumefantrine) up to day 21 of lactation. In rats the M/P ratio was estimated from distribution of radioactivity in mammary gland after oral administration of radiolabelled artemether and lumefantrine. The potential amount of each drug moiety excreted in mother's breast milk in 24 hours was estimated by M/P ratio × maternal plasma Cmax concentration × 150 mL/kg/day (volume of milk consumed per day per kg body weight of infant). The maximum M/P ratios observed over 24 hours for artemether and lumefantrine were 1.04 and 1.3, respectively. Over the recommended six doses of AL, the mean maximum plasma concentrations of artemether and lumefantrine were 186 ng/mL and 25.7 µg/mL, respectively, in malaria patients. Based on the M/P ratio and plasma levels of artemether and lumefantrine, the estimated daily cumulative consumption of artemether and lumefantrine by infants through breast milk following recommended AL doses in nursing mothers is 0.03 and 5.01 mg/kg, respectively, which is ~270 and ~10 fold lower than the recommended daily dose (40 mg artemether and 240 mg lumefantrine dose) for 5 kg body weight infants.

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TRANSMISSION-BLOCKING ACTIVITY IS A FUNCTION OF OOCYST INTENSITY IN A CONTROL AND TRANSMISSION-REDUCING ACTIVITY OF A TEST IN THE STANDARD MEMBRANE-FEEDING ASSAY (SMFA)

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For transmission-blocking vaccine development against ***Plasmodium falciparum*** malaria, the standard membrane-feeding assay (SMFA) has been utilized widely to evaluate the functionality of vaccine-induced antibodies. However, there is no consensus whether to use % inhibition in oocyst intensity (transmission reducing activity, TRA), % inhibition in prevalence (transmission-blocking activity, TBA), or both as the main readout(s) of SMFA. We show that TBA is a function of mean oocyst intensity in the control. To establish this relationship, we examined 104 experiments where anti-Pfs25 monoclonal antibody (4B7 mAb) was tested at the same 94 µg/ml dose.The experiments with the 20 lowest control mean oocysts (range: 0.1 - 5.9) were compared with the 20 highest (range: 30 - 74). The TRAs were not statistically significantly different (93 vs. 95, p-value=0.25), but the TBAs were significantly different: 80.0 in the low group vs. 43 in the high group (p-value < 0.001). To address this dichotomy, we developed a model that adjusts TBA estimates to a specified normalizing target control intensity. We used our SMFA data that contained 105 independent feeding experiments with 11,838 mosquitoes to build the model, and two other data sets were utilized to test the model: one set used 4B7 mAb (104 feeds with 14,079 mosquitoes), and another set used polyclonal antibodies against multiple antigens (33 feeds with 6,826 mosquitoes). When observed TBA and model-based TBA (adjusted with control target mean oocyst intensity set to the observed value) were compared, there was a strong concordance between the two TBA values (concordance correlation=92.4 (95CI: [91.3, 93.4]) for 4B7 mAb; 97.6 (95CI: [97.2, 98.0]) for polyclonal) in both data sets. The results indicate that TBA cannot be interpreted without mean number of oocysts in the controls, and TRA and TBA are not independent readouts. Moreover, this study establishes that with appropriate data (i.e., mean number of oocysts in the control and the TRA of a test sample), this model can be used to predict TBA at specific target control oocyst mean values in SMFA.

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THE ROLE OF HUMAN MOBILITY ON VECTOR-BORNE DISEASE DYNAMICS

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The emergence and continued persistence of various vector-borne diseases into human populations represents a significant global health risk and disease burden. Accurate predictive models identifying changing patterns of spatial vulnerability to both malaria and dengue will be critical to control and elimination measures. Key to defining spatial vulnerability is the role of infected human travelers on disease dynamics. Here we compare and contrast the role of human mobility on two vector-borne disease: malaria and dengue in both Asia and Africa. We find that the type of mobility relevant for transmission is both pathogen and location specific. We show that although the modeling framework varies between diseases, human mobility in combination with transmission suitability maps are able to estimate disease importation to generate fine-scale dynamic risk maps.

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WHERE TO START MALARIA ELIMINATION: CORE OR FRINGES?

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Malaria and some other tropical diseases are currently targeted for elimination and eventually eradication. In many areas where diseases have been eliminated, it is often concluded that it would have been more efficient to focus efforts earlier in the places with the highest disease burden and transmission. These areas remained a threat after transmission was interrupted elsewhere, leading to the need to maintain potentially expensive surveillance activities in peripheral areas after the disease has been eliminated from them. We use a mathematical model to compare the implications of prioritisation choices in reducing overall burden and costs. We consider the implications of various assumptions of the relationships between burden, risk of importation, and the required duration of elimination program to transmission potential to show that (i) when the duration of the elimination program is independent of the transmission potential, burden is always reduced most by targeting high transmission areas first; (ii) the optimal ordering to reduce costs depends on the actual transmission levels; and (iii) in general, when overall transmission potential is low and the surveillance cost per secondary case is low compared to the cost per imported case, targeting the higher transmission area first is favoured.

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ESTIMATED INCREASE IN MALARIA MORBIDITY AND MORTALITY IN EBOLA-AFFECTED COUNTRIES DUE TO DECREASED HEALTHCARE CAPACITY AND THE POTENTIAL IMPACT OF MITIGATION STRATEGIES

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At its peak the Ebola epidemic in West Africa overwhelmed healthcare systems making adequate care for malaria impossible and threatening the gains in malaria control achieved over the previous decade. Using a mathematical model we attempted to quantify the potential additional indirect burden this may have caused. We estimated the number of cases and deaths from malaria in Guinea, Liberia and Sierra Leone from DHS survey data on malaria prevalence and malaria-intervention coverage prior to the Ebola outbreak. We then removed the impact of treatment and hospital care to estimate additional cases and deaths from malaria due to reduced healthcare capacity. In the worst case scenario of malaria care ceasing as a result of the Ebola epidemic, we estimate increases in untreated malaria cases of 45%, 88% and 140% in Guinea, Liberia and Sierra Leone respectively in 2014. This represents 3.5 (95% CrI 2·6-4·9) million additional untreated cases and 10,900 (5,700-21,400) additional malaria-attributable deaths in 2014. As there is a large asymptomatic reservoir of infection in these highly endemic countries we found that the absence of treatment would have only a limited impact upon transmission, suggesting that the additional burden of this worst-case scenario can be scaled according to any intermediate level of health-care capacity when more detailed data of the impact of Ebola upon malaria care are available. Any disruption to ITN delivery during the Ebola epidemic is also likely to have contributed substantially to the additional indirect burden of the disease, although due to the cyclic nature of transmission we found the main impact upon burden due to this factor may not occur until the 2015 transmission season. We found that emergency MDA and ITN campaigns timed to coincide with the 2015 transmission season could largely mitigate any future impact of Ebola on malaria and to substantially decrease the burden of non-Ebola-related febrile patients in affected areas.

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MODELING THE IMPORTANCE OF THE INFECTIOUS RESERVOIR IN DESIGNING EFFECTIVE MALARIA ELIMINATION CAMPAIGNS

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Elimination of malaria can only be achieved through removal of all vectors or complete depletion of the infectious reservoir in humans. Mechanistic models can be built to synthesize diverse observations from the field collected under a variety of conditions and subsequently used to query the infectious reservoir in great detail. The EMOD model of malaria transmission is calibrated to prevalence, incidence, asexual parasite density, gametocyte density, infection duration, and infectiousness data from nine study sites. The infectious reservoir is characterized by diagnostic detection limit and age group over a range of transmission intensities with and without case management and vector control. The composition of the infectious reservoir by diagnostic threshold is similar over a range of transmission intensities, and higher intensity settings are biased toward infections in children. Recent ramp-ups in case management and use of insecticide-treated bednets reduce the infectious reservoir and shift the composition toward submicroscopic infections. Mass campaigns with antimalarial drugs are highly effective at interrupting transmission if deployed shortly after ITN campaigns. Proper timing of vector control, seasonal variation in transmission intensity, and mass drug campaigns allows lingering population immunity to help drive a region toward elimination.

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CASE-MANAGEMENT FOR COMPLETE CURE OF *PLASMODIUM VIVAX* MALARIA: A MARKOV COST-EFFECTIVENESS MODEL

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Plasmodium vivax elimination, now on the agenda in some regions, requires treatment not only of the blood stage infection but the hypnozoites in the liver that cause relapse. This requires use of primaquine, or another 8-aminoquinoline, which can cause severe haemolysis in patients with Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, an X-linked enzymatic mutation positively associated with malaria prevalence. In this cost-effectiveness model, we analyse the relative costs, effects and risks associated with three alternative ***P. vivax*** case management strategies: 1. chloroquine only treatment for vivax, 2. chloroquine and primaquine treatment without G6PD testing or 3. chloroquine, G6PD testing and primaquine treatment for those testing normal. Known heterogeneity is explored such as G6PDd prevalence, probability of relapse as a proxy for regional vivax strain and sex-specific risk. The results of this conceptual exploration show that uncertainty surrounding key parameters such as adherence to primaquine, likelihood of severe vivax infection, likelihood of drug-induced haemolysis in G6PD deficient individuals is important for decision making and more data are needed. This analysis shows the potential cost-effectiveness of G6PD test contingent primaquine treatment for many settings, under the chosen baseline assumptions.

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MODELING AND SENSITIVITY ANALYSIS OF THE EFFICACY OF LONG-LASTING MICROBIAL LARVICIDING ON MALARIA TRANSMISSION IN WESTERN KENYA HIGHLANDS

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Recent studies have showed that rising insecticide resistance and a relative increase in outdoor transmission have greatly hampered the effectiveness of insecticide-treated nets (ITN) and indoor residual spraying (IRS) in further reducing malaria disease burden in Africa. Microbial larvicides have been suggested as a supplemental intervention tool that may tackle outdoor transmission and pyrethroid insecticide resistance. Long-lasting microbial larvicides (LLML) are particularly attractive because they may be potentially more cost-effective. In order to determine the efficacy and cost-effectiveness of EPA-approved LLML in reducing malaria transmission and clinical malaria incidence, the Epidemiological Modeling (EMOD) model for Malaria Transmission developed by Institute for Disease Modeling was employed to determine optimal larviciding strategies. We simulated malaria transmission dynamics using parameters from study sites in western Kenya highlands under several scenarios: 1) LLML as a supplemental tool in the context of different levels of pyrethroid resistance, 2) different killing efficacy of LLML, 3) varying duration of the effective period of larviciding, and 4) resistance to microbial larvicides. The results show the beneficial effect of ITNs and IRS can be gradually being reduced due to increased insecticide resistance and outdoor transmission in the absence of other supplemental interventions. After introducing LLML as a supplemental intervention, EIR in the highland sites can be reduced by up to 95%. Sensitivity analysis found that 50% larval killing efficacy by LLML in combination with ITNs can still lead to an overall 91% reduction in EIR. On the other hand, an 80% killing rate by LLML can lead to an 98% EIR reduction. Re-treatment of aquatic habitats every 5 months or less would lead to consistent reduction in transmission for the default duration of LLML. In summary, the modeling analyses suggest that LLML has the potential to provide significant added benefits to malaria control.

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MODELING THE EFFECTIVENESS OF POPULATION-LEVEL MALARIA INFECTION DETECTION STRATEGIES FOR OPTIMAL CAMPAIGN SCOPING

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Recent geo-tagged data express significant variation of prevalence and parasitemia, both across spatial regions and sociological units of analysis. Correspondingly, the relative benefits of tools for malaria infection detection - informing drug campaigns planning, execution and outcome analysis - differ across geographical locations and social scales. We investigate the relative effectiveness of infection detection methods in drug campaigns to reduce malaria incidence. Utilizing surveillance and remote sensing data, we re-construct the environmental covariates, vector life cycle, human mobility, and infectious reservoirs at distinct locations characterizing various population units: individuals, households, villages, and districts. We develop a calibrated multi-node spatial model within the EMOD framework accurately capturing the dynamics of malaria transmission and prevalence across hundreds of distinct population units and geographical sites. Within the model, we study five drug campaign strategies: mass drug administration, focal drug administration, mass screen and treat, focal screen and treat, and "snowball" reactive case detection. We evaluate campaign performance over a set of four well-established diagnostics - RDT, PCR from filter paper bloodspots, thick film microscopy, high-volume qPCR - as well as the less commonly used aggregate high-volume qPCR. We derive the optimal combination of infection detection method and campaign strategy for different infectious reservoirs and transmission intensities spanning distinct geographies. In light of ongoing and future malaria elimination efforts comprising regions of greatly varying infectious reservoirs, our results provide a framework for improved drug campaign design via optimal infection detection strategies.

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CATEGORIZING THE SEASONAL PATTERNS OF MALARIA TRANSMISSION FROM CLINICAL OBSERVATIONS AND SATELLITE SURFACES OF CLIMATE DATA

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It is generally accepted that planning of malaria control measures requires proper accounting of local seasonal variation. Where elimination is concerned, it is of even greater importance. That said, many analyses are conducted using gross definitions of transmission intensity, usually in the form of an annual EIR or year-average prevalence, either because more detailed information is unavailable or is difficult to incorporate. Mathematical models have shown that seasonality is an important input to simulations that aim to represent clinical incidence and the impact of interventions that act on either the vector or human populations. In carrying out these analyses, modelers are faced with the need to balance the accuracy of results simulated using spatially and temporally detailed descriptions of seasonality with the efficacy of assuming that large, often arbitrarily defined, regions are similar. We introduce a general framework for assigning regional seasonality profiles in simulations of ***Plasmodium falciparum*** malaria that balances fidelity to observed patterns of clinical data and the computational cost of accurately capturing high resolution climate data. Our approach couples the Temperature Suitability Index (TSI), which quantifies the monthly propensity for transmission across Africa from 2000 to 2012, with the EMOD open-source model of malaria, a mathematical description of the vector life cycle coupled to within-host parasite and immune dynamics. Fourier analysis of the continental TSI data was used to separate latitude-dependent phase shifts from other seasonality characteristics, and dynamic mode decomposition was then applied to identify a basis set of profiles that spans the diversity of observed shapes while limiting redundancy. Finally, a calibration was performed to determine the translation of these profiles into model inputs, in the form of larval habitat descriptions. The broad user base of EMOD can practically leverage this mapping in their own simulations of the disease in sub-Saharan Africa while the seasonality basis set itself can be adapted for use with the wider family of available malaria models.

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COVERAGE AND EFFECTIVENESS OF MALARIA CASE MANAGEMENT IN SUB-SAHARAN AFRICAN COUNTRIES

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Malaria case management provides individual benefits by curing infection and preventing progression to severe disease as well as community-level benefits by reducing the infectious reservoir and averting emergence and spread of drug resistance. Despite the availability of efficacious drugs, the failure to treat malaria episodes effectively probably contributes much to the still unacceptably high burden of the disease. Many patients with malaria do not have access to or delay appropriate treatment, providers do not always comply with treatment guidelines, and patients do not necessarily receive the correct regimen. Following systems effectiveness literature we propose a framework to identify failures along the service delivery path and quantify their impact on patient outcomes. Populated with data from nationally representative surveys from across SSA countries the model yields important insights for malaria control policy.

Effective coverage, captured as a function of treatment seeking, provider compliance, adherence, and quality of medication remains low. While on average treatment is sought for about 60% of fevers, only 35% of episodes are treated effectively; that is nearly 40% of treatments fail to cure parasitemia. Effective coverage is particularly low in Somalia, South Sudan, Chad, Ethiopia, Central African Republic, and North and South Sudan. The counterfactual analysis indicates the potential improvement in effective coverage that could be achieved by scaling-up each of the service indicators. The analysis highlights the constraints that the downstream factors impose on treatment outcomes. Expanding access to treatment translates into higher levels of effective coverage in settings where other service indicators perform well; switching to a more efficacious treatment regimen can only improve effective coverage if treatment is sought and the drug is taken. This suggests that policy efforts targeting marginal improvements across multiple dimensions of service provision will result in a greater improvement in effectiveness than interventions focused on scaling up only one aspect of malaria case management.

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SOCIOECONOMIC-RELATED HEALTH INEQUALITY IN RURAL WESTERN KENYA: EVIDENCE FROM A HOUSEHOLD MALARIA SURVEY ON BURDEN AND CARE-SEEKING BEHAVIOR

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Health inequality is recognized as a barrier to achieving health-related international development goals. Health equality data are essential for evidence-based health planning and assessing the effectiveness of current initiatives to promote equity. Such data have been captured but aren't always analysed or used for managing program efforts. In a malaria endemic site in western Kenya, we examined health inequality data and the societal impact of malaria control initiatives on reducing the equity gap at the micro-level. We analysed data from a malaria cross-sectional survey among 2,719 people in 1,063 households in Siaya County, Kenya in mid-2012. Data were collected on socioeconomic (SES) and demographic factors, history of fever, malaria medication usage, and household expenditure on medications. A composite SES score was created from multiple correspondence analyses of household assets and households were classified as poor (lowest 3 wealth quintiles) or less-poor (highest 2 wealth quintiles). Comparing the two groups, we calculated the odds of diagnostically-confirmed malaria, medication use and care seeking using logistic regression. Medication costs were compared using a generalized linear model. The odds of having malaria was significantly higher in households in the poor category (OR=1.4; 95% CI=1.2-1.7, p=0.001) than less poor. Poor households spent mean of $1.36 (95%CI=0.29-2.42) to purchase malaria medications 2 weeks prior to survey compared to $1.53 (95%CI=1.08-1.98) spent by less-poor households. Poor households spent 13% more to purchase non-recommended medications for malaria. Odds of care seeking and medication usage was lower amongst poor households. In multivariate analysis, the odds of malaria infection were significantly higher in poor versus less-poor households. The data demonstrate the existence of inequalities in malaria indicators between poor and less-poor households in an impoverished rural setting in Kenya. The findings contribute to a strategy for assessing and monitoring micro-level health-equity impact of malaria prevention and control efforts

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AVAILABILITY OF MALARIA PRODUCTS AT THE LAST MILE: AN ANALYSIS OF FACILITY- AND COMMUNITY-LEVEL LOGISTICS DATA

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Learning Objectives: Participants will describe relationships between supply chain data on malaria commodities at community- and facility-levels, specifically looking at stockout data and consumption data. Community health workers (CHWs) are a major entry point to the health system for detection and treatment of malaria. As a first point of contact and common source of artemisinin-based combination therapy (ACT), consumption at the community level should be included for an accurate picture of health service delivery. Exploring the relationship between consumption at the health facility level and commodity level can better ensure availability of malaria products at the last mile. In 2008, Malawi rolled out a new strategy for integrated community case management of childhood illness to improve treatment given by health surveillance assistants (HSAs), who treat malaria cases in the community with ACTs. As the number of HSAs has increased, overall consumption has significantly increased. Compared with health-facility consumption, community-level consumption accounts for a larger percentage of cases in the dry season and up to 50 percent of overall pediatric case treatment. Health facilities supply products to HSAs. From 2011 to 2014, the number of stockouts increased overall, reflected both in the number of days that HSAs stocked out each month and the percentage of HSAs reporting a stockout of a commodity each month. At the national level, the trend of increasing stockouts among HSAs does not reflect on stockouts at the facility level. Overall facility-level stockouts during the dry season are low; and, in general, have decreased over time. In fact, the percentage of HSAs stocked-out increased significantly in 2013, but the facility-level stockouts of pediatric artemether/lumefantrine declined. Although the percentage of facilities stocked-out has decreased overall, this is not consistently reflected in HSA resupply. When stock is available at the facility level, it does not necessarily correlate to a high order fill rate. For most of 2012 and 2013, order fill rates ranged from around 50 percent to 90 percent, averaging around 65 percent.

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WHO GOES WITHOUT? AN ASSESSMENT OF GAPS IN MALARIA INTERVENTION COVERAGE USING STANDARDIZED NATIONAL SURVEYS

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While there have been impressive increases in the coverage of malaria interventions over the past decade, there are millions that do not receive life-saving services. Gaps in service coverage are evident in all countries that have National Malaria Control Programs. To design programs that fill these gaps, it is important to have a good understanding of the factors responsible for differential coverage levels of insecticide-treated nets (ITNs), Intermittent Preventative Therapy in pregnancy (IPTp), a malaria diagnostic test, and malaria treatment with an Artemisinin-based combination therapy (ACT). Some insight can be gained by examining nationally-representative household surveys (which document the characteristics of people who do not receive services), and by decomposing the explained variance in regression models to identify factors that are associated with gaps in coverage. Across the most recent household surveys, a median: 45% of households do not have an insecticide-treated net (IQR 36-66%), 36% of children do not receive any care for fevers (IQR 28-41%), 78% of febrile children do not receive a diagnostic test (IQR 70-88%), and

43% of pregnant women do not receive at least one dose of Intermittent Preventative Therapy. Poverty, represented by belonging to the lowest wealth quintile, was the strongest factor associated with a household not having an ITN. Other important factors were being in a rural area and a head of household without a formal education. Poverty was also the most important factor associated with a pregnant woman not receiving IPTp. Poverty and living in a rural area were most strongly associated with receiving a diagnostic test, whereas the strongest predictor for not receiving an ACT was low educational attainment, followed by living in a rural area and being poor. To build on the increases of the past decade, it is important to identify and fill specific gaps in intervention coverage. The monitoring of malaria intervention coverage should not only include an assessment of where we have come from, but also identify areas where future gains are possible.

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USE OF MALARIA SURVEILLANCE DATA TO DRIVE SUPPLY CHAIN DECISION-MAKING TO RESPOND TO MALARIA OUTBREAKS IN THE LAO PEOPLE'S DEMOCRATIC REPUBLIC (PDR)

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Southern Laos experienced a prolonged malaria outbreak in 2013 and 2014, with the highest number of cases reported in a decade in June 2014. The need for a rapid response was critical to ensure that quality malaria rapid diagnostic test kits (RDTs) and artemisinin-based combination therapy (ACT) treatments were available at health facilities to test and treat the surge in malaria cases expected until the end of the rainy season in October. The United States Agency for International Development | DELIVER PROJECT helped plan and carry out an emergency distribution of RDTs and ACTs in the five southern provinces of the country that were the ***foci*** of the outbreak. In the absence of a regular reporting and resupply system, and lack of data on the availability of these critical commodities at provinces and districts, the Centre for Malaria, Parasitology, and Entomology (CMPE) utilized data from the country's Malaria Information System and from sentinel surveillance sites to quantify and target the emergency distribution to those provinces and districts most heavily affected by the malaria outbreaks. Within four months, the project successfully distributed sufficient stocks to the southern provinces where the outbreak was contained.

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PLASMODIUM CHABAUDI MALARIA IN MICE STRAINS PHENOTIPICALLY SELECTED FOR MAXIMAL OR MINIMAL ACUTE INFLAMMATORY RESPONSE

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AIRmax (high) and AIRmin (low) mouse strains diverged in their acute inflammatory response and they were obtained by the bidirectional phenotypic genetic selection, which allows the concentration of alleles of several loci of genes involved in the acute inflammatory response. Rodent malaria models are ideal and useful for the study of immunity and immune response to malaria, especially those with spontaneous control of the infection, as ***Plasmodium chabaudi***. Here, we studied the evolution of ***P. chabaudi*** malaria and some aspects of the immune response min those strains, using as standard BALB/c mice. Parasitemia, serum cytokines and interleukins or their mRNA production by spleen cells were also determined, as well as liver and spleen histology. Groups of mice of each strain were infected ip with 10^6 parasitized erythrocytes. ***P. chabaudi*** CR malaria was more severe and with slower control in phenotypically selected AIRmin with low acute inflammatory response, a fact more intense in early stages of the infection. The spleen enlargement, evaluated by weight and histology, was notable and similar in all strains, without differences, but the limits between white and red spleen pulps was destroyed in latter periods in the 8th and 12th days after infection, due to cell proliferation. The liver showed fewer portals infiltrates in AIRmin models, which was abundant in BALB/c and AIRmax mice, with extra medullary erythropoiesis ***foci*** more intense in AIRmax mice. There are significant increment in mRNA or inflammatory cytokines, as IFNγ e TNFα, in AIRmin mice, with sustained elevated levels and a delay in the increment of regulatory cytokines IL-4 and IL-10. AIRmax mice appear to produce less inflammatory or regulatory cytokines but in a more orchestrated production, as suggested by histology. Our data support that rodent malaria was more severe in acute inflammatory response phenotypically deficient AIRmin mice as compared to BALB/c or AIRmax strains, the latter associated with organized spleen response with lower levels of cytokines. Those data support the participation of acute inflammatory response in the control of blood stages of malaria infection.

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NEW EFFORTS AIMED AT REPLACING ACTS: THE IDENTIFICATION OF NOVEL, DRUG-LIKE, AND FAST-ACTING ANTIMALARIAL COMPOUNDS FROM A PHENOTYPIC SCREENING

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Malaria is a major global infectious disease that causes nearly 700,000 deaths per year, mostly amongst pregnant women and young children. Drug resistance to the most used current tratments (ACTs) is widespread and the last antimalarial introduced into clinical practice was in 1996. Consequently, the next generation of antimalaria drugs is urgently required. Novel chemotypes with new mechanism of action must be identified to deliver new drugs that not only cure the disease, but that could also block the transmission of it. Unfortunately, in the last few years target-based approaches have proven to have limited success in delivering new antimalarial therapies. This fact has forced the antimalarial scientific community to be innovative and to find other ways of identifying new drugs to tackle this disease. Phenotypic screening has become the preferred approach across the antimalarial community for the identification of novel hit series to support these drug discovery efforts. In 2010 we built and published the Tres Cantos Antimalarial Set (TCAMS): 13,533 compounds that are the result of a whole cell screening of nearly two million compounds from the GSK corporate collection. In the last years, TCAMS along with other antimalarial tool sets of compounds have been our source of new hits. Different ways of analyzing, prioritizing and selecting the best molecules have successfully led to deliver different promising series with potential to become the future antimalarial drugs. With a particular focus on fast-acting antimalarial compounds, we selected hits displaying good physicochemical properties and an encouraging developability profile. Hit optimization efforts and additional profiling data around one of these series will be presented.

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COSTS OF MALARIA TREATMENT BETWEEN PUBLIC, COMMUNITY AND PRIVATE HEALTHCARE PROVIDERS IN SUBSIDIZED INPUT CONDITIONS

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Malaria is one of the main leading causes of healthcare demands in endemic areas. Governments are subsidizing or making free of charge the inputs of fighting against malaria in order to make malaria treatment affordable for their respective populations. Public and private sectors use subsidized and free inputs provided by the government for their customers. The aim of this work was to compare the costs of malaria treatment between public, community and private sectors. In West African Malaria Initiative framework, we carried out a cross-sectional study covering all healthcare center categories in 7 Regions and the Capital city of Mali in 2011. The heads of selected healthcare centers were asked on the fee for medical consultation and the unit cost for the treatment of uncomplicated and severe malaria. For variance comparisons Anova tests were used as appropriate. We surveyed 19, 18 and 16 from the public, community and private healthcare centers, respectively. The average costs for uncomplicated malaria treatment were $4.6, $3.6 and $10.3 (p-value = 0.0031). The treatment of severe malaria was also more expensive within the private sector with $48.2 than public sector with $18.5 or community healthcare providers with $16.7 (p-value = 0.021). When we removed the fees of medical consultation bills the average costs of malaria treatment remained higher in private healthcare providers than public or community providers. There was inequity in the costs of malaria treatment between private, public and community healthcare consumers while all care providers used subsidized malaria control inputs.

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GREATER IMPACT AT A LOWER COST: PRIORITIZING SUPPORT TO PATENT AND PROPRIETARY MEDICINE VENDORS FOR INCREASED QUALITY FEVER CASE MANAGEMENT IN EBONYI STATE, NIGERIA

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Recent ACT watch data shows the importance of the private health sector in Nigeria as a provider of antimalarial drugs to patients. This comes with concerns over the correct use of drugs, as well as the overall quality of care offered by Patent and Proprietary Medicine Vendors (PPMVs). Large-scale support systems available for PPMVs are currently incomplete, and often do not emphasize quality of care aspects. Traditionally, improving healthcare providers' quality of care has relied on training and supervision, which includes performance assessments and coaching. This can be expensive, especially when engaging large networks such as PPMVs. Furthermore, introducing real-time observation of case management can also increase costs, especially if all providers are routinely visited. In order to study better ways to allocate available resources to support PPMVs, an integrated community case management project is being implemented in the Ebonyi State of Nigeria by Society for Family Health, MalariaCare and Population Services International. The project uses two data sources to classify providers: (i) case management performance observations to classify providers into three different groups (good, average and poor) and (ii) commodity stock tracking as a proxy of caseload, to separate PPMVs into two groups according to productivity (high= PPMVs accruing 80% of the overall caseload and low= the remaining 20%). The analysis yields a performance vs. productivity matrix that helps prioritize providers for support, focusing first on those with a higher caseload and lower performance. This classification enables a more focused and better-tailored quality monitoring support effort towards those providers who will benefit the most. This innovative approach leads to more cost-effective resource allocation and by targeting providers with the biggest caseload, ensures populations most in need benefit from a better quality case management service.

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HIGH FOLATE LEVELS ARE NOT ASSOCIATED TO INCREASED RISK OF MALARIA BUT TO REDUCED ANEMIA RATES IN THE CONTEXT OF HIGH DOSED FOLATE SUPPLEMENTS AND SP-IPTP IN BENIN

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Gestational anemia is the first cause of morbidity in pregnancy worldwide, and nutritional deficiencies and pregnancy associated malaria (PAM) are its main causes. Therefore, WHO recommends an intermittent preventive treatment in pregnancy (IPTp) of 1500/75 mg sulphadoxine-pyrimethamine (SP) at each antenatal care visit (ANC) to tackle PAM and its effects. WHO also recommends 200 mg ferrous sulfate and 0.4mg folate daily to fight gestational anemia. However, national guidelines in Benin still recommend 5mg per day. SP is an effective anti-folate and, hence, high dose folate supplements might diminish the efficacy of IPTp, especially in the context of some resistance to SP. To evaluate this possible effect, we analyzed hematological and P.falciparum parasite indicators during the entire pregnancy in the context of the MiPPAD clinical trial comparing IPTp regimes of SP and mefloquine. Between 2010 and 2012, 1,005 pregnant women were followed in a prospective longitudinal cohort until delivery in Allada (Benin). At inclusion, socio-demographic status and gyneco-obstetric history were investigated. Extensive medical and biological exams were realized on the occasion of each dose of IPTp and at delivery. Further exams were realized at each unscheduled visit. All patients were treated in case of disease. Random coefficient models assessed the relationship between the different hematological and parasitological measures and other variables. High folate levels and low ***Plasmodium falciparum*** parasite density had a protective effect on gestational anemia in the context of SP-IPTp. Furthermore, high folate levels were not associated with increased malaria risk (measured by blood smear), nor with increased ***P. falciparum*** parasite density. On the contrary, high iron levels were statistically linked to increased odds of PAM and increased ***P. falciparum*** parasite density. Albeit some resistance to SP, benefits of folate are undeniable and do not seem to entail an increased risk of malaria in the context of IPTp with anti-folates and high dosed folate supplements in Benin.

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IMPROVING PROVISION OF MALARIA SERVICES THROUGH PROVIDER TRAINING IN BURKINA FASO

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In 2013, malaria was the main reason for consultation (53.90%), hospitalization (63.20%) and death (49.60%) in health facilities; children under 5 and pregnant women are most affected. Recent revisions to the World Health Organization's (WHO) guidance to maintain effective approaches to defeating malaria, include monthly dosing of intermittent preventive treatment for pregnant women (IPTp), starting from the 13th week of gestation. To align with the latest WHO guidance, the Burkina Faso Ministry of Health, with support from the United States Agency for International Development-funded Improving Malaria Care (IMC) project, revised national malaria guidelines in March 2014. 68 trainers from 9 health regions were trained on the revised national malaria guidelines. From June to September 2014, 744 providers from 524 health facilities in 21 districts (33%) were trained on the prevention and management of malaria cases. To ensure updated guidance reaches all health workers, the training included a module on how to update colleagues in their respective facilities. During supervision visits, most trained providers were using the new guidelines and pregnant women are increasingly receiving the third and higher doses of SP before delivery. In the first six months after the training sessions, pregnant women, who received the third dose of SP (IPTp3) increased from 0% to 12%. Three months later, that proportion rose to 30%. Comparatively, in the remaining 42 districts who received only the copies of the new guideline without training, IPTp3 was 5% nine months after receiving the guidelines. The training sessions contributed to improving the implementation of revised IPTp guidelines and uptake of IPTp 3 and higher better than distribution of the new guidelines alone. As a result the IMC project will scale-up the training in Year 2 to 600 more providers from 464 health facilities, and other partners have also agreed to support the National Malaria Control Program to reach remaining facilities. Challenges in increasing IPTp uptake include commodity distribution and inadequate engagement of private health facilities to update their practices and reporting of SP distribution.

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NRH: QUINONE REDUCTASE 2 (NQO2) PROTECTS AGAINST HEMOLYTIC TOXICITY INDUCED BY PRIMAQUINE ANTIMALARIAL IN G6PD DEFICIENT HUMAN ERYTHROCYTES

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8-Aminoquinolines (8-AQs), including primaquine (PQ), are important class of anti-protozoal drugs but have limited utility due to hemotoxic effects of one or more metabolites. Hemolytic episodes are of major concern in G6PD deficient populations in states of oxidative stress. Erythrocytes from G6PD deficient Individuals also display depletion of reduced glutathione (GSH) due to compromised capability to produce NADPH. Phenolic metabolites generated through cytochrome P450-dependent pathways appear to be responsible for hemolytic effects of primaquine. The hemotoxic response of the redox active metabolites of PQ could be measured ***in vitro*** by accumulation of methemoglobin and kinetic measurement of increase in oxidative stress. NQO2, a FAD-linked oxidoreductive enzyme, catalyzes mandatory two-electron reduction of quinones to hydroquinones, without accumulating semiquinones and free radicals. Human erythrocytic NQO2 is the only protein target identified for PQ. NQO2 seems to be a detoxification enzyme for quinones. This suggests the role of NQO2 in detoxification of reactive metabolites of PQ in erythrocytes, as redox cycling of quinone and quinone-imine metabolites has been implicated in hemotoxicity of 8-AQs. 5-Hydroxy PQ (5HPQ), and 6-methoxy-8-hydroxylaminoquinoline (MHQ) are among the putative hemotoxic metabolites of PQ. 5HPQ is highly unstable, which is spontaneously converted to 5, 6-orthoquinone (5, 6-o-QPQ) and quinone-imine. 5HPQ and MHQ produce dose-dependent methemoglobin accumulation, oxidative stress and depletion of GSH in G6PD deficient human erythrocytes. The NQO2 inhibitors namely, melatonin, resveratrol and quercetin were tested for their effects on PQ metabolites-induced hemolytic toxicity. The NQO2 inhibitors synergistically increased 5HPQ- and MHQ-induced methemoglobin accumulation, oxidative stress and depletion of GSH in G6PD deficient human erythrocytes. The results suggest a protective role of NQO2 in hemolytic toxicity of PQ. More precise knowledge on interactions of these metabolites with NQO2 should help in understanding the mechanism of PQ-induced hemolytic toxicity.

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TRANSFORMING THE PRIVATE SECTOR MARKET FOR QUALITY MALARIA CASE MANAGEMENT IN KINSHASA: RESULTS FROM BASELINE SURVEYS AND MONITORING ACTIVITIES.

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Malaria remains one of DRC's foremost public health and development challenges. There were an estimated 11.3 million malaria cases and almost 31,000 malaria-related deaths reported through health facilities in 2013, making the DRC the second most malaria-affected country in the world after Nigeria. With support from DFID, PSI and ASF (PSI's local affiliate) are embarking on one of the most significant malaria control pilot projects in the world today. The capital city, Kinshasa, is home to an estimated 12 million people, all of whom at risk from malaria. 97% of antimalarials are distributed through the private sector in Kinshasa, and yet the quality of that treatment is unacceptably poor. In 2013 only 4% of antimalarials distributed in the private sector were quality-assured artemisinin-based combination therapies (QAACTs), compared to 32% SP and 19% quinine. One of the most important current challenges in malaria control is finding a sustainable and replicable approach to transforming the private sector from a public health threat for malaria sufferers to an opportunity to access affordable, high-quality malaria case management services. Such services include the availability of diagnostic testing and QAACTs at private health facilities and drug shops, the most common private sector sources of care. The project comprises a combination of interventions to target identified market constraints including the high ex-factory price of QAACT, poor supply chain coordination, low consumer demand for QAACT and the poor quality private sector malaria case management. A comprehensive M&E plan guides project learning, course correction and evaluation. Here we present the M&E plan, and baseline results on QAACT price from mystery shopper activities and consumer informed demand and use for QAACT and diagnostic testing from a representative population-based survey. In addition, we present quality of care and rationale treatment outcomes from medical detailing visits and client exit interviews at a subset of private facilities included in an RDT pilot embedded within the wider project.

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A TALE OF TWO PROVIDERS: DIFFERENCES IN FEVER CASE MANAGEMENT PRACTICES AND PERFORMANCE AMONG PRIVATE CLINICIANS AND PRIVATE PHARMACY PROVIDERS ON THE KENYAN COAST

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The private health sector is universally recognized as a key player for effective fever case management in malaria-endemic countries. The term 'private health sector' encompasses a variety of providers, with varying competences and behaviours. This inherent heterogeneity of the private health sector is often poorly understood. Population Services International is implementing a project to improve fever case management in the private health sector. In the coastal region of Kenya the project enrols private clinics and pharmacies. In order to better understand differences in provider performance, exit interviews were conducted in Q4 2014 with 534 clients seeking treatment for fever from 86 private clinics and 44 pharmacies. Routine competency assessments were conducted with 75 private physicians and 52 pharmacists in Q3/4 2014. Clients were more likely to be tested for malaria in private clinics than pharmacies (84.1% vs. 39.7%, $p<0.0001$). Overall, 84.9% of 216 malaria test-positive clients received any antimalarial, with no difference between facility types ($p=0.7$). However, test-positive clients were more likely to receive a recommended ACT at pharmacies (86.8%) than they were at private clinics (75.5%, $p=0.06$). Clients at private clinics were twice as likely to receive an antibiotic independently of test outcomes. From the routine assessments 54.4% of pharmacists and 57.7% of physicians scored over 80% when assessed on RDT procedure. Levels of patient counselling were similar in the two groups (pharmacists: 91.5%, physicians: 98.2%). However, no more than one-in-five providers assessed patients for danger signs (pharmacists: 14.1%, physicians: 20.6%). Pharmacists performed worse than physicians in correct fever case management practices (30.9% vs. 42.4%, respectively) and in correct disposal of lancets (24.7% vs. 38.1%, respectively). Provider heterogeneity should be taken into account when conceiving projects within the broad private health sector in order to better define provider profiles and design tailored interventions that improve cost-effectiveness.

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COSTS ASSOCIATED WITH MALARIA IN PREGNANCY IN THE BRAZILIAN AMAZON

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Information on costs associated with malaria in pregnancy (MiP) in low transmission areas where ***Plasmodium vivax*** (Pvivax) predominates is so far missing. This study estimates health system and patient costs of MiP in the Brazilian Amazon. Methods/principal findings: Between January 2011 and March 2012 patient costs for the treatment of MiP were collected through an exit survey at a tertiary referral hospital and at a primary health care centre in the Manaus metropolitan area, Amazonas state. Pregnant and post-partum women diagnosed with malaria were interviewed after an outpatient consultation or at discharge after admission. Seventy-three interviews were included in the analysis. Ninety-six% of episodes were due to Pvivax and 4% to ***Plasmodium falciparum*** (Pfalciparum). In 2010, the total median costs from the patient perspective, were estimated at US$ 45.91 and US$ 216.29 for an outpatient consultation and an admission, respectively. When multiple Pvivax infections during the same pregnancy were considered, patient costs increased up to US$ 335.85, representing the costs of an admission plus an outpatient consultation. Provider direct and indirect costs (overheads) data were obtained from several sources. The provider cost associated with an outpatient case, which includes several consultations at the tertiary hospital was US$ 103.51 for a Pvivax malaria episode and US$ 83.59 for a Pfalciparum malaria episode. The cost of an inpatient day and average admission of 3 days was US$ 118.51 and US$ 355.53, respectively. Total provider costs for the diagnosis and treatment of all malaria cases reported in pregnant women in Manaus in 2010 (N=364) were US$ 17,038.50, of which 92.4% due to Pvivax infection. Conclusion: Despite being an area of low risk malaria transmission, MiP is responsible for a significant economic burden in Manaus. Especially when multiple infections are considered, costs associated with Pvivax are higher than costs associated with Pfalciparum. The information generated may help health policy decisions for the current control and future elimination of malaria in the area.

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EVALUATION OF A MOBILE PHONE-BASED MALARIA ROUTINE SURVEILLANCE SYSTEM IN AMHARA REGION, ETHIOPIA

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Ethiopia is a malaria epidemic-prone county learning to transition across the control to elimination continuum in selected districts. Surveillance systems for tracking morbidity and monitoring systems to decide on positioning of anti-malaria commodities based on village level spatiotemporal fluctuations are crucial parts of the program. A rapid reporting system was established in health posts (HPs) in 209 villages in eight districts in Amhara Region, Ethiopia. Community based surveillance assistants (CBSAs) were trained and deployed to report weekly morbidity and commodity data using mobile phones supported by the web-based DHIS2 platform. Reporting includes the counts of seven morbidity and behavioral elements and six commodity data elements. Weekly reporting data were extracted over a 20-month period (September 2013-April 2015). We explored whether reported surveillance data could enable health workers to visualize accurate data to follow the oscillation of morbidity and trigger local response decisions. Across the 209 HPs, the overall report submission rate was 91%; 89% of submitted data was considered complete; and 89% of HPs reported data parameters free of errors. Due to challenges with the DHIS2 system, mobile reporting timeliness could not be captured in the system. In the 209 HPs, the mean weekly rapid diagnostic test (RDT) positivity rate was 30% (weekly range: 13-49%). This initial system assessment demonstrated that for the existing data collection system in rural Ethiopia, it is feasible to use smartphones to report from rural HPs into platforms such as DHIS2 to inform decisions at any level. Data validation procedures are critical to assess and ensure the accuracy of data reported. Factors influencing data quality include the training and follow-up of the CBSA in each HP catchment area, existence and use of job aides, and the number and quality of source documents for reporting cases. Lessons were documented on specific improvements to the rapid reporting system including better measurements of reporting timeliness.

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MASS WEEKLY FEVER SCREENING, TESTING AND TREATMENT FOR MALARIA IN LOW TRANSMISSION AREAS IN MATAM AND LOUGA REGIONS, SENEGAL

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The PECADOM+ strategy (prise en charge à domicile), a weekly active case detection for malaria conducted by community health workers, is ongoing in Southeast Senegal since 2012 and has shown promising results to decrease the malaria burden. The MOH and MACEPA implemented an enhanced PECADOM++ strategy in Kanel, Linguère and Ranérou districts as part of malaria elimination activities conducted in these low-transmission areas during the 2014 transmission season. All households were visited weekly to screen for fever cases and all individuals with a fever or a history of fever in the last 7 days were tested with a rapid diagnostic test (RDT). Positive individuals were treated with dihydro-artemisinin-piperaquine and all individuals in their households were tested and treated if positive (household focal testing and treatment). A quasi-experimental study was conducted in six intervention health post catchment areas, with seven adjacent health posts with similar characteristics selected as controls. Villages within the catchment areas were stratified according to the 2013 incidences of passively detected malaria cases and targeted with different interventions by strata. Those with an incidence ≥5 cases/1000/year received PECADOM++ from October 2014 to January 2015. To evaluate the impact of the interventions, the incidence of passively detected, RDT-confirmed malaria cases at the health posts will be compared before and after the intervention and between intervention and control villages. Preliminary results show that 94% (3100/3304) of households received at least one weekly visit, but the field work was logistically challenging, resulting in inconsistent coverage over time. On average 9% of households had at least one individual with a history of fever each week and 6% of those were RDT-positive (ranging from 2 to 14% by health post). The household focal testing and treatment positivity rate was also 6% (range 0-9% per health post). Final results of the impact evaluation, assessment of geographical clustering of infections over time, characteristics of malaria cases and estimates of implementation costs will be available in mid-2015.

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SHARED EPITOPES BETWEEN *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN (PVDBP) AND *P. FALCIPARUM* VAR2CSA INDUCE CROSS-REACTIVE ANTIBODIES IN COLOMBIAN POPULATIONS

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Antibodies against the VAR2CSA antigen of ***Plasmodium falciparum*** are associated with improved outcomes of pregnancy-associated malaria. In a recent study, we demonstrated that contrary to what was described in African studies, men and children from Colombia exposed to ***P. falciparum*** and ***P. vivax*** had functional antibodies against VAR2CSA. Here we identified cross-reactive epitopes between VAR2CSA and the Duffy binding protein from ***P. vivax*** (PvDBP). We showed by confocal microscopy that a PvDBP monoclonal antibody generated in mice recognized the VAR2CSA protein expressed by the CS2 ***P. falciparum*** lab strain. Both the PvDBP antibody and anti-VAR2CSA antibodies from an immunized rabbit cross-recognized PvDBP and VAR2CSA proteins by western blot. We identified two peptides within the DBL5ε domain of VAR2CSA that are selectively recognized by sera from Colombian, but not Beninese, men and children. Furthermore, we identified one peptide from PvDBP that shares homology with one of the DBL5ε peptides. Antibodies against these two peptides from different species were highly correlated. Our findings suggest that epitopes from ***P. vivax*** antigens induce antibodies that cross-react with VAR2CSA from ***P. falciparum*** and may be associated with protection against pregnancy-associated malaria.

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A MALARIA VACCINE FOR TRAVELERS AND MILITARY PERSONNEL

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The growth of global travel to potentially malarious regions increases the risk of malaria infection among non-immune travelers. Despite the availability of highly effective malaria protective measures, including chemoprophylaxis, malaria cases among persons who travel from malaria-free countries to endemic areas continue to occur, likely due to suboptimal rates of compliance and other related factors. Two non-immune groups who are at a particularly high risk of acquiring severe malaria infection are international travelers to Africa who visit friends/relatives and military personnel deployed to malaria endemic areas. These two groups would benefit greatly from a vaccine to protect them against malaria infection and constitute a substantial market for such a vaccine. Desirable attributes of a vaccine to protect non-immune travelers and military personnel include a protective efficacy of at least 80% and preferably greater than 90%, adequate level of protection achieved within 14 days of completing the immunization series, durability for at least 6 months, acceptable safety/tolerability profile, and compatibility with current malaria protective measures. Phase 1 clinical studies have shown that a vaccine protective efficacy of at least 80% against controlled human malaria infection (CHMI) was achievable with the RTS,S vaccine with delayed fractional dose and the PfSPZ Vaccine. Clinical studies are being conducted on both vaccine candidates to obtain valuable data on the optimum regimen that affords protection against heterologous strains of ***P. falciparum***, and greater than 6 months durability of protection to support licensure of an effective vaccine for travelers and military members.

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RELATIONSHIP OF MALARIA PARASITEMIA TO PFAMA-1 VACCINE-INDUCED IMMUNOGENICITY

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Many clinical trials of malaria vaccines have included pre-vaccination curative antimalarial therapy and others have not. The impact of parasite clearance on vaccine immunogenicity for both pre-erythrocytic and erythrocytic antigens is not known. In Phase 2 testing in Malian children, an apical membrane antigen 1 (AMA1) malaria vaccine showed strain-specific efficacy against clinical malaria caused by ***Plasmodium falciparum***, and the increase in anti-AMA1 antibody from baseline

was associated with protection. To evaluate the effect of ***P. falciparum*** asexual parasitemia at vaccination on subsequent immunogenicity, we compared the anti-AMA1 antibody levels at baseline and 30 days after the last vaccination in AMA1 vaccinees positive for asexual parasites by PCR or by microscopy to AMA1 vaccinees negative for parasitemia using an unpaired t test. A subset of 40 vaccinees was also evaluated using a protein microarray that measured seroreactivity to 264 unique AMA1 ectodomain variants at baseline and at 30 days after the last vaccination. Among AMA1 vaccinees with immunogenicity measured, 47/194 had detectable ***P. falciparum*** parasites on vaccination days. Mean anti-AMA1 antibody levels at baseline and 30 days after last vaccination in parasite-positive children were 56.3 µg/mL and 563.2 µg/mL, respectively. In parasite-negative children, these values were significantly lower at 6.0 µg/mL ($p<0.0001$) and 398.8 µg/mL ($p=0.0045$), respectively. Of 40 vaccinees with seroreactivity measured by protein microarray, 11 were parasitemic on days vaccinations were given. Among these 11, seroreactivity against 23 AMA1 variants was higher at baseline than for the 29 participants who were malaria negative ($p<0.01$). At 30 days post-last vaccination when seroreactivity had increased for all variants, no differences in microarray seroreactivity were observed for participants positive vs. negative for malaria at vaccination. These findings suggest that malaria parasitemia at vaccination did not negatively impact, and may have enhanced, humoral immunogenicity for a blood stage vaccine that showed strain-specific efficacy.

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GENOME-WIDE IMMUNOSCREENING OF ANTIBODIES RESPONSIBLE FOR *PLASMODIUM FALCIPARUM* GROWTH INHIBITORY ACTIVITY OF MALIAN ADULT IGG

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Residents in malaria endemic area acquire resistance against the disease by repetitive infection with ***Plasmodium falciparum***. Experiments have demonstrated that malaria symptoms in children infected with the parasite can be alleviated by passive transfer of antibodies from malaria semi-immune individuals. This passive transfer provides strong evidence that antibodies have important roles in malaria immunity. Furthermore, antibodies from people residing in a malaria endemic area have been demonstrated to recognize antigens of merozoite, the erythrocyte invasive form of the parasite, and exhibit growth inhibitory activity (GIA) against ***in vitro*** cultured ***P. falciparum***. We therefore hypothesized that identification of antigens responsible for GIA may support the discovery of novel blood-stage vaccine candidates. In this study, we used Malian adult IgGs (n=51) that had GIA against ***P. falciparum*** 3D7 strain ranging from 17 to 84%. Genome-widely selected 1,848 proteins of the 3D7 strain were synthesized by wheat germ cell-free system; a system that can express natively-folded malaria proteins. AlphaScreen, a high-throughput homogeneous system that retains natural conformation of proteins in solution, was modified to detect antigen-antibody reaction. By setting the reactivity cut-off point as mean plus 3SD of the AlphaScreen count of negative control, we selected 905 immunoreactive proteins against Malian IgGs. Of those, we selected 39 antigens which showed significant positive correlations between their reactivity and the GIA. GIA has been attributed to antibodies targeting multiple antigens. Therefore, in addition to the analysis of individual antigens, we statistically analyzed combinations of 3 antigens that could better explain the GIA observed with Malian adult IgGs. To validate this approach, GIA assays were performed with a mixture of 3 rabbit antibodies against ***P. falciparum*** antigens selected by the analysis. The mixture showed higher GIA value than any of individual IgG. From these results, we conclude that our AlphaScreen system is effective to identify novel malaria vaccine candidates.

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COMPLEX SYNTHETIC MINIGENE VACCINES: AN ALTERNATIVE APPROACH TO ANTIGEN DISCOVERY

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In the whole ***Plasmodium*** sporozoite model in BALB/c and C57BL/6 mice, we have attempted to modify dosing regimens, intervals, dosages, degree of parasite attenuation and sporozoite species in order to increase the potency of sporozoite-based vaccination. Our efforts continue to show that whole sporozoite hyperimmunization results in 'boostable' CD8 T cell responses to high-abundance, pre-formed antigens and 'unboostable' responses to antigens newly expressed in the liver stage. Since modifications to traditional sporozoite immunization could not dramatically increase diversity the immune repertoire or the intensity of responses against newly expressed liver-stage antigens, we turned to a novel synthetic DNA vaccination approach. We performed proof-of-principle experiments in mice by testing a rapid synthetic "minigene" technology for highly parallel synthesis of multi-antigen ***Plasmodium***-derived DNA vaccines. We utilized microarray-based oligonucleotide synthesis technology and ligation-independent cloning to rapidly synthesize two DNA vaccines, each encoding the complete peptide compliment of 50 secreted or transmembrane ***P. yoelii*** proteins. Using different DNA gene gun vaccination regimens, we induced responses to both known and previously unknown novel antigens. Antigenic responses are being investigated for their ability to protect mice singly or in combination with other DNA vaccine-induced responses. Interestingly, while most but not all DNA vaccine-induced responses can be recalled by later sporozoite exposure, a minority of these responses could be induced by sporozoites alone. The utility of such sub-dominant antigens in multi-antigen vaccines is under evaluation. These data show that rapidly produced, complex experimental DNA vaccines are capable of identifying multiple subdominant T cell antigens. We believe that, with appropriate automation, this approach represents a high-throughput system for discovery of vaccine subunits that cannot be identified by conventional vaccinology approaches.

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A SYNTHETIC TLR4 AGONIST ENHANCES BI-FUNCTIONAL ANTIBODIES AND MULTI-FUNCTIONAL CD4+ T-CELL RESPONSES AGAINST A *PLASMODIUM FALCIPARUM* MULTI-STAGE PROTEIN VACCINE

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The development of a multi-stage subunit vaccine targeting both transmission and disease-causing asexual blood stages of ***Plasmodium falciparum*** (Pf) is considered a focus area for malaria vaccine development. We have previously explored a combination vaccine composed of the N-terminal portion of Pf GLURP (R0) genetically fused to the C-terminal 10C fragment of Pfs48/45. Since the GMZ2 vaccine candidate is in advanced clinical development, it was of interest to investigate whether a combination of GMZ2 and Pfs48/45 can elicit an immune response against both transmission and asexual blood-stage parasites. GMZ2 is a hybrid protein consisting of conserved domains of the two asexual blood-stage antigens GLURP27-500 and MSP3212-380 aimed at mimicking pathogen components that induce premunition, a state of NAI against Pf malaria. Here, we have produced the GMZ2.6C chimera between GMZ2 and the 6 Cys-fragment (6C) of Pfs48/45 containing a major epitope for transmission blocking (TB) antibodies.

Fusion molecules containing properly folded 6C were affinity purified using a rat monoclonal antibody. A preclinical study screening of a series of adjuvant formulations (stable emulsions, liposomes, and alum) containing the immune modulators GLA, SLA, and/or QS21 plus GMZ2.6C was performed in C57BL/6 mice to identify a vaccine suitable for further human clinical studies. Those adjuvant formulations containing the synthetic TLR4 agonists GLA or SLA elicited the highest parasite-specific IFA titers, the greatest IFN-γ responses in CD4+ TH1 cells, and the highest percentage of multi-functional CD4+ T cells expressing IFN-γ and TNF in response to GMZ2.6C. GMZ2.6C combined with GLA or SLA formulated with QS21 provided the strongest TB activity four weeks following the last immunization. Furthermore, SMFA activity correlated strongly with the titer of antibodies recognizing sexual-stage parasites as measured in a gametocyte-extract ELISA. Vaccines combining GMZ2.6C with an adjuvant formulated with a synthetic TLR4 agonist show considerable promise, and scale-up manufacturing of the components is underway.

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A FRAMEWORK FOR EVALUATING DIFFERENT MALARIA TRANSMISSION BLOCKING VACCINES

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Transmission blocking vaccines (TBVs) against malaria are intended to induce immunity against the stages of the parasites which infect mosquitoes. Used within a community they protect the immediate neighbourhood of vaccinated individuals and could be a key tool for malaria elimination. Various TBV candidates are currently under evaluation. Their efficacy at reducing the number of infectious mosquitoes is dependent both on the level of parasite exposure (measured as the mean oocyst number in the control group, which may vary widely between locations) and on antibody titre (which decays with time between vaccination campaigns). This makes it important to understand the shape of the 3D relationship between efficacy, exposure and titre for each TBV candidate in order to compare them and predict their long term effectiveness in the field using early clinical trial data. Here we present a new mathematical framework for understanding this 3D relationship which takes into account the high variability generated by the membrane feeding assay. A variety of different functional forms are fit to the direct and standard membrane feeding assay data simultaneously for each TBV candidate, in order to generate smooth curves that allow the different candidates (with different titres) to be directly compared. Efficacy estimates from 4 different monoclonal antibodies (Pfs230, Pfs25, Pfs 48/45.1 and Pfs 48/45.5) are generated that allow their respective strengths and weakness in different conditions of malaria exposure and antibody titre to be identified. For example results indicate that pfs230 causes transmission blockade at a lower IgG titre than pfs25 and is more sensitive to changes in parasite exposure. This framework procures a comprehensive, easily accessible method of evaluating TBV candidates and can be combined with Phase II clinical trial data to predict their public health benefit in different field settings.

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TRANSMISSION BLOCKING ACTIVITY OF ANTIBODIES TO *PLASMODIUM FALCIPARUM* GLURP-PF10C CHIMERIC PROTEIN FORMULATED IN DIFFERENT ADJUVANTS

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Plasmodium falciparum (Pf) is transmitted to a human host by bites of infected Anopheles mosquitoes after completion of parasite reproduction and sporogony. Clinical development of vaccines against transmission stages is critical for effective control and eradication of malaria. We generated a chimeric protein composed of ***Pf***-sub-unit fragments Glutamate-rich Protein (R0) fused in frame to a correctly folded fragment of ***Pf***s48/45 (10C). R0-10C was expressed as a recombinant protein in ***Lactococcus lactis*** and purified by affinity-chromatography. The soluble protein generated strong transmission blocking antibodies in rodents as determined in the Standard Membrane Feeding Assay (SMFA). Potency of different adjuvant/R0.10C combinations was tested in mice and rats using Freund's adjuvant, aluminium hydroxide (Alum), Alum with addition of GLA (TLR4-agonists), Stable Emulsion (SE)/GLA and AbISCO-100. All formulations produced high antibody titres recognizing the native Pfs48/45 protein in macrogametes/zygotes. Interestingly, Alum adjuvated combinations were the more potent inducers of transmission blocking antibodies. Moreover, SMFA activity correlated strongly with the titer of antibodies recognizing the native antigen as measured in a gametocyte-extract ELISA. The combined data provide a strong basis for entering the next phase of clinical grade R0-10C production and testing.

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SCREENING FOR HIGHLY IMMUNOGENIC REGION OF PYGM75, A NOVEL TRANSMISSION-BLOCKING VACCINE CANDIDATE

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Malaria transmission-blocking vaccines (TBVs) are intended to induce antibodies that inhibit parasite mating or further development inside mosquito midgut. Thus, TBV-immunized individuals cannot transmit malaria to mosquito vector, which could be one of good strategies to eradicate malaria. The number of candidate antigens for TBV is limited, and up to now, one leading vaccine-candidate, Pfs25, expressing on the ookinete surface is under phase I clinical trial. Thus, we urgently need to discover more vaccine targets. Previously, we reported that a novel male specific protein named PyGM75, is localized to the surface of microgametes in ***Plasmodium*** yoelii and that anti-PyGM75 antibodies had strong transmission-blocking activity. Since genomic database demonstrates that orthologue genes for PyGM75 are existed in human malaria parasites, ***Plasmodium falciparum*** and ***P. vivax***, this could be a promising candidate for TBV development. In this study, our aim is to determine which region of PyGM75 contains suitable epitopes for effective transmission-blocking antibodies. We produced 5 truncated PyGM75 recombinant proteins, excluding transmembrane domain in C-terminal, using wheat-germ cell-free expression system, and designated them as regions I, II, III, IV and V. Region-specific antibodies were collected by affinity purification from anti PyGM75full rabbit serum. Then the transmission-blocking efficiency of purified region-specific antibodies was examined by the membrane-feeding assay. As a result, specific antibodies against region V significantly reduced the numbers of oocysts on the mosquito midgut as efficiently as PyGM75full antibodies. These data suggested that the major epitopes for transmission-blocking antibodies

locate in region V, nearly C-terminal of PyGM75. This study might provide useful information for TMV development targeting to Pf or Pv orthologue of PyGM75.

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ARE TWO MALARIA TRANSMISSION BLOCKING VACCINES BETTER THAN ONE? SAFETY AND IMMUNOGENICITY OF PFS25M- EPA/ALHYDROGEL® PLUS PFS230D1M- EPA/ ALHYDROGEL® IN MALARIA NAÏVE ADULTS

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A vaccine to interrupt malaria transmission would be a valuable tool for local elimination or eradication of this disease. Pfs25 and Pfs230, surface antigens of zygotes and ookinetes in the mosquito stage of ***Plasmodium falciparum***, are currently the lead candidates for a malaria transmission blocking vaccine. A phase 1 clinical trial to assess the safety and immunogenicity of Pfs25 has recently been completed in malaria naïve adults in the US and in malaria-exposed adults in Mali with promising results. Here, we hypothesized that a combination of Pfs25- and Pfs230-based vaccines would enhance functional activity by targeting sequential phases of parasite development in the mosquito. A dose-escalating phase 1 study has been initiated to determine safety and immunogenicity of these vaccines in US adults prior to testing in Malian adults. A total of 35 subjects have been enrolled in the US to receive two doses of Pfs25M-EPA/ Alhydrogel®, two doses of Pfs230D1M-EPA/Alhydrogel®, or simultaneous administration of two doses of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® on Day 0 and on Day 28, escalating between groups. Enrollment within each group was staggered for additional safety, and subjects were enrolled into the simultaneous administration group once each individual dose had been administered and reviewed for safety. Vaccinations have been well tolerated with the majority of the reported AEs being mild (Grade 1) and the most commonly reported AE being local site injection pain. There have been few reported related Grade 2 AEs (local site injection pain, fatigue, hemoglobin decreased) and a few reported mild (Grade 1) laboratory abnormalities. No Grade 3 AEs have been reported. Specific anti-Pfs25 and anti-Pfs230 antibodies and functional activity are scheduled to be completed in the upcoming months.

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VIRUS LIKE PARTICLES CONTAINING PFS25

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Transmission-blocking malaria vaccines which target the sexual-stages of the malaria parasite in the mosquito midgut are widely considered to be an essential tool for malaria elimination. In order to achieve effective transmission-blocking activity high-titer functional antibodies are required against target antigens. We have fused Pfs25 and EGF2, the Pfs25 domain responsible for the majority of transmission blocking antibodies to three virus like particle (VLP) platforms. Genetic fusion to the Hepatitis B surface antigen (HbSAg) and the coat protein of bacteriophage AP205 as well as chemical conjugation to the coat protein of bacteriophage QBeta (Qβ) generated correctly-formed visible particles under electron microscopy (EM). Mice were immunized with a prime-boost vaccination regime using protein-in-adjuvant (Alhydrogel). The poster will present data on immunogenicity and transmission blocking activity.

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COMPARATIVE ASSESSMENT OF VIRAL VECTORED AND PROTEIN-IN-ADJUVANT PLATFORMS FOR DELIVERY OF TRANSMISSION-BLOCKING VACCINE CANDIDATES AGAINST *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum malaria transmits infection between the human host and mosquito vector via its sexual stages. This transition between drastically different host environments exerts a bottleneck effect, making these stages particularly alluring for transmission-blocking vaccine (TBV) interventions. Indeed, in a vaccination model, antibodies to sexual-stage antigens, ingested in the mosquito blood meal, can inhibit parasite growth in the insect mid-gut as judged by functional ***ex vivo*** experiments such as the standard membrane feeding assay (SMFA). Despite the availability of promising antigenic targets and multiple vaccine delivery strategies, pre-clinical development has lagged in rationally ranking these antigens and platforms in head-to-head comparisons. In this study, the current best-characterized panel of TBV candidates, Pfs230C, Pfs48/45, Pfs28 and Pfs25, were delivered as vaccine antigens in both viral vectored and protein-in-adjuvant formulations to (1) rationally rank the candidates in both delivery platforms and (2) to compare vaccine strategies for each antigen. Recombinant chimpanzee adenovirus 63 (ChAd63) and modified vaccinia virus Ankara (MVA) were generated to express each of the above antigens. The same gene sequences were cloned into a customized plasmid for heterologous protein expression in transiently transfected HEK293 cells followed by affinity-tag purification. Groups of BALB/c mice were immunized in parallel with either a ChAd63-vectored prime followed by MVA-vectored boost or recombinant protein in Addavax™ adjuvant to generate anti-sera for determining antibody immunogenicity. Purified whole IgG from both regimes exhibited a hierarchy of inhibitory activity by SMFA, with anti-Pfs230C and anti-Pfs25 antibodies giving between 99.8 and 100% blockade in both the viral vectored and protein-in-adjuvant delivery platform. This study serves to inform future clinical development by providing the first head-to-head comparative analysis of current leading TBV candidate antigens using two different antigen delivery platforms in the same study.

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IMMUNOGENICITY OF TWO CHIMERIC RECOMBINANT CIRCUMSPOROZOITE PROTEIN EXPRESSED IN PICHIA PASTORIS CANDIDATES FOR THE DEVELOPMENT OF A GLOBAL VACCINE AGAINST *PLASMODIUM VIVAX*

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Recently a recombinant vaccine was developed against the deadly ***Plasmodium falciparum*** based on the Circumsporozoite protein (CSP). These optimistic results boosted effort for using the CSP to develop of a vaccine against ***P. vivax***, the most widely distributed malaria worldwide. Toward that goal, we generated two chimeric recombinant proteins

based on the ***P. vivax*** CSP merging the three central repeat regions of the different allelic forms (VK210, VK247 and vivax-like). The full-length (FL) construct contains the fused repeat regions flanked by N- and C-terminal regions. The second construct contains the fused repeat regions and the C-terminal domain. Both proteins were expressed as a soluble form in the supernatant of cultures of the yeast Pichia pastoris. The recombinant proteins were purified by Ni-affinity and ion exchange chromatography, with high yield and purity confirmed by RP-HPLC. Groups of C57BL/6 mice were vaccinated with formulations containing each recombinant protein in the presence of the adjuvants Poly (I: C) or Montanide ISA-720. In all groups of vaccinated mice, high titers of IgG (>106 as measured by ELISA) against all the three allelic variants were observed after three doses. We concluded that both chimeric constructs are interesting candidates to develop a global vaccine formulation against malaria caused by ***P. vivax***.

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A WHOLE PARASITE VACCINE AGAINST MULTIPLE STAGES IN *PLASMODIUM FALCIPARUM* LIFE CYCLE FOR INTERRUPTING MALARIA TRANSMISSION FROM THE HUMAN HOST TO THE MOSQUITO VECTOR

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A vaccine that interrupts malaria transmission (VIMT) would be a valuable tool for malaria control and elimination. Sanaria is exploiting its capacity to manufacture ***Plasmodium falciparum*** (Pf) gametocytes under GMP conditions to develop a whole parasite (WP) vaccine against the sexual and mosquito stages (SMS) of Pf, to be used to induce antibodies that interdict parasite transmission from the human host to the mosquito vector. This PfSMS-WP-VIMT approach increases the diversity of antigens presented as compared to subunit vaccine approaches. This should improve the chance of protective coverage due to vaccine and reduce the likelihood of parasite evasion of the immune response and evolution of vaccine resistance. We have purified SMS from an ***in vitro*** cultured mixture of gametocytes, gametes, zygotes and ookinetes, and purified the parasites to significantly reduce the presence of human erythrocytes. Mice were immunized with this enriched-SMS preparation in GLA-LSQ adjuvant (Infectious Disease Research Institute, Seattle, GLA, QS21, liposomes), each receiving 5×10^6 ookinetes per dose by IM injection on days 0, 14, 36 and 57. Anti-sera were collected 2 weeks after the last immunization. The pooled sera from the immunized mice completely (100%) inhibited transmission of parasites to mosquitoes by standard membrane feeding assay (SMFA). A PfSMS-WP-VIMT will be an ideal complement to Sanaria PfSPZ Vaccine, which have shown complete protection against Pf in human volunteers at the pre-erythrocytic stage of the life cycle.

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MALARIA INFECTION AND GAMETOCYTE CARRIAGE RATE, ASSESSED IN A COHORT OF ADULTS DURING MALARIA TRANSMISSION BLOCKING ASSAY DEVELOPMENT IN BANCOUMANA, MALI

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For malaria elimination/eradication, transmission blocking tools are key interventions to be integrated in existing control strategies. Epidemiological characterization of transmission reservoirs are being evaluated in targeted populations prior to the start of phase 1 or 2 transmission blocking vaccine clinical trials in order to support study designs and analysis plans. From 2011 to 2014, we have followed a dynamic cohort of adults aged from 18 to 50 years in Bancoumana, Mali, in order to improve assays that support trials of transmission blocking vaccines. After community permission, an individual informed consent was obtained for each volunteer. The study protocol and informed consent document were approved by both National Institute of Allergy and Infectious Diseases/NIH in USA and FMPOS IRB in Mali. The volunteers were screened monthly for malaria parasite and gametocyte carriage. Passive surveillance and care were also provided to volunteers in case of illness throughout the duration of the study. Malaria smears were stained using Giemsa and slides were read by two different readers to assess the presence of malaria parasites.Considering November, the month where parasite carriage peaks, the ***Plasmodium falciparum*** infection rates were: 22.58 (14/62), 25.79 (57/221), 32.94 (28/85), respectively in 2011, 2012 and 2014. Likewise, considering October, the month where the gametocyte carriage peaks, the ***gametocyte carriage*** rates were: 8.57 (6/70), 10.07 (30/298), 6.82 (6/88), respectively in 2011, 2012 and 2014. There were no statistically significant differences seen, for the ***Plasmodium falciparum*** infection rates or for the gametocyte carriage rates, between the different years of the study during the peak months of carriage. Malaria infection and gametocyte carriage rates are sufficiently prevalent in the adult population at this study area to conduct trials that assess the activity of transmission blocking vaccines.

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EVALUATION OF PROTEIN CONJUGATE NANOPARTICLES FOR MALARIA TRANSMISSION BLOCKING VACCINES

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Antigen delivery technologies play an important role in enhancing the immunogenicity of antigens and are critical in the development of effective vaccines. The Laboratory of Malaria Immunology and Vaccinology has pioneered the use of protein-protein conjugates for product development, and specifically shown that chemical conjugation of antigens to carrier proteins creates nanoparticles that enhance the antibody response and functional activity of serum after vaccination with the malaria transmission-blocking candidate antigen Pfs25. We sought to evaluate features of our conjugated nanoparticles that might further enhance immunogenicity. In comparative studies of different carriers, conjugation of Pfs25 to Tetanus Toxoid (TT) or CRM-197 induced similar Pfs25 antibody titers when compared to our standard conjugates of Pfs25 to ExoProtein A (EPA). We also explored nanoparticle size, as this may have an effect on the uptake of the antigen complex. Pfs25-EPA conjugates were subjected to fractionation by Size Exclusion Chromatography and different molecular weight fractions were collected and characterized by DLS and electron microscopy. Mice were immunized with fractions with an average diameter of 16, 37, 52 and 73 nm, to evaluate the anti-Pfs25 antibody titer. Immunizations were carried out both formulated on Alhydrogel (AH) and in saline alone. In the absence of AH, conjugates with 16 nm average size gave the highest level of antibody titer. This was not significantly higher than the others except 73 nm. Immunogenicity in other groups did not vary with size. In the presence of AH, average titers of various groups were similar, again with no significant difference between the groups, suggesting that adsorption to the large AH particles may nullify any effects based on the size of individual conjugates. In summary, the use of different established carrier proteins, or of nanoparticles of sizes above 16 nm, did not enhance the immunogenicity of our Pfs25-EPA conjugate vaccines.

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A NEW SET OF *PLASMODIUM FALCIPARUM* GAMETE-SURFACE REACTIVE MONOCLONALS

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Monoclonal antibodies (mAb) are valuable tools to identify vaccine candidates as well as assess the structure and function of specific epitopes. These features of mAb have been key to monitoring the production of recombinant vaccine candidates, which can be challenging to generate with native structure using heterologous expression systems. Disulfide-rich, membrane-associated antigens, such as the malaria transmission-blocking vaccine candidates, are particularly difficult to produce. To increase the current library of monoclonal antibodies against transmission-blocking targets in ***Plasmodium falciparum***, we generated a new panel of hybridomas that produce IgG molecules that recognize the surface of intact gametes. An efficient flow cytometry-based immunofluorescence assay (IFA) was developed to screen the 594 hybridomas generated from gamete-immunized mice. From this analysis, 59 hybridomas (10%) produced IgGs that recognized the gamete surface. The target antigens were further evaluated by immunoblot and immunoprecipitation. Supernatants from 39% of the 59 hybridomas recognized a protein corresponding in size to Pfs230, 7% recognized a ~48-45 kDa protein and 24% recognized a ~25 kDa protein. Forty six of the hybridoma supernatants with a range of IFA patterns and antigen reactivity profiles were tested for transmission-blocking activity in a SMFA and 8 have significant transmission-reducing activity (TRA) ($p<0.05$) at 375 µg/ml concentration. IgG from two of the supernatants with TRA recognized a 25 kDa protein and six recognized a protein corresponding in size to Pfs230. This work reconfirms the 3 established transmission-blocking vaccine candidates as immunodominant gamete surface antigens and provides an important toolset of mAb to evaluate the structure and role of specific epitopes. Moreover, the target antigens recognized by 30% of the gamete surface-reactive IgGs remain to be determined and should provide insight into the composition of the external surface of the gamete during fertilization and early zygote development in the mosquito midgut.

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CHEMICAL PROBE PLATFORMS IDENTIFY TARGETABLE MOLECULES AND PATHWAYS THAT ARE INVOLVED IN *PLASMODIUM* GAMETOCYTE-TO-OOKINETE TRANSITION IN THE MOSQUITO

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Malaria parasite transmission cycles require an obligatory developmental stage in the ***Anopheles*** mosquito vector. In the era of global malaria elimination and eradication, there is emergent emphasis on the development of interventions that break the transmission cycle. While there are several existing antimalarials that have been shown to be effective in blocking the parasite's jump from humans to mosquitoes, to prevent parasite breakthrough resulting from overlapping resistance mechanisms, new pathways that can be targeted by small molecules (and eventually drugs) need to be identified. We used four natural product compounds, two usnic acid derivatives as well as parthenin and parthenolide as chemical probes to explore and identify drug-susceptible pathways during gametocyte-to-ookinete transition. We measured efficacy by a battery of quantitative, functional approaches including high-content fluorescence image capture, imaging flow cytometry and standard membrane feeding assay (SMFA). Two usnic acid (UA) derivatives, BT-122 and BT-37 were extremely potent in blocking ***Plasmodium falciparum*** and ***P. berghei*** zygote-to-ookinete maturation ***in vivo*** and ***in vitro***. We further modified BT-37 with a UV-crosslinking probe and identified its putative targets in zygotes by mass spectrometry. We also observed that parthenin appeared to be more effective in blocking gamete-to-zygote formation than parthenolide; although the latter compound has a more promising pharmacological profile based on Phase I clinical trials. Importantly, we noted that exposure of day 15 stage V gametocytes to parthenin (1 µg/ml) for 24 hours, followed by drug wash out and incubation in parthenin-free culture medium for another 24 hours resulted in the complete blockade of mosquito infection as measured by SMFA. Chemical derivitizations of parthenin are being explored to develop a new crosslinking probe to permit subsequent identification of its candidate target molecules in ***Plasmodium*** stage V gametocytes. We envision that these studies will illuminate the potential mechanism of action that results in the inactivation of this important transmission stage.

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CAN INSECTICIDE RESISTANCE HINDER COMMUNITY RETENTION AND UTILIZATION OF LONG LASTING INSECTICIDAL NETS?

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The use of long-lasting insecticidal nets (LLINs) remains the mainstay for malaria prevention. Although different human behaviors and misconceptions hamper the use of LLINs, the evolution of insecticide resistance is a major threat facing malaria control. Here we investigated LLINs retention, utilization perceived efficacy and bio-efficacy in areas with and without insecticide resistance. A cross-sectional survey was carried out by interviewing household heads in six villages selected based on a priori report on insecticide resistance in the major malaria vector: ***Anopheles gambiae*** s.s. Two villages without resistance were used as control group. Clusters of houses were selected within each village for selection of households. 50 households from each village that had received either permaNet 2.0 or olyset nets in 2013 were randomly chosen from each cluster. 50 used permaNet 2.0 and 50 used olyset nets were collected after 12 months field usage for bio-efficacy tests using standard WHO procedures. A total of 400 household heads were interviewed during the study. Although there was no significant difference in the side effect reported by both groups, the retention rate of LLINs after 12 month of nets distribution was 45% for Olyset and 52% for permanent 2.0, but > 85% in the control group. 20% Olyset nets and 25% permaNet 2.0 were observed to be hanged inside houses when used as proxy indicator for usage in the resistant villages as against 75% in the control group. Bioassay mortality of field strain of Anopheles on LLINs from resistant villages was 22 -38% as against 98% in the control group. While 80-90% respondents in villages without resistance perceived the use of LLIN beneficial, > 50% in the resistant villages found LLIN ineffective with a significant number preferring the use of aerosol. The respondents have little or no knowledge of insecticide resistance but the presence of resistance correlated to net usage in the villages. To maintain the highest level of net usage, resistance tests should be an integral component of LLINs campaign program.

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THE COMPLEMENTARY ADVANTAGE OF COMBINING SPATIAL REPELLENT TREATED SISAL DECORATIVE BASKETS WITH LONG-LASTING INSECTICIDE TREATED NETS

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Spatial repellents (SRs) interfere with host attractiveness of mosquitoes consequently preventing bites and may substantially reduce malaria transmission. The goal of this study was to determine the advantage of combining Transfluthrin treated sisal fiber decorations with long lasting treated nets (LLINs). Efficacy of Transfluthrin treated sisal baskets against malaria vectors that bite in the early evening before humans are protected by LLINs was investigated in experimental huts. The proportion of mosquitoes in experimental huts with Transfluthrin treated sisal baskets and LLINs was compared to huts that had LLINs only using a 3 x 3 Latin square design. Treatments included: 1) untreated sisal baskets and LLINs, 2) 2.5ml Transfluthrin treated sisal baskets and LLINs and 3) 5.0ml Transfluthrin treated sisal baskets and LLINs. One male volunteer was allocated to each hut to conduct human landing catches at 1900hrs-2300hrs and retired to bed until 0600 hours. Mosquitoes were collected from exit traps, the floor and resting surfaces inside huts. Results indicate that 2.5ml and 5.0ml Transfluthrin sisal baskets reduced the proportion of Anopheles arabiensis mosquitoes inside huts by almost three quarters (Relative Rate (RR): 0.26 [0.22, 0.29]; $z = -19.00$, $p < 0.000$ and RR: - 0.31 [0.27, 0.36]; $z = -16.97$, $p < 0.000$) respectively. Both 2.5ml and 5.0ml Transfluthrin baskets treatments prevented more than three quarters of An. arabiensis mosquitoes from biting humans(RR: - 0.17 [0.11, 0.24]; $z = -9.78$, $p < 0.000$). This study shows that combining spatial repellents with LLINs inside huts significantly reduces house entry as well as biting rate of An. arabiensis. This is especially useful where residual malaria transmission occurs in the early evening before people go to bed and are under the protection of LLINs. Nevertheless, there is need to conduct further studies that determine the epidemiological impact of combining spatial repellents with LLINs.

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WHAT IS THE CONTRIBUTION OF AESTIVATION TO THE PERSISTENCE OF *ANOPHELES* MOSQUITOES AS VECTORS FOR *PLASMODIUM FALCIPARUM* MALARIA?

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Plasmodium falciparum malaria, transmitted by the *Anopheles* mosquito, remains a leading cause of death in tropical regions of the world. Despite efforts to drive transmission down, rebound epidemics associated with the persistence of malaria vectors has remained a major impediment to local elimination. One critical area that remains poorly understood is how Anopheles populations survive long dry seasons to re-emerge following the onset of the rains. We developed a matrix population projection model of the mosquito lifecycle to explore the impact of different survival/persistence strategies on the dynamics of the mosquito population. Both longevity and hibernation (mosquito aestivation or adaptive plasticity) allow persistence of the mosquito population through the dry season and can reproduce patterns observed in field data from the Sahel region. We therefore conclude that both may be attributes for vector persistence. We use these results to demonstrate the importance of practical ecological methods to control vectors in both the dry and wet seasons if malaria transmission is to be interrupted.

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CAREGIVER CHARACTERISTICS AND BED-NET USE AMONG UNDER-FIVE CHILDREN IN RURAL GHANA

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In Ghana, malaria accounts for 44% of outpatient cases and 22% of mortality among children less than five years of age. Mass free distribution of insecticide-treated nets (ITNs) to caregivers with children less than 5 yrs of age is one the interventions in the control of malaria in Ghana. An improved understanding of how caregiver characteristic influences ITN can inform the design of interventions to promote ITN use. Between April and July 2013, we conducted a survey among caregivers of children aged 3-59 months who presented with fever to 18 primary care facilities in five districts in the Brong Ahafo Region of Ghana. Caregivers were asked if their children had slept under an ITN the previous night. The data was analyzed to explore how caregiver socio-demographic and socioeconomic factors influenced ITN use. A total of 4111 caregivers were interviewed. Most (95%) were females. Their average age was 29 years (SD=9). About 37% had no formal education. Presenting children were more likely to have slept under an ITN if their caregivers were least poor (P-value<0.001), had more than one child (P=0.008) and had no formal education (P<0.01). Use was lower among children whose caregivers were between 13-22 years (78.8 %,) compared to carers of 33-42 years (83.0%) (P=0.01). Where it becomes necessary, the characteristics of caregivers can be explored to improve the targeting of ITN distribution and likelihood that they will be used.

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COMMUNITY KNOWLEDGE, VIEWS AND EXPERIENCES ON OUTDOOR MALARIA TRANSMISSION AND ITS CONTROL IN SOUTHERN TANZANIA

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The massive applications of Indoor Residual Spraying and Insecticide Treated Nets in Africa have significantly contributed to the reduction of malaria transmission yet residual malaria transmission persists in many parts of Africa, partly driven by mosquitoes that bite people outdoors. It is essential to consider knowledge, perspectives and behavior of people in this transmission before embarking in introduction of outdoor intervention tools. We assessed views, knowledge and experiences of people in rural and peri-urban communities in southern Tanzania, regarding outdoor mosquito bites and malaria prevention. A qualitative cross sectional study was conducted in four villages within the Kilombero Valley in southern Tanzania, using semi-structured interviews and structured observation. A total of 40 participants were selected for interview and participant observations were also conducted in their households. The key areas of focus were; a) explore their knowledge, view and experience on malaria and outdoor malaria transmission b) whether malaria vectors also bite outdoors and whether transmission can also occur, c) means of protection from mosquito bites while they are outdoors performing their daily activities. Majority knew about malaria but little is known on outdoor malaria transmission. Although they regularly experienced outdoor mosquito bites, but most still believed that transmission occurs indoors from midnight. The increasing proportions of outdoor transmission pose risks to these communities and challenge to the control efforts. Malaria is a threat to people and still rated as one among the leading cause of illness. It is also still surprising to them that despite all the efforts and interventions in place, people still suffer from the disease. Majority of use bednets as indoor protection while using long sleeve cloths, smoke from burning leaves or cow dung and cloth to chase mosquitoes away while

outdoors during the evening. In conclusion, community education on outdoor malaria transmission is of vast importance before embarking into provision of outdoor interventions tools

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ACCEPTABILITY AND PREFERENCE OF INSECTICIDE-TREATED CLOTHING FOR MALARIA PREVENTION AMONG RUBBER TAPPERS IN MYANMAR

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Insecticide-treated clothing (ITC) is needed in situations where, for occupation or necessity, people (primarily mobile and migrant populations-MMP) are beyond the reach of core vector control interventions. The success of ITC as a strategy against residual transmission of malaria hinges on acceptability and adherence, as there is limited information to inform policymakers regarding targeted distribution to MMPs. 234 male and female rubber tappers in 16 rubber plantation clusters were enrolled in a two-arm (ITC versus non-treated clothing-NTC) cluster-randomized non-inferiority crossover trial to investigate preference and acceptability of ITC in Mon State, Myanmar. Clusters were randomly allocated to the order of clothing distribution. Quantitative questionnaires and 32 focus group discussions were conducted at baseline and three follow-up intervals. Preliminary findings up to the second follow-up (FU2) revealed high acceptability and adherence to ITC and NTC: in both arms, 94.3% (95% CI: 92.1-97.3*) of respondents at follow-up 1 (FU1) and 96.0% [94.0-98.6] at FU2 reported liking the clothing overall and more than 63% (range 63.0%-83.2%) reported wearing the clothing every night. One explained, "After wearing the [ITC], no other measures are needed. It is perfect." 92.0% [85.4-95.7] of respondents at FU1 and 93.0% [86.3-96.6] at FU2 reported that ITC reduced mosquito bites, compared to 92.2% [85.8-95.8] of respondents at FU1 reporting that NTC reduced mosquito bites and 89.1% [81.5-93.8] at FU2. This unexpected equivalence between the two clothing types may be explained by low mosquito biting pressure during the dry season. The results suggest that ITC could be effective for outdoor malaria prevention among nighttime workers, however, a reassessment of acceptability of ITC versus NTC is needed during the rainy season when mosquito density is higher.*CIs not adjusted

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MORTALITY OF *ANOPHELES GAMBIAE* AFTER EXPOSURE TO SULFADOXINE AND PYRIMETHAMINE

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Vector control tools are major component of malaria control/elimination programmes (LLINs, IRS). In recent years, pyrethroid resistance has emerged in many countries and previously endophilic mosquitoes are turning into biting outdoors. The development of new methods of vector control is critical for malaria elimination. We investigated the mortality of ***Anopheles gambiae*** fed on different concentrations of sulfadoxine (S) and pyrimethamine (P) corresponding to physiological concentrations of 3 days (S3 = 61 µg / mL blood, P3 = 154.7 ng / ml), 7 days (S7 = 33.8 µ g / ml, P7 = 66.6 ng / ml) and 14 days (S14 = 14.2 µ g / ml, P14 = 15.7 ng / ml) after an oral dose of sulfadoxine-pyrimethamine. Adults ***Anopheles gambiae*** (N = 1000 per dose) aged at least 5 days and fasted for 24 hours were membran-fed with two solutions, one containing the drug at the above concentrations and one without drug. The number of dead mosquitoes was counted 24 hours, 48 h, and 72 h after feeding. At H24 the mortality rates of ***Anopheles gambiae*** fed on S3, S7, and S14 compared to controls were 12% (n = 455) vs. 2% (n = 487), p <0.001; ; 2% (n = 477) vs. 5% (n = 474), p >0.05; ; 1% (n = 487) vs 1% (n = 487), p >0.05. The mortality rates of mosquitoes fed on each of the pyrimethamine concentrations were similar to the mortality of those fed on controls. We show that Sulfadoxine kills ***An. gambiae*** at day 3 physiological concentrations. The implications of these observations for vector control will be discussed.

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THE VITAL ROLE OF VOLUNTEERS IN CONTAINING ARTEMISININ RESISTANT MALARIA: LESSONS FROM WESTERN CAMBODIA

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Evidence of artemisinin-resistance in Western Cambodia has drawn national and international attention to contain and eliminate resistant parasites. Cambodian national strategic interventions have relied heavily on health facilities and village malaria workers (VMWs) for early diagnosis and prompt treatment. The Control and Prevention of Malaria Project (CAP-Malaria) conducted a study to assess the contribution of VMWs on case management, focusing on 104 VMWs from 52 villages under 6 health facilities in Samlot District, Battambang Province. A retrospective analysis was done on data retrieved from 2009 to 2014 of malaria cases treated by health facilities and VMWs. The number of malaria cases during this time increased from 1,273 cases in 2009 to 2,127 in 2011 and has since declined to 977 in 2014. During that time, the proportion of malaria cases treated by VMWs increased from 37% in 2009 to 81% in 2014. The results clearly indicate that VMWs have made an enormous contribution to the detection and treatment of malaria in Western Cambodia where artemisinin resistance has been documented.

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HOW NET CARE AND REPAIR INFLUENCE NET DURABILITY IN SÈMÈ-KPODJI COMMUNE, SOUTHERN BENIN

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In Benin, The Center for Entomologic Research of Cotonou (CREC) conducted studies on bed net durability that showed low net retention and integrity after 6 months of use in 4 communes randomly selected at national level with perennial (2communes) and seasonal (2communes) transmission. Some net manufacturers are improving fabric durability and others are increasing bio efficacy, but these strategies are more likely to succeed if communities become successfully engaged in net care and repair. The project to advance the durability of long-lasting insecticide treated bed nets (PADNET) is investigating community-based interventions that prolong the useful life of nets. PADNET used an experimental design with two intervention groups =cohorts: C1 -Behavior Change Communication (BCC) on net durability, C2 -BCC+ bed net repair kits, and C3 - a control group. The monitoring measured between a quite number, two main indicators of LLINs durability, the survivorship: Deltamethrin retention and physical integrity through proportional Holes Index pHI. Households received in Jan-Feb 2014 one of two different types of nets both deltamethrin treated and, labeled "A" and "B". In November 2014, 9 months after net distribution, we conducted a monitoring round By net type and cohort: Retention of nets "A": C1=94%, C2=92%, C3=90%. Retention of nets "B": C1=93%, C2=93%, C3=85%. Percentage of nets "A" without holes: C1=96%, C2=94%, C3=96%. Percentage of "B" nets without holes C1=91%, C2=95%, C3=97%. Average (Avg.) proportional hole index (pHI) for nets "A": C1=13.2, C2=25.4, C3=48.6. Avg. pHI for nets" B": C1= 35.1 C2=11.3, C3=51.5. After 9 months of intervention, for nets used by children, pregnant women or the remaining household

members There were statistically significant differences between the "B" brand cohorts for retention (X^2 =3.77 p= 0.004) and also in net holes found (X2 = 8.9 p= 0.012).

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USE OF A TABLET-BASED CAMPAIGN INFORMATION MANAGEMENT SYSTEM TO PLAN, MANAGE AND MONITOR LLIN DISTRIBUTION ON BIOKO ISLAND THROUGH MASS TOP-UP CAMPAIGN

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Between December 2014 and June 2015, the Bioko Island Malaria Control Project (BIMCP) set out to distribute 150,000 LLINs to 220,000 people through a door-to-door mass top-up campaign. In order to efficiently plan, implement, and monitor the distribution, the BIMCP used a tablet-based Campaign Information Management System (CIMS) that contains a georeferenced listing and mapping of all households by community and an ODK-based form to record within each household the number of sleeping areas, the supply of existing LLINs, and the number of new LLINs provided (based on the top-up quantity calculated by the CIMS to ensure 1 LLIN per sleeping area). At the end of each distribution day, data collected by all field workers was synchronized to a main server, and then analyzed to track the quantity of LLINs distributed, determine community coverage, assess daily distributor productivity, and map the exact location of houses that had not yet received nets. Through the use of the CIMS, field teams were able to quickly locate houses that needed nets, achieve very high supply coverage, improve distribution efficiency particularly during mop-up days when targeting houses that had not yet received nets, and track in real time the cumulative coverage achieved. In addition, use of the CIMS reduced the cost and burden of paper-based data collection and processing, improved turnaround time for analysis and reporting, and improved the quality of data through incorporation of validation rules and data input constraints. Between December 2014 and March 2015, 87,633 nets had been distributed to 35,017 households. Complete coverage and distributor prodcutivity data will be presented, as well as an analysis of impact of the CIMS on distribution. It is anticipated that the CIMS will be used to plan, manage and monitor keep-up efforts planned through antenatal clinics, child immunization clinics and through annual distributions to all primary school attenders.

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ANTIBIOTIC RESISTANCE PATTERNS AND β-LACTAMASE IDENTIFICATION IN *E. COLI* ISOLATED FROM YOUNG CHILDREN IN RURAL LIMPOPO, SOUTH AFRICA: THE MAL-ED COHORT

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Antibiotic resistance is a growing problem worldwide. Mechanisms of resistance vary, and can confer resistance to multiple classes of antibiotics. In this study, we sought to characterize the antibiotic resistance profiles of E. coli isolates obtained from stool samples. These samples were collected from children between the ages of 4 to 12 months who were participants in the MAL-ED study at the South Africa research site. We isolated 87 E.coli samples (clones) from 65 individual participants, all of which were subjected to the disk diffusion assay to determine resistance. We characterized the minimum inhibitory concentration of antibiotics in a subset of strains as well as the mechanism by which these strains were resistant to β-lactam antibiotics. Our results revealed high resistance rates to co-trimoxazole (54.02%), penicillin (47.13%), and tetracycline (44.83%) in our isolates, and indicated that the β-lactamase TEM-1 is a prevalent source of β-lactam resistance. We also identified two isolates with the extended-spectrum β-lactamase CTX-M-14.

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ENTEROAGGREGATIVE *E. COLI* (EAEC) IN PATIENTS WITH TRAVELERS' DIARRHEA, COMMUNITY ACQUIRED DIARRHEA AND NON-DIARRHEAL GASTROINTESTINAL ILLNESS

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Enteroaggregative E. coli (EAEC) is a diarrheal pathogen epidemiologically linked to residence in a developing country or international travel. The finding of EAEC in travelers unassociated with diarrhea has been documented and certain genetic markers seem to predict risk for developing diarrheal disease. As most U.S. clinical laboratories have historically not tested for this microbial pathogen, local epidemiology is largely unknown. With the advent of culture independent diagnostic techniques such as high throughput multiplex DNA extraction PCR technology, we have been able to identify a broad array of enteric pathogens in patients with travelers' diarrhea, community-acquired diarrhea, as well as in patients in whom stool aspirates were obtained at the time of colonoscopy for screening or for non-diarrheal gastrointestinal complaints. Stool specimens obtained between May 2014 and March 2015 were analyzed with the BioFire FilmArray multiplex platform. Of 394 stool specimens, 40 (10%) tested positive for EAEC making it the most common microbial finding of the 22 pathogens tested for with this assay. Of the 40 positive specimens, 20 (50%) were from patients who traveled, 17 (42.5%) were from patients who acquired EAEC in the community, and 3 (7.5%) were obtained from patients at the time of colonoscopy. Of the 40 specimens which tested positive for EAEC, 19 (47.5%) were positive for co-infecting pathogens. Enteropathogenic E. coli (EPEC) was the most common co-infecting pathogen and was detected in 14 (35%) of specimens positive for EAEC. Using culture independent diagnostic techniques we have found EAEC to be more common than previously recognized, especially in community acquired diarrhea and in patients in the community without diarrhea. Further studies and characterization of clinical symptoms related to EAEC are needed to more fully understand this pathogen.

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EVIDENCE THAT CHILDHOOD UNDERNUTRITION IS CAUSALLY RELATED TO IMPAIRED DEVELOPMENT OF THE GUT MICROBIOTA

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We have tested the hypothesis that perturbations in postnatal development of the gut microbiota are causally related to childhood undernutrition. Applying machine-learning methods (Random-Forests) to fecal bacterial 16S rRNA datasets generated from samples collected during the first three postnatal years from healthy Malawian twins, we identified age-discriminatory bacterial strains. The relative abundances of these strains were used as microbial signatures to define microbiota maturity in Malawian infants/children with varying degrees of undernutrition compared to healthy controls of the same chronological age. Infants/children with undernutrition have immature microbiota. Transplantation of 19 fecal microbiota samples from donors with healthy growth phenotypes and with varying degrees of undernutrition (defined by anthropometry) into young germ-free mice consuming a Malawian diet, and 16S rRNA analysis of recipient animals' fecal microbiota as a function of body mass gain revealed that the age-indicative taxa are growth indicative. Co-housing young coprophagic mice shortly after receiving microbiota from healthy versus undernourished infants demonstrated invasion of age/growth indicative taxa from the former into the latter's microbiota, prevention of growth faltering, and altered host metabolic phenotypes. We have established that impaired development of the gut microbiota is a factor in the pathogenesis of childhood undernutrition and that the developing microbiota is a target for disease treatment and prevention.

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WHAT DOES CLIMATE CHANGE MEAN FOR DIARRHEAL DISEASES?

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Environmental association of diarrheal diseases is well documented and inspite that diseases such as cholera, Shigella, rotavirus etc continue to be a global public health threat. The uncertainty in environmental processes affecting diarrheal diseases can significantly be affected by changing climate at different temporal and spatial scales-either through amplification of hydroclimatic cycle or by enhanced variability of large scale geophysical processes. Using cholera as key signature diarrhea diseases, the goal of this study was to understand intricacies on conditions that may impact outbreak of cholera across tropical regions. Our previous research categorized cholera into two forms: epidemic (sudden and unexpected) and endemic (persistent over time). Endemic cholera in Bengal Delta region of South Asia, as an example, has a unique pattern of two seasonal peaks and are associated with asymmetric and episodic variability in river discharge. The first cholera outbreak in spring is related with intrusion of bacteria laden coastal seawater during low discharge seasons while cholera occurring in fall season is hypothesized to be associated with high river discharge that aids in cross-contamination of water resources, thus leading to second wave of disease, primary in the inland regions. On the other hand, epidemic cholera in several inland regions of Africa were strongly associated with anomalous conditions of temperature followed by precipitation. Using simulations from three global climate models (GCM) and combination of data mining technique that includes support vector regression, we will show that endemic cholera is expected to increase in the deltaic coastal regions. Several parts of Africa are prone to sudden and unexpected cholera outbreaks in the next 50 years.

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ASSOCIATION OF ENTERIC PATHOGENS WITH POOR GROWTH IN CHILDREN IN A RURAL DISTRICT OF PAKISTAN

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Malnutrition in early childhood has adverse effects on long term physical and cognitive growth of individuals. Environmental Enteropathy (EE), characterized by villous blunting, improper permeability and lymphocytic infiltration of small intestinal mucosa, is considered to be an important factor in the development of malnutrition. Major determinants leading to EE include poor diet, hygiene and frequent enteric infections. However, little is known about the contribution of individual enteric pathogens (both symptomatic and asymptomatic) towards the development of EE and the resulting long term growth compromise. A total of 328 children were enrolled in a prospective study in a rural district of Sindh, Pakistan to test novel biomarkers of EE. Stools specimen from the enrolled children were collected at 6 and 9 months of age, and were tested for enteric pathogens using high throughput TACMann Low Density Array (TLDA). Logistic regression was carried out to determine association of stool pathogens at 6 and 9 months of age with malnutrition and growth faltering at 12 and 18 month of age. We found that the presence of ***Campylobacter*** [1.95(1.10-3.47);p=0.02], ETEC [2.33 (1.22-4.44); p=0.01] and ***Giardia*** [1.93 (1.12-3.32); p=0.02] at 6 month stool samples was associated with higher odds of stunting at 12 month. Whereas presence of ***Campylobacter*** [1.79 (0.92-3.48);p=0.09] and Sapovirus [2.04 (0.89-4.70);p=0.09] at 9 month stool sample were associated with stunting (HAZ <-2.0) at 18 month. However, infection with ***Cryptosporidium*** [0.36 (0.18-0.71);p=0.003], ***Shigella*** [0.43 (0.19-0.99); p=0.05] and Adenovirus [0.27 (0.08-0.89);p=0.03] were associated with lower risk of stunting at 18 and 12 month respectively. Our data suggests that certain pathogens like ***Campylobacter, Giardia*** and ETEC may play a disproportionately important role in causing malnutrition. This finding needs to be confirmed by other studies of similar design.

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VIRULENCE GENES OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* (EAEC) AND THEIR ASSOCIATION WITH DIARRHEA IN CHILDREN

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Enteroaggregative ***Escherichia coli*** (EAEC) are a group of enteric pathogens that causes acute and persistent diarrhea among children, human HIV-infected patients, and travelers to developing countries. EAEC pathogenesis involves adherence to intestinal mucosa, elaboration

of enterotoxins and cytotoxins, and induction of mucosal inflammation involving several virulence genes. However, the pathophysiology of EAEC infection is not completely understood. In this study we analyzed 18 virulence genes associated with aggregative adherence, biofilm, dispersin, and toxins in 172 well characterized EAEC strains (aggR+) isolated from stool samples of 97 children with diarrhea and 75 healthy controls, from a passive diarrhea surveillance study in peri-urban communities in Lima, Peru. 81 different genetic profiles were identified; 56 profiles among diarrhea samples and 44 among control samples. The most frequent was aggC+aatA+aap+shf+fyuA+, present in 19% of all strains. Of all genes evaluated, the most frequent were aatA (CVD 342) present in 159 strains (92%), followed by fyuA in 157 (91%) and aap in 149 (87%). When co-infection cases were not considered and EAEC strains were analyzed as a single pathogen, only pic was found more frequently in diarrhea than in control samples (64% vs. 44%, $p<0.05$), and was associated with prolonged diarrhea (≥ 7 days) (83% vs. 50%, $p<0.05$). In summary, EAEC strains isolated from children living in endemic countries are highly heterogeneous. Further studies are needed to elucidate the exact role of each virulence factor and its association with diarrheal episodes.

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IMPACT OF SOCIOECONOMIC STATUS ON ANTIMICROBIAL RESISTANCE PATTERNS OF ENTERIC PATHOGENS ISOLATED FROM BANGLADESHI CHILDREN WITH DIARRHEA

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Antimicrobial resistance is a global problem affecting countries of all income levels. Knowledge of how socioeconomic status affects antimicrobial resistance patterns could critically inform interventions targeting this problem in low-income countries. The objective of this study is to determine differences in antimicrobial resistance patterns of enteric pathogens between high- and low-income patients presenting for care at a diarrheal hospital. We used data collected from 2009 to 2012 by the diarrheal disease surveillance system at the icddr,b Dhaka Hospital to examine the relationship between socioeconomic status and antimicrobial resistance patterns of enteric pathogens. We identified children ≤5 years of age who presented with diarrhea and had ***Shigella*** spp. (***Shig***, n=163) or ***Salmonella*** spp. (***Salm***, n=43) isolated from stool culture. We found that 47% (76/163) of ***Shig*** isolates were resistant or had reduced susceptibility (R/RS) to ciprofloxacin, and 33% (14/43) of ***Salm*** isolates had R/RS to ciprofloxacin. We calculated family income as the combined reported income of the patient's father and mother. We found that children from families in the top income quartile were more likely to have ***Shig*** R/RS (27/41 61%) than those who came from families in the bottom income quartile (14/43, 31%, P = 0.002). In the smaller group with ***Salm*** infection, we also found that those in the top income quartile had higher rates of R/RS (6/12, 50%), compared with those in bottom quartile (5/17, 29%, P = 0.26), but the difference was not statistically significant. We also found that patients who lived in homes with brick walls were more likely to have ***Salm*** with R/RS (12/25, 48%) than those living in corrugated tin walls (1/14, 7%, P = 0.009). In this preliminary analysis, we show that children from wealthier families have an increased risk of antimicrobial resistant enteric bacteria. We plan to complete a multivariable regression model analysis to include factors such as presence of domestic animals, education level, and household property ownership, and anticipate that results will be available at time of presentation.

1011

ARCOBACTER SPP. VIABILITY IN STOOL SAMPLES OF HUMANS AND ANIMALS (PIGS AND CATTLE)

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Arcobacter spp. is currently considered emerging zoonotic pathogen. Not found reports about its viability in fecal samples of humans and animals such as cattle and pigs. In this study we report the feasibility of ***Arcobacter spp.*** mixed in fecal samples with physiological serum (FS) in sealed plastic vials at room temperature for a period of one year. We study 100 samples positive for ***Arcobacter*** spp., 4 human fecal samples and 96 fecal samples from animals obtained from colon freshly slaughtered cattle, 48 pigs and 48 cattle. Samples were mixed with FS in plastic vials with screw cap and seal. Monthly plantings are made for the isolation of ***Arcobacter*** with filter method on Columbia blood agar plates, incubated in microaerophilic with the method of the ***Klebsiella***, the plates were sealed with rubber bands without talc obtained from latex gloves, Incubated at 28 ° C for 24 to 48 hours and phenotypically identified only. During the first 10 months the feasibility of ***Arcobacter spp***. was shown in 100% of samples; at 11 and 12 months was found feasible the organism in 95% of samples. Therefore, ***Arcobacter spp.*** viability remained one year between 95% to 100% in fecal samples mixed with FS.

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THE ROLE OF VIRULENCE-RELATED GENES IN THE PATHOGENESIS OF MALNUTRITION ASSOCIATED WITH ENTEROAGGREGATIVE *ESCHERICHIA COLI* INFECTION: THE MAL-ED CASE-CONTROL STUDY SITE IN FORTALEZA, CEARA, BRAZIL

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Enteroaggregative ***Escherichia coli*** (EAEC) infection is associated with intestinal inflammation and it leads to childhood malnutrition and growth impairment. This case-control study investigated the association of EAEC virulence-related genes (VRGs), isolated or combined, with malnutrition in Brazilian children. A total of 125 children positive for EAEC, 66 malnourished (WAZ<-2) and 59 nourished (WAZ>-1), had their stool samples analyzed by five multiplex-PCRs targeting 20 DNA sequences encoding VRGs. The EAEC HilA homolog (eilA) was the most frequently detected (78%, 98/125). The presence of AAF/II fimbria assembly unit gene (aafC) was associated with malnutrition among the tested samples (P=0.01, OR=6.33, 95%CI=1.35-29.63). This association was stronger in the absence of agg4A gene (P=0.005, OR=11.60, 95%CI=1.45-92.91). The isolated absence of aafC was significantly correlated with nourished children carrying EAEC, as well as in combination with two sets of genes: 1) Presence of enteroaggregative immunoglobulin repeat protein (air, P=0.04, OR=0.45, 95%CI=0.22-0.94); and 2) Absence of air, agg3A, sigA (***Shigella*** IgA-like protease homolog), aafA and shiA (shiA-like inflammation suppressor) (P=0.02, OR=0.20, 95%CI=0.05-0.79). The presence of sigA in the absence of aafC, air, agg3A, and aafA was sufficient to associate this combination of genes with malnourished group (P=0.001, OR=10.36, 95%CI=1.28-83.63). These results are consistent with the heterogeneity of VRG profiles of EAEC in malnourished versus nourished children. The data also showed that aafC, alone or in combination with other VRGs, were associated with malnourished children and their absence were protective in nourished children.

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MULTI-SITE SURVEY OF DIARRHEAGENIC PATHOGENS WITHIN THE WHO GLOBAL ROTAVIRUS SURVEILLANCE NETWORK USING TAQMAN ARRAY CARDS

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We have previously developed a customized TaqMan Array Card (TAC) that enables sensitive and quantitative detection of a wide range of enteropathogens. The card was customized to test for 36 pathogens and controls using 57 molecular targets, including 8 G (VP7 gene) and 6 P (VP4 gene) targets for genotyping rotavirus A (RVA). In this work we deployed the technology to 4 WHO Global Rotavirus Network Laboratories and tested stool samples from RVA EIA- positive (n=396) and RVA EIA-negative (n=681) diarrheal cases collected from 11 countries. Using quantitative cutoffs for diarrhea association that we have determined previously, five countries showed rates of Norovirus GII of at least 15% with Ct values indicating that it was a probable cause of diarrhea. Three of five African countries showed rates of Cryptosporidium of at least 12% with Ct values indicating a probable cause of disease. Country-specific results included: Myanmar (n=121) revealed high rates of adenovirus 40/41 (45%) and norovirus (21%, both GI and GII); from Melbourne (n=185), the RVA negative specimens (n=108)revealed >5% rates of adenovirus, ***B. fragilis, C. difficile***, norovirus, and sapovirus; from Brazil (n=303), >10% rates of adenovirus, enteroaggregative ***E. coli***, enterovirus, and ***Giardia***. The RVA typing results from the card matched the networks' standard testing results in most cases, however the sensitivity of the TAC assay was lower than the networks' nested PCR/electrophoresis assay. Additional optimization may be necessary before these card-based genotyping assays are deployed in surveillance activities. Operationally we learned that best results required well-maintained specimens, and correlations with RVA enzyme immunoassay and genotyping were better when specimens were retested prior to the use of the card versus archived results. In summary, this technology can be deployed to yield information on a range of diarrheagenic pathogens and allow geographic comparisons throughout the world.

1014

IMMUNIZATION WITH SALMONELLA ENTERICA SEROVAR TYPHI TY21A STABLY PRODUCING ANTHRAX PROTECTIVE ANTIGEN PROTECTS MICE AND RABBITS FROM INHALATIONAL ANTHRAX-- A BIVALENT, ORAL ANTHRAX-TYPHOID VACCINE

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We are developing an anthrax vaccine intended to be distributed for public use without refrigeration, self-administered orally, durably protective following a one-week immunization regimen, safe, and well tolerated. We integrated a codon-optimized, protective antigen (PA) gene of ***Bacillus anthracis*** (plus extracellular secretion machinery) into the chromosome of the licensed, oral, live-attenuated typhoid fever vaccine Ty21a to form Ty21a-PA-01 and demonstrated excellent expression of PA. We produced the vaccine in a 10 L fermenter; foam-dried, vialed and characterized the dried product, which retained ~50% viability for 20 months at ambient temperature. Sera from immunized animals had high levels of anti-PA antibodies and anthrax lethal toxin-neutralizing activity. Immunized mice were fully protected against intranasal challenge with ~5 LD_{50}s of Sterne spores, and immunized rabbits were protected against aerosol challenge with 200 LD_{50}s of ***B. anthracis*** Ames spores with significant correlation between protection and threshold antibody titers by both ***in vitro*** assays.

1015

STAPHYLOCOCCUS AUREUS IN BOTSWANA: PREVALENCE, RISK FACTORS, ANTIBIOTIC RESISTANCE, AND MOLECULAR EPIDEMIOLOGY

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Carriage of ***Staphylococcus aureus***, an opportunistic pathogen of concern in Africa and elsewhere, is a primary risk factor for disease. Understanding carriage risk factors is critical for identifying who is at greatest disease risk. Individuals with HIV suffer more severe ***S. aureus*** disease and are at risk of treatment failure and death. Despite the high HIV burden, data on ***S. aureus*** carriage in southern Africa are sparse. To describe ***S. aureus*** nasal carriage in Botswana, we evaluated outpatients in and around Gaborone. From March-June, 2013, 473 adults (404 HIV+ /69 HIV- and 56 children (<18 yrs) (14 HIV+/42 HIV-), had 2 nasal swabs, 4 weeks apart, and interviews about health status and potential risk factors. Carriers (≥1 swab positive for ***S. aureus*** by standard microbiologic techniques) were classified as intermittent or persistent carriers. We tested antibiotic susceptibility to 19 antibiotics and performed ***spa***-typing of isolates. ***S. aureus*** nasal carriage prevalence was 39.3%, of which 36.0% was persistent; 3.97% had MRSA, but none persistently. Rural patients (Prevalence Ratio [PR] 2.19, p<0.01), those in households with ≥1 child (PR 1.36, p=0.06), or with viremia (>399 copies/mL) (PR 1.88, P=0.02) were more likely to be persistently carriers. HIV+ participants were less likely to have MRSA (p=0.049), while children (PR 1.9, p<0.01) and those with eczema (PR 5.72, p=0.001), asthma (PR 3.8, p=0.037), or a history of tuberculosis (PR 3.26, p=0.03) or pneumonia (PR 3.6, p=0.03) had more MRSA. Resistance was low to TMP-SXT (10.6%) but high to cloxacillin (87.0%). Persistent carriers harbored more resistance,

and 14.2% of carriers were multiply resistant. A large genotypic diversity was present, including new ***spa***-types (analysis ongoing). Rural and large households and children with HIV are high-risk ***S. aureus*** carriage groups, and adults with viremia are more likely persistent reservoirs. Children and outpatients with comorbidities or history of respiratory disease are major MRSA risk groups. Cloxacillin resitance is noteworthy, as it is the empiric antistaphylococcal. Our findings may guide indicators of carriage risk in a clinic setting.

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ISOLATION AND CHARACTERIZATION OF BROAD SPECTRUM BACTERIOPHAGES WITH LYTIC ACTIVITY IN *STAPHYLOCOCCUS AUREUS* METHICILLIN RESISTANT (MRSA) STRAINS

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Infections by antibiotic resistant ***Staphylococcus aureus*** are common on the clinical and community settings. Bacteriophages (phages) are prokaryotic virus that infect and multiply using bacteria as a host. In an effort to develop an alternate therapy against MRSA infections, we used our collection of nosocomial and community acquired MRSA strains to identify phages with lytic activity. We have isolated and identified eight ***S. aureus*** phages from various environmental sources and tested for their lytic activity against 170 MRSA isolates. The results indicate that these phages are highly virulent and can effectively prevent the growth of 95% of the MRSA isolates. Phage K was previously isolated as a broad spectrum phage with lytic activity against ***S. aureus*** and was available from American Type Culture Collection (ATCC). Testing of phage K against our MRSA collection showed that it can prevent the growth of 81.7% of the isolates. One of our phages designated as SA0414$^{\Phi}$1 in our laboratory was further analyzed by electron microscopy. Results indicate that like phage K, SA0414$^{\Phi}$1 belongs to the ***Myoviridae*** family of virus. The eight phages were characterized via restriction endonuclease digestion and protein profile and determined to be distinct from one other and from phage K. Furthermore, minimal inhibition concentration (MIC) assay indicates that combination of the phage K, with three of our isolates have an enhanced phage-mediated lytic effect on MRSA strains. These results indicate that these phages combination can produce a cocktail preparation which can be used to treat ***S. aureus*** infections in human wounds.

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QUANTIFYING THE INDIRECT EFFECTS OF *HAEMOPHILUS INFLUENZAE* TYPE B VACCINATION IN CHILDREN UNDER 5 YEARS-OLD

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Haemophilus influenzae type b (Hib) is a significant cause of meningitis and pneumonia among children under 5 years. A conjugate vaccine has been available since the mid-1980s, leading to disease reductions well above what would be expected from direct protection of the vaccine alone. This indicates indirect effects provide substantial protection against Hib disease. Indirect effects are rarely investigated because it requires large, cluster randomized trials; instead we used pre-post vaccine introduction studies to model the effects. Three methods to perform this analysis were identified in the literature. Wolfson, et al. described a method to compare the observed disease reduction to the reduction expected only from direct effects, resulting in an indirect effect multiplier based on vaccine coverage. Samandari, et al. estimated a multiplier for the number of people effectively protected by vaccination using vaccine coverage and incidence rates before and after vaccination. Lastly, Adegbola, et al. used average ages of infection and vaccination to calculate an alternative estimate of direct protection from vaccination. Eleven studies were used for the Wolfson and Samandari methods and three were used for the Adegbola method. All three methods suggest a robust indirect effect against Hib disease, with > 90% disease reduction predicted at only 70% coverage. Indirect effects were more influential at lower vaccine coverage, as vaccinating one child protected anywhere from two to six others. Direct effects dominated at vaccine coverages above 60%. Validating these results against an infectious disease theoretical framework and a study that examined indirect effects on an individual level confirmed the accuracy of our results. Predicted protection varied between the methods, but all demonstrated the importance of indirect effects at low vaccine coverage. These results can be used to better estimate the expected disease reduction prior to beginning a vaccine program and can impact policy decisions regarding vaccination. The models used in this analysis can also be applied to other vaccines, such as pneumococcal conjugate vaccine.

1018

MARKED IMPROVEMENT OF *NEISSERIA MENINGITIDIS* CARRIAGE DETECTION USING PCR AFTER OVERNIGHT BROTH CULTURE WITH PARALLEL QUANTIFICATION USING FILTER PAPER SAMPLES

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Conventional methods used to detect pharyngeal carriage of ***Neisseria meningitidis*** (Nm) involve multiple tests which are time consuming, expensive and a source of potential errors. There is a need to develop better methods for large-scale studies. Prior to field surveys, we demonstrated on spiked samples that Todd-Hewitt broth (THB) was optimal for the overnight growth of Nm prior to direct PCR after DNA extraction. We demonstrated also that blots on filter paper can be used to detect a few copies of Nm DNA. 1000 school-children aged 10 to 18 years were sampled once with 3 different swabs and detection of carriers was compared using three different methods randomly chosen: (i) plating on Thayer-Martin selective medium and testing by conventional microbiology; all suspected Nm were confirmed by PCR. (ii) seeding in THB and, after overnight culture, processing using the same PCR (iii) compression of the swab on filter paper and processing after DNA concentration by the same PCR. Conventional microbiology detected 57 Nm carriers. Most of these (84%) were also detected after THB culture and 44% were detected on filter papers. In comparison, direct PCR after DNA extraction of THB samples detected 139 carriers, an improvement of 243%. Filter papers detected 54 carriers, 53 of which were also detected through broth culture and 25 by conventional microbiological methods. DNA copies from a first central filter paper punch varied between 1 and 25,000 DNA copies. 26 among the 57 Nm carriers (46%) detected by the conventional method were non-groupable on genogrouping and the capsule null intergenic region was identified, whereas 62% of the Nm identified from broth culture were non-groupable as were 30% obtained from filter papers. No genogroup A Nm was detected; one fifth of the capsulated bacteria were genogroup W, followed by X, B and Y in decreasing prevalence. For large-scale studies, where standardized methods are needed, simple molecular detection after broth overnight culture in an appropriate medium allows improved detection of Nm. We also demonstrated the feasibility of using filter paper followed by quantitative PCR for carriage studies.

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SERUM BACTERICIDAL ANTIBODIES AGAINST GROUP W *MENINGOCOCCI* IN NIGERIAN HOUSEHOLDS

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The African Meningococcal Carriage Consortium (MenAfriCar) was established in 2009 to study the epidemiology of meningococcal carriage across the African meningitis belt. The Centre de Recherche Médicale et Sanitaire (CERMES) in Niger was one of the seven African research centres within MenAfriCar. In 2010 and 2011, following cross-sectional carriage surveys, households in which a carrier was identified were recruited and followed up for 6 months. In Niger, 61 households, including 466 individuals were recruited, although not all consented to a blood sample. Blood samples collected at the first household visit were tested for serum bactericidal antibody (SBA) activity to group W (strain M01 240070; W:NT:P1.18-1,3 cc22). A validated complement-mediated SBA assay, utilising baby rabbit complement was used. Serum from 330 individuals was tested for SBA activity against group W. SBA titres were analysed overall, and by carrier status. The overall SBA geometric mean titre (GMT) was 18 (95% CI 13,24) and GMTs did not vary significantly by age or sex. There were 31 index carriers of a group W meningococcus; GMTs in this group were significantly higher (350, 95% CI 138, 890) than in individuals who were not carriers (13, 95% CI 10,18; p<0.001) at the time the blood sample was taken. Individuals in the same household as group W index carriers, but who were not carriers themselves, had similar SBA titres to individuals in households without a group W carrier. Fifty-three individuals who were not index carriers acquired group W meningococci during the 6 month follow-up period; GMTs at baseline were modest in this group (15, 95% CI 7,35) but not significantly different to individuals who did not carry group W at any time (13, 95% CI 9, 18). Thus, while we can observe a clear immunological response to group W carriage, we are not able to elucidate a correlate of protection against group W carriage.

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MENINGOCOCCAL CARRIAGE IN NORTHERN GHANA

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The African Meningococcal Carriage Consortium (MenAfriCar) was established in 2009 to study the epidemiology of meningococcal carriage across the African meningitis belt. The Navrongo Health Research Centre in northern Ghana was one of the seven African research centres within MenAfriCar. Three cross-sectional carriage studies were undertaken in 2010, 2011 and 2012, in both urban and rural areas, with a target sample of 1000 participants in each area and survey. A total of 5209 oropharyngeal swabs were collected and tested for the presence of meningococci. The overall carriage prevalence of ***Neisseria meningitidis*** was 3.7%, but prevalence increased with each survey. Most meningococci (79%) were genogroup W. Factors associated with carriage included age, with the highest prevalence in 5-14 year olds, and sex with males having a higher prevalence of carriage than females. In 2010, blood samples were collected from a sub-set of participants to allow sero-epidemiological investigations. The seroprevalence of meningococcal serogroup-A specific IgG antibodies was determined by ELISA in 765 age-stratified samples. The overall geometric mean concentration (GMC) was 9.09 (95% CI 8.29, 9.97) and 87% of participants had antibody concentrations above 2ug/ml. Antibody concentrations were higher in the urban compared to rural area and increased with increasing age. In 2011 and 2012, households in which a carrier was identified were recruited and followed up for 6 months. Twenty-three households and 167 individuals were studied and the patterns of transmission among these households will be described. Studies of carriage, such as this, are central to understanding the epidemiology of meningococcal infection in the African meningitis belt.

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DOES *STAPHYLOCOCCUS AUREUS* NASAL COLONIZATION INDUCE T-CELL AND INFLAMMATORY CYTOKINE RESPONSES? EVIDENCE FROM MILITARY TRAINEES AT HIGH-RISK FOR *S. AUREUS* COLONIZATION AND DISEASE

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Staphylococcus aureus is a major cause of skin and soft tissue infection (SSTI). Colonization of the anterior nares, the reservoir for ***S. aureus***, is thought to be a risk factor for SSTI. Numerous studies have shown that both humoral and cellular immunity are involved in the host response to SSTI. By contrast, few studies have evaluated the host immune response to colonization among individuals without SSTI, and none to our knowledge have evaluated the impact of colonization on distributions of T-cell subsets and pro-inflammatory cytokines in serum. From 4/2013-12/2014, we conducted a case-control study of SSTI among US Army Infantry trainees, a group known to be at increased risk for ***S. aureus*** colonization and disease. Trainees presenting to the medical clinic for non-infectious conditions (e.g. musculo-skeletal injury) were recruited as healthy controls. A nasal swab and blood sample was collected at the time of enrollment. Swabs were processed by standard methods to assess colonization status. T-cell populations in peripheral blood were assessed by flow cytometry. Serum concentrations of pro-inflammatory cytokines were assessed by 25-plex Luminex panel. Forty-seven participants without SSTI were included in the analysis. Of these, 20 (42.5%) were colonized with ***S. aureus***. The proportion of $\gamma\delta$ T-cells was higher among colonized as compared to non-colonized individuals ($p<0.01$). Moreover, proportions of Th1-cells ($p<0.05$) and $\gamma\delta$ T-cells producing IFN-γ ($p<0.01$) were higher among colonized vs non-colonized individuals. There were no differences between groups with respect to proportions of IL-17 producing T-cells ($p=0.30$). Colonized individuals also had higher serum concentrations of pro-inflammatory cytokines, specifically IFN-γ ($p=0.004$), MCP-1 ($p=0.01$), IL-1RA ($p=0.02$), Eotaxin ($p=0.03$), and MIP-1α ($p=0.05$). Concentrations of IL-17 did not differ between groups ($p=0.14$). In conclusion, these findings suggest that nasal acquisition of ***S. aureus*** elicits a systemic immune response, namely an induction of pro-inflammatory cytokines and proliferation of Th1 and IFNγ-secreting cells.

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GLOBAL ELIMINATION OF LEPROSY BY 2020: ARE WE ON TRACK?

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Annually more than 200,000 new leprosy cases are registered. This number has been fairly stable in the past 8 years. WHO has set a target to eliminate leprosy globally by 2020.The aim of this study is to investigate whether this is feasible given the current control strategy. We focus on three endemic countries, India, Brazil and Indonesia, which together account for nearly 80% of all leprosy cases. We used the existing individual-based model SIMCOLEP to predict future trends of leprosy incidence given the current control strategy in each country. SIMCOLEP simulates life histories of individuals, structured in households, and the

natural history of infection with M. leprae. Current control consists of passive case detection, some active case detection, and multidrug therapy (MDT). Also, BCG vaccination of infants against tuberculosis is known to be effective against leprosy. Predictions of leprosy incidence were made for each country as a whole and for one high-endemic region in each country: Chhattisgarh (India), Para (Brazil) and Madura (Indonesia). Data for model quantification came from the National Leprosy Elimination Program (NLEP, India), SINAN database (Brazil) and the Netherlands Leprosy Relief (Indonesia). Our projections of future leprosy incidence all show a downward trend. In 2020, the country-level leprosy incidence has decreased to 6, 7 and 4 per 100,000 in India, Brazil and Indonesia, respectively, meeting the elimination target of less than 10 per 100,000. However, elimination may not be achieved in the high-endemic regions. The leprosy incidence in 2020 is predicted to be 17, 19 and 24 per 100,000 in Chhattisgarh, Para and Madura, respectively. Although it seems that country-level elimination is reached by 2020, leprosy is likely to remain a problem in the high endemic regions, which account for most of the cases in a country. We therefore conclude that elimination may only be reached by 2020 with additional control measures.

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CO-INFECTION OF GONORRHEA AND CHLAMYDIA AMONG PATIENTS ENROLLED WITHIN A STI SURVEILLANCE STUDY IN GHANA

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Gonorrhea and chlamydia are the most prevalent bacterial sexually transmitted infections (STI) and they often coexist. Common manifestations of these diseases include urethritis in men and cervicitis in females. This study provides data on co-infections collected during June 2012 and November 2014 in five health facilities in Accra and Sekondi/Takoradi. Eligible patients presenting with dysuria and or genital discharge were consented and enrolled. After enrolment, urine samples were obtained and tested by nucleic acid amplification test (NAAT) for ***Neisseria gonorrhea*** and ***Chlamyia trachomatis***. Gram stain and culture for ***N. gonorrhea*** was also performed. A total of 679 specimens were tested. Males and females enrolled were 313 and 361 respectively One hundred and seventy-two cases (25.3%) were positive for gonorrhea and 72 (10.6%) for chlamydia. There were 18 co-infections (2.7%). Among patients who tested positive for gonorrhea, 10.4% were positive for chlamydia. Males who tested positive for gonorrhea were 39.3% and females 13.6%. Only 16 males were co-infected, however, for those between 25 and 31 years of age, 9 of 57 (15.7%) with ***N. gonorrhea*** also tested positive for chlamydia. Co-infection among females in the same group was 2 of 22 (9.1%). For patients who tested positive for chlamydia, 25% of them tested positive for gonorrhea. Symptoms seen in co-infections were as follows; dysuria (61.1%), genital discharge (83.3%), malodorous urine (16.7%), genital ulcers (5.6%) and genital warts (5.6%). Co-infection rates were higher among males than females. Males who tested positive for chlamydia were 13.7% and females 8.0%. Gonorrhea infection rates for chlamydia positive cases were higher in males (37.2 %) than in females (6.9%). The majority of patients with co-infections presented with genital discharge. Genital ulcers and warts observed in co-infected patients could be indicative of STIs caused by viral and other bacterial pathogens which may warrant further surveillance of other STIs in Ghana.

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ISOLATION OF BACTERIOPHAGES SPECIFIC FOR *ACINETOBACTER BAUMANNII*, A PROSPECTIVE ALTERNATIVE THERAPY AGAINST MULTI-DRUG RESISTANT BACTERIA

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Antimicrobial resistant organisms complicate the successful treatment of wound infections and are a threat to both military and civilian populations. The U.S. Naval Medical Research Center (NMRC) along with the Naval Medical Research Unit No. 6 (NAMRU-6), Peru and NAMRU-3, Egypt, has initiated development of phage therapy as an alternative therapeutic strategy against multi-drug resistant bacterial infections. To provide proof of concept and to generate a viable bacteriophage therapy, we focused on isolation of phage specific to ***Acinetobacter baumannii*** due to the recent mergence of carbapenemase expression and multi-drug resistance that is becoming commonplace in wound and nosocomial infections. Because of capsular phase variation and the highly variable surface phenotype associated with ***A. baumannii*** strains, it is important to generate a robust library of different ***A. baumannii*** phage specific to different receptors that can be compounded into an effective therapeutic cocktail. To generate the phage library, environmental water samples were collected from different sites in Frederick, Maryland, Lima and Iquitos, Peru, and Cairo, Egypt. Using a standardized diversity set of ***A. baumannii*** isolates, we screened each of the water samples for the presence of ***A. baumannii***-specific phage with phage isolates obtained from 9% of water sources tested in Frederick, 50% in Lima, 31% in Iquitos, and approximately 50% in Cairo. All purified phage isolates were sent to NMRC for further characterization and sequencing. Once completely characterized, a cocktail of broad spectrum phage against a large variety of ***A. baumannii*** strains will be generated for evaluation in pre-clinical trials. These preliminary findings demonstrate high success rates of identifying bacteriophage from common environmental water sources providing initial feasibility to generate phage cocktails for alternative therapies against drug-resistant bacteria.

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ZOONOTIC CESTODES OF WILD AND DOMESTIC CANIDS IN SOUTHERN KAZAKHSTAN

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Cystic echinococcosis (CE) and alveolar echinococcosis (AE) caused by the zoonotic cestodes ***Echinococcus granulosus*** and ***E. multilocularis***, respectively, are considered neglected tropical diseases by the World Health Organization and other international entities. Both ***Echinococcus*** species primarily use domestic and/or wild canids as definitive hosts. Humans are at risk of becoming infected via the ingestion of parasite eggs shed in the feces of an infected definitive host. In order to obtain information on the frequency of infection in wild and domestic canids, field surveys were conducted from 2008-2012 in 3 regions (Almaty, Zhambyl, and South Kazakhstan) of southern Kazakhstan. In total, 41 wolves (***Canis lupus***), 128 red foxes (***Vulpes vulpes***), 60 jackals (***Canis aureus***), 39 corsac foxes (***Vulpes corsac***), and 101 stray dogs were evaluated via necropsy

of the intestinal tract. ***E. granulosus*** was identified in 6.9% (7/101) of dogs and 19.5% (8/41) of wolves while ***E. multilocularis*** was found in 4.7% (6/128) of red foxes, 2.6% (1/39) of corsac foxes, and 2.0% (2/101) of dogs. Neither parasite was found in jackals. Findings from this study indicate that there is an ongoing zoonotic risk from both ***E. granulosus*** and ***E. multilocularis*** for the population of southern Kazakhstan.

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THE EXPRESSION AND POSSIBLE ROLE OF TH1/TH2/TH9/TH17/TREG TRANSCRIPTION FACTORS IN LIVER TISSUES FROM HEPATIC ALVEOLAR ECHINOCOCCOSIS PATIENTS

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Human alveolar echinococcosis continues to be a major public health issue in China. The role and expression profile of T helper cell subsets in the hepatic lesion in hepatic alveolar echinococcosis patients are still not quite clear. In this study, we are aiming to investigate the expression and effect of T-bet / GATA-3 and Foxp3 / RORrt in liver tissue from alveolar echinococcosis patients, and then reflect the function and effect of the cell Th1/Th2 and Th17/Treg in AE. All liver tissues were selected from 14 patients with hepatic AE who were treated and divided into 3 groups: lesion tissues (L group), para-lesion tissues (P group) and liver tissues (N group). The mRNA expression of T-bet / GATA-3 and Foxp3 / RORrt were measured by real-time PCR (qRT-PCR). For statistical analysis,interquartile test and Spearman correlation were used. Both T-bet and GATA3 mRNA levels were significantly higher in group L than those in group N. There was no significant difference in those between group L and group P. However, there was significant difference in those between group P and group N. While, the expression of GATA-3 and T-bet mRNA were positively correlated in different liver tissue. As for the Foxp3 and RORrt mRNA expressions, the hepatic Foxp3 levels were significantly higher in group L than that in group P and group N. However, there was no significant difference in those between group P and group N. Meanwhile, RORγt mRNA expression in group P were also significantly higher than that in group N. Significant difference was found between group L and group N. The expression of RORγt and Foxp3 mRNA were positively correlated in different liver tissues. Th9 related transcription factor PU.1 mRNA levels elevated in group L and group P with statistical significance when compared to group N. The increased expression of Th1/Th2/Th9/Th17/Treg transcription factors in different hepatic tissues from human alveolar echinococcosis may indicate a close involvement of T cell subsets during the process of parasitic granuloma formation in alveolar echinococcosis patients.

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THE POSSIBLE ROLE OF TLR2 AND TLR4 WITH THEIR RELATED TRANSCRIPTION FACTORS/CYTOKINES IN INTRAPERITONEALLY INFECTED MICE WITH *ECHINOCOCCUS MULTILOCULARIS*

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The crosstalk between host and parasite ***Echinococcus.multilocularis (E.m)***, which causes alveolar echinococcosis (AE), is highlighted by the immune response. Toll-like receptor (TLR) 2 and 4 are known to play crucial role in other parasitic infections. Therefore, in this study, we explored the expressions of TLR2 and TLR4 with related cytokines in ***E.m*** infected mices. 24 subjects were enrolled and divided into three groups: ***E.m*** infection with CMC (***E.m*** + CMC, n=8) group, ***E.m*** infection with albendazole (***E.m*** + ABZ, n=8) group, and controls with CMC (C + CMC, n=8) group. The splenic cells and peritoneal exudates cells were prepared and real-time fluorescent quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and enzyme linked immune-sorbent assay (ELISA) analyses were carried out to detect the levels of TLR2, TLR4 and a series of transcription factors and cytokines. The mRNA expression levels of TLR2 and TLR4 and relative transcription factors and cytokines including GATA-3 , IFN-γ and IL-10 in splenocytes were significantly increased in ***E.m***+CMC group comparing with both ***E.m***+ABZ and C+CMC group. Simultaneously, T-bet mRNA expressions were elevated in ***E.m***+ABZ and C+CMC group compared to ***E.m***+CMC group. In addition, T-bet / GATA3 ratios were elevated in ***E.m***+ABZ group when comparing with ***E.m***+CMC group and were higher in C+CMC group than those in ***E.m***+CMC group. The concentration levels of IFN-γ, IL-10, and IL-5 in abdominal exudates cells as well as splenocyte supernatants were extremely lower, interestingly, they were found to be significantly elevated after stimulating with Con A for 36h, resulting in higher concentrations in ***E.m***+CMC group comparing with both ***E.m***+ABZ and C+CMC group. TLR2 mRNA expressions in splenic cells had a positive correlation with splenocyte IL-10 concentration levels. The present study provided evidence on the possible role of TLR2 in the process of immune tolerance during ***E.m*** infection. Further, our study also suggests that albendazole treatment might reverse the immune tolerance situation and improve parasite clearance process.

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DEVELOPMENT OF A SIMPLE IDENTIFICATION OF HUMAN *TAENIA* SPECIES BY USING MULTIPLEX LAMP AND DOT-ELISA

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Differential detection of ***Taenia solium*** from ***T. saginata*** and ***T. asiatica*** is a key strategy for the control and prevention of human cysticercosis in endemic areas. For this purpose, we have recently developed a sensitive and specific LAMP assay targeting the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene and have showed its usefulness. However, to achieve differential identification, three reaction mixtures containing ***T. solium***-, ***T. saginata***- and ***T. asiatica***-primer set separately per one specimen must be set up, which is complicated and time-consuming. In this study, we developed a simple identification of human Taenia species by using multiplex LAMP (mLAMP) and dot-ELISA. FIP primers of ***T. solium***, ***T. saginata*** and ***T. asiatica*** labeled with FITC, DIG and TAMRA, respectively, and biotin labeled-BIP primers were used in mLAMP. mLAMP assay succeeded in specific amplification of each respective target gene in single tube. Furthermore, LAMP product from each species was easily distinguished by using dot-ELISA with antibody specific for FITC, DIG or TAMRA. This method specifically identified cysticerci, proglottids and eggs of Taenia from endemic areas. mLAMP in conjunction with dot-ELISA will make real-time identification of human ***Taenia*** species in the field more practicable.

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FIRST TIME SEROLOGICAL EVIDENCE OF *TAENIA SOLIUM* LARVAL INFECTION IN ORANG ASLI COMMUNITIES - THE ABORIGINES FROM MALAYSIA

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Till today Malaysia is being presumed to be non-endemic for ***Taenia solium*** cysticercosis. Being a Muslim majority country, pork consumption by Muslims is prohibited. While considering non-Muslim communities in Malaysia who are pork eaters, possibility of its underlying prevalence is a matter of concern. Though there may be strict regulations in pig farming practice particularly in Peninsular Malaysia, however, the true estimate of the underlying prevalence, and risk factors of its transmission in this country remains unexplored. We initiated this study to understand the scenario on the native populations of Malaysia; 'Orang Asli' communities are known as aborigines of Malaysia who are underprivileged and also well

known to be carriers of many different parasitic infections. But the possible burden of ***T. solium*** cysticercosis was never explored in these aborigines. By using a commercial IgG-ELISA kit, the sera from a total of 522 randomly chosen Orang Asli individuals including either genders (age range between 1 to 68 years) were screened for ***T. solium*** larvae specific antibodies. A total of 3.8% subjects were diagnosed positive for anti-Cysticercus antibodies (95% CI: 2.5% - 5.8%; $\chi 2=17.8$; $p<0.05$). The prevalence of antibody positivity ranged between 0.9% (Semelai subgroup) to 9.9% (Orang Kuala subgroup). Statistical significance was observed between the low income status of family and seropositivity for cysticercosis ($p=0.041$) based on univariate analysis. Study findings indicated exposure to ***T. solium*** larval infection might have occurred in the aborigine communities in Peninsular Malaysia. For the first time our study findings could highlight that cysticercosis is an under recognized public health problem in Malaysia. Its actual prevalence can be estimated if a larger population is targeted in this country including both Peninsular and East Malaysia. Also a public health surveillance program must verify further on the risk factors for this neglected parasitic disease transmission either among the natives or imported from neighbouring endemic countries of Southeast Asia.

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MOLECULAR DETECTION OF TAENIID CESTODE EGGS IN DUNG BEETLES FROM ENDEMIC AREA TO PORCINE CYSTICERCOSIS

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The complex taeniasis/cysticercosis by ***Taenia solium*** is an important zoonotic disease mainly in development countries. The adult ***T. solium*** tapeworm localizes in the small intestine of humans, the definitive host. The eggs of ***T. solium*** are eliminated into gravid proglottids with the faeces, and then they are ingested by pigs, the intermediate host. Dispersal of cestode eggs of the Taeniidae family can occur in various manners, resulting in different ways in which they can be ingested by the corresponding host. The aim of this study was to confirm molecularly the presence of ***T. solium*** eggs in dung beetles collected from environmental, to confirm they are potential vectors of porcine cysticercosis. In this study, dung beetles were collected from 3 endemic villages of porcine cysticercosis in Piura, Peru. Dung beetles were collected in 41 different geographic point. For molecular study, we used a pool sample from each geographic point, each pool contained between 3 to 10 beetles. The DNA of the 41 pools was extracted using the FastDNA spin kit for soil DNA extraction according manufacture protocol. Then, mitochondrial cytochrome c oxidase subunit I (COXI) gene was amplified to identify occurrence of tapeworm. Finally, the positive samples were sequenced to know the species of tapeworm. Fifteen positive samples were obtained to PCR amplification. Two pools were compatible to ***T. solium*** and 3 pools were compatible to ***T. hydatigena***. Our findings suggest that the dung beetles likely to play a role in the dynamic of transmission of cysticercosis by ***T. solium*** and ***T. hydatigena*** in the endemic areas.

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GENERATION AND CHARACTERIZATION OF A PROLIFERATING *TAENIA CRASSICEPS* CELL LINE

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Neurocysticercosis, caused by the larval stage of the flatworm ***Taenia solium***, is the most frequent helminth brain infection in humans and the most common cause of adult onset seizures worldwide. ***Taenia crassiceps***, a closely related tapeworm, is a useful model for ***T. solium*** infection and has the advantage of supplying large numbers of larval cysts that multiply in mice following passage of cultivated cysts. We used ***T. crassiceps*** larvae to isolate, identify and grow neoblasts, the only platyhelminth-specific proliferating cells. Neoblasts have been studied in free-living planaria and later in cestodes like Echinococus. To isolate neoblasts, ***T. crassiceps*** (ORF strain) larval cysts were harvested from female BALB/C mice 8-10 weeks after intraperitoneal inoculation. These were washed, physically disrupted and placed at 27ºC in RPMI 1640 (with fetal calf serum, amino acids, mercaptoethanol and antibiotics) for one week before digestion with 1% trypsin and agitation. After cultivation and further growth, individual cells were isolated by limiting dilution and allowed to proliferate. Growth was enhanced by culture under a 5% N2 atmosphere and by addition of Concanavalin A, gonadotropin or b-estradiol, each at 1 µg/ml. Proliferation was confirmed by BrdU uptake, observed by immunofluorescence and immunohistochemistry assays detecting incorporated BrdU. Cells were maintained in serial passage over several months and grew at doubling time of 4.5 hr. These cells contained ***T. crassiceps*** antigens as determined by immunoblots and reactivity only against ***T. crassiceps*** infected mouse serum. Cells were cryopreserved using RPMI with 10% DMSO or 10% glycerol with full recovery of viable neoblasts. Since these are the only cells proliferating in cestodes and they show ***T. crassiceps*** antigens, they fulfill the definition of neoblasts. The successful isolation and ***in vitro*** culture of ***T. crassiceps*** neoblasts as a stable cell line with remarkable proliferating capacity allows for systematic studies on the development and biology of a helminthic parasite, including the search for novel drugs, and provides the basis for similar assays in ***T. solium***.

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DESCRIPTION AND PREDICTORS OF NEUROCYSTICERCOSIS CYST EVOLUTION AND RESOLUTION: A CYST-LEVEL ANALYSIS

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Neurocysticercosis (NC) is an infection of the central nervous system by the larval stage of the pork tapeworm. In the human brain, NC cysts evolve through three distinct phases, the active phase in which the parasite is viable, the transitional phase in which the parasite is degenerating and is targeted by the host's immune system, and in some cases the parasite leaves a calcified cyst instead of resolving completely. Almost all research on NC describes the cysts at the patient level, presenting summaries of the cyst burden within patients (e.g. number or presence of cysts in a

phase or location). To date, these analyses have been unsatisfactory in explaining the different patterns of cyst evolution between patients. In this study we disaggregated data from the patient to the cyst level for 62 patients who had 1 or 2 NC cysts in the active or transitional phase who were participating in a randomized clinical trial of albendazole treatment in Ecuador 2001-2003. We describe the evolution of 73 cysts from baseline to months 1, 6 and 12 through serial imaging and look at possible predictors of complete cyst resolution, cyst calcification and phase trends over time. By month 12, 59.4% of the cysts had completely resolved. Cysts in the parenchymal region of the brain and those in the transitional phase at baseline were more likely to have resolved completely by 12 months. Cysts in patients who developed new cysts (either due to new infection or cysts becoming newly apparent on imaging due to decreased inflammation) were also more likely to have resolved by 12 months. Age and gender, presence of calcified cysts at baseline, number of cysts at baseline and treatment group had no impact on cyst resolution by month 12. The associations were similar after adjusting for covariates and taking clustering into account using generalized estimating equations. Host immune response to cysticercosis varies widely across individuals, as well as between different cysts within the same individual. Understanding the patterns and predictors of cyst evolution within as well as between individuals may help us better identify treatments for those in whom current options are not working.

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EOSINOPHILIA IS NOT ASSOCIATED WITH NEUROCYSTICERCOSIS: RETROSPECTIVE ANALYSIS OF CASES SEEN AT THE NATIONAL INSTITUTES OF HEALTH 1985-2015

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Neurocysticercosis (NCC) has been generally cited as a parasitic infection associated with eosinophilia, but little evidence has been published to establish this relationship. A retrospective analysis was done of 106 patients with confirmed neurocysticercosis referred to NIH for evaluation and treatment between 1985 and January 2015. Historical records from referring providers, and from NIH were reviewed to identify the earliest available eosinophil count at the time of diagnosis. Eosinophilia was defined as an absolute eosinophil count ≥ 500/mm3. Patients with current or recent corticosteroid use (within two weeks) were excluded from further analysis (31%). The remaining cases (73) were reviewed for medical history including type of NCC, presence of a viable cyst(s) at diagnosis, current and past surgical and medical treatments and evidence of concurrent parasitic infections as judged by stools for ova and parasites and serologies for ***S. stercoralis*** (performed in most) and other helminthes when indicated. In this cohort, only 2 had an absolute eosinophilia of 500/mm^3 at the referring hospitals but were likely due to strongyloidiasis based on positive serology. The advantage of this study is the low likelihood of confounding eosinophilia due to migrating gastrointestinal helminthes not commonly endemic in the U.S. and routine serologic testing for ***Strongyloides stercoralis***, a common cause of occult eosinophilia in immigrants. Limitations include treatment prior to referral to NIH in many and incomplete historical records for some patients seen prior to 2000. In summary eosinophilia in patients with NCC was rare and when present could be attributable to other helminth infections.

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COMMUNITY-BASED SURVEILLANCE AND TREATMENT TO CONTROL TRANSMISSION OF *TAENIA SOLIUM*

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Taenia solium is a common zoonosis and a leading cause of preventable epilepsy worldwide. Focusing screening and treatment for taeniasis within households located around pigs that are heavily-infected with cysticercosis can reduce parasite transmission over time. This control approach, known as ring-strategy, reduced the seroincidence in pigs by 41% over one year in a prior study. The objective of this on-going randomized community trial is to apply ring-strategy in a community-based approach to evaluate whether control gains can be replicated by villagers working in coordination with the local Ministry of Health. This approach requires significant community collaboration and capacitation including formation of a functioning surveillance and response system at the local level. We randomized 4 villages (pop. 1117) to intervention and 3 villages (pop. 1287) to control. The intervention consists of villager surveillance for infected pigs followed by Ministry of Health response including case investigation and presumptive treatment of humans and pigs in nearby households. The seroincidence of cysticercosis is monitored serially as the outcome over a period of one-year. We collaborated with the Ministry of Health so that local health workers could provide treatment of humans and pigs in response to disease reports. We trained 33 healthcare workers in case investigation, diagnosis and treatment, and another 12 health promoters to promote and document cysticercosis reports from residents. Collaboration with the Ministry of Education provided access to primary and secondary classrooms where students were given interactive instruction regarding cysticercosis prevention and surveillance. This collaboration also facilitated treatment of children at the schools provided parent consent. Twelve cases of porcine cysticercosis were reported within the first 6 months of the study resulting in presumptive treatment of 249 humans and 169 pigs in at-risk households. The final effect of the program on pig seroincidence in the intervention communities versus control will be presented at the 2015 ASTMH meeting.

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IDENTIFICATION, ISOLATION, CULTURE AND CHARACTERIZATION OF *TAENIA SOLIUM* PROLIFERATING CELLS

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Proliferation, regeneration and differentiation in flatworms are functions restricted to a particular group of cells, called neoblasts, which are also involved in the enormous plasticity typical of planarians. Despite some diferences at the morphologic and genetic level, neoblasts are also found in parasitic flatworms and are the only proliferating cells in platyhelminths. These cells have been identified in and isolated from Echinococcus and ***Taenia crassiceps***. We identified proliferating cells in ***T. solium*** cysts collected from an infected pig; neoblasts were identified by their ability to replicate and incorporate bromodeoxyuridine (BrdU), detected with

a BrdUspecific monoclonal antibody. Cells were extracted from cysts by digestion with trypsin followed by limiting dilution cultures, proliferating cells were abundant in the neck of the parasite, and were more evident after bile-induced evagination of the cysts. Their identity as neoblasts was confirmed after incubation with BrdU. ***In vitro*** growth of isolated neoblasts was enhanced with different supplements and incubation at two temperatures. Plates were incubated in the presence of 5% N2. Proliferating cells in stable cell cultures have been achieved. T solium neoblasts reacted with sera from pigs naturally infected with ***T. solium***, and not with sera from healthy pigs gave in immunohistochemical assays. Given the urgent need for novel, more effective and faster cysticidal drugs without deleterious side effects, efficient and scalable ***in vitro*** systems are essential to perform systematic testing and further research. Therefore we utilized the neoblast to test new compounds for cytotoxicity as a first step towards development of therapeutic agents.

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CORRELATION OF EVANS BLUE STAINING AND GADOLINIUM ENHANCEMENT IN MAGNETIC RESONANCE IMAGING OF PIGS NATURALLY INFECTED WITH *TAENIA SOLIUM*

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How well Evans blue (EB) extravasation into the brain correlates with MRI studies employing gadolinium (Gd) enhancement was analyzed in pigs harboring brain cysts of ***Taenia solium***. Previous experiments documented that after intravenous injection of EB, there was increased leakage of the dye into the cyst capsules that was associated with the enhanced presence of pericystic inflammation. Twelve naturally infected pigs were injected with EB two hours prior to evaluation by coronal, sagittal and axial T1-weighted, Gd-enhanced magnetic resonance imaging (T1-MRI), after which the animals were euthanized and their brains analyzed. Histological examination allowed for classification of pericystic (capsular) inflammation as minimal, moderate or extensive, according to kind and distribution of immune cells. Ten-mm coronal sections of each brain were photographed and cyst capsules were scored according to absence or presence of EB (0 or 1: clear or blue capsules) and distribution of the color per cyst (EB categories were 0: 0%; 1: up to 50% of the capsule was blue; 2: over 50% of the capsule was blue; 3: 100% of the capsule was blue). The open access software packages IMAGE J and R were used for bidimensional analysis of MRI images, which were normalized, assigned spacial sectors and converted to 3-D data. The occurrence and spacial distribution of Gd enhancement (GE) were determined for each capsule identified on MRI using continuous normalized histogram values from 0 (black) to 256 (brightest). GE scores had a 100% success and sensitivity for discriminating between the presence of EB in clear (mean: 40.46) and blue capsules (mean: 64.03) (Student's t test; $p<0.001$); the cutoff value for GE was 42.5. According to EB distribution per capsule, 0% of blue had statistically lower GE from the other three categories (Bonferroni corrected Anova; $p<0.01$). Distribution of Gd correlated with the presence and distribution of EB and both correlated with the severity of the inflammation as observed histologically. These data demonstrate that EB staining can be a surrogate for Gd enhancement in MRI and may be applied to study inflammation in the pig model of NCC.

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THREE-DIMENSIONAL DISTRIBUTION OF *TAENIA SOLIUM* CYSTS IN PORCINE NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is the common major cause of seizures in Latin America. The symptoms of NCC depend on different characteristics, among them the anatomical location of the cysts. In domestic pigs, the middle or rostral cerebral artery has 10 main branches on the surface of the cortex, 7 of which are oriented towards the occipital lobe. In order to know if cysts are distributed following the vascular network at the central nervous system, we analyzed the three-dimensional spatial point pattern distribution of 414 ***Taenia solium*** cysts in the brains of 15 naturally infected pigs. Photos of coronal brain sections were used to obtain the X, Y, Z coordinates of the center of each cyst with the ImageJ program. The coordinates were corrected by the size and center of each brain to normalizate the data. We analyzed the distribution patterns of each cyst through the Ripley's K function, G-function and F-function for three-dimensional data, using the statistical program R and Spatstat library. The spatial point pattern was categorized in three main classes, namely aggregation (defined by a distribution with the shortest distances between cysts), regularity and complete spatial randomness (CSR), according to the interpretation of functions graphs. Aggregation of cysts was confirmed only if indicated by the three functions. Cyst distribution in the occipital, parietal and frontal lobe followed an aggregation pattern in 100%, 10% and 22% of pigs, respectively. Only in the temporal lobe the distribution pattern observed was random in all pigs studied. Parenchymal cysts follow a CSR distribution in 20% of the pigs and the other 80% follow a regular distribution. The meningeal and corticomeningeal cysts showed aggregation in 20% of the pigs studied, and the zones with the highest aggregation (i.e., the shortest distance between cysts) followed the branching of the rostral cerebral artery in 4 temporal branches oriented to the occipital lobe. According to these results we confirm that the distribution of cysts in the brain tends towards aggregation following the distribution of blood vessels.

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IN VITRO CULTURE ASSAY AND *IN VIVO* RAT NEUROCYSTICERCOSIS MODEL TO STUDY DIFFERENCE BETWEEN *TAENIA SOLIUM* AND *T. SAGINATA*

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Why can larval-stage ***Taenia solium*** infect humans while larval-stage ***T. saginata*** does not have this ability? It has not yet been answered. The aim of this work was to use an ***in vitro*** culture assay and a rat neurocysticercosis model to study and compare the development of ***T. solium*** and ***T. saginata*** from oncosphere to cysticerci. In the first experiment ***T. solium*** and ***T. saginata*** activated oncospheres were cultured in INT–407 intestinal cells for up to 180 days in order to evaluate the difference in the morphology during the development. Both of them developed to postoncospheral stage and the morphology was similar up to 60 days of culture, then ***T. solium*** postoncospheral stage started to die and ***T. saginata*** grew for up to 180 days and developed to cysticerci. In the second experiment, we inoculated rats, intracranially, with oncospheres and postoncospheral stage of ***T. solium*** and ***T. saginata***. Rats that were infected with ***T. solium*** oncosphere and postoncospheral stage developed neurocysticercosis whereas rats infected with ***T. saginata***

oncosphere and postoncospheral stage did not develop neurocysticercosis. In conclusion, an ***in vitro*** assay could permit in the future to compare the molecules and proteins that are expressed by ***T. solium*** and ***T. saginata***. An ***in vivo*** model could permit in the future to study at which moment ***T. saginata*** loses the ability to cause neurocysticercosis.

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TRANSMISSION DYNAMICS OF *TAENIA SOLIUM* AND CYSTICERCOSIS IN PIGS AND HUMANS IN BURKINA FASO

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A transmission dynamics model of ***Taenia solium*** and the resulting infections of taeniasis and cysticercosis is explored using a mixed model which combines both density dependence and a frequency dependent Who Acquires Infection From Whom matrix similar to those used to model sexually transmitted infections. This approach aims to address the differences in the way that pigs acquire infection versus the way that humans become infected and the interaction between the two in a way that, to our knowledge, has yet to be addressed. This model assumes that a pig's likelihood of acquiring infection is dependent on the density of the human population, and that seasonality plays an important role in the rate that porcine cysticercosis is acquired. The model also assumes the contact of humans with infected meat; and then subsequently each other; is not homogeneous. Individuals of a lower socio-economic status are modeled to be more frequently in contact with infected meat, as well as additionally at higher risk for spreading the disease through contamination to others following food preparation. Verification of the model using serum samples from pigs and humans testing for cysticercosis and taeniasis collected from Burkina Faso will allow for not only the verification and more complete understanding of the transmission dynamics of this disease, but also the feasibility of this novel modeling approach.

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INJECTIONAL ANTHRAX AMONG HEROIN USERS: A VIEW FROM THE AMERICAS

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Anthrax is caused by ***Bacillus anthracis***, a Gram-positive, rod-shaped bacterium that is the only obligate pathogen in the large genus ***Bacillus***. The disease can manifest in four forms including: cutaneous, inhalational, gastrointestinal, and, more recently, injectional. "Injectional anthrax is a form of anthrax infection that is receiving increasing attention after a single strain caused an outbreak in Europe left over 130 infected and at least 19 individuals dead." Cases involving this new form of anthrax involve people who inject drugs (PWIDs) who have been found to use anthrax-contaminated heroin. "Important differences between cutaneous and injectional anthrax have included an increased risk of shock and a higher mortality rate despite antibiotic therapy (<1% versus 34% respectively)." Literature on the European outbreak highlight the belief that natural contamination of the heroin supply occurred as it was transported and/or processed through anthrax endemic regions of the Middle East. Surveillance of the illicit heroin supply for future anthrax contamination is therefore of concern. The United Nations Office on Drugs and Crime (UNODC) estimates that in 2011 a total of 14.0 million (range: 11.2 million to 22.0 million) people injected drugs worldwide, which corresponds to 0.31 per cent (range: 0.24-0.48 per cent) of the population aged 15-64." These people are potential at-risk individuals for injectional anthrax. This study will specifically look into the possibility of anthrax contamination of the illicit heroin supply within North America using the European outbreak as a model. The importance of this study comes from the fact that North America has a large percentage of the world's PWIDs, having 2 million people vulnerable to a lethal form of anthrax infection. As there is gap in the literature as to how anthrax contamination could affect other illicit heroin markets other than Europe, this hypothetical piece views the possibility of anthrax contamination within North American PWIDs.

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ETIOLOGY OF PEDIATRIC FEVER IN WESTERN KENYA: A CASE-CONTROL STUDY OF FALCIPARUM MALARIA, RESPIRATORY VIRUSES, AND STREPTOCOCCAL PHARYNGITIS

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In Kenya, >10 million episodes of acute febrile illness are treated annually among children under five. Most are clinically managed as malaria without parasitological confirmation. There is an unmet need to describe pathogen-specific etiologies of fever. We enrolled 370 febrile children and 184 healthy controls. We report demographic and clinical characteristics of patients with ***Plasmodium falciparum***, group A streptococcal (GAS) pharyngitis, and respiratory viruses (influenza A&B, respiratory syncytial virus, parainfluenza types 1-3, adenovirus, human metapneumovirus), as well as those with undifferentiated fever. 79.7% of febrile children were treated for malaria. However, ***P. falciparum*** was detected infrequently in both cases and controls (14/268 [5.2%] vs. 3/133[2.3%],p=0.165), whereas 41%(117/282) febrile children had a respiratory viral infection, compared to 24.8%(29/117) controls (p=0.002). Only 9/515(1.7%) children had streptococcal infection. Of febrile children, 22/269(8.2%) were infected with >1 pathogen, and 102/275(37.1%) had fevers of unknown etiology. Respiratory viruses were common in both groups, but only influenza or parainfluenza was more likely to be associated with symptomatic disease (attributable fraction 67.5% and 59%, respectively). Malaria was over-diagnosed and over-treated. Few children presented to the hospital with GAS pharyngitis. An enhanced understanding of carriage of common pathogens, improved diagnostic capacity, and better-informed clinical algorithms for febrile illness are needed.

1042

THE PREVALENCE OF ANAEMIA IN CHILDREN UNDER FIVE YEARS OLD LIVING IN THE AREA OF INTERVENTION OF THE AMAZON HOPE MEDICAL PROGRAM, PERÚ

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The Amazon Hope medical boat health program offers primary medical care and health promotion to 167 communities of the Peruvian Amazon in four of its river regions (Amazonas, Tigre, Puinahua, and Ucayali). The subsistence farming and fishing communities in these regions have high rates of poverty and infant morbidity, including diarrhoeal disease and stunted growth. Despite this, there is limited research on anaemia and parasite infection in the area. We aimed to assess the prevalence of anaemia in children from rural Peruvian Amazonian communities who access care from the Amazon Hope medical boat. 1421 children in the study site were randomly selected to participate. Inclusion criteria were being a child from 6 to 59 months whose guardian gave informed consent to participate. All consenting participants underwent a haematocrit analysis undertaken by a laboratory technician aboard the Amazon Hope. Anaemia was defined as a haematocrit percentage <33%. Prevalence of anaemia in participants was high (89%), regardless of gender. The highest prevalence of anaemia was found in children aged between 6-11 months

(96.9%) followed by 12-33 months (90.8%). The prevalence of anaemia in children aged two, three, and four years was 86.6%, 87.4%, and 83.7% respectively. The Rio Tigre had the highest rate of anaemia (93%) followed by the Ucayali (90%), Puinahua (89%) and Amazonas (83%). This study demonstrates a previously undocumented high prevalence of anaemia in the children of isolated Peruvian Amazonian communities attending a medical boat clinic. The reasons behind this high prevalence are likely to be inter-related with the extreme poverty found in the region, which can cause: poor nutrition (including iron deficiency) and high burden of gastrointestinal parasites. Interventions are required to combat this previously unidentified health need and thus prevent associated long-term sequelae such as stunted growth and impaired immunity.

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LOW-COST SYSTEM FOR PERFORMING A WHITE BLOOD CELL COUNT AND 3-PART DIFFERENTIAL AT THE POINT-OF-CARE

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The ability to perform a white blood cell (WBC) count and differential is an important laboratory diagnostic test. However, the current methods for performing a WBC count and differential in high-resource settings are not feasible for use in low-resource settings due to high cost and infrastructure requirements. There is a particular need for affordable tools to measure the WBC count and differential at the point-of-care in such settings. To meet this need, we developed a portable microscope and disposable cartridge that can be used at the point-of-care to perform a WBC count and 3-part differential using 20 uL of blood obtained from a fingerprick. The device is inexpensive, can be deployed at the bedside, and results are available within 60 seconds. The cartridge, which is composed of a glass slide, a layer of transfer tape, and a glass cover slip with laser cut inlet and outlet ports, contains a well in which acridine orange is pre-dried. Blood from a fingerstick is drawn into the well via capillarity and WBCs are stained with acridine orange, staining nuclei green and granules red. The cartridge can then be imaged using a portable fluorescence microscope; the image is analyzed automatically to report the WBC count and the percentages of granulocytes, monocytes, and lymphocytes to the user in less than one minute. This is achieved by a novel image analysis program that identifies the WBCs in the field of view and classifies them into the WBC subtypes based on the red and green pixel intensity within each cell. Preliminary results from testing the cartridge and microscope device with 9 normal capillary samples have shown promising results with all samples tested falling within ±15% the true WBC value. Further, the differential data also correlates strongly with the true values with an overall R-squared value of 0.85. We estimate that, at production scale, the portable microscope can be developed for under $800 and the cartridge device for less than $0.50.

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EPIDEMIOLOGY OF REINFECTION BY LEPTOSPIROSIS IN NEW CALEDONIA: A FIVE-YEAR PROSPECTIVE STUDY

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In endemic areas, recurrent episodes of leptospirosis remain scarcely described. The objective of the study was to assess incidence, characteristics and risk factors of reinfection by leptospirosis in a large at-risk population. We performed a longitudinal study to capture patients admitted for leptospirosis more than once over a 5-year period (2007-2011) in New Caledonia. Of 716 episodes of confirmed leptospirosis recorded among 707 individuals, 9 cases of reinfection (incidence rate 1.3%) were identified. All recurrent episodes occurred in adults who presented with fever (100%), jaundice (100%), conjunctival suffusion (55%), headache (78%), and myalgia (78%). Renal function impairment was noted in 5 (55%) cases. No Weil disease nore fatality case were observed. The mean time elapsed between the two episodes was 23 months. The serogroup was identified in 8 recurrent cases and was similar to the previous episode in 6 (75%) cases. There was no difference in behavioural changes after the first infection between people experiencing recurrent episodes and the other. Although recurrent episodes of leptospirosis appeared to be rare and non severe, prompt administration of standard antibiotic therapy is required in reinfection. Similar environmental factors for both reinfected and non reinfected patients suggest the potential role played by individual factors in the physiopathology of the disease. However, reinforced public health campaigns should help preventing both infection and reinfection to occur.

1045

FIRST ESTIMATES OF THE DISEASE BURDEN OF PODOCONIOSIS IN ETHIOPIA

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Ethiopia has the highest burden of podoconiosis globally, yet the geographical distribution and burden of the disease is poorly understood: no up-to-date, nationwide survey data were available and the appropriate measure of disease burden has yet to be defined. A commonly used standardized measure of disease burden is the disability-adjusted life years (DALYs) metric which combines years lost due to premature mortality (YLLs) and years lived with disability (YLDs). Currently there are no YLDs or DALYs estimates for podoconiosis. We report the results of a recent nationwide mapping of podoconiosis in Ethiopia, plus additional work providing estimates of YLDs for podoconiosis - both provide an estimate for the population at risk and the disease burden of podoconiosis in Ethiopia, and will be the first ever countrywide estimate of disease burden due to podoconiosis. Our analyses are based on data arising from the integrated mapping of podoconiosis and lymphatic filariasis (LF) conducted in 2013, supplemented by data from an earlier mapping of LF in western Ethiopia in 2008-2010. Data are available for 141,238 individuals from 1,442 communities in 775 districts from all regional states in Ethiopia. The proportion of people with podoconiosis was 4.0 % (95% CI; 3.9-4.1%) overall and ranged from 0% to 8.6% in regional states. Using boosted regression tree modelling and a suite of environmental variables we estimated that 34.9 million people are at risk of podoconiosis in Ethiopia. Using the Bayesian meta-regression method, DisMod-MR, employed by the Global Disease Burden (GBD) 2010 study, we estimate the YLDs due to podoconiosis. Results will be reported on the number of people affected with podoconiosis in Ethiopia, and YLDs will be calculated as prevalence of podoconiosis by age, sex and weighted by disability weights for lymphoedema. Estimates of uncertainty will be presented at all stages of the analysis. Finally, we will compare the YLDs for podoconiosis with other priority health problems in Ethiopia and discuss the implications for policy and planning.

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CANDIDA AND BACTERIA RELATED VAGINITIS AMONG FEMALE SENIOR HIGH STUDENTS IN NAVRONGO, GHANA

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Vaginal candidiasis affects most females during their lifetime, with approximately 50% having two or more episodes. Most cases are caused by ***Candida albicans*** (85%-90%). It is the second most common cause of vaginitis. On the other hand, Bacterial vaginosis is an imbalance of the vaginal bacterial microbiota and its etiology is still unknown. It is a common condition afflicting mostly women in their reproductive age. The study was conducted to assess the incidence of ***Candida*** and bacteria related vaginitis among female senior high students. Seventy-two high vaginal swabs were obtained from consenting students for laboratory analysis and a structured questionnaire administered to assess symptoms, risk factors and demographic information. Methods employed in laboratory analysis included wet mount, whiff test, clue cell test, pH test, Gram staining and culture. The results revealed 40% (29) of participants were infected with ***Candida*** vaginitis while 14% (10) were diagnosed with bacteria vaginosis. Lactobacilli which are supposed to be a normal flora in a healthy vagina were isolated in only 29% (21) of the participant who were between the ages of 14 to 22 years. For symptoms, 100% (72) of the participants had vaginal discharge. 68% (49), 63% (45) and 18% (13) had itching, irritation and burning sensation respectively. Among the risk factors sexual activity recorded the highest 76% (55), 61% (44) douched and 32% (23) were on antibiotics. The incidence of ***Candida*** vaginitis especially is high in the study population and this may be due to few participants having Lactobacilli isolated from their vagina and may also explain the incidence of bacteria vaginosis.

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'IT COULD BE VIRAL BUT YOU DON'T KNOW, YOU HAVEN'T DIAGNOSED IT': CHALLENGES IN MANAGING NON-MALARIA PEDIATRIC FEVERS IN THE LOW TRANSMISSION SETTING OF MBARARA DISTRICT, UGANDA

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In 2012, Uganda initiated nationwide deployment of malaria rapid diagnostic tests (mRDT) as recommended by revised national guidelines. The shift from presumptive malaria treatment of pediatric fevers to test-based management has great potential to improve quality fever care. Yet studies show common mRDT non-compliance, which has spurred calls to deploy mRDT as part of enhanced training packages that include integrated fever management protocols. An understanding of how health workers currently manage non-malaria fevers and challenges faced in this work should also inform efforts. A qualitative study was conducted in the low-transmission area of Mbarara District in Uganda where most fevers are not due to malaria. In-depth interviews with a purposive sample of 20 health workers at lower level clinics focused on mRDT perceptions, strategies to differentiate non-malaria fevers, influences on clinical decisions, desires for additional diagnostics, and dilemmas faced in this work. Differential diagnosis strategies included studying fever patterns, taking histories, assessing symptoms, and analyzing contextual factors (e.g. child's age or home environment). If no alternative cause was found, malaria treatment was often prescribed despite a negative result. Other reasons for malaria over-treatment included mRDT mistrust; mRDT misperceptions ('for RDTs to become positive it might take like three days [from illness start]'); caregiver demands and system constraints, notably working alone without opportunity to confer on difficult cases. Many health workers expressed uncertainty about how to manage non-malaria fevers, feared doing wrong and patient death, worried caregivers would lose trust, or felt unsatisfied without a clear diagnosis. Enhanced support is needed to improve mRDT adoption at lower level clinics that focuses on how to manage non-malaria fevers. This includes facilitating peer-learning networks, correcting mRDT misperceptions, building trust in negative results, and reinforcing integrated care initiatives (IMCI/iCCM) to improve mRDT compliance, rational drug use and quality fever management.

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COMPARING THE USE OF PERSONAL PROTECTIVE MEASURES FOR MOSQUITO-AVOIDANCE DURING TRAVEL TO REGIONS WITH DENGUE OR CHIKUNGUNYA ACTIVITY, AND REGIONS ENDEMIC FOR *PLASMODIUM FALCIPARUM* MALARIA

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The recent outbreak of chikungunya in the Caribbean highlights the importance of personal protective measures (PPM) in preventing mosquito-borne febrile illnesses that lack effective treatment. We compared the use of PPM in Department of Defense beneficiaries traveling to areas in the Caribbean with dengue or chikungunya activity, to those traveling to areas of Africa with high ***Plasmodium falciparum*** endemnicity. Individuals presenting to 4 US military travel clinics were prospectively enrolled. Participants completed a post-travel survey documenting the use of PPM, and the frequency and timing of mosquito bites. Participants traveling to regions in Africa with high malaria endemnicity (***P. falciparum*** parasite rate [PfPR2-10] > 35%) or regions in the Caribbean with minimal or no risk of malaria, but with documented chikungunya or dengue activity were included. Compliance with PPM was defined as frequent or daily use of insect repellant on skin, and use of a repellant on clothes. Multivariate regression was performed to evaluate factors associated with PPM compliance. Between 2010 and 2015, 1723 enrolled travelers completed a post-travel survey, of which 186 traveled to highly-endemic malarious regions of Africa, and 148 traveled to regions in the Caribbean with chikungunya or dengue activity. A significantly greater proportion of participants traveling to malaria-endemic regions reported seeing mosquitoes (87% vs 56%) and experiencing mosquito bites (65% vs. 47%). Travelers to the Caribbean reported mosquito bites during the day more frequently than travelers to malaria-endemic regions (76% vs. 39%) ($p < 0.05$ for all comparisons). Compliance with PPM was associated with travel to areas with chikungunya or dengue activity (24% vs. 13%; OR: 1.58 [95% CI 1.05-2.39]), and travel for a military mission (49% vs. 8%; OR: 5.52 [95% CI: 3.11-9.79]) on multivariable analysis. Overall, we observed poor compliance with PPM in travelers, although compliance was significantly higher in travelers going to areas with dengue/chikungunya activity. Additional studies and interventions aimed at improving compliance with PPMs are needed.

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USE OF ELECTROCARDIOGRAM TO EVALUATION SAFETY OF ANTIMALARIAL DURING POST LICENSURE SURVEILLANCE IN AFRICA-MODELLING OF PATIENTS ECG

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Current use of Dihydroartemisinin/piperaquine [DHA/PQP] for the treatment of uncomplicated malaria infection in vulnerable groups with cardiogenic problem requires more supportive safety data. One of the most outstanding side effect of DHA/PQP is its negative electrocardiographic (ECG) effect on heart rhythm through prolongation of the QT intervals. There is at present scarce toxicity data of ECG to warrant greater use of DHA/PQP in cardiogenic arrhythmic predisposed individuals. PQP is the fat soluble part of DHA/PQP that is responsible for belated ventricular depolarization hence QT prolongations. We conducted an ECGs safety assessment at baseline before intake of DHA/PQP and during subsequent doses as well on day seven in a 7 months multicentre antimalarial study. Laboratory analyses included haematology, biochemistry and population pharmacokinetics. We hereunder report a descriptive details of the QTc intervals changes during the trial together with modelling of QTc outcome using a linear mixed model. Overall 1315 patients gave consent of which only 1002 (76.2%) were eligible. A comparison of mean QTcf at baseline (383.3 ms) and during subsequently follow up records was 395.1 before drug intake and 402.3 after drug intake on day 3 and the resultant QTcf on day 7 was restored to baseline of 387.3 ms. Mean QTcf in patient from Ghana was significantly higher compared to other three countries (Burkina Faso, Tanzania and Mozambique). In a multivariate model a unit increase in total bilirubin and body mass index was associated with a decrease for nearly 10% and 50% of mean QTcf intervals respectively. Plasma drug concentration on day 3 and day 7 was recorded preliminary results is expected before ASTMH in October. This study demonstrated DHA/PQP is not cardiogenic unsafe in children and adults at normal dose with uncomplicated malaria in Africa.

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A CLINICAL PREDICTION MODEL OF IN-HOSPITAL MORTALITY DERIVED FROM PATIENTS ADMITTED WITH ACUTE INFECTION TO A REGIONAL REFERRAL HOSPITAL IN MBARARA, UGANDA

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Clinical early warning scores (EWS) used in resource-rich settings (RRS) to predict mortality due to sepsis may not be applicable to resource-limited settings (RLS) due to a lack of laboratory infrastructure and population differences. Therefore, we created a prediction model of in-hospital mortality for use in RLS based on data from patients admitted with suspicion of infection to Mbarara Regional Referral Hospital (MRRH) located in Southwestern Uganda. We imputed missing data (range<0.5-30%) for temperature, white blood cell count (WBC), hemoglobin, platelets, and lactic acid using a k-nearest neighbors algorithm. We used multivariable logistic regression with 10-fold cross validation to create the model. We compared our model to the Modified Early Warning Score (MEWS) and CRB-65 which have been validated in RRS. The study included 1089 patients with a median (IQR) age of 34 (27-45) and which was 47% female. There were 755 (69%) HIV-infected patients with a median (IQR) CD4 count of 80 (20-201). Tuberculosis was suspected or confirmed in 67%, 36% had suspected meningitis, and 35% had a suspected pulmonary source. In-hospital mortality was 26% and 30-day mortality was 61%. Median (IQR) length of hospital stay was 6 days (3-10). Glasgow coma score ($p<0.0001$), respiratory rate ($p<0.001$), temperature ($p<0.001$), mean arterial pressure ($p=0.001$), WBC ($p=0.001$), hemoglobin ($p=0.001$), and lactic acid ($p=0.001$) were associated with increased mortality in the multivariable model. The AUC for the model without laboratory values was 0.70 and improved slightly to 0.71 with laboratory values. The AUC was 0.66 for MEWS and 0.64 for CRB-65. The AUC for a model that included HIV sero-status (n=992) was 0.77. In conclusion, our derived clinical model showed good ability to predict in-hospital morality in this special population of alarmingly young and critically ill patients. Improved predictive models for mortality from critical illness in RLS are needed to accelerate early interventions and improve morbidity and mortality in this population.

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ADMISSION EEG FINDINGS IN AFRICAN CHILDREN WITH CEREBRAL MALARIA ARE ASSOCIATED WITH MORTALITY, MORBIDITY, AND MALARIAL RETINOPATHY

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The spectrum and implication of electroencephalographic (EEG) findings in children with cerebral malaria (CM) has been described in case reports and small case series, but to date there has been no large study assessing these findings in children in more than one site or comparing findings in children with and without malarial retinopathy . In the present study we characterized and compared admission EEG findings in Malawian and Ugandan children with CM, identified EEG factors associated with mortality or adverse neurological outcome in survivors, and compared EEG findings in patients of differing malarial retinopathy status. We reviewed admission EEG tracings from 281 hospitalized children with clinical CM, 122 from Uganda and 159 from Malawi, admitted between December 2008 and January 2012. Tracings were interpreted by neurologists blinded to the patient's outcome and retinopathy status. The risk of death was lower in those with EEG vertex waves (odds ratio (OR)= 0.15, 95% confidence interval (CI): 0.03, 0.87), higher maximal voltage, and higher average background frequency. EEGs with an increased proportion of epochs with spindles were associated with a lower likelihood of death. A generalized voltage attenuation to painful stimulation decreased the odds of death compared to those with no EEG reaction to stimulation (OR= 0.33, 95%CI: 0.11, 0.98). The presence of electrographic seizures was not associated with mortality but was associated with an increased odds of a neurologic deficit in survivors at discharge (OR= 12.5, 95%CI: 2.8, 56.2). Children with malarial retinopathy were more likely to have EEG variability, absence of spindles, and a non-attenuated reaction to painful stimulation (all $p < 0.05$). Specific EEG findings in children with CM are associated with mortality, neurologic deficits, and the presence of malarial retinopathy. In pediatric CM, brain pathways associated with death likely differ from those associated with neurologic morbidity in survivors.

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REPRODUCTIVE HEALTH: BURDEN OF SEXUALLY TRANSMITTED INFECTIONS IN MACHALA, ECUADOR

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Over one million people acquire a sexually transmitted infection (STI) every day worldwide. Sexually transmitted infections are a major threat to public health in Latin America. The objective of this analysis was to examine the burden of sexually transmitted infections, including viral, protozoan, and bacterial infections, in Machala, Ecuador, a coastal city located in southern Ecuador. A total of 398,919 records were analyzed from a citywide epidemiological database of patient clinic visits in Machala, Ecuador, in 2014. Binomial regression was used to examine the associations of sexually transmitted infections with socio-demographic factors. There were a total of 1,432 cases of sexually transmitted infections in Machala in 2014. Viral infections, including herpes, hepatitis B, HIV, and human papillomavirus (HPV), were the most commonly reported, comprising 46.8% of the total STI cases, followed by protozoan (38.1%) and bacterial (15.1%) infections. Trichomoniasis, HIV, and herpes accounted for more than half of all cases of sexually transmitted infections. Although the burden of syphilis was relatively low, it accounted for 86.6% of bacterial infections. Women had a two-fold greater risk of presenting with sexually transmitted infections, compared to men (RR: 1.91, 95% CI: 1.71-2.12, p<0.0001); the risk was even higher for women of reproductive age (15-49y) (RR: 5.50, 95% CI: 4.75-6.36, p<0.0001), compared to women of other age groups. Although men had a lower risk of presenting with a sexually transmitted infection (p<0.05), they had a three-fold greater risk of HIV infection (RR: 3.10, 95% CI: 2.48-3.89, p<0.0001), compared to women. The burden of sexually transmitted infections is relatively high in coastal Ecuador, particularly among women of reproductive age. Future surveillance efforts are needed among women of reproductive age that integrate sexually transmitted infection screening with reproductive and perinatal health care in this setting.

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IDENTIFICATION OF *RICKETTSIA* SPP INFECTION IN PATIENTS WITH CLINICAL SUSPICION OF LEPTOSPIROSIS: APPLICATION OF MOLECULAR TECHNIQUES IN A CASE SERIES

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Leptospirosis is a public health problem on the Colombian Caribbean Coast. In Cartagena, a major city in this region, this disease is endemic with high annual lethality. In clinical practice, differential diagnosis of leptospirosis with others icteric febrile syndromes is crucial to an appropriated therapeutic approach. Nevertheless, high complexity hospitals employ only serological test to confirm suspicious cases, with an elevated frequency of undetermined results. Therefore, the aim of this study was to apply molecular techniques in patients under leptospirosis suspicion. It was carried out a case series study with adults in 2013 last trimester. Patients with clinical manifestations and positive/undetermined results in serological test for leptospirosis were included. We used Polymerase Chain Reaction to detect ***Leptospira spp*** from blood samples with specific primers that amplify a DNA segment from the ***lipl32*** gen. A total of 9 patients were included (3 women, 6 men). IgM test for leptospirosis was positive in 5 subjects and undetermined in 4 others. A 423pb product was expected, however only 3 samples showed an amplicon of 358pb. When those DNA fragments were sequenced, none of them were part of ***Leptospira spp***. genome. Thereafter, samples were amplified for ***Riskettsia spp.*** by nested PCR for the ***OmpB*** gene. Seven samples were positive (420pb amplicon). These samples were also positive for ***Rickettsia spp*** 17KDa gene (230pb amplicon). According to these results, in spite of high clinical suspicion in a scenario were physicians are familiarized with leptospirosis, lacking of specific tests could lead to misdiagnosis. On this particular situation, considering that therapeutic approaches between leptospirosis and rickettsiosis are mainly divergent, a permanent protocol for differential diagnosis would be a major advance in reducing diseases burden. Also, this case series study revealed an apparently silent public health threat by rickettsiosis.

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CHIKUNGUNYA FEVER IN THE CARIBBEAN: CLINICAL FINDINGS FROM GRENADA

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Chikungunya virus (CHIKV) made its debut in the Americas during a large outbreak in the Caribbean from 2013-2014. The tri-island nation of Grenada was severely affected. The objective of this study was to evaluate the demographic features and presenting symptoms of those patients with suspected and/or confirmed CHIKV infection in Grenada. Patients with fever (>38.0°C) and symptoms consistent with CHIKV were enrolled at health facilities throughout Grenada. Survey data and serum samples were collected. Sera were tested for CHIKV and dengue virus using real-time PCR and for anti-CHIKV IgM by ELISA. Sera from 493 suspected cases were collected from mid-July until early October 2014 from Grenada, Carriacou, and Petit Martinique. Sixty-six percent were female and the mean age was 37.3 (range 0.1-92) years. The most common reported symptoms included joint pain (89%), fever (88%), myalgia (66%), headache (54%), chills (50%), and rash (43%). On exam, petechia (5%), gingival bleeding (2%), hematuria (1%), bruising (1%), and epistaxis (1%) were also noted. Of the 487 sera tested by both PCR and IgM ELISA, 87% (N=425) had a positive test: 22% (N=94) were PCR positive, 66% (N=282) were IgM positive, and 12% (N=49) were positive for both. Those positive by PCR had 2.2 days of illness vs. 8.1 days if positive by IgM (p<0.001). No DENV was detected by PCR in any sample. Confirmed CHIKV cases (aged 0.1-89 years) were more likely to present with fever, rash, joint swelling, joint pain, and chills (p<0.05) than those who tested negative. Grenada suffered an explosive CHIKV outbreak in 2014. IgM testing detected 78% of test positive patients while PCR detected only 34%. Fever, rash, joint swelling, joint pain, and chills were associated with CHIKV disease. Hemorrhagic signs were rare.

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SEROPREVALENCE STUDY TO IDENTIFY AGE OF GROUPS THAT ARE MOST AT RISK OF DENGUE INFECTION IN PIEDECUESTA, AN ENDEMIC MUNICIPALITY OF COLOMBIA

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Dengue has become an important public health problem because of its increased burden and geographic expansion over the last years. Taking into account that a vaccine may become available shortly, it is critical to characterize the transmission dynamics of the virus at local scales in order to design appropriate vaccination policies. The purpose of this study was to estimate the age-specific dengue seroprevalence in the population of

Piedecuesta, Santander, Colombia, and to explore risk factors for infection. Between July and October 2014, we conducted a household based cross-sectional survey among 1037 individuals aged 2 to 40 years living in 40 randomly selected locations in urban Piedecuesta. In addition, we also enrolled 246 indviduals living in rural "veredas". Participants were asked to answer a questionnaire that included demographic, socioeconomic and environmental questions and to provide a 5ml blood sample. Sera were tested using the IgG indirect ELISA (Panbio) kit to measure past infection by dengue. The average age of participants was 20.73 years (CI 95%=20.12; 21.33). The overall dengue seroprevalence was 69.8% (CI 95%= 67.43122; 72.45949), but was significantly higher in urban (81%) as compared to rural (22.3%) locations. Age was a major predictor of seropositivity, consistent with endemic circulation of the virus. We estimate that on average, 11% (95%CI 8%-16%) of susceptible individuals are infected by dengue each year. Preliminary analyses also suggest that health insurance, occupation and migration are risk factors for infection in both rural and urban settings. Additional analyses will explore other factors associated with seropositivity. These results show that Piedecuesta is an endemic area of DENV transmission, with large heterogeneities between urban and rural settings. Findings from this study will inform the design of a prospective cohort study among children between 2 and 15 years of age. Enrollment for this study is expected to be completed by late 2015, and follow-up will continue for 2.5 years.

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CLINICAL COMPARISON OF RETINOPATHY-NEGATIVE AND RETINOPATHY-POSITIVE CEREBRAL MALARIA

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Cerebral malaria (CM), defined as coma in an individual with ***Plasmodium falciparum*** on blood smear and no other defined cause for coma, is the most severe and lethal complication of ***P. falciparum*** malaria. A classic malaria retinopathy is seen in some (retinopathy-positive, RP) children but not others (retinopathy-negative, RN), and is associated with increased parasite sequestration. It is unclear whether RN CM is a severe non-malarial illness with incidental parasitemia or a less severe form of the same malarial illness as RP CM. Understanding the clinical differences between RP and RN CM may help shed light on the pathophysiology of malarial retinopathy. We compared clinical history, physical exam, laboratory findings, and outcomes of RP (n=170) and RN (n=91) children admitted to Mulago Hospital, Kampala, Uganda. Compared to RN children, RP children presented with a longer history of illness, as evidenced by longer duration of coma before presentation (p=0.021), time since first convulsion (p=0.047), and time since last meal (p<0.001). In addition, RP children presented with physical exam and laboratory findings indicative of more severe disease and organ damage when compared to RN children, including higher levels of respiratory distress, pallor, and jaundice; elevated creatinine, BUN, and BUN/Cr ratios; and lower hemoglobin levels. The hospital course of RP children was complicated by longer coma duration (p<0.001) and a greater transfusion burden (p<0.001) than RN children. Mortality did not differ significantly between RP and RN children (14.1% vs 8.8%, p=0.21). RP CM is associated with a longer history of illness and greater evidence of end-organ damage than RN CM. The data suggest that RP and RN CM may reflect the spectrum of illness in CM, and that RN CM could be an earlier, less severe form of disease.

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TREATING CHAGAS DISEASE IN SPAIN: THE EXAMPLE OF A "NON-NEGLECTED" TROPICAL DISEASE IN A NON-ENDEMIC COUNTRY

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Population from endemic areas of ***Trypanosoma cruzi*** represents in Spain around two million people. Thus, Spain is the most affected country of Chagas disease in Europe, and the second globally in terms of infection among migrants (after US). Several estimations launch a figure between 45,000 and 65,000 migrants infected with ***T. cruzi*** living in Spain. Healthcare access was universal for all people in Spain. Nevertheless, since a Royal Decree launched in September 2012, barriers of access to the health system have been enhanced, and many migrants are currently in a situation of vulnerability. Benznidazole (the first-line treatment option for Chagas disease) shortage occurred at the end of 2011 could have made Chagas disease a neglected tropical disease also in developed countries. We describe the process and results of the re-availability of benznidazole in Spain, a non-endemic country of Chagas disease. Database with the requested orders of benznidazole from the Spanish hospitals, managed by Fundación Mundo Sano and the laboratory that imports the drug from Argentina, was analyzed. The drug started to be produced by a different laboratory in Argentina in 2012. Since November 2012, benznidazole is available in Spain as foreign medication, through the Spanish Drug Agency (Agencia Española de Medicamentos y Productos Sanitarios). Fundación Mundo Sano is contributing since then to facilitate the access to the treatment. Globally, 134 healthcare centers in Spain have requested this drug for their patients. In the first 4 months of the re-availability of the drug in Spain, around 900 treatments were given (1,738 bottles of 100mg and 12 of 50mg). From November 2012 to February 2015, around 3,450 treatments were administered to patients with Chagas disease (6,703 bottles of 100mg and 228 u. of 50mg). Fifty-eight percent of the treated patients are living in four regions of Spain: Catalonia, Madrid, Murcia and Valencia. These data show how Spanish healthcare professionals are attending a Neglected Tropical Disease in a non-endemic country, contributing to its visibility.

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INTERRUPTION OF LYMPHATIC FILARIASIS FOLLOWING MASS DRUG ADMINISTRATION IN NORTHEASTERN TANZANIA: TRANSMISSION ASSESSMENT SURVEYS (TAS) IN LUSHOTO AND MUHEZA DISTRICTS, TANGA

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Lymphatic filariasis (LF) is one of the major tropical diseases affecting people living in poverty. It is estimated that LF infects 120 million people in more than 80 countries throughout the tropics and sub-tropics. One third of those infected live in India, one third in Africa and the remainder in South Asia the pacific and the Americas. Mapping was done in 2000 in Lushoto and Muheza and Circulating Filarial Antigen (CFA) prevalence was 10% and 62% respectively. Tanga region has been implementing consecutive Ivermectin Mass Drug Administration (MDA) for Onchocerciasis control since 2000 while Ivermectin and Albendazole MDA for LF have been conducted since 2004 with reported coverage of above 65%. Pre-Transmission Assessment survey conducted in 2012 revealed that, Muheza had CFA prevalence of 1.6% and Lushoto was 0.3% and these results warranted Transmission Assessment Survey.A community-based household survey was conducted in Lushoto per WHO -TAS guideline. A cluster-sampling approach was employed and children aged 6-7 years old were sampled in 78 hamlets. For Muheza district, since the school attendance rate appeared to be above 75%, sampling was done in schools. Children of Grade 1 and 2 were recruited for the study.

A blood sample was collected from each child, from finger-prick using a 100-microlitre capillary tube and tested with ICT. Children found positive for CFA were traced to their home for night (between 2100 and 2300 hours) blood collection for further evidence of filarial infection. Data were immediately entered in a mobile smart phones loaded with a simple survey questionnaire. In Muheza; A total of number of children enrolled for study was 1664, from 33 schools and 13-tested positive for CFA (0.78%). Mf count was done to all 13 cases and non-was found positive. For Lushoto;a total of 1843 children of 6-7 years old children were tested and non tested positive for CFA. These findings warrant both districts of Muheza and Lushoto to stop MDA according to the WHO guidelines as they indicate interruption of Disease transmission.

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LYMPHATIC FILARIASIS MASS DRUG ADMINISTRATION IN SEVEN COMMUNES IN AND AROUND METROPOLITAN PORT-AU-PRINCE, HAITI, MARCH MAY 2014: HAS COVERAGE BEEN ATTAINED?

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Lymphatic Filariasis (LF) is a parasitic infection endemic to Haiti causing lymphedema and hydrocele. The World Health Organization called for LF elimination using at least five yearly rounds of mass drug administration (MDA) using Albendazole and Diethylcarbamazine with >65% coverage of the population at risk. Achieving good coverage in urban settings is challenging. In May 2014, Port-au-Prince (PAP) completed its third MDA and two nearby communes completed their fourth. The study objectives were to assess MDA coverage and knowledge, attitudes and practices (KAP) of the population regarding LF MDA. Five communes inside and two communes outside PAP were selected using a two-stage cluster sample design. Thirty clusters per commune were selected with probability proportional to estimated size with 10 households per cluster. A coverage questionnaire was administered to everyone age ≥2, and a KAP questionnaire was administered to one randomly selected household member age ≥18. Results were weighted according to cluster and household size and analyzed using SAS 9.3. Out of 5145 individuals, 74% (95% CI 72-76) reported having swallowed medication during the 2014 MDA. Coverage was 72% in females and 76% in males (p<.0101). Coverage was highest in the rural communes (Grand Goâve 81%) and Petit Goâve 79%) while Pétionville in the metro area had the lowest (64%). Top reasons for noncompliance were drug safety concerns (20%) and not being present (15%) or aware (9%) of MDA and losing the pills (7%). KAP results revealed that 9% of participants knew LF elimination requires 5 years of MDA. The commonest routes for community notification of MDA were radio, TV, megaphone and health workers. Potentially useful but unexploited messaging routes include community meetings, church meetings and public health officials. Results indicate that except for Pétionville, the 2014 MDA achieved satisfactory coverage in both urban and rural settings. Increased awareness of the duration of MDA campaigns may reduce inconsistent participation. Use of newly identified routes of communication for awareness of the MDA campaigns may assist in improving future compliance.

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HOW LONG WILL IT TAKE TO ERADICATE LYMPHATIC FILARIASIS? A MODEL-BASED ASSESSMENT ON THE IMPACT OF SCALING UP MASS DRUG ADMINISTRATION PROGRAMS

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Lymphatic filariasis (LF) is a neglected tropical disease primarily prevalent in poor populations in 73 countries. LF is caused by ***Wuchereria bancrofti***, ***Brugia malayi*** or B. timori transmitted by a variety of mosquito genera. Infection with the filarial nematodes can damage the lymphatic vessels, leading to lymphedema, hydrocele and elephantiasis. Administration of albendazole with ivermectin or diethylcarbamazine (DEC) has been shown to reduce circulating microfilariae (MF) to levels that cannot sustain transmission. For this reason, LF is one of six diseases considered to be potentially eliminable. Accordingly, in 1997 the World Health Assembly adopted Resolution WHA 50.29, which calls for the elimination of LF as a public health problem, and in 2000, the World Health Organisation (WHO) established the Global Programme to Eliminate Lymphatic Filariasis (GPELF). However, current efforts appear to aim for elimination in some but not all endemic areas. With the 2020 goal of elimination looming, we set out to develop plausible scale up scenarios to reach elimination and eradication. We predict the duration of MDA necessary to reach local elimination for a variety of transmission archetypes using an existing model of LF transmission and consider implications of rapid scale up. We define four scenarios that differ in their geographic coverage and rate of scale up. For each scenario, country specific simulations and calculations were performed that took into account the pre-intervention transmission intensity, different vector genera, drug regimen, previously achieved coverage, previous progress towards elimination, and programmatic delays. Our results indicate that eliminating LF by 2020 is uncertain. If MDA programmes are drastically scaled up and expanded, the final round of MDA could be delivered by 2029. However, if the current rate of scale up is maintained, the final round of MDA may not occur until 2050. While rapid scale up of MDA will decrease the amount of time required to reach eradication, it may also propel the programme towards success, as the risk of failure is likely to increase with increased programme duration.

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MONITORING COVERAGE OF LYMPHATIC FILARIASIS MASS DRUG ADMINISTRATION (MDA) IN TANZANIA

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Successful interruption of the transmission of lymphatic filariasis through mass drug administration (MDA) is dependent on achieving a high rate of annual consumption of preventive chemotherapy (PCT) among at-risk populations. Currently, WHO estimates that 5 years of MDA, achieving 65% coverage can result in disease elimination in most populations. Inadequate coverage rates result in continued disease transmission, and likely additional years of MDA. Thus, the Tanzania Neglected Tropical Diseases Control Program (TZNTDCP) closely monitors MDA coverage. In October 2014 the TZNTDCP carried out an integrated Albendazole and Ivermectin MDA campaign in 16 regions of Tanzania jointly with the national immunization program. A post-MDA coverage survey was conducted in nine randomly selected regions to compare success of campaign styles, validate coverage rates reported by PCT distributors, determine age and gender specific coverage, and determine reasons for noncompliance among community members. Coverage rates ranged from 81% to 52%, with the lowest rate of coverage occurring in Dar es Salaam. Most regions achieved over 65% coverage. Lack of compliance in all regions was primarily associated with absence during the dates of

the MDA, and lack of awareness about the campaign. Coverage rates reported by the survey varied from rates reported by PCT distributors, reflecting both under- and over-reporting. An important limitation for reported coverage is imperfect census data. Further, in Dar es Salaam, PCT distribution centers included transit centers for day laborers, many of whom are not city residents. MDA coverage among different age groups was not statistically significant, however, women were more likely to have received PCT in almost every district in each region. Survey findings provided valuable information that TZNTDCP will use to tailor MDA campaigns in coming years to achieve higher coverage rates in low-performing districts, including intensified pre-campaign education where lack of awareness limited participation, and finding innovative ways to reach people, especially men, who are temporarily absent during campaigns.

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SHRINKING THE LYMPHATIC FILARIASIS MAP: A NEW TOOL TO ASSESS ACTIVE TRANSMISSION OF LF DURING MAPPING

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The Global Program to Eliminate Lymphatic Filariasis (GPELF) recommends mapping to determine if each district, or implementation unit (IU), requires mass drug administration (MDA) based on the presence of active filarial transmission. Current guidelines require that 2 sites be selected per IU and within each site a convenience sample of 100 adults be tested for antigenemia. The presence of 1 or more positives in either site is interpreted as an indicator of potential transmission, prompting MDA at the IU-level. While this strategy has worked well in high-prevalence settings, imperfect diagnostics, the limited geographical representation of just two sites within an IU, and the fact that a single antigenemia positive adult may not provide evidence of disease transmission, have raised concerns about its use in low-prevalence settings. A statistically rigorous re-mapping strategy was designed. Schools are selected by either systematic or cluster sampling, and within each selected school, children (9-14 years old) are sampled systematically. Children are tested for antigenemia, and the number of positive results is compared against a critical value to determine whether the prevalence of LF is likely below a threshold of 2%. It was applied to 14 IUs in Ethiopia and Tanzania that had qualified for MDA during mapping. In 13 IUs, the number of antigen-positive identified in the remapping surveys was below the critical cutoff, suggesting that in these IUs transmission of LF is not ongoing. In one IU, 10 positives were identified, suggesting that there is on-going transmission and MDA should be initiated. Whereas the current guidelines would have recommended MDA in all 14 IUs based on the traditional strategy, the results of the new strategy suggest that only 1 has on-going transmission. In low-prevalence settings where initial mapping results are uncertain, this inexpensive, rigorous and representative re-mapping strategy may help to generate data on LF transmission to guide programmatic decisions, enabling the GPELF to focus resources where they are truly needed to achieve global LF elimination by 2020.

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ASSESSING THE PREVALENCE OF LYMPHATIC FILARIASIS THROUGH SENTINEL AND SPOT CHECK SITES IN TANZANIA

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Lymphatic filariasis (LF) is endemic in all districts in Tanzania putting over 47 million people at risk of infection. The causative agent, ***Wuchereria bancrofti***, is spread by vector mosquitos, particularly anopheles spp. Control efforts have largely focused on interruption of transmission through mass drug administration (MDA) and vector control. Along the coastal belt, initial mapping reported infection levels (circulating filarial antigen, measured by ICT tests) up to 68%. With over 6 rounds of Ivermectin (IVM) and Albendazole (ALB) MDA, infection levels have started to go down. Two communities from each of the 38 implementation units, which have had over five rounds of IVM and ALB MDA, were selected for the assessment. Approximately 300 people aged 5 years and above were selected and tested for circulating filarial antigen (CFA) for Wulchereria Bancrofti using ICT cards. All ICT positives were followed up and night blood was tested for microfilaraemia using a counting chamber technique. Of the 38 districts surveyed, 31 districts had CFA prevalence between 0-1%, and 7 districts had CFA Prevalence of 2.0-3.7%. These results are in line with expectations following six or more rounds of IVM and ALB MDA. Infection levels have been significantly reduced. Districts with higher baseline prevalence (Morogoro and Tanga regions) reported higher than expected CFA level. This may due to higher baseline/pre-intervention LF infection levels, and or sub-optimal MDA coverage in certain areas.

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LYMPHATIC FILARIASIS IN UGANDA: PROGRESS TOWARDS ELIMINATION TARGETS

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Uganda launched its Lymphatic Filariasis (LF) Elimination program in 2002, in line with World Health Assembly resolution of 1997, with a strategy of annual Mass Drug Administration (MDA) to all at-risk populations with ivermectin and albendazole, co-administered. The first MDA covered 2 highly endemic districts in northern and eastern Uganda, with an eligible population of 1 million, achieving ~75% program coverage. Plans to scale up MDA to 8 districts in 2003 did not materialize due to civil war, instability due to cattle rustling in the north and Eastern regions and lack of funds. In 2004, MDA covered 5 districts and then 10 districts in 2005. In 2007, LF joined other vertical MoH Programs in an integrated program under the umbrella of Neglected Tropical Disease Control Program, funded by United States Agency for International Development. As a result of this program, LF mapping was completed by 2008 and MDA scaled up to 38 districts. By 2010, all 54 LF endemic districts were covered under MDA and to date, all endemic districts have had 3 or more MDAs. Maintenance of effective coverage of 65% is still a challenge, especially in the post-conflict districts in the north. Impact assessments indicated dramatic reductions in both antigenemia and microfilariae prevalence. Transmission Assessment Surveys (TAS), based on WHO protocol, commenced in 2012 in 6 districts. In each evaluation unit, at least 1550 individuals, either aged 6-7 years in communities or primary one and two in schools where feasible were examined for circulating filarial antigens (CFA) using Immunochromatographic Test (ICT) cards. CFA positivity ranged from about 0.06% to 0.6%. From 2014 to 2015, TAS surveys were done in 27 districts, where positivity for CFA ranged from 0% to 0.1%, with many districts registering 0% CFA. Current data indicate that LF transmission

in more than 33 districts has been interrupted, and these districts are eligible for stopping MDA. WHO Regional Program Review Group recently approved the stopping of MDA in 16 districts and more districts are under the same consideration. PELF Uganda is optimistic that by or before the year 2020, most of the evaluation units would have achieved elimination target of CFA below 1%.

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SPATIAL CLUSTERING OF FILARIASIS AND RISK FACTORS ANALYSIS IN BENGO PROVINCE, ANGOLA

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The implementation of lymphatic filariasis (LF) and onchocerciasis elimination programmes in areas where ***Loa loa*** is co-endemic is problematic because individuals with heavy ***L. loa*** infections may develop severe adverse events when treated with ivermectin. This has implications for mass drug administration campaigns and alternative strategies may be required. The aim of this study was to determine the distribution of filariasis and associated risk factors in Bengo Province using rapid field survey methods including clinical observations, serology and molecular analysis. RapidEye (5m resolution) satellite data was also employed to provide the foundation for empirical information on vector and parasite populations. A total of 1616 individuals were surveyed in 22 villages across peri-urban and rural areas during August- September 2014. A total ***L. loa*** prevalence of 6.2% using the Rapid Assessment Procedure for loiasis (RAPLOA) was observed, with village prevalence ranging from 0 to 18%. Molecular analysis of blood samples for ***L. loa*** is under analysis with Real Time PCR, and the results will be presented. No positive infection of LF were found by the rapid ICT card or Real Time PCR. However, 13 lymphoedema (0.8%) and 16 hydrocele cases (1.7%) were observed. For onchocerciasis, an overall prevalence of 4.7% was found using the Ov16 ELISA, with village prevalence ranging from 0 to 35%. Regarding intervention use, a low bed net ownership (46.1%) was reported, with only 33.8% sleeping under a bed net the previous night. There was little or no evidence of ivermectin consumption for onchocerciasis. When asked about the main ***L. loa*** vector Chrysops spp. 12.1% of individuals could identify the fly by a photo. Individuals with loiasis were twice as likely, and males with loaisis were four times as likely to recognize the fly, than those without. Filariasis is endemic at different prevalences and spatial patterns throughout the region, and the presence of ***L. loa*** suggests that alternative strategies for elimination are required, which may include scaling up vector control with key information on the best targets directed by local community knowledge.

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"RIVER EPILEPSY" IN DEMOCRATIC REPUBLIC OF THE CONGO? PREVALENCE OF EPILEPSY SYMPTOMS IN A ZONE ENDEMIC FOR ONCHOCERCIASIS, KATANGA PROVINCE, 2015

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Nodding syndrome is a particular type of epilepsy epidemic in some regions of South Sudan and east Africa. Its cause is unknown, but epidemiological data suggest an association with onchocerciasis. Increased levels of epilepsy have been observed in other regions endemic for onchocerciasis, and nodding syndrome may be only one manifestation along a continuum of onchocerciasis-associated neurological syndromes. A surveillance system for epilepsy cases in different onchocerciasis-endemic regions is planned to be rolled out in the Democratic Republic of the Congo (DRC) in 2015. The relationship between prevalence of epilepsy, degree of onchocerciasis endemicity, and ivermectin coverage will be investigated. In April 2015, we conducted a pilot study in the health zone of Kafubu, outside the city of Lubumbashi in Katanga province, DRC. We surveyed 500 households with 2359 people. Reported ivermectin uptake since annual MDA started in 2006 ranged between 26 and 46%. There were 63 (2.7%) cases consistent with epilepsy (reporting either a previous diagnosis of epilepsy, or 2 or more episodes of loss of consciousness with or without incontinence or drooling; absence seizures; convulsions; or sudden-onset, brief auditory, visual, or olfactory hallucinations). Mean age of these cases was 16.9 years, and mean age of onset was 6.4 years; 54% were female. Four of the 63 cases consistent with epilepsy also reported nodules consistent with onchocerciasis. The results of this pilot study indicate that the prevalence of reported epilepsy in this onchocerciasis endemic region in the DRC is well above the WHO average prevalence estimate of 4-10 per 1000, and also suggest the need to strengthen ivermectin distribution programs and epilepsy care in this region. Determining how much of this high prevalence of epilepsy is due to "river epilepsy" -- epilepsy caused by onchocerciasis -- will require laboratory testing and the exclusion of cysticercosis and other potential causes.

1070

AN IMPLEMENTABLE AND SUSTAINABLE QUALITY ASSURANCE PROGRAM FOR OV16 SEROLOGY-BASED RAPID TESTS

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The availability of an Ov16 serology-based rapid test has highlighted important issues around uptake and appropriate use. Confidence in the testing process, including test quality, performance, and accurate results, are important factors to consider when national onchocerciasis control and elimination programs (NOCPs) use test results for action. A quality assurance (QA) program will help to assure NOCPs that the tests are functioning as intended, results are of high quality, and additionally provide an opportunity for feedback to the test manufacturer. Six modules have been developed to support a QA program: 1. Operator training to familiarize those involved in the testing process with the proper use of the test; 2. Operator proficiency to qualify trained individuals to conduct the test; 3. Quality assessment to demonstrate performance with country-specific samples, as necessary; 4. Lot testing to confirm that the tests perform as intended when received in-country from the manufacturer; 5. Quality control to increase confidence that the test system is working as

intended at the point of use; and 6. Post-market surveillance to monitor the performance of the product during routine use. The QA program is intended to be utilized by and implemented completely or by module with NOCPs in conjunction with test manufacturers and other key international stakeholders. Early adopters of Ov16 serology-based rapid testing have piloted modules of the QA program. Test users were trained by a variety of mechanisms including in-person training, remote training by teleconferences and/or video conferences, and QA program material review. Feedback from QA program implementers has indicated that the methods are acceptable. The feedback is also used to iteratively improve the QA program and will seed future implementation. Implementation of the QA program or modules by NOCPs should be simultaneous with the adoption of Ov16 rapid testing. The elements and reagents required for such a QA program are presented here. Stakeholder support for the QA program and reliable availability of the sample panels and supplemental materials are needed to make it sustainable.

1071

SCREENING CASCADE FOR DISCOVERY PROGRAMS AIMING AT THE IDENTIFICATION OF NEW MACROFILARICIDE AGENTS FOR TREATMENT OF ONCHOCERCIASIS

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Existing medicines to treat filarial infections primarily kill microfilaria and temporarily sterilize adult nematodes. There remains an urgent need to design and develop modern anti-filarial treatments that are cidal to adult worms (macrofilaricides). Recently, a small pipeline of new drug candidates has been created by PDPs, academic groups and pharmaceutical companies. Given the attrition rate in drug discovery and development, it is important to strengthen the pipeline by adding new chemical entities into the drug development pathway. To identify compounds with such potential, DNDi has accessed commercial libraries and worked with companies to access focused libraries from their drug discovery programs. These libraries were evaluated through a screening cascade encompassing three steps: phenotypic screen against Onchocerca spp, determination of ***in vivo*** ADME, and proof of principle testing in murine models using L. sigmodontis. Through this process, several existing drugs and advanced clinical candidates were identified for potential repurposing as antifilarial agents. These include compounds that have activity against several human kinase targets, as well as a set of nitroheterocyclic molecules. This strategy has also been adapted to the screening of pharma collections. A prioritized subset of the AbbVie compound collection was clustered based on structure, and sampled for screening against adult ***Onchocerca gutturosa*** worms. Back-screening of hits, facilitated by cheminformatic analysis, led to a dramatically improved second-round hit rate. Several of the primary hits have demonstrated ***in vivo*** activity. The combination of structure activity relationships and ***in vivo*** proof of principle identified three distinct chemical series. These novel anti-filarial scaffolds will be advanced to hit expansion and lead optimization studies.

1072

IN VITRO ANTIFILARIAL ACTIVITIES AND METABOLOMIC PROFILING OF EXTRACTS OF *DANIELLIA OLIVERI* AND *PSOROSPERMUM FEBRIFUGUM*

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Subcutaneous filariasis (onchocerciasis) caused by ***Onchocerca volvulus***, and lymphatic filariasis caused by ***Brugia malayi*** are among the neglected tropical diseases predominant in the developing world. These infections cause debilitating pathologies, including blindness, severe skin itching and lymphedema. Currently, the approved drugs for treatment of filariasis; ivermectin, diethylcarbamazine and albendazole, are only microfilaricidal. Effective control is therefore hindered by the lack of macrofilaricides, leaving the adult worms, which may live for over 15 years. Additionally, the repeated use of ivermectin, diethylcarbamazine and albendazole has generated concerns of resistance development. Clearly, there is the need for the development of new drugs that will be effective against the macrofilariae, while more effective microfilracidal compounds are also desirable. In an endeavor to contribute to the search for new antifilarial drugs, we screened extracts of the plants; ***Daniellia oliveri and Psorospermum febrifugum***, for activity against both macrofilariae and microfilariae of ***O. ochengi***, the best known animal model of ***O. volvulus***; and adult ***B. malayi***, using ***in vitro*** quantitative biochemical and motility assays. Extracts of ***D. oliveri*** and ***P. febrifugum*** were effective in killing both ***O. ochengi*** and ***B. malayi*** adult worms, and also ***O. ochengi*** microfilariae. Remarkably, the active extracts of these plants had IC_{50s} as low as 4µg/mL, and the majority of them showed little or no toxicity on N27 rat neuronal cells. Metabolomic profiling of active extracts using GC-MS revealed identifiable peaks for compounds which can be isolated and further tested for enhanced activity. Based on our findings, ***D. oliveri and P. febrifugum*** have potential as sources of lead compounds for the development of novel antifilarial drugs. Presently, we are investigating the effects of our most promising extracts on the motility of ***Caenorhabditis elegans***, a model for parasitic nematodes, using the WormLab system. Results obtained will be combined with mutagenesis studies in ***C. elegans*** to elucidate the mode of action of our filaricidal extracts/compounds.

1073

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE STABILITY OF DRY BLOOD SPOTS: EVALUATION OF ANTIBODY REACTIVITY AND COLOR

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Dried blood spots (DBS) are an inexpensive and robust format to preserve blood specimens for public health evaluations and surveys, including programmatic monitoring and evaluation of neglected tropical diseases. DBS have been demonstrated to preserve antibody reactivity for long periods; however, procedures to determine the specimen quality in DBS are not always available or performed. We conducted a 12-month DBS antibody stability study using a reference control for lymphatic filariasis (LF). DBS cards were split into groups and exposed to 4°C, 25°C or 37°C, and low (<40%) or high (>99%) humidity. DBS were collected biweekly and stored frozen until all samples were analyzed. At the end of 12 months, all DBS were eluted and simultaneously analyzed for IgG antibody responses against 7 antigens: Bm14, Bm33, Wb123 and BmR1 for LF, and ***Streptococcus***, ***E. coli*** toxin, and ***Tetanus*** toxoid. Additionally, coloration of the eluted DBS was quantified using image analysis. DBS kept at 37°C and 27°C under high humidity conditions lost all detectable antibody reactivity by days 56 and 112, respectively. DBS stored at 37°C and 25°C

under low humidity conditions had decreased reactivity at 101 and 154 days, respectively, with total loss of reactivity by 264 and 364 days, respectively. Meanwhile, DBS stored at 4°C had minor losses in reactivity, with greater reductions seen with exposure to high humidity. Color of eluted DBS was measured using colorimetric RGB codes; values closer to 255 in each channel correlated with higher antibody reactivity. This study shows the importance of storage at low temperature conditions and the key role of desiccants when refrigeration is not immediately available, In addition, it illustrates the potential use of color analyses of eluted DBS as a simple surrogate method for specimen quality control.

1074

COMBINATION OF CONVENTIONAL PARASITOLOGICAL DIAGNOSTIC METHODS AND QPCR ASSAYS FOR DETECTING *ONCHOCERCA VOLVULUS* INFECTIONS

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Commonly used methods for diagnosing ***Onchocerca volvulus*** infections (microscopic detection of microfilariae in skin snips and nodule palpation) are insensitive. Improved methods are needed for monitoring and evaluation of onchocerciasis elimination programs and for clinical diagnosis of individual patients. We have developed a sensitive probe-based qPCR assay for detecting ***O. volvulus*** DNA, and this assay was tested with samples collected from an endemic area in eastern Côte d'Ivoire. The new test was evaluated with dried skin snip pairs from 369 subjects and compared to routine skin snip microscopy and nodule palpation results from the same individuals. Onchocerciasis prevalence for these samples by qPCR, skin snip microscopy, and nodule palpation were 56.9%, 26.0%, and 37.9%, respectively. Furthermore, the combination of all three tests produced an infection prevalence of 72.9%, which was significantly higher than the 53.1% detected by microscopy plus nodule palpation without qPCR. The qPCR assay was also compared to conventional PCR: while qPCR did not detect significantly more positive individuals, the limit of detection of the qPCR assay was 100-fold lower than that of conventional PCR. In conclusion, the new qPCR assay could be a useful tool for detecting residual ***O. volvulus*** infections in human populations as prevalence decreases in areas following community-directed treatment with ivermectin.

1075

A NOVEL BIPLEX RAPID TEST FOR THE SIMULTANEOUS DETECTION OF *WUCHERERIA BANCROFTI*- AND *ONCHOCERCA VOLVULUS*-SPECIFIC ANTIBODIES: A NEXT GENERATION INTEGRATED SURVEILLANCE TOOL

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In recent years, there has been a global push for an integrated approach to neglected tropical disease (NTD) control and elimination. In Africa, there is the opportunity to integrate programs for onchocerciasis and lymphatic filariasis (LF) due to similarities regarding surveillance activities, control mechanisms, and programmatic overlap. To facilitate the integrated surveillance activities for onchocerciasis and LF, PATH and NIAID are collaborating to develop a biplex rapid diagnostic test that detects IgG4 antibodies against both the Ov16 antigen, specific for ***Onchocerca volvulus***, and the Wb123 antigen, specific for ***Wuchereria bancrofti***. Concurrent to the 2014 launch of an Ov16-only antibody detection rapid test, a proof-of-concept onchocerciasis/LF biplex test prototype has been developed. Laboratory data using well-characterized clinical specimens indicate the sensitivity and specificity to be 92.6% and 96.0%, respectively for Wb123 and 90.3% and 98.1%, respectively for Ov16, similar to the performance of the Ov16-only test. The technology has been transferred to a commercial manufacturing partner for optimization. Early beta prototypes are undergoing further performance evaluation and stability studies to ensure a field-ready test. Supplemental materials that have been developed include a human monoclonal IgG4 antibody positive control for Wb123 and user-friendly training materials for use by field teams. The test and supplemental materials will be used in product verification and field validation, leading up to activities in support of programmatic use of the test.

1076

IN VIVO FILARIASIS MODEL PREDICTS HIGH DOSE RIFAMPICIN CAN ACHIEVE RAPID ELIMINATION OF WOLBACHIA FROM FILARIAL NEMATODES

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The Anti-Wolbachia (A•WOL) programme aims to find safe macrofilaricides for lymphatic filariasis and onchocerciasis through targeting the Wolbachia bacterial endosymbiont. Doxycycline demonstrates proof of concept to achieve depletion of Wolbachia leading to macrofilaricidal activity when administered for a period of 4-6 weeks. The prolonged period of treatment and exclusion of key target groups (pregnancy and children <8) restricts the widespread scale-up of doxycycline for use in elimination programmes. The A•WOL programme aims to identify new drugs and regimens that can reduce the treatment period to 7 days or less and be used in currently restricted groups. In this study we show that clinically relevant high doses of rifampicin can lead to Wolbachia elimination rates from adult worms that are several times faster than elimination rates achieved by doxycycline. Using systemic dose escalation studies in the A•WOL mouse screening model we show that Rifampicin can achieve >90% Wolbachia elimination in time periods of 7 days or less resulting in marked macrofilaricidal activity at later stages. Using pharmacokinetic/pharmacodynamic (PK/PD) modelling and mouse-human bridging analysis, we show that high doses of rifampicin, which could be safely administered to eligible populations should reduce treatment times to 7 days or less. Clinical trials are planned to test these findings in human lymphatic filariasis and onchocerciasis.

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ESTABLISHMENT OF A DISEASE MODEL OF LYMPHATIC FILARIASIS

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In this study, we sought to establish the small mammal model of lymphatic filariasis (LF) initially developed in the 1980s. Ferrets were infected with 150 L3 stage ***Brugia malayi*** or ***Brugia pahangi*** larvae by subcutaneous injection into the right footpad. Blood was drawn every two weeks for white blood cell differential counts and microfilaria quantification. With both ***B. malayi*** and ***B. pahangi*** microfilaremia began at 12-14 weeks after infection with a mean peak of 85 mf/ml and 300 mf/ml, respectively. Eosinophilia developed at 12 weeks. Animals were euthanized four months post infection and dissections were performed to localize adult worms. The mean number of adults recovered was 13 for ***B. malayi*** and 16 for ***B. pahangi***. 85% of recovered worms were located in the lymphatics draining the injected limb; most of the other worms were found in the lymphatics of the opposite leg. While there were no differences in adult worm numbers between male and female

ferrets, females exhibited greater numbers of microfilariae and less peripheral eosinophilia. Subcutaneous Evans Blue dye (injected into both legs one hour prior to euthanasia) demonstrated expanded networks of tortuous and dilated lymphatic vessels on the side of the initial infection. Motile worms were observed within these vessels. To visualize changes in lymphatic function over time, we developed an 18F-FDG PET/CT lymphography protocol using the Siemens Inveon Multimodality scanner for imaging. With this technique, we are able to assess both lymphatic anatomy and functionality. Preliminary results support those of the Evans Blue studies, demonstrating aberrant anatomy and disrupted lymphatic flow. Histology and immunology studies are also in progress. In conclusion, we have successfully re-established the small mammal disease model of lymphatic filariasis. We expect it will be a robust model for 1) testing the efficacy of new antifilarial medications, 2) ensuring new antifilarial medications do not worsen clinical disease, 3) evaluating novel means of decreasing lymphatic disease morbidity in LF, and 4) understanding the mechanisms by which lymphatic dysfunction occurs in LF.

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TYLOSIN ANALOGS AS ANTI-FILARIAL AGENTS. PART A: MEDICINAL CHEMISTRY OPTIMIZATION AND CANDIDATE SELECTION

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Onchocerciasis and lymphatic filariasis (LF), diseases resulting from filarial nematode infections, together affect nearly 150 million people worldwide, with over 1.5 billion at risk. These diseases create an enormous burden of morbidity and lost productivity, in addition to the social stigma associated with their clinical manifestations. Current treatment options (ivermectin, albendazole, and diethylcarbamazine, alone or in combination) focus on eliminating microfilaria and temporarily sterilizing adult worms, but do not clear the infection. New agents with macrofilaricidal activity are needed. Many filarial nematodes, including those responsible for onchocerciasis and LF, carry an obligate symbiont, the bacterium ***Wolbachia***. Anti-***Wolbachia*** therapy using doxycycline has demonstrated clinical benefit by clearing adult worms; however, the developmental toxicity of this agent and the long treatment courses required for efficacy limit its scale-up for programmatic use. Through a screen of a representative sample of the AbbVie anti-infectives collection, we identified the veterinary antibiotic tylosin A (TylA) as a potent anti-***Wolbachia*** agent. ***In vivo*** testing confirmed that this agent is an effective macrofilaricidal agent; however, the pharmacokinetic properties of TylA make it unsuitable for oral administration. We have prepared a set of over 150 analogs of tylosin, optimizing anti-***Wolbachia*** activity as well as drug exposure upon oral delivery. During the course of these structure-activity studies, potency has increased by over 1,000-fold, while oral drug levels have improved by as much as 40-fold. The optimized analogs A-1535469 and A-1574083 are highly effective in several models of filarial worm infection, meeting Target Product Profile regimens of less than 7 days. Their efficacy and safety profiles make them attractive candidates for therapeutic use, and they are currently being advanced toward clinical evaluation.

1079

FEASIBILITY STUDIES TOWARDS A VIRTUAL SKIN BIOPSY FOR ONCHOCERCIASIS

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The gold standard test for onchocerciasis diagnosis is the skin biopsy procedure, including removal of around 2 mg of skin, incubate in standard saline at rt for 24 hours, and count microfilariae (mf) under light microscopy. Although the skin snip is highly specific, its sensitivity can be limited in case of low microfilaridermia. Skin biopsies are invasive, painful, require sterilization of equipment and examination by experienced microscopists. In this study, we evaluated optical coherent tomography (OCT) as a non-invasive alternative to generate a virtual biopsy in an artificial setting. Onchocerca lienalis mf kept in culture on a monkey kidney feeder cell line were examined in three successive steps, using two visualization techniques: light microscopy (control) and VivoSight multi-beam dermatological OCT (Michelson Diagnostics, UK). First, O. lienalis mf were placed in 20 µL on a Petri dish and imaged, conclusively demonstrating that the mf could be imaged by OCT at the limit of the device's resolving power. Next, O. lienalis mf were placed in 20 µL medium at the surface of a sample of human abdominal skin (4cm x 4cm) collected from surgery (Tissue Solutions, UK). It was found to be impossible to find the mf in-situ using optical microscopy. However, a parasite could be seen in the OCT images and positively identified from its characteristic wriggling motion, illustrating that a parasite could be detected based on motion, but not because of optical contrast against the skin tissue. Finally, mf were injected in the upper dermis (250 - 310 µm under the skin surface). After 30 min at 37°C, the skin sample was imaged with Dynamic OCT. The algorithm successfully picked out the areas in the skin where movement was seen, demonstrating that the mf had migrated from the injection site into the tissue of the dermis. These initial results show promise for the technology to be developed into a practical method for the field diagnosis and quantitation of Onchocerca microfilaria present in a skin tissue in-vivo. The imaging processing could be further enhanced to automatically count the number of individual parasites.

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A VISUAL ISOTHERMAL NUCLEIC ACID AMPLIFICATION ASSAY FOR DIAGNOSIS OF ONCHOCERCIASIS IN HUMANS

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Onchocerciasis or "river blindness" is a parasitic infection caused ***Onchocerca volvulus***. The standard method to diagnose infection relies on the detection of microfilariae in human skin biopsies. This technique lacks sensitivity particularly when samples are collected from drug-treated individuals. Nucleic acid-based tests provide specificity and sensitivity however the currently used assays, such as polymerase chain reaction (PCR), and real-time PCR assays are difficult to implement in low resource settings. Loop-mediated isothermal amplification (LAMP) is a one-step, nucleic acid amplification technique that can be performed under isothermal conditions. Its simple visual detection format, and no need for equipment or post amplification processing, offer considerable advantages over PCR in the field. In the present study a LAMP assay targeting the genus-specific O-150 repeat sequence was developed. Amplification was detected by measuring turbidity through real-time monitoring using a turbidimeter, or visually using either hydroxylnaphthol blue (HNB) or a newly developed pH sensitive dye. The performance of LAMP and PCR

were evaluated using human skin snips spiked with varying concentrations of ***O. volvulus*** genomic DNA or unrelated filarial DNAs. Both PCR and LAMP (regardless of detection method used) assays were highly specific. However, higher levels of sensitivity were achieved in LAMP assays detecting DNA equivalent of less than one parasite within 60 minutes. Of the various LAMP detection methods evaluated, the pH sensitive dye provided easier discrimination between positive and negative results and the color remained stable. In summary, we have developed a rapid and highly specific and sensitive LAMP assay to detect ***O. volvulus*** infection in humans. Colorimetric visualization of amplification using a pH sensitive dye facilitates its use in less equipped laboratories and in rural settings. The test has the potential to be developed further as a field tool to assist in the management of control programs.

1081

MNSODTG MICE EXHIBIT IMPROVED HEART AND MITOCHONDRIAL FUNCTION DURING CHRONIC CHAGAS DISEASE

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We observed that mitochondrial reactive oxygen species (mtROS) plays very important roles in the pregression of chagesic disease. In this study, we utilized genetically-modified mice to scavenge mtROS to investigate the impact of improved ROS scavenging capacity on heart function in Chagas disease. C57BL/6 mice (wild-type, MnSODtg, MnSOD+/-) were infected with ***Trypanosoma cruzi*** (Tc***)***. Chronically infected mice (≥120 days post infection, dpi) exhibited a substantial decrease in heart tissue MnSOD gene expression, MnSOD protein level, MnSOD enzyme activity and antioxidant level; decrease of heart dysfunction via lower of stroke volume (SV), cardiac output (CO), ejection fraction (EF), fractional shorting (FS) and left ventricular posterior wall of systolic, and increase of end-systolic/diatolic volume (ESV/EDS) and left ventricular internal diameter of systole(LVID;s); enhancement of hypertrophy by increase of left ventricular septum (IVS), left ventricular mass (LV mass) and areas duo to augmentation of collagen expressions. One of our novel observations was that sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2) lost its role of maintenance of low cytoplasm free calcium and mediated calcium uptake to intracellular store in Tc-induced chronic chagasic disease. Studies of fresh heart slices using O2K confirmed that Tc diminished heart mitochondrial function like decrease of oxygen flux and respiratory control ratio (RCR), which were caused by enhancements of ROS. Chronically infected MnSODtg mice exhibited a marginal decline in Tc-induced heart function, heart hypertrophy, mitochondrial dysfunction. In conclusion, overexpression of MnSOD inhibited Tc-induced oxidative damage od heart tissue, suggesting that enhancing the mitochondrial ROS scavenging capacity was beneficial in controlling the inflammatory and oxidative pathology, and cardiac remodeling responses that are hallmarks of chronic Chagas disease.

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ENHANCING PGC-1A ACTIVITY IMPROVES HEART FUNCTION THROUGH ACTIVATING MITOCHONDRIAL BIOGENESIS AND INHIBITING INFLAMMATORY PATHWAYS IN CHAGAS DISEASE

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Chronic chagasic cardiomyopathy (CCM) is presented with ventricular hypertrophy and contractile dysfunction that can lead to heart failure. I have found that a substantial decline in mitochondrial biogenesis and SIRT1/PGC-1α activity ensue in chronic chagasic mice. It was evidenced by the decline in mitochondrial DNA content as well as mRNA levels of mitochondrial encoded genes and mtDNA replication machinery. Further, the activity of SIRT1 (required for PGC-1α activation) was decreased and associated with decreased nuclear levels of PGC-1-regulated NRF1 transcription factor in chagasic hearts. The mitochondrial size and number were also reduced in chagasic heart, determined by electron microscopy. Therefore, we hypothesized that enhancing the SIRT1/PGC-1α activity by SIRT1 agonist would improve heart function through activating mitochondrial biogenesis and inhibiting inflammatory pathways in Chagasic disease. Mice were infected with ***Trypanosoma cruzi***, and beginning at day 90 post-infection (pi), treated with resveratrol (SIRT1 agonist) or metformin (AMPK agonist, can enhance SIRT1 activity) for 21 days; and then heart function was monitored at 150 days pi. We found that treatment with resveratrol partially attenuated the heart dysfunction (stroke volume, cardiac output, ejection fraction, heart rate) and cardiac hypertrophy in chagasic mice. These benefits were associated with improved expression of the mitochondrial DNA encoded genes and mtDNA content though the expression of genes involved in mtDNA replication was not improved. Treatment with metformin was not significantly beneficial in improving the CCM outcomes. The partial beneficial effects of resveratrol could be due to inefficient activation of SIRT1 or delayed start of the treatment. We plan to treat mice with SIRT1 agonist SIRT1720 (10 fold more active than resveratrol) during the indeterminate phase of ***T. cruzi*** infection in next set of experiments. This study will improve our understanding of the molecular and immune mechanisms of chagasic heart disease and will provide a novel treatment for chronically-infected chagasic patients.

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HISTOPATHOLOGICAL AND ULTRASTRUCTURAL FINDINGS IN SKIN BIOPSIES OF PATIENTS WITH EARLY AND LATE CUTANEOUS LEISHMANIASIS INDUZED BY *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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Cutaneous leishmaniasis (CL) is the most frequent clinical form of leishmaniasis, which affects the skin and is an important health problem in Brazil. ***Leishmania (V.) braziliensis*** infection induces a large spectrum of lesions that clinically may manifest as a single skin lesion localized, generally in uncovered parts of the body, initialized with a papule until the development of an ulcer or which may regress spontaneously. Patients with early cutaneous leishmaniasis (ECL) have 46% failure to standard treatment, while the cure rate for patients with late cutaneous leishmaniasis (LCL) is until 90%. We aim to investigate the cellular inflammatory aspects in situ biopsies of human infection by ***L. braziliensis*** in ECL while papule comparing them with LCL. We describe tissue cells from inflammatory infiltrate different stages of infection and feature the immune response based on histophatological and ultrastructural analysis, as well the total extent of inflammation and cellular profile, changes in the epidermis and dermis, amount and quality of amastigotes. Hyperkeratosis is more frequent in ECL while giant cells were observed during the classical ulcer. Macrophages and lymphocytes were the predominant cells in the ulcers of localized CL and the inflammation increased with diseases evolution. By other hand, the quantity of amastigotes decreased with the time of disease. Some parasites righteous were viewed by transmission electron microscopy. Although the attempt of the macrophages to kill some Leishmania in the earlystage, we also observed the development strong inflammatory response with extensive cellular infiltrate and other histological changes that may be involved with pathogenesis and progress lesion in CL, also may contribute to treatment failure.

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TRYPANOSOMA CRUZI REDOX NETWORK AS A VIRULENCE FACTOR: ROLE OF TRYPAREDOXIN PEROXIDASE IN THE PATHOGENESIS OF CHAGAS DISEASE

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The aim of our study is to gain insight the involvement of ***Trypanosoma cruzi*** redox system in the pathogenic mechanism of Chagas disease. Many groups have attributed to this pathway a role in the evasion of the host innate immune response. Previously, we described the up regulation of enzymes of this network, particularly tryparedoxine peroxidase (TXNPx), in trypomastigotes of DTUI isolates. In order to characterize its participation in the cellular response to infection, we employed a site-directed mutagenesis approach to construct the active site mutants of cytosolic (CPX^{C52S}, CPX^{C173S}) and mitochondrial (MPX^{C81S}, MPX^{C204S}) isoforms of TXNPx. The pRibotex plasmid encoding the cDNAs for mutant TXNPx isoforms was electroporated into Sylvio X10/4 and transfectants were selected under drug pressure (250-400µg/ml G418). Overexpression of the mutant proteins and the wild type protein in the transfectants was confirmed by western blotting with specific antibodies against CPX and MPX isoforms. Also, the differential peroxidase activity of the recombinant parasites was detected with the Peroxidase Assay Kit (Invitrogen). Evaluation of viability by Alamar Blue®, revealed that ***T. cruzi*** expressing mutant isoforms of CPX and MPX were significantly more sensitive ($p<0.05$) to oxidizing species, e.g. H_2O_2 and $ONOO^{\bullet-}$, when compared to the wild type parasites or ***T. cruzi*** transfected with CPX wild type isoform. ***In vitro*** infection studies in RAW macrophages showed that intracellular replication of $Syl.MPX^{C81S}$ and $Syl.CPX^{C173S}$ determined by qPCR was decreased, compared to that noted for SylWT and Syl.CPX ($p<0.01$). This capability was associated with their inability to prevent intracellular ROS accumulation (measured by H_2DCF-DA probe) and oxidants released into the culture media (Amplex red and Griess assays). In summary, we demonstrate that via TXNPx decomposition of macrophage derived free radicals, ***T. cruzi*** ensures its survival and persistence in the host. Further characterization of the mutants' biological behavior by murine infection is ongoing, and these data will be presented in further support of TXNPx role in the pathogenesis of Chagas disease.

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DIVERSITY OF *TRYPANOSOMA CRUZI* INFECTION IN PATIENTS CO-INFECTED WITH HIV AND CHAGAS DISEASE

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Chagas disease, caused by the protozoan ***Trypanosoma cruzi***, can cause severe neurological syndromes in immunocompromised patients such as those infected with HIV. We characterized the genetic diversity of ***T. cruzi*** infection in several HIV-infected Bolivian patients with high-level parasitemia. Samples were derived from HIV-positive patients recruited into epidemiological studies of HIV/Chagas disease coinfection in Cochabamba and Santa Cruz, Bolivia. DNA was extracted from whole blood or guanidine-preserved samples, and ***T. cruzi*** infection was confirmed with RT-PCR. We used high-fidelity nested PCR to amplify a 327-base pair fragment of the TcSC5D gene, which has been used for strain typing, and amplicon deep sequenced the region using an Ion Torrent PGM. We determined multiplicity of infection and genotyped the strains of ***T. cruzi*** using deep sequencing and conventional restriction fragment length polymorphism (RFLP) methods. Sequences were clustered to predict genotypes using a heuristic clustering algorithm. Within-host and within-population diversity indices were calculated using EstimateS and/or QIIME. We have shown that deep sequencing of ***T. cruzi*** from clinical samples is possible and explored the diversity of ***T. cruzi*** infections in HIV-infected patients, which may have implications for the pathogenesis of the disease in this population. Future studies will examine parasite diversity in a larger sample size and compare ***T. cruzi*** strains found in blood and cerebrospinal fluid.

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CEREBRAL VASCULATURE AND COGNITIVE IMPAIRMENT IN ACUTE *TRYPANOSOMA CRUZI* INFECTION

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The brain is sheathed with a blood-brain barrier (BBB) system, which tightly regulates the passage of substances from the blood to the Central Nervous System. Certain organisms, however, including ***Trypanosoma cruzi***, are able breach this selectively permeable unit. Damage to the cerebral vasculature and the BBB are important features tied to neurological impairments in Chagas disease (CD). Endothelin-1 (ET-1) has been shown to mediate BBB permeability, inflammation, and vascular tone, thus may be important in the pathogenesis of ***T. cruzi*** infection. ET-1 reduces the expression of angiopoietin-1 (Ang-1), a growth factor that promotes endothelial quiescence and expression of tight junction proteins responsible for maintaining BBB integrity. We postulate that ET-1 contributes to the pathogenesis of neuro-CD by disrupting BBB integrity and potentiating endothelial activation and inflammation. C57BL/6 mice were infected with 10,000 trypomastigotes of the Tulahuen strain of ***T. cruzi*** to induce experimental CD (ECD). Cognitive function, degree of illness, and levels of inflammatory mediators were assessed in the brains of ***T. cruzi*** infected mice and controls. Acute ECD caused an increase in parasitemia, which correlated with blood glucose as well as the level of illness as determined by a decrease in rapid murine coma and behavior scale (RMCBS). Object recognition and placement tests revealed significant impairments in the visual and spatial memory of infected mice. These deficits were associated with elevated levels of pro-inflammatory cytokine IL-1β and cell adhesion molecules, E-selectin and ICAM-1. Ang-1 has been shown to suppress these molecules on endothelial cells, whereas Ang-2 promotes their expression. qRT-PCR performed on whole brain homogenates revealed elevated levels of ET-1 in the brains of ***T. cruzi*** infected mice in conjunction with decreased Ang-1, resulting in an increased ratio of Ang-2 to Ang-1. An imbalance in the angiopoietin system illustrates endothelial dysfunction and cerebral vasculature disruption, which may contribute to the neurological impairments and activated endothelium observed in ECD.

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INHIBITORS OF NA+, K+-ATPASE AND RELATED ION ANTI-PORTER FUNCTIONS AS NEW ANTILEISHMANIAL DRUG LEADS

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Visceral leishmaniasis, caused by protozoan parasite ***Leishmania donovani,*** is fatal if left untreated. Extreme toxicity, increasing resistance of the parasite to currently available drugs and absence of a suitable vaccine necessitate the discovery of new anti-leishmanial drugs. The Na^+-K^+-ATPases and related anti-porter functions may be necessary for survival of the leishmania parasite in extreme acidic environment of phagolysosomal vacuoles in macrophages. A P-type ion-motive ATPase, with predicted function as a potassium and/or sodium efflux pump, has been identified in ***Leishmania donovani*** genome. Random screening of a library of natural products has indicated Na^+,K^+-ATPase or related ion transports as potential antileishmanial drug targets. To validate this, a few selected standard inhibitors namely, N-ethylmaleimide, omeprazole, ouabain, amiloride, bufalin, quercetin, oleandrine, digoxin, mansonin, sanguinarine, cinobufagin and procillaridin A, known to inhibit Na^+,K^+-ATPases or proton pumps, were evaluated ***in vitro*** against different forms of ***L. donovani*** namely, promastigotes, axenic amastigotes and intracellular amastigotes growing in differentiated THP1 human acute monocytes cells. The inhibitors were also tested for cytotoxicity against differentiated THP1 cells. Except omeprazole and amiloride, all inhibitors showed highly potent and selective anti-leishmanial effect on intracellular amastigotes, with no significant effect on promastigotes and axenic amastigotes. Bufalin, a cardiotonic steroid was the most active inhibitor with IC_{50} value in sub-nano molar range. This selective action of inhibitors indicate essential role of Na^+,K^+-ATPase and related proton transporters in survival of intracellular ***L. donovani*** amastigotes. Further analysis of selective susceptibility of intracellular amastigotes, molecular/functional characteristics of leishmanial Na+,K+/H+-ATPase and their role in leishmania growth and survival would be useful in identification of selective leads, devoid of cardiotonic functions as potential novel antileishmanial drug leads.

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LEISHMANIA SPECIES IDENTIFICATION BY HIGH RESOLUTION MELTING ANALYSIS (HRMA) IN PERU

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Leishmaniasis is a disease characterized by the presence of different clinical manifestations depending on ***Leishmania*** species. Our aim was develop of a High Resolution Melting Analysis (HRMA) for the identification of ***Leishmania*** species, amplifying a region of minicircles of kDNA which presents polymorphism. Two hundred and thirteen DNA samples from skin biopsy of the lesion from patients confirmed with the disease and the following reference strains: ***Leishmania (Viannia) braziliensis, L. (V.) peruviana, L. (V.) guyanensis, L. (Leishmania) amazonensis and L. (L.) mexicana*** were evaluated. 10 ng/ul of DNA and the following primers: OL1: GGGGAGGGGCGTTCTGCGAA and OL2: CCGCCCCTATTTTACACCAACCCC were used in a Real-time PCR protocol using 1X of Eva Green dye in a Rotor Gene Q thermocycler (Qiagen®). The thermal cycling profile was: Initial denaturation at 95°C by 5 min followed by 35 cycles of denaturation at 95°C x 10s, annealing at 55°C x 30s and an extension at 72°C x 10s. Finally, the temperature was increased 1°C between 75 to 90°C. The HRMA analysis was done using Rotor Gene Q Software v2.1.0. This method allowed identifies five different pattern curves corresponding to each one of the reference Leishmania strains. The samples evaluated were identified as ***L. braziliensis*** (52), ***L. peruviana*** (59), ***L. guyanensis*** (54), ***L. amazonensis*** (29), ***L. lainsoni*** (15) and ***L. mexicana*** (4). Twenty six samples (12%) were confirmed by partial sequencing of cytochrome B gene using GeneBank/DDBJ/EMBL databases. Of these samples, we found four discordances between HRMA and sequencing of Cyt B gene: Two strain of ***L. braziliensis*** which were identified previously as ***L. guyanensis***; one strain of ***L. amazonensis*** and ***L. guyanensis***, were identified both as ***L. (V.) braziliensis*** by HRMA respectively. It is necessary the evaluation of this HRMA with a greater number of samples to determine the real power of this technique as an identification method of ***Leishmania*** species.

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IMMUNO-ENZIMATIC EVALUATION OF THE TCTASV PROTEIN FAMILY OF *TRYPANOSOMA CRUZI* AS A TOOL TO DIAGNOSE NATURAL INFECTION OF DOGS FROM ENDEMIC AREAS

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Chagas disease, produced by ***Trypanosoma cruzi***, affects millions of people worldwide. High incidences of human infection and vector transmission are still reported in endemic regions like the Gran Chaco, due to partial success of triatomine control programs. In this area, dogs represent important reservoirs of the parasite in domestic environments. Canine infection correlates with the risk of transmission to humans, therefore, proper methods to detect the parasite in dogs are needed. The novel TcTASV protein family (subfamilies A, B, C) has no orthologs in other species and is differentially expressed in bloodstream trypomastigotes, suggesting that these antigens could be useful to diagnose ***T. cruzi*** infection. The aim of this study was to assess the antibody response to TcTASV-A, B and C proteins in dogs naturally infected with ***T. cruzi*** using ELISA. We produced a representative member of each subfamily (Ags: A, B and C) with a GST-tag, and GST. Three groups of sera were evaluated. GI: 30 healthy dogs from non-endemic area, with negative ELISA-epimastigote protein extract (ELISA-HT); GII: 35 infected dogs from Chaco, ELISA-HT positive; and GIII: 35 infected dogs with positive ELISA-HT that also had ***T. cruzi*** detection by xenodiagnoses and/or PCR. Each sample was tested in duplicate against TcTASV antigens individually or combined and against GST (background control). Sensitivity (Se), Specificity (Sp), AUC ROC and Kappa Index (KI) were estimated for all assays, considering GI and GIII as controls. A-ELISA and C-ELISA, obtained the highest sensitivities: 65.7% and 74.3%, with 0.83 and 0.86 AUC ROC, respectively, indicating high accuracy of these tests. All assays were 100% Sp, except for B-ELISA (50%) that also had a poor reactivity in GII and GIII sera. The ELISA with the A+C mixture obtained 94.3% Se and 0.99 AUC ROC, proving that the combined use of these antigens has desirable diagnostic performance that could be useful to detect the infection in dogs in endemic regions. The mean OD in GIII samples was significantly higher than in GII samples, in C-ELISA and A+C-ELISA, suggesting that TcTASV-C antigen could have a role in the acute phase of infection.

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QUANTIFICATION OF MACROPHAGE MARKERS NEOPTERIN AND CHITOTRIOSIDASE ACTIVITY TO MONITOR VISCERAL LEISHMANIASIS TREATMENT RESPONSE

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As a 6 month follow-up is required to establish final cure, pharmacodynamic (PD) biomarkers are potentially useful to monitor treatment response in visceral leishmaniasis (VL), but have not been identified yet. ***Leishmania*** parasites replicate within host macrophages, thereby increasing the overall macrophage biomass, decreasing again with waning parasitic infection. Neopterin and chitotriosidase activity are markers of macrophage activation and their potential use as PD markers in VL was evaluated in this analysis. Plasma samples were collected from VL patients in Sudan and Kenya, receiving 2 different treatments: (i) combination therapy (liposomal amphotericin B, L-AmB + miltefosine) or (ii) miltefosine monotherapy. Neopterin was quantified by ELISA (133 samples, 31 patients) and chitotriosidase activity by an enzymatic fluorescent assay (116 samples, 29 patients), during and after treatment. All values are reported as mean ±95% CI. Baseline neopterin levels were elevated (90.6±11.4 nmol/L) compared to normal (<10 nmol/L) and decreased during treatment to 35.9±13.1 nmol/L for combination and 23.3±8.05 nmol/L for monotherapy. On day 7, neopterin concentrations halved for the combination therapy, while for monotherapy they only started to decrease after day 7. Mean neopterin concentrations one day after L-AmB infusion were significantly higher for cured (124±30.1 nmol/L) than for relapsed patients (79.3±14.3 nmol/L, P<0.01), indicating neopterin levels exhibit a prognostic value for the combination therapy. The initial surge in neopterin in cured patients, could imply an instant immunomodulatory effect of L-AmB. The baseline mean chitotriosidase activity was 317±169 nmol/mL/hr, with large between-subject variability, and decreased in response to both treatments. 1-month follow-up activity increased significantly in relapsing patients (n=10), while cured patients (n=9) retained stable levels (P<0.001), compared to end of treatment. However, a chitotriosidase deficiency-rate of 26.3% was found in Kenyan patients (~5% in Caucasians,) which causes chitotriosidase activity to lack sensitivity as a biomarker.

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EFFECTIVENESS OF ORAL PENTOXIFYLLINE PLUS SODIUM STIBOGLUCONATE THERAPY VERSUS SODIUM STIBOGLUCONATE MONOTHERAPY IN MUCOSAL LEISHMANIASIS: A RETROSPECTIVE COHORT

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Treatment failure in mucosal leishmaniasis occurs in 7-30% of patients treated with antimonials. Pentoxifylline is a xanthine oxidase inhibitor with anti-inflammatory effects that if it is used in addition to antimonials may improve the effectiveness of mucosal leishmaniasis therapy. The aim of the study was to determine the effectiveness of sodium stibogluconate therapy plus oral pentoxifylline compared to sodium stibogluconate monotherapy in mucosal leishmaniasis. Data was collected retrospectively from clinical records of consecutive patients treated and with complete follow-up at a reference center in Lima, Peru. Sodium stibogluconate was administered intravenously at 20mg per kg of body weight per day and pentoxifylline was administered orally at 400 mg 3 times daily. Effectiveness was defined as clinical cure with complete reepithelization of the mucosal tissue and absence of inflammatory activity 180 days after 30 days of treatment. Treatment assignment depended on pentoxifylline availability without random assignment. A retrospective cohort analysis was conducted calculating crude and adjusted relative risks (RR) of treatment effectiveness using a robust Poisson regression. A total of 234 eligible patients were enrolled and 205 (90%) were evaluated at six months. Combined therapy and monotherapy groups were only different by sex (proportion male: 78% versus 91%, p=0.022). Effectiveness of monotherapy and pentoxifylline combined therapy was 61% (85/139) and 79% (52/66), respectively (p=0.011). The difference remained significant (RR: 1.34, 95% confidence interval: 1.10-1.56, p=0.002) after adjusting for severity and sex. Adjusting for therapy, better effectiveness was observed when the oral mucosal was also affected compared to nasal tissue only, without a significant interaction between therapy and oral tissue involvement. In conclusion combined therapy of sodium stibogluconate plus oral pentoxifylline is more effective than sodium stibogluconate monotherapy for mucosal leishmaniasis. Randomized, experimental trials may give additional validity to this finding.

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BACTERIAL CELLULOSE BIO-CURATIVES CONTAINING DIETHYLDITHIOCARBAMATE (DETC) FOR THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA BRAZILIENSIS*

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Leishmaniasis remains a worldwide public health problem. The limited therapeutic options, drug toxicity and reports of resistance reinforce the need for the development of new treatment options. Herein, we tested a topical formulation of bacterial cellulose (BC) membranes containing Diethyldithiocarbamate (DETC), a superoxide dismutase 1 inhibitor. Exposure of leishmania-infected murine macrophages to BC-DETC resulted in a dose-dependent killing of intracellular parasites, without pronounced toxic effects to host cells. Parasite killing was associated with decreased SOD1 activity paralleled by the increased production of superoxide and pro-inflammatory mediators. Topical application of BC-DETC to dermal lesions significantly decreased ear thickness and parasite load at the infection site. Additionally, expression of IFN-γ and TNF-α, was down modulated in situ as well as in recall responses employing draining lymph node cells. BC-DETC also decreased parasite load following exposure to human macrophages infected with ***Leishmania braziliensis***, an effect reversed in the presence of anti-oxidants. These results highlight the feasibility of using BC-DETC as a topical formulation for chemotherapy of cutaneous leishmaniasis caused by ***L. braziliensis***.

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REPURPOSING OF FINGOLIMOD FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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Human African trypanosomiasis (HAT) is a parasitic disease caused by the protozoan parasites ***Trypanosoma brucei rhodesiense*** and ***T.b. gambiense***. Currently prevalent in 36 sub-Saharan countries the disease inflicts a significant degree of morbidity and mortality accounting for the loss of over 560,000 disability adjusted life years each year. An estimated 20,000 cases of HAT occur annually, nearly all of which are fatal without adequate treatment. However, treatment of HAT is currently reliant on a small number of drugs nearly all of which have poor safety profiles and complicated, prolonged administration schedules. New drugs are desperately needed for HAT. The high-throughput screening of an inhouse compound library containing licensed drugs and compounds that had reached the late stages of clinical development led to the identification

of Fingolimod (Glienya®) as a promising lead compound for HAT. Fingolimod is an orally available drug currently licensed for the treatment of relapsing multiple sclerosis (MS). The compound exhibited an IC_{50} of 0.59 µM against ***T.b. brucei*** with a selectivity index (SI) of 17 against the mammalian cell line HEK293. Importantly the compound also displayed potent activity against the human infective subspecies, ***T.b. rhodesiense*** and ***T.b. gambiense*** with IC_{50} values of 0.69 and 0.021 µM, respectively. Fingolimod is cidal in action with ***T.b. brucei*** parasites being eliminated within 48 hours following exposure to the minimum inhibitory 90 (MI90) concentration. Disappointingly, Fingolimod exhibited no in-vivo activity in acute or CNS murine models of HAT. These poor in-vivo results led to the evaluation of commercially available analogues of Fingolimod and the initiation of a medicinal chemistry program in order to produce analogues of Fingolimod with in-vivo trypanocidal activity. In this presentation the identification of Fingolimod is discussed along with the results obtained to date against ***T. brucei*** spp and the steps taken to optimise and progress the drug along the drug discovery pipeline for HAT.

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USING A CONSENSUS TECHNIQUE TO IMPROVE THE METHODOLOGY OF CLINICAL TRIALS ASSESSING TREATMENTS FOR CUTANEOUS LEISHMANIASIS (CL)

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The term Cutaneous Leishmaniasis (CL) includes a variety of disease manifestations caused by ***Leishmania*** species in the old and the new world. Despite an estimated 0.7-1.3 million new cases each year, CL is not life-threatening and is not assigned a high burden of disease; however, it causes visible, generally long-lasting lesions leaving potentially life-long scars. There are few treatment options, and recommendations have a weak evidence base: systematic published reviews pointed to a lack of methodological standardization in the conduct and analysis of clinical trials of CL interventions. In order for both current and newer treatments to be adequately assessed, standardised trial methodologies are needed which can be applied generally, while allowing the flexibility required to cover the diverse disease manifestations. This would provide clinical investigators with guidance for the design, conduct, analysis and report of future clinical trials of CL treatments, including the definition of measurable, reproducible and clinically meaningful outcomes. A guidance document is available. To further broaden the base for consensus, we involve a range of stakeholders (researchers, care-providers (physicians, nurses) and patients) to define key methodological questions regarding whom to treat and how to measure treatment effects (core eligibility criteria, core outcome measures). An iterative online Delphi-type consensus methodology is used. A call for expression of interest in February 2015 was answered by around 120 researchers and care-givers. Additional input is being sought from patients via interviews (56 applications received; selected candidates will also receive specific training in patient interviews). CL is used as a case-study to assess whether the key stakeholders can be identified and encouraged to collaborate using an innovative, participatory approach. The project is in progress; results from the first two Delphi rounds will be presented.

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DETECTING *TRYPANOSOMA CRUZI* ANTIGENS WITH NANOPARTICLES

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Chagas disease, caused by the protozoan parasite ***Trypanosoma cruzi***, is one of the major health problems affecting the Latin American population. The World Health Organization estimates that approximately 16 to 18 million people are chronically infected, and 120 million are at risk of contracting the disease. Currently there is no gold standard test for the diagnosis of Chagas disease, and immunological diagnosis is based on the detection of anti-***T. cruzi*** antibodies. However, these tests have poor specificity and have been known to cross react with other endemic diseases. Moreover, the efficacy varies depending on the genetic makeup of both the parasite and the human population studied, and even despite successful treatment and parasite eradication, the immunologic tests may remain positive. Alternatively, antigen detection tests may provide a solution to these issues, as they are able to directly detect the presence of the parasite in a sample. This work aims to develop a screening test for the detection of ***T. cruzi*** antigens. For this purpose we have developed specific antibodies against a panel of ***T. cruzi*** antigens (membrane, flagella, and excretory/secretory proteins), from rabbits, chickens and alpacas. The purified antibodies have been evaluated by Western blot, and have shown different banding patterns, even when presented with the exact same antigen. These antibodies will next be tested in an ELISA format. We plan to perform a pilot study using nanoparticles in an ELISA platform to increase the conventional assay's sensitivity in samples from chronic Chagas disease patients. One goal of this work is to determine the potential of this antigen detection test to be used as a rapid diagnostic test (RDT) that can be transferred to laboratories in endemic areas as a way to reliably detect and thereby treat Chagas disease.

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AN IMAGE-BASED, HIGH THROUGHPUT ASSAY FOR PROFILING THE ACTIVITY OF SELECTIVE INHIBITORS AGAINST *TRYPANOSOMA CRUZI* FROM THE MMV MALARIA BOX COLLECTION

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Treatment of Chagas disease caused by ***Trypanosoma cruzi*** is limited to drugs with known side effects and questionable efficacy against the chronic phase of the disease. The search for more effective, less toxic drugs is imperative for improved treatment of ***T. cruzi*** infection. To identify new compounds with anti-***T. cruzi*** activity, a high throughput, image-based assay was developed to visualise the effect of compounds against the intracellular amastigote life cycle stage. A prototype of the Medicines for Malaria Venture (MMV) Malaria Box containing 641 molecules, with previously demonstrated activity against ***Plasmodium falciparum***, was screened against ***T. cruzi***. The activity of compounds against the parasite and the host cells were estimated in one well by the use of fluorescent markers. Compounds with an IC_{50} value of <10 µM and a selectivity index of >10 were considered for further evaluation. Nine molecules with activity against ***T. cruzi*** amastigotes were identified. Of these, six compounds had previous reported activity against ***T. cruzi***, supporting the use of this assay, whilst three remaining compounds were not reported in the literature. These were evaluated for activity against the mammalian cell line HEK293, and against ***T. cruzi*** trypomastigotes in whole cell viability-based assays utilising the redox dyes, Resazurin and PrestoBlue, respectively. Lack of activity in these assays suggested selective activity against amastigotes

with IC_{50} values of 1.2 and 1.7 μM and 3.6 μM and selectivity indices of 59 and 42 and 21, respectively toward the parasite over 3T3/ HEK293 cells. As compounds did not remove 100% of parasites from host cells at the EC_{100}, modification of the image-based assay was undertaken to determine the clearance of parasites following removal of compound, important for the chronic phase of the disease. The incubation time was separately extended, to determine efficacy over time. These 3 structures, from different chemical classes, serve as chemical starting points for ***T. cruzi*** research.

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PROBING A SMALL MOLECULE SCAFFOLD TO IDENTIFY NOVEL INHIBITORS OF *TRYPANOSOMA BRUCEI, TRYPANOSOMA CRUZI,* AND *LEISHMANIA DONOVANI*

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Three of most devastating Neglected Tropical Diseases - African Trypanosomiasis, Chagas Disease and the Leishmaniases - are caused by vector-borne, flagellated protozoa. An estimated 30,000 people are infected and up to 70 million are at risk of developing African Trypanosomiasis (also known as African Sleeping Sickness,) which is caused by ***Trypanosoma brucei***. ***Trypanosoma cruzi*** is the causative agent of Chagas Disease, which affects an estimated 7-8 million people. Although Chagas is primarily classed as a disease of poverty endemic to Latin and South America, it is considered an emerging disease in the United States. The Leishmaniases, which are caused by various subspecies of ***Leishmania,*** affect an estimated 10-12 million people in almost 100 countries. All three can be extremely debilitating and even fatal if untreated. However, current drug therapies have issues with toxicity and there are growing concerns about developing resistance. There is an urgent need to develop new drugs that are safer and more effective to treat these devastating diseases. In this study, derivatives of a small molecule with known antimicrobial properties were synthesized and screened for inhibitory activity against the bloodstream form of ***T. brucei***. Compounds were also screened against a rat skeletal myoblast line (L6) and macrophages differentiated from a human monocyte line (THP-1) to gauge cytotoxicity. Compounds with low toxicity were further screened against the intracellular forms of ***T. cruzi*** and ***L. donovani***. These screens identified several novel compounds with IC_{50} values in the low micromolar range and low cytotoxicity that have potential to be developed into new drug therapies for these diseases.

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COST-EFFECTIVENESS OF DIAGNOSTIC-THERAPEUTIC STRATEGIES FOR PEDIATRIC VISCERAL LEISHMANIASIS IN MOROCCO

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Visceral leishmaniasis (VL) is a neglected parasitic disease that is fatal if left untreated. VL is endemic in Morocco and other countries in North Africa were it mainly affects children from rural areas. In Morocco, the direct observation of ***Leishmania*** parasites in bone marrow aspirates is used to diagnose VL and antimonials (Sb for 20 days) is the first line of treatment. In this study we evaluate the cost and cost-effectiveness of alternative diagnostic-therapeutic strategies for pediatric VL in Morocco. In particular we evaluate the use of liposomal amphotericin B (L-AmB), the safest and most efficacious anti-leishmanial drug. A decision-analysis model was used to estimate the cost-effectiveness of using RDT and/or short course L-AmB to manage VL pediatric cases in Morocco compared to the current clinical practices. Incremental cost-effectiveness ratios (ICERs), expressed as cost per death averted, were estimated by comparing costs and effectiveness of the alternative algorithms with the current practices. This study shows that using RDT and/or implementing short course L-AmB treatments would be cost-effective in the Moroccan context according to the World Health Organization (WHO) criteria. In particular, if L-AmB is purchased at a preferential price (18 US$ per vial) the use of this drug to treat pediatric VL cases would be less expensive than antimonials. The results of this study should encourage the implementation of RDT and/or short course L-AmB treatments for pediatric VL in Morocco and other countries in North Africa facing similar challenges.

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IGG PRODUCTION, CIRCULATING IMUNE COMPLEXES AND IGG AVIDITY MATURATION DURING EXPERIMENTAL HAMSTER VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a neglected disease according World Health Organization, with about 300,000 cases and 20,000 deaths annually. Hypergammaglobulinemia is found in VL but is not associated to effective control of the disease. Circulating immune complexes (CIC) were associated with hypergammaglobulinemia and we showed that CIC could interfere in the positivity of serology in infected hamsters, dogs and humans. Widely used in epidemiology, serology does not distinguish active disease and may be inconclusive due to the presence of CIC. We study ***Leishmania*** CIC during experimental infection in hamsters and its impact in conventional serology. Serum samples from ***L. (L.) infantum chagasi*** infected hamsters were collected at 15, 30, 45, 60 and 90 days after $5x10^7$ amastigote infection. For detecting IgG and its avidity and CIC we used solid phase conventional ELISA with or without 6M Urea washing (cELISA), an antigen capture ELISA assay using anti promastigote rabbit IgG in solid phase and a biotinylated anti ***Leishmania*** rabbit IgG as conjugate (DAC) and a dissociative pH shock ELISA, which is performing by diluted sample brought at pH 2,5 added to well, followed by neutralization, reaction and subsequent conjugate incubation (dELISA). Using cELISA specific IgG showed gradual increase, with higher levels found after 30 days of infection. Detection of circulating antigen by DAC and the IgG increment in dELISA identified positive CIC samples in most periods of infection. Highest frequency of positive samples was found at 30 days of infection (28% and 54.1%, respectively). ELISA IgG avidity showed the same proportion of high-avidity antibodies in all periods of infection, suggesting absence of avidity maturation of anti-***Leishmania*** IgG antibodies. The association of these results indicates that during the experimental VL in hamsters, CIC were present and interfere with conventional serology, and could be also associated to immunopathology due to the absence of antibody avidity maturation. Antibodies and circulating immune complexes could be involved in the establishment of ineffective immune response associated to B cell activation in experimental VL.

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A SIMPLE COLORIMETRIC ASSAY FOR HIGH-THROUGHPUT SCREENING OF POTENTIAL DRUG COMPOUNDS AGAINST INTRACELLULAR AMASTIGOSTES OF *LEISHMANIA*

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There is a pressing need to identify new drugs (leadcompounds against ***Leishmania*** parasites as current medication is old, often toxic and failing due to resistance. Critical to the search for new anti-leishmanial drugs (or lead compounds) is the availability of high-throughput screening (HTS) methods to test chemical compounds against the relevant stage for disease pathogenesis, the intracellular amastigotes. Recent progress in automated microscopy and genetic recombination have produced powerful tools for drug discovery. Nevertheless, a simple and efficient

test for measuring drug activity against ***Leishmania*** clinical isolates is lacking. At KIT Biomedical Research (Parasitology Unit), we have developed a quantitative colorimetric assay, whereby the activity of a ***Leishmania*** native enzyme is used to assess parasite viability. Enzymatic reduction of disulphide-trypanothione, monitored by a microtiter plate reader, was used to quantify the growth of ***Leishmania*** parasites. An excellent correlation was found between the optical density, as measured at 412nm, and the number of parasites inoculated. Pharmacological validation of the assay was performed against the conventional AlamarBlue® method for promastigotes or standard microscopy for intracellular amastigotes. The activity of a selected-compound panel, including several anti-leishmanial reference drugs, demonstrated high consistency between the newly developed assay and the reference method, and corroborated with previously published data. Quality assessment with standard measures confirmed the robustness and reproducibility of the assay, which performed in compliance with HTS requirements. This simple and rapid assay provides a reliable, accurate method for screening anti-leishmanial agents, at high-throughput. The basic equipment and manipulation required to perform the assay makes it easy to implement, simplifying the methodology for scoring inhibitor assays.

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BENZNIDAZOLE-RELATED ADVERSE DRUG REACTIONS IN BRAZILIAN PATIENTS WITH CHAGAS DISEASE

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There are only two drugs approved for the treatment of Chagas disease: benznidazole (BZN) and nifurtimox. Both present a wide spectrum of toxicity. BZN is the only one available in Brazil and the response rate and adverse drug reactions (ADRs) are extremely variable. There are few studies describing in details the ADRs during the treatment of adult patients with BZN. We have established a prospective cohort study that followed 93 ***Trypanosoma cruzi*** infected adults, (age between 18 - 65 years), with the chronic indeterminate form of the disease, with mild to moderate cardiac or digestive involvement, without advanced forms during BZN treatment (5mg/kg/day, up to 300mg/day for 60 days). Patients were evaluated in 3 schedules visits for adhesion and ADRs (approximately 15th, 30th and 60th day after treatment initiation). They were informed to call a dedicated line if they presented with ADRs, and they received prompt medical evaluation if needed. Of the 93 patients, 63 (67.7%) informed at least one ADR and 18 (19.4%) had to interrupt the treatment (4 temporally and 14 permanently). The majority of ADRs, 57 (90.5%) occurred during the first 15 days of treatment, 22 (34.9%) patients related ADRs on schedule visit day 30. The most frequent ADRs were related to skin (47%), gastrointestinal (47.6%) and peripheral nervous system (31.7%). ADRs were less common among individuals receiving a proton pump inhibitor, omeprazole (p=0.0002), OR=0.02 (CI 0.001-0.45), and more common among individuals using hydrochlorothiazide (p=0.047), OR=4.5, (CI 0.97-21.5). ADRs were not associated with age, gender, skin color, education and comorbidities, such as diabetes and hypertension. The ADRs symptoms disappeared after BZN interruption and the administration of drugs to manage the skin manifestations and gastrointestinal symptoms. Two patients with severe ADRs were hospitalized in the emergency for less than 24hs to receive intravenous glucocorticoids. There was no fatal event in this cohort. In conclusion, ADRs are common during BZN treatment, but they can be manageable with orientation and easy access to the physicians.

1102

CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF CUTANEOUS LEISHMANIASIS IN A GROUP OF MILITARY PERSONNEL IN SRI LANKA

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Cutaneous leishmaniasis (CL) is a newly established vector-borne parasitic disease in Sri Lanka. Military personnel have an occupational risk for CL due to being stationed in endemic areas and exposure to vectors outdoors. This study describes the clinical and histopathological features of CL in a group of military personnel. Thirty five patients with smear positive for ***Leishmania*** amastigotes were included, their data analyzed for clinical features and skin biopsies processed routinely for histology, examined at a conference microscope and classified into 4 groups using modified Ridley criteria for Leishmaniasis as: I-parasitized macrophages with variable lymphocytes and plasma cells; II-parasitized macrophages with lymphocytes, plasma cells and ill formed histiocytic granulomata; III-a mixture of macrophages (with or without parasites), lymphocytes, plasma cells and epithelioid granulomata; IV-epithelioid granulomatous response with a few lymphocytes and plasma cells but no amastigotes. Lesions were categorized by duration, as acute (< 6 months) or chronic (≥ 6 months). Study group composed of all males with a mean age of 32.6 years (range 22-47) and lesion duration of 5.6 months (range 1-24). Number of lesions varied from 1 to 6 with majority (71.4%, n= 25) having a single lesion. Nodular (37.1%, n=13) and nodulo-ulcerative (25.7%, n=9) lesions in upper limbs (68.6%, n=24) was the commonest presentation. Twenty nine (82.9%) of the biopsies were positive also by histology. Twenty two (62.9%) were acute and 13 (37.1%) chronic. Group I, II, III and IV patterns were seen in 14 (40%), 12 (34.3%), 5 (14.3%) and 4 (11.4%) respectively and 9 (40.9%), 9 (40.9%), 2 (9.1%) and 2 (9.1%) of acute lesions and 5 (38.5%), 3 (23.1%), 3 (23.1%) and 2 (15.4%) of chronic lesions respectively. Necrosis was not seen in any of the lesions. Majority in this group of military personnel with CL had single lesions affecting the upper limbs and sought treatment within 2 years of appearance of lesions. The histological picture varied from diffuse infiltration of parasitized macrophages admixed with chronic inflammatory cells to ill-formed histiocytic granulomata.

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EVALUATING THE COST-EFFECTIVENESS OF DIFFERENT SCREENING STRATEGIES FOR HUMAN AFRICAN TRYPANOSOMIASIS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Human African trypanosomiasis (HAT), caused by the protozoan ***Trypanosoma brucei gambiense*** is a neglected tropical disease that is endemic in many areas of sub-Saharan Africa. Interruption of transmission requires the early diagnosis and treatment of cases among suspects that are identified using a screening test. Screening for HAT has been performed using the card agglutination test for trypanosomiasis (CATT), but recently, rapid diagnostic tests (RDTs) have been developed that, unlike CATT, are thermostable and do not require electricity. In a clinical

trial carried out in the Democratic Republic of the Congo (DRC), the SD BIOLINE HAT RDT had a higher sensitivity, but a lower specificity than CATT in active screening (by mobile teams) and passive screening at healthcare facilities. This study estimates the cost-effectiveness of using the RDT and CATT in different algorithms and diagnostic infrastructures. Data on HAT prevalence and performance of diagnostic tests were collected during the RDT clinical trial. These data informed a stochastic epidemiological model incorporating the number of people presenting for screening, infection rates, the number of cases detected at screening, and the number subsequently confirmed and cured. Into this we incorporated the costs incurred when screening was done by CATT or by RDT in passive or active screening. The RDT was the most cost-effective screening test, costing 406 and 394 USD per case cured in active and passive screening respectively, cheaper than CATT by 131 USD in active and 118 USD in passive screening. Sensitivity analysis demonstrated that these conclusions were robust to a number of assumptions, and that the results could be scaled to smaller or larger healthcare facilities and a range of prevalences of HAT. By analyzing the cost-effectiveness of different screening tests in different diagnostic settings, we have provided valuable information on diagnostic options to the HAT control program in DRC. Crucially, the RDT remains the most cost-effective test as prevalence declines and HAT nears elimination. We conclude that the RDT should be recommended as the routine screening test in the DRC.

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ENZOOTIC TRANSMISSION AND ECOLOGY OF ARBOVIRUSES IN SYLVATIC REGIONS OF KENYA

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Prompted by the re-emergence of chikungunya virus [CHIKV] originating in Kenya in 2004 (which subsequently spread into the Indian Ocean and beyond, with adaptation to transmission by Aedes albopictus), we investigated potential enzootic sources of CHIKV and similar arboviruses in East Africa that could lead to similar spillover events of medical or veterinary importance. Sylvatic cycles e.g. circulation between non-human primates and arboreal Aedes spp. mosquitoes, are hypothesized based on studies in West Africa and Asia. By sampling sylvatic mosquito vectors at multiple forest sites across Kenya, and potential vertebrate reservoir hosts (including recent surveys of non-human primates) at two regions (coastal and western Kenya), we assessed possible ***foci*** for outbreak scenarios and evidence for enzootic circulation of arboviruses. Virus detection methods and serological assays were used to screen for infection in 1187 pools of mosquitoes of 43 species, and for antibody presence in 41 rodents (2 species) and 77 non-human primates (4 species), as well as in banked primate samples. We discuss isolation of viral agents, and seroprevalence for o'nyong nyong virus, dengue (1-4) and chikungunya viruses, as well as characterization of vector species diversity. Understanding of the enzootic ecology of arboviruses, and where human contact with sylvatic pathogen cycles occur, is critical to limit future disease outbreaks.

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MIXED METHODS SURVEY OF ZOONOTIC DISEASE AWARENESS AND PRACTICE AMONG ANIMAL AND HUMAN HEALTHCARE PROVIDERS IN MOSHI, TANZANIA

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Zoonotic diseases are common yet under-recognized causes of morbidity in northern Tanzania. We conducted a mixed methods study to assess healthcare provider knowledge, perceptions, and practices about endemic zoonoses in the formal animal and human healthcare sectors. We administered a questionnaire with questions on knowledge, local cases, and testing for zoonoses to 52 human health practitioners and 10 livestock health providers in Moshi, Tanzania. We also conducted 61 post-questionnaire interviews to discuss a previous study that demonstrated that bacterial zoonoses are common among febrile patients in this region. Sixty (96.8%) of the 62 questionnaire respondents had heard of brucellosis, 26 (41.9%) of leptospirosis, and 20 (32.3%) of Q fever. Respondents in the animal sector were more likely to have heard of leptospirosis than those in the human sector (Odds Ratio: 7.3, 95% Confidence Interval: 1.3-77.6, p=0.013). There was no statistically significant difference between sectors in awareness of brucellosis or Q fever. The zoonoses that respondents reported seeing cases of included brucellosis (19, 30.6%), rabies (13, 21.0%), and anthrax (9, 14.5%). No respondents reported cases of leptospirosis or Q fever. Nineteen (38.0%) of the 50 human sector providers who were aware of brucellosis gave details of a human brucellosis diagnostic test available locally, and one gave details of tests for leptospirosis (5.6%) and Q fever (6.7%). One (10.0%) animal sector respondent gave details of an animal brucellosis diagnostic test available locally, and none described tests for leptospirosis or Q fever. During interviews, few respondents were surprised by the high human health burden of bacterial zoonoses, and many agreed that it was consistent with local livestock keeping practices. Several respondents highlighted an absence of knowledge of zoonoses among both healthcare providers and the general public, and many cited a lack of testing facilities for diagnosis of zoonoses. These findings reveal clear differences in knowledge of different zoonoses and a need for improved diagnostic capacity for multiple zoonoses in northern Tanzania.

1106

EXPRESSION OF CATHEPSIN L-LIKE AND ENOLASE FROM *TAENIA SOLIUM* IN THE BACULOVIRUS SYSTEM FOR DIAGNOSIS OF HUMAN NEUROCYSTICERCOSIS

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Abstract: Neurocysticercosis (NCC) is the main cause of adult epilepsy in developing countries. It is caused by the infection of ***Taenia solium*** larvae in the central nervous system. The available immunodiagnostic test is the Western Blot test composed by 7 partially purified lentil-lectin glycoproteins; with sensitivity of 40-60% for patients with single cyst NCC. In the search for new antigens for immunodiagnostic, we have expressed ***T. solium*** cathepsin L-like and enolase with their respective posttranslational modifications in the baculovirus-insect cell expression system (BES). Cathepsin L-like gene and enolase were anotaded from the ***T. solium*** genome. Coding sequences were cloned in both pFastBac

HT and E. coli DH5alpha. The gene rearrangement was made in the baculovirus genome (bacmid) in E. coli DH10 Bac. Bacmids were used for production of viral particles, and these were used for cathepsin L-like and enolase expression in Sf9 cells. Western blot with polyclonal rabbit antibodies specific for cathepsin L and Enolase was made to verify the expression of the proteins. We expressed cathepsin L (27 kDa) and enolase (52 kDa) of ***T. solium*** in BES. Expression was confirmed by western blot with specific rabbit polyclonal antibodies (1/5000) for cathepsin L-like and enolase. The BES is efficient to express low levels of cathepsin L-like enolase and its posttranslational glycosylation. This was suggested by a 5 kDa difference between the molecular weights of the proteins expressed in BES and expressed in E. coli BL21 DE3 pLys. Lower titers of antibodies to recognize and cathepsin L-like and enolase suggest that they are highly antigenic. Also, in the next three months we will evaluate the usefulness of recombinant proteins in the diagnosis using sera from patients with NCC.

1107

RISK OF ZOONOTIC DISEASE TRANSMISSION IN WORKERS WITH WILDLIFE CONTACT: CHINA

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Wildlife zoonoses are a significant source of emerging infectious disease, but much remains unknown the factors driving species jumps of pathogens at the human animal interface. Here we report on a longitudinal cohort study conducted in Guangdong, China, to characterize the transmission of pathogens with pandemic potential in highly exposed human populations at the human-animal interface. The study was conducted in 12 prefecture level locations in Guangdong, targeting high-risk individuals- primarily hunters, persons working in wet markets and restaurant workers who process bushmeat. A risk factor/exposure survey was constructed and administered to these workers following informed consent and enrollment. Serology testing was performed at baseline for evidence of past infection with hanta virus, SARS CoV, and novel Bunyavirus. Follow up serology was performed on a subset of the cohort as well as close contacts of individuals testing positive on initial baseline serology. Bivariate and multivariate analysis was performed to examine association of reported risk factors with infection status. In our survey, cooks or kitchen workers were the most commonly reported occupation (reported by approximately one third of participants), while approximately 17% engaged in agriculture, 8% worked as butchers. However, hunters were rarely reported. Our data shows an overall low seropositivity to Hantavirus, SARS CoV, or new Bunyavirus (less than 1%), but higher positivity rate among certain subgroups such as butchers (approximately 10%). When reported tasks were analyzed, ever butchering a wild animal was associated with an elevated risk of seropositivity (approximately 5%), while ever hunting was associated with a lower rate (approximately 1%). Our results indicate that working as a butcher appears to be associated with an increased risk of infection with wildlife pathogens. Targeted educational programs for prevention, monitoring of high risk populations and comprehensive evaluation of sick persons with wildlife contact appears warranted.

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PREVALENCE OF *MYCOBACTERIUM BOVIS* INFECTION IN CATTLE FROM WESTERN KENYA

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Bovine tuberculosis is a zoonotic disease with worldwide distribution affecting a broad range of mammalian hosts.It is caused by the bacterium ***Mycobacterium bovis*** causing pulmonary tuberculosis in cattle,leading to chronic debilitation and coughing,and the potential for systemic spread to other organs.In humans, ***M. bovis*** infection results mainly in extra-pulmonary tuberculosis especially among HIV positive and immune-compromised individuals. Asembo,Western Kenya, is an area with an estimated HIV prevalence of 16%. However there are limited data about the prevalence of ***M. bovis*** in cattle in Asembo.In livestock hosts, the single intradermal comparative cervical tuberculin test (SICCTT) detects infection with ***M. bovis*** before the onset of clinical disease. To determine the prevalence of cattle reacting to a SICCTT in Asembo, a cross-sectional survey was conducted among herds in 64 compounds randomly chosen from 33 villages. SCCITT was performed;animals were classified as reactors if their M bovis tuberculin reaction was 4mm greater than an avian tuberculin reaction. We tested 258 cattle from 64 herds and observed an individual cattle and herd-level prevalence of 1.6% (4/258) and 6.3% (4/64), respectively. Three of the four reactors were females. The median age of the reactors was 60 months, compared with 54 months for non-reactors. All reactors had been within their herds for more than 12 months, and 2 (50%) were born in their current herds, compared with 218/254 (85.8%) and 89 (35%) respectively of the non-reactors. All reactors (4/4, 100%) were herded together with other ruminant species. Among the non-reactors,213/254(83.9%) were commingled with other ruminant species. All households with reactor-cattle (100%) as well as 57/60 (95%) with non-reactor cattle also kept chickens. Similar studies from African countries have reported a prevalence range of 0.9-21%, with a higher prevalence in regions with high cattle: human ratio. This is within the range observed in the current study. This study demonstrates that ***M. bovis*** is present among cattle in a high-HIV burden area in Western Kenya, indicating a threat to both cattle and human health.

1109

AN EPIDEMIOLOGICAL APPROACH TOWARDS MASSIVE BOTULISM OUTBREAK IN CATTLE FARMS IN SOUTH KOREA

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Botulism (types A to G) is neuroparalytic disease caused by a toxin produced by anaerobic bacteria ***Clostridium botulinum***. The bacteria are commonly present in the environment and become highly toxic in decaying vegetable compound and animal carcasses. Diagnosis is determined based on clinical signs and compatible history because standard confirmatory laboratory tests are not useful due to low sensitivity. The aim of this study is to provide knowledge on the recent outbreak of Botulism in Pocheon and Yeoncheon, Gyeonggi-do between August 2011 and July 2012 which specifically occurred in 24 cattle farms causing 431 cattle deaths. Further to this, possible risk factors were examined to find out the cause of the outbreak that the findings could be applied to establish bio-security policies. ***C. botulinum*** toxins were detected in 5 farms among 24 farms. Toxin type B and D detected farms were 1 and 2 respectively. Toxin type B and D, type B, C and D were detected at the same farm each. ***C. botulinum*** type D was confirmed in poultry litter specimens from 2 of 15 poultry farms situated nearby the affected

farms. With the consideration of diagnostic difficulty in detection of the bacteria, it is hard to ignore possibilities that other test negative poultry farms might be actually affected in real. From the fact that large number of livestock farms, agricultural farms using animal feces, burying livestock carcasses around the farms, and factories dealing with food leftover and livestock feces are densely populated in Pocheon, the area is assumed to have been already polluted with the toxin. The long rainy season (52 days, precipitation of 1,318mm), intensive heavy rain (675mm for 4 days) and flood then seemed to act as a carrier of potential risk elements from the environment to the farms. It was the first official diagnosis of ***C. botulinum*** in South Korea.

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BAT EXPOSURE RISK TO THE INDIGENOUS POPULATION OF THE PERUVIAN AMAZON

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Surveillance activities have provided recent evidence that multiple ***Chiroptera*** (bats) species in Asia, Africa, and America serve as reservoirs for previously unrecognized pathogens affecting domestic animals and humans. Community education and enhanced surveillance activities among these at-risk populations are necessary to identify the level of exposure due to contact between bats and humans. This study aimed to examine the frequency of bat exposures among indigenous and non-indigenous populations, and describe the knowledge, attitudes, and practices of individuals with regard to possible bat exposure in Peru. This study was a collaborative effort between the Centers for Disease Control and Prevention, the Peruvian Ministry of Health, and the Virology and Emerging Infectious Department of the U.S. Naval Medical Research Unit No. 6 in Peru (NAMRU-6). A participant questionnaire gathered information about any history of bat exposures (e.g. bites, scratches, skin contact), actions taken following a bat exposure, perceived risk towards bats, and knowledge of bat-borne zoonotic diseases. The study population was comprised of residents from two rural villages around Iquitos, the largest city in the Peruvian Amazon (non-indigenous), and four riverside indigenous communities in Datem del Marañon province in Loreto, Peru. A venous blood sample was taken from the respondent from each household as well as anyone else in the household who claimed to have been exposed to bats. During four nights in each community, we captured ***Chiroptera*** using 6-20 mist nets around houses and caves near the villages. We found that the indigenous populations had more bats exposures (391/790, 49.5%) than the non-indigenous population (25/154, 16.2%). Riverside communities, indigenous, and non-indigenous populations had more bats exposure than the roadside community, 48.7% (407/836) and 8.3% (9/108) respectively. Three genuses of vampire bats reported in the amazon region were captured (***Diphylla, Diaemus***, and ***Desmodus***) primarily in the indigenous communities. Laboratory analyses will be conducted to determine the role of bats in the transmission of bat borne diseases.

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PREVALENCE AND DISTRIBUTION OF *ANGIOSTRONGYLUS CANTONENSIS* (CHEN, 1935) IN RATS AND FRESHWATER SNAILS OF MUÑOZ, NUEVA ECIJA, PHILIPPINES

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Angiostrongylus cantonensis or rat lungworm is the major cause of eosinophilic meningitis in humans. Humans can be infected through ingestion of infected mollusks, paratenic hosts such as shrimps, crabs, or frogs, and vegetables which may be contaminated with infective stage. This study was conducted to determine the prevalence of ***A. cantonensis*** in rats and freshwater snails of Muñoz, Nueva Ecija, Philippines. Rats and freshwater snails were collected from both residential and agricultural areas in five selected barangays of Muñoz, Nueva Ecija. Rat tissues, e.g. heart and lungs, and snails were examined and subjected to artificial digestion for parasite collection. Results revealed that 30.62% of the rats examined were positive with ***A. cantonensis***. ***Rattus norvegicus*** and ***R. tanezumi*** showed prevalence of 45.83% and 28.65%, respectively. Also, prevalence was compared between sexes and age groups, however, only the latter showed significant difference among ***R. tanezumi*** ($P < 0.001$), e.i., adults, sub-adults, and juveniles showed prevalence of 57.14%, 44.62%, and 8.70%, respectively. Meanwhile, out of 781 freshwater snails belonging to eight different species, only ***Pomacea canaliculata*** (1.50%) and ***Melanoides maculata*** (1.12%) from irrigations and rivers were infected with larval stages (L3). This study revealed that rat species ***R. norvegicus*** and ***R. tanezumi*** and snail species ***P. canaliculata*** and ***M. maculata*** are susceptible hosts for ***A. cantonensis***. Furthermore, analysis of the host distribution showed an overlap in their habitats between residential and agricultural areas. Their presence on and near human settlements indicates risks of transmission to humans. The present study can be used in promoting public health awareness regarding rats and snails that can serve as sources of infection for both humans and other animals.

1112

IMPROVING RISK MAPPING FOR VECTOR-BORNE, ZOONOTIC DISEASES

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Geospatial mapping of infectious diseases is important for targeting and assessing various public health activities. By combining information on locations where diseases have been recorded with geographic data on environmental and socioeconomic covariates known to affect disease transmission using machine-learning models, niche modelling can generate fine-resolution, evidence-based risk maps for a variety of diseases of public health importance. To understand spatial variation in risk for diseases with reservoirs in multiple host species, transmitted by multiple vector species, we need to develop this mapping approach further. For spatial epidemiology to capture the full complexity of these disease transmission systems, it is important to consider the niches and distributions of all component species. Prime examples of human diseases involving multiple vector and animal reservoir species include a number of important arboviruses and ***Plasmodium knowlesi*** malaria. Japanese encephalitis, West Nile fever and yellow fever all have reservoirs in multiple animal species and are transmitted by a wide range of mosquito genera. ***P. knowlesi*** is a malaria parasite found in wild monkey populations which is transmitted to humans via anopheline mosquitoes. It is the most common cause of clinical malaria in high transmission regions of Malaysia. Using a boosted regression tree modelling framework, species distribution maps were produced for putative reservoir and vector species of ***P. knowlesi*** malaria. Methods were developed to account for various sources of bias in the available data. These maps were used to assess whether distribution maps of putative reservoir and/or vector species explain the distribution of ***P. knowlesi*** infections in humans. A consensus map incorporating the distributions of multiple vector and reservoir species was subsequently generated and used as a covariate for disease occurrence mapping of ***P. knowlesi*** malaria. The application of this approach to investigate variation in risk from arboviruses with multiple reservoirs and vectors is discussed, and preliminary results for key arboviruses are presented.

1113

DIRECT BLOOD ANALYSIS OF *BARTONELLA BACILLIFORMIS* MULTI LOCUS SEQUENCE TYPING FROM PATIENTS WITH OROYA'S FEVER DURING A PERUVIAN OUTBREAK

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Bartonella bacilliformis is the etiological agent of Carrion's disease, which is a neglected disease, affecting Mountain Andean valleys. This disease in absence of antibiotic treatment presents a high mortality during the acute phase (Oroya's Fever) between 44-80%. The second phase is characterized by the development of dermal eruptions, known as "Peruvian wart". Asymptomatic carriers also has been described in the population from endemic areas (0.5-40%). ***B. bacilliformis*** is a fastidious slow growing microorganism, being difficult and cumbersome to culture and isolate from clinical sources. Then, the available data about phylogenetic relationship in clinical samples are really scarce, but suggesting high variability. The aim of the study was to perform direct blood analysis of ***B. bacilliformis*** Multi Locus Sequence Typing (MLST) from diagnosed patients with Oroya fever. Almost the majority of the samples (8 out of 10) were collected during an outbreak in 2009 in Cajamarca. All samples were confirmed by PCR for B.bacilliformis. In addition 2 collection strain were included. Phylogenetic relationship was performance by CLUSTAL W. A total of 8 blood samples isolated during a Carrion outbreak in the period March-April 2009 in Cachachi (Cajamarca) in Northern Peru were analyzed. One blood sample was added in 2011 from Condebamba (Cajamarca), only 50Km of distance and finally another blood sample from Sihuas (Ancash), 500 Km of Cachachi in the center of the country. All strains from the outbreak belong to the same ST group, ST1. The strain of Condebamba is closely related to ST1, and finally the strain of Ancash is belonging to ST4. The collection strains from Pasteur Institute isolated in 1941 and 1949 belong to ST3. The present study demonstrate that the direct blood MLST PCR is a technique useful for application apply in the phylogenic characterization of this fastidious microorganism's endemic from Andean regions. In this study, we demonstrate that the Cajamarca outbreak of Oroya's fever is caused by the same Sequence Typing group, it might suggest that variability of strains from a region is not as variable as previously described.

1114

INFLUENCE OF DAIRY PRODUCTION PRACTICES ON RISKS FOR BOVINE VACCINIA EXPOSURE IN CATTLE: BRAZIL

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Vaccinia-like viruses have been observed in natural circulation in Brazil since 1965. Outbreaks of bovine vaccinia (BV) have been recurrent in agricultural areas of the country for the last 15 years. A genetically diverse set of vaccinia-like strains have been recovered during these outbreaks and from related biological surveys aimed at virus discovery. Some strains have been observed to persist in specific regions for years and even decades. The purpose of this study was to identify environmental features of dairy farms or farming practices that are associated with risk for bovine exposure to vaccinia-like viruses. To accomplish this we collected sera from a cohort of domestic bovine (N=745) in an area with a history of outbreaks in order to assess associations between the environmental features and work practices of farms and virus exposure in bovines. Over the course of eight field visits which took place during June 2010--February 2012, 18 dairy farms within Minas Gerias state, Brazil, were visited by investigators. The properties surveyed during this study varied both in size and relative sophistication. A standardized data-collection instrument was used to record information from each property (i.e., cattle feed, utilization of mechanized ***vs.*** manual milking, pasture characteristics, presence of other domestic animals, water sources, non-domestic animals seen on property, etc.) Forty-eight presumptive predictor variables were assessed for association with ***Orthopoxvirus*** sero-status using univariate logistic regression methods; 10 were found to be significantly associated (p-value ≤ 0.050). A subset of these variables (relating to farming practices and to the presence of other domestic animals on the property) were determined to be independently associated with virus exposure in bovines.

1115

NOVEL ABUNDANT HEMOTROPIC PARASITISM OF NIGERIAN DOGS

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Dogs are an understudied reservoir host in Nigeria to potentially recognize blood-borne parasitic diseases that could be zoonotically transmitted to human. Most ill dogs brought to the University of Ibadan Veterinary Teaching Hospital (UIVTH) are tick-infested and anemic. Due to resource limitations, light microscopy (LM) is the only diagnostic means used in UIVTH for detection of haemoparasites. Quantitative PCR (qPCR)-based assays have been reported to a the gold standard for accurate diagnosis of haemoparasitism among others. In this study, we used LM and qPCR-based assays on 117 blood samples from dogs brought to the UIVTH for detection of haemoparasites. In addition, next generation sequencing was performed on positive samples for characterization of detected parasites. Six different haemoparasites were detected by LM and qPCR, including novel ***Mycoplasma haemobos.*** Analysis of thirty-eight of 117 dogs that were clinically suspected to be infected with haempoarasites confirmed, 7 (18.4%) and 31(81.6%) by LM and RT-PCR, respectively. qPCR and sequencing analysis revealed 89 (76.1%) positive samples, among which 75 (64.1%) for ***Babesia canis***, 43 (36.7%) for ***Ehrlichia canis,*** 12 (10.3%) for ***Hemoplasma canis,*** 2 (1.7%) for ***Hepatozoon canis*** and novel ***Mycoplasma haemobos*** (0.8%)***.*** Of these, single, double and triple parasite infections occurred in 49 (55.1%), 35 (39.3%) and 5 (5.6%) respectively. Overall, we outline the first report of ***Mycoplasma haemobos*** in dogs in Nigeria. We showed significant abundance and common occurrence of potentially zoonotic haemoparasitic infections in Nigerian dogs. These findings call for development of molecular diagnostic procedures for routine screening of sick companion animals, and further studies to understand the mechanisms of coinfection in altering parasite load and transmission and the relative risk of human infection when cohabitating with haemoparasite-infected dogs.

1116

KNOWLEDGE MANAGEMENT SYSTEM FOR NEGLECTED ZOONOTIC DISEASES IN PERU: A PROPOSAL FOR A ONE HEALTH APPROACH

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The One Health approach seeks synergy between human, animal and environmental health. If typically each of these fields is an island, a Knowledge Management System (KMS) can help bridge them. Universities are well positioned to build such bridges by facilitating collaboration and providing data sharing platforms. We propose an inter-institutional KMS for animal, human, and environmental health in Peru. The objectives of the National Service of Agriculture Health in Peru (SENASA) do not include to improve public health. However, in working toward its goal to increase animal productivity, SENASA is making important contributions to prevent transmission of zoonoses. Similarly, the MOH addresses zoonoses through human case management or preventive education, but no attention is paid to animal hosts. Despite overlapping interests, effective

communication between these 2 governmental agencies, to integrate work on humans and animals did not exist in Arequipa, Peru. Currently, the Zoonotic Disease Research Group in Peru of UPenn and Universidad Peruana Cayetano Heredia is bridging both agencies to share information, coordinate actions, and develop projects to leverage available data. The university role in the KMS is catalyzing exchange. Fasciola hepatica, known for its economic impact on livestock, has long been studied by SENASA. Through animal screening and awareness campaigns, it has dramatically reduced the prevalence in herds, and the impact on public health is being analyzed by our group and shared with the MOH for controlling human fascioliasis. Similarly, hydatid disease was not considered an important livestock illness in Arequipa. On medical records, we found over 150 hydatid disease patients in the last 2 years, most residing in Arequipa. These data is now being used to discover animal transmission ***foci***. Using a KMS, the data produced by SENASA's activities to evaluate animals are now being used for public health and MOH medical records are being used to identify animal hosts.

1117

HUMAN MONKEYPOX TRANSMISSION DYNAMICS THIRTY YEARS AFTER SMALLPOX ERADICATION IN THE SANKURU DISTRICT, DEMOCRATIC REPUBLIC OF CONGO

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Reports from the first monkeypox (MPX) active surveillance program in the Democratic Republic of Congo (DRC) in the 1980s determined that the disease was not of epidemic potential, with R_0<1. However, during an active surveillance period from 2005-2007, researchers found a 20-fold increase during the last 30 years. The purpose of this study was to analyze the contact data from 2005-07 and compare characteristics to those of the 1980s. Results were used to assess the current R_0 of MPX, and to determine whether there has been a significant change since the first program. Contact tracing information and samples from active lesions were collected. Samples were screened by PCR and positive cases were ranked by generation and grouped into chains of transmission according to date of rash onset, contact tracing, and location. R_0 was determined using calculations provided in the 1980s study and chain size distribution was compared. Of 1407 suspected cases of MPX investigated in 2005-07, 570 provided contact information with an average of 6.4 (range, 1-21) contacts each. Among the 733 positive cases, 435 distinct chains of transmission were identified. Average chain size was 5.3 cases (range, 1-13), with the longest reaching six generations. Among confirmed cases, 293 reported contact tracing information, and the crude secondary attack rate (AR) was 0.067. Stratified into household and non-household contacts, AR was 0.070 and 0.49, respectively. R_0 was found to be between 0.419-0.570 as opposed to 0.815. Contact characteristics and types of contacts differed from those of the 1980s program. This analysis found a higher crude secondary attack rate, but a lower number of contacts on average, as well as a smaller difference in the attack rates within household and non-household members. This could be the result of a higher proportion of unvaccinated contacts, or that the virus is better able to transmit between humans with a more limited amount of contact. Further analysis of R_0 should be continued in order to evaluate the many variables potentially involved in the transmission differences.

1118

PREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN VETERINARY STUDENTS AT WESTERN UNIVERSITY OF HEALTH SCIENCES AND EVALUATION OF RISK FACTORS: PRELIMINARY RESULTS

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Toxoplasmosis is a parasitic, zoonotic infection causing a significant One Health impact globally. In recent history, it was thought that ***Toxoplasma gondii*** infections were asymptomatic, but new research suggests that the outcome of these infections have been fatal for both animals and people. Over the last two decades, toxoplasmosis has been associated with neurological disorders in mice and humans. The infected individuals are at a higher risk for car accidents, suicide and schizophrenia. Currently, the prevalence of infection among the population in the United States is largely unknown. This study aims to determine the seroprevalence of ***T. gondii*** in a group of students attending the College of Veterinary Medicine at Western University of Health Sciences. Blood samples have been collected and tested for ***T. gondii*** immunoglobulin G (IgG). The participants have also completed a survey aimed to identify risk factors associated with acquiring the ***T. gondii*** infection. The preliminary results will be presented and discussed. The results of this study will direct the development of public health education and outreach efforts to reduce the transmission of ***T. gondii*** among animal patients and veterinarians. Considering that toxoplasmosis has been selected by the Center of Disease Control and Prevention as one of the neglected diseases for public health action, this project could structure future prevention measures for the general public of Southern California.

1119

CO-PREVALENCE OF AIDS AND OTHER DISEASES AND THE DEMOGRAPHIC CHARACTERISTICS OF TUBERCULOSIS PATIENTS IN A MUNICIPALITY OF SOUTHEASTERN BRAZIL

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In the second half of the twentieth century, the eradication of tuberculosis in the world was believed to be possible. The emergence of AIDS in the 1980s which changed the behavior of tuberculosis. In the twenty-first century, tuberculosis remains a serious public health concern in Brazil, with 75,120 cases reported in 2014. Araraquara is a medium-sized city located in the state of Sao Paulo, a region with the highest Human Development Index in Brazil. The prevalence of tuberculosis in Araraquara was 30% lower compared with the national average in Brazil in 2011 (27.0 cases and 38.4 cases per 100,000 inhabitants respectively). This can be explained in large part by the high average income and other social, economic and health factors of the city. 533 new cases of tuberculosis were recorded in Araraquara between 2002 and 2011 of which 72.6 % were male and the rest female (67.4 % were men and 32.6 % were women in Brazil in 2011). The predominant age group consists of young adults between 20 and 49 years old, contributing to 66.0 % of the total. 4% of the patients were under the age of 20 and 4.9 % were 70 years and older. AIDS was diagnosed in 20.4 % of male TB patients and 29.4 % of female TB patients in the same period, well above the national average of 10.4 % in men and 9.3 % in women. AIDS was also diagnosed in 5 children with less than 5 years of age who had TB-HIV co-prevalence. Following the trend that is present throughout Brazil of which hepatitis C is the most common, hepatitis C was diagnosed in 10.1% of men and 8.9 % of women with tuberculosis and hepatitis B in 5.9 % of men and 4.1% of women with TB in the period between 2002 and 2011. Throughout the period of study,

cure rates of almost 80 % were obtained in the municipality while the mortality rate was 12.3 %. The findings of the study present a worrying situation for the city of Araraquara and there is a need to improve the diagnostic routines and medical care of TB patients. The co-prevalence of tuberculosis and AIDS, much higher than the national average (3 times higher in women and 2 times in among men), is also very alarming and calls for further studies.

1120

THE IMPACT OF ANTI-TUBERCULOSIS TREATMENT ON THE ELABORATION OF PRO-INFLAMMATORY CYTOKINES IN HIV SERO-NEGATIVE TUBERCULOSIS PATIENTS

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Tuberculosis has continued to be a major public health challenge in Sub-Saharan Africa and some other regions of the world even though the causative bacterium was discovered more than one century ago. The emergence and spread of drug resistant strains of the bacteria, concurrent HIV/AIDS epidemics, lack of better biomarkers, and unavailability of more effective vaccines have all contributed to the persistence of the disease in the world. Pro-inflammatory cytokines are known to play significant roles in the pathogenesis of tuberculosis. In a prospective observational study we investigated the serum concentrations of three cytokines in adult men and women suffering from tuberculosis of the lungs. The research protocol was approved by the research ethics committee of the University of Nigeria Teaching Hospital Enugu. Each patient was followed up from diagnosis to completion of 6 months WHO approved treatment. Forty two patients and 18 healthy controls participated in the study after giving informed consent. They were all HIV sero-negative. Serum Interleukin-1 β, tumor necrosis factor α, and interleukin 6 were measured in serum of the patients at the time of diagnosis, after eight weeks of treatment and after 6 months of treatment using human ELISA technique (www.ebioscience.com). Data analysis was done using Graphpad prism software, and statistical significance was determined at $p = 0.05$. All three cytokines were found to be very high compared to healthy controls at the time of diagnosis. After eight weeks of treatment there were significant reductions in the serum levels of the cytokines compared to levels recorded before treatment. Subsequently, there were further reductions in the serum concentrations of the 3 cytokines with statistically significant differences between the values at 2 months and those at 6 months. The values recorded at 6 months were close to but not identical with those of healthy controls at any time. These findings could find important applications in tuberculosis drug and vaccine development. Larger studies to validate these findings are needed.

1121

SPATIAL EPIDEMIOLOGY OF TUBERCULOSIS IN KISUMU AND SIAYA COUNTIES, WESTERN KENYA

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The occurrence of tuberculosis (TB) in Kenya is characterized by spatial variations across the country. Nyanza region in western Kenya has the highest TB burden with an estimated prevalence of 500-600 cases per 100,000. Effective management of TB and reduction of TB incidence rates relies on knowledge of where, when and to what degree the disease is present. Objective The objective was to determine the spatial variability of TB disease within a holoendemic region and investigate the factors associated with the observed distribution. Methods Data on TB occurrence was obtained from the combined registers of 233 TB clinics in Kisumu and Siaya Counties. Locator information in the registers was used to link TB cases to lowest formal administrative units referred to as sub-locations. The ArcView application was used to generate maps showing the spatial distribution of TB. Results Of the 5,528 patient's records in the TB registers, 5,468 (99%) were geo-coded to a geographical location. A total of 4, 537 (82%) were residents of the study area. Significant high- and low-rate spatial and space-time clusters were identified in the two counties. A significant secondary cluster was also identified in one of the densely populated areas of the study area. Discussion and conclusion Results from this study can guide targeted TB screening and control efforts with the goal of reducing TB incidence.

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VARIABILITY OF THE RESPIRATORY RATE MEASUREMENTS IN CHILDREN SUSPECTED WITH NON-SEVERE PNEUMONIA NORTHEAST TANZANIA.

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With so little time left for completion of the Millennium Development Goals (MDG), World Health Organization (WHO) and others are giving increased attention towards respiratory rate (RR) measurement in children in order to identify and reduce pneumonia mortality. The introduction of new devices raises the need for validity assessment and this raises the need for a 'gold standard' measure of RR. There is no such standard that is internationally accepted but one option is the use of video of respiratory movement in children that can be later reviewed by experts and results compared with those obtained by primary care workers in their daily practice. We assess the use of such video recordings taken under controlled conditions and comparing results from two independent paediatricians. Using WHO recommendation of observation for 60 seconds, RR were recorded in children aged 2 - 59 months presenting with cough or difficulty breathing in a busy clinic and then repeated at 10 minute intervals over 1 hour in a quiet setting. Random effects linear regression was used to assess variability in RR measurements. A total of 167 children were studied, the mean age was 17.5 (SD±13.8) months and 97(58.1%) were males. Half of the children were awake or calm during the one hour of observation and about a quarter of children were agitated making video review difficult. The mean RR was 35.9 (SD± 9.7) breaths per minute (bpm) and 36.4 (SD± 10.1) bpm by first and second video reviewer respectively compared to 35.8 (SD± 10.7) by the research nurse. On multivariate analysis, the video reviewers recorded high RR than the research nurse (video recorder 1= 0.53, 95% CI=0.23-0.84, video recorder 2=0.85, 95%CI=0.53-1.17). Inter-observer variability in RR between the 2 video recorders and the research nurse was high (SD=3 breaths per minute) after accounting for differences among children and over time. RR is an important clinical sign in identifying children with pneumonia who require antibiotic. Video does not appear to be a reliable gold standard of RR quality checks. Alternative tools need to be identified to facilitate correct and proper assessment of pneumonia in children.

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PNEUMOCOCCAL VACCINE RESPONSE IN MID-CHILDHOOD IS NOT AFFECTED BY PREVIOUS PARASITE EXPOSURE *IN UTERO* OR DURING THE FIRST THIRTY-SIX MONTHS OF LIFE IN COASTAL KENYA

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Children in developing countries respond poorly to life-saving vaccines. ***Streptococcus pneumoniae*** causes vaccine-preventable invasive disease and is currently a leading cause of mortality under age five. Increasing evidence suggests that exposure to chronic parasitic infections can significantly contribute to poor vaccination response. Our goal was to measure Str. pneumoniae vaccine response in a well-characterized cohort of Kenyan children who had been exposed to parasitic infections ***in utero*** and/or in the first 36 months of life.In 2014, the cohort, now aged 4-7 years old, were tested for malaria, soil transmitted helminths, ***Entamoeba histolytica, Giardia lamblia***, and ***Schistosoma haematobium*** infection. Participants received a 10-valent pneumococcal conjugate vaccine.Post-vaccination serum was collected after 4 weeks.Our multiplex fluorescent bead assay determined pre- and post-vaccination IgG concentrations to the ten vaccine PnPs serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. Ranked post-vaccination anti-PnPS IgG concentrations and pre-post increments were compared using non-parametric statistics to determine the impact of subject demographic and parasite exposure factors.Maternal infection with more than one parasite at delivery was associated with a lower PnPS 19 vaccine response. Children exposed to more than two infections ***in utero*** had elevated post-vaccine IgG response to PnPS 14.Experiencing multiple parasitic infections by 30 months of age was significantly associated with elevated PnPS 1 and 6B IgG levels.No association was found between vaccine responses and subject sex or the presence of hookworm, Trichuris, Giardia, or malaria at the time of vaccination. Anti-PnPS responses were not correlated with anti-***Haemophilus influenzae*** type b (Hib) responses earlier in infancy.Although children in this cohort who had been exposed to prenatal maternal parasitic infections demonstrated impaired response to Hib vaccination as infants, there was no consistent impairment of pneumoccocal vaccine response later in life.Mid-childhood vaccine response to PnPS 19 is lower in children born to mothers with polyparasitism at delivery.

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STREPTOCOCCUS PNEUMONIAE CARRIAGE RATES AND SEROTYPE PREVALENCE IN COASTAL KENYA

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Streptococcus pneumoniae (SP) is an important cause of many vaccine preventable invasive diseases, including bacterial meningitis, pneumonia, and sepsis, and is the leading cause of mortality in children under 5 worldwide. In industrialized countries, routine SP conjugate vaccination has decreased the incidence of invasive SP disease in vaccinated infants and unvaccinated children due to herd immunity. Epidemiological data on SP colonization rates and serotype prevalence in the developing world are relatively scarce, thus the effect of SP vaccination is less clear in these regions. The 10-valent SP vaccine was introduced into the childhood vaccination schedule in Kenya in 2012. Because serotype carriage affects immunization responses to corresponding vaccine antigens, localized characterization of colonization is important now and as the vaccine is further rolled-out, to maximize its clinical effectiveness. The purpose of this study is to measure nasopharyngeal (NP) carriage of SP in unvaccinated children. In addition, we plan to evaluate the impact of the 10-valent SP vaccine on carriage rates and serotype prevalence. Beginning in January 2014, children aged 4-7 years old were enrolled in Msambweni, Kenya. NP swabs were performed, and children then received a single dose of a 10-valent SP vaccine. Repeat swabs were obtained 4 weeks post immunization. SP carriage was determined by bacterial culture and multiplex PCR for the SP capsular gene cpsA and the serotype gene wzy. 354 samples (257 pre- and 97 post-vaccine) have been tested. 38/257 (14.7%) pre-vaccine samples were positive for SP, compared to 12/97 (11.6%) of the post-vaccine samples (p=0.61). These preliminary data suggest that the overall SP carriage rate is not greatly impacted by the vaccine. However, sample collection and molecular identification is ongoing, and analysis of paired (pre- and post- vaccine) samples will be informative in the evaluation of the vaccine impact on vaccine-serotype colonization, while also monitoring for post-vaccination replacement serotypes and emergent strains.

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CLINICAL AND EPIDEMIOLOGICAL CHARACTERIZATION OF TUBERCULOSIS IN CENTRAL REGION OF THE STATE OF SÃO PAULO, BRAZIL

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Brazil is one of the 22 countries that accounts for 80% of all TB cases in the world. So it is important to have a better understanding of the clinical and epidemiological distribution of TB in the country. It is also important to know the performance of the disease control programs in the country. Brazil is a very heterogeneous country from an economic and social point of view. We conducted this study in one of the wealthiest regions of the country, the central region of São Paulo. This region has 24 municipalities and approximately one million inhabitants. We conducted a retrospective descriptive study using the TB Web System, which is used for TB control, as our data source. The study period was from 2006 to 2012. The incidence of TB was lower in the region studied than the average incidence in São Paulo state: 19.7 and 38.0 cases / 100,000 inhabitants, respectively. The mortality rate in the region was one death / 100,000 in 2012. As for patients who were co-infected with TB / AIDS, the mortality rate was 8.4. In 2012, there was a cure rate in 81.1% of the patients, 7.0% of abandonment and 10.8% of deaths. For TB patients co-infected with AIDS, the prognosis was worse: low cure rate (55.6%), high abandonment rate (11.7%) and high mortality rate (32.7%). From a demographic point of view, males (74%), patients aged between 20 and 49 years (62.5%) and low schooling, defined as illiterate or up to 7 years of schooling (59.9%) predominated. Supervised treatment was offered to almost 80% of patients in 2012, compared to only 5.4% in 2006. Were examined 75.6% of contacts of TB cases in period 2006-2012. Pulmonary disease was predominant: 83.6%. Diagnosis of TB in the region is still carried out late: 16.9% during hospitalization and 1.2% after death. Culture for Mycobacterium tuberculosis was performed only in 52% of cases with this epidemiological indication. The results demonstrate serious deficiencies in TB control in one of the wealthiest and most developed region in Brazil. It is essential the early diagnosis of tuberculosis and AIDS, to reduce mortality from these diseases.

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IMPROVING ADHERENCE TO CHILDHOOD PNEUMONIA TREATMENT: THE DESIGN AND DEVELOPMENT OF PATIENT INSTRUCTIONS AND A JOB AID FOR AMOXICILLIN DISPERSIBLE TABLETS

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Pneumonia is the leading cause of death from infection in children worldwide. Despite global treatment recommendations which call for children with pneumonia to receive amoxicillin dispersible tablets (DT), only one-third of children with pneumonia receive any antibiotics and many do not complete the full course of treatment. Poor adherence to antibiotics may be driven in part by low literacy. In order to improve pneumonia treatment adherence at the community level, we developed a user-friendly product presentation for caregivers and healthcare providers (HCP). This poster presentation aims to document the development process and offers a model for future health communication tools. We employed an iterative design process which included document reviews, key stakeholder interviews, engagement with a graphic designer, and pre-testing design concepts among target users in India and Kenya through focus groups and usability testing. Though educational resources for pneumonia treatment are available in some countries, their content is incomplete and inconsistent with global recommendations. Document reviews and stakeholder interviews identified the critical information to convey to caregivers and recommendations for how to best differentiate between treatment regimens. Target users in India and Kenya confirmed the need to support better treatment adherence, recommended specific modifications to design concepts, and suggested the development of a companion job aid. There was a consensus among caregivers and HCP that these tools would be helpful and improve adherence behaviors. The output of this process is a user-friendly product presentation and job aid for amoxicillin DT that will be evaluated in a health care setting in Bangladesh. The development of new pictogram instructions for medications for low-literacy populations is a critically important but time and resource intensive process that should involve engagement with target audiences.

1127

METHODS OF A DOUBLE-BLIND RANDOMIZED CONTROLLED CLINICAL TRIAL OF THREE VERSUS FIVE DAYS AMOXICILLIN DISPERSIBLE TABLETS FOR CHEST-INDRAWING PNEUMONIA AMONG CHILDREN 2-59 MONTHS OF AGE PRESENTING TO KAMUZU CENTRAL HOSPITAL IN LILONGWE, MALAWI

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Pneumonia is the leading cause of mortality from infection in under-5 children. World Health Organization's (WHO) Integrated Management of Childhood Illnesses (IMCI) guidelines recommend 5 days of twice-daily oral amoxicillin for children with chest-indrawing pneumonia. Limited data suggest 3 days of therapy, the standard of care for fast-breathing pneumonia, may be sufficient for chest-indrawing pneumonia as well. A shorter regimen could simplify prescriptions, enhance treatment adherence and reduce health system costs. To generate evidence for shorter therapy, we are initiating a double-blind randomized non-inferiority trial to compare 3 vs 5 days of amoxicillin dispersible tablets (DT) for children 2-59 months old with chest-indrawing pneumonia from an outpatient clinic in an endemic malaria region of Malawi. This is the first known study of this research question. Children will be excluded if they have WHO-defined severe pneumonia, HIV-seropositivity/exposure, severe acute malnutrition, possible tuberculosis, severe anemia, severe malaria, unresolved wheeze, or a non-pneumonia illness requiring antibiotics. Children will be randomized to receive 3 days of amoxicillin DT plus 2 days of placebo or 5 days of amoxicillin DT. Upon enrollment children will be hospitalized 48 hours for observation with follow-up on days 2, 4, 6 and 14. The primary endpoint is the proportion of children failing treatment before or on day 6, defined as: WHO danger signs, hypoxia, fever, vomiting ≥3 doses, antibiotic regimen change, prolonged hospitalization/readmission or death. A total of 2,000 children will be enrolled. We assume a treatment failure rate of 10% in the control arm and an absolute 4% non-inferiority margin. In the final analysis, there is 97.2% power to rule out a 4% increase in failure rate, even if the true difference is a 1.3% increase. We will present descriptive statistics of the enrolled study population to date and reasons for exclusion. At the time of presentation, the trial will be in progress, with an anticipated end date in January 2017. This is a novel investigation that has the potential to improve childhood pneumonia treatment.

1128

RARE CASE OF MEDIASTINAL TUBERCULOSIS

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A 27 year old Burmese female presented with recurrent fevers for greater than 2 months. Upon immigrating to the United States 4 years ago her tuberculin skin test (TST) was negative. She had a previous admission 1 month prior for fevers, and at that time a computed tomography (CT) scan of the chest revealed a large anterior mediastinal mass and lytic/sclerotic lesion of the manubrium. A CT-guided biopsy of the mass had chronic necrotizing granulomatous inflammation. BAL and mediastinal biopsy stains for fungi and AFB were negative. An HIV test was negative. This admission she had repeat imagining which had a new moderate right pleural effusion. Pleural fluid studies including culture and stains for bacterial, fungal and AFB pathogen were negative. TB was high on the differential and she was empirically started on 4 drug therapy. An Interferon γ release assay (IGRA) was elevated at 9.16 (normal <0.34) as were ESR and CRP. During this work up her prior mediastinal biopsy culture had growth at 3.5 weeks and TB DNA probe by PCR was positive. She had significant improvement with resolution of fevers within a week of initiating TB therapy. Mediastinal TB is rare, is decreased with increasing age, is more common in high incidence areas and is uncommon in immunocompetent hosts. Tissue culture is essential and remains the diagnostic gold standard with a sensitivity >90%. While TB PCR probes can be used on culture negative specimens, they have lower sensitivity than culture. It is essential in patients with suspected active TB disease to initiate treatment while awaiting results.

1129

MOLECULAR CHARACTERIZATION OF THE SPIKE GENE OF THE MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS ISOLATED IN EGYPT IN 2014

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The Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in a patient in Saudi Arabia (KSA) in 2012 and has since been detected across the Middle East, Europe and the USA. Egypt reported its first case in late April, 2014 from an adult patient who had been in

contact with a previously-confirmed case of MERS-CoV in Jeddah, KSA. We sequenced the spike (S) gene of the virus to investigate genetic changes indicative of positive strain selection or altered virulence. Sequencing of the S gene was performed on nucleic acid extracted directly from a PCR-confirmed sputum sample. Six overlapping DNA fragments covering the entire S gene were generated using specific primer sets. Sanger sequencing was performed. The generated sequence (3500 nucleotides) was aligned with other MERS-CoV sequences from GenBank, and phylogenetic analysis conducted. The S gene showed 99.9% nucleotide similarity to MERS-CoV samples isolated from a health care worker who resided in KSA (GenBank accession number: KJ829365), and a camel in KSA (KJ713296). The Egyptian MERS-CoV case clustered within the Riyadh_3 group in genetic clade B, forming a distinctive lineage with other MERS-CoV cases collected in the period of April-May 2014 from individuals who visited Jeddah, and traveled to Florida, USA and Greece. The S gene of the MERS-CoV from Egypt showed seven nucleotide differences from the prototype strain, resulting in two amino acid mutations; Q1020R in the heptad repeat region of the S protein which is present in many sequences of clade B viruses and Q833R. The close similarity between the S gene of the Egyptian case and that found in the camel indicates phylogenetic relatedness. The sustained presence of the Q1020R mutation is suggestive of a positive strain selection, concordant with previous report that arginine substitution in this position provides a potential endosomal protease cleavage site which may influence the membrane fusion activity of the S protein.

1130

CLINICAL AND ECONOMIC BURDEN OF ADULT HOSPITALIZATIONS OF DRIFTED INFLUENZA A (H3N2) VIRUS 2014-2015

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Global surveillance of influenza strains help to make recommendation for Northern Hemisphere influenza vaccine composition. Morbidity and mortality is higher when influenza viruses have antigenically drifted from vaccine strain, as noticed in 2014-15 flu season. The study enumerates the clinical and economic impact of laboratory-confirmed influenza hospitalizations of adult patient subset in a community hospital with tertiary care. Patients who tested positive for influenza A in rapid flu PCR test were studied by medical chart abstraction. Clinical spectrum was characterized with ICD-9-CM codes:Influenza with pneumonia (487.0), Influenza NOS, (487.1), Influenza with gastrointestinal symptoms(487.8); Encephalopathy due to influenza (487.8), acute respiratory failure(518.81), cardiovascular symptoms, (785) rapid heart rate, hypotension, shock. Data including, age, length of stay, underlying medical conditions, total cost of inpatient stay with imaging, medications, room and board were obtained for each patient, and was tabulated weekly for November 2014 to February 2015, A total of 218 adult hospitalized patients tested positive for influenza during this period. Influenza activity peaked in third week of December 2014(week 51),majority were adults aged 65-85years. Average length of stay was 5 days. Average inpatient cost ranged from 14,000USD for uncomplicated flu cases to 66,000USD in patients with respiratory failure. Most common underlying medical condition for this patient sample was chronic pulmonary disease, followed by cardiac diseases and diabetes mellitus.23% of adults had no underlying medical conditions to place them at risk of complications. Largest subset in clinical spectrum was Influenza with upper respiratory sympotms, followed by flu cases with respiratory failure.Six patients died of influenza complications. Effectiveness of vaccine is reduced with drifted viruses, hence in cases of clinical suspicion, anti-viral agents should be instituted early even before laboratory confirmation.

1131

CORRELATION BETWEEN LOW TEMPERATURE AND ACUTE RESPIRATORY ILLNESS IN IQUITOS, PERU

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Acute respiratory illness (ARI) is a major cause of morbidity and mortality worldwide. In Peru, ARI incidence in the Loreto Region typically exceeds the national annual average incidence. In 2013, the Ministry of Health reported 139,278 cases of respiratory infection in Iquitos, from which 104,126 and 35,152 were upperand lower respiratory tract infections, respectively. It is generally believed by clinicians in Loreto that low temperatures are closely followed by increases in ARIs. To test this perception, we evaluated minimum temperature and clinical data between January and December of 2013 in Iquitos, the largest city in Loreto. Low temperatures, as defined as temperatures below 22°C, occurred in January, April, July, and August of that year. Apparent increases in ARI incidences were observed after these episodes. Data from the U.S. Naval Medical Research Unit No.6's respiratory virus surveillance programs was used to identify the etiologies of circulating viruses and the number of cases. Correlation between low temperature in one week and ARI incidence in the following week was estimated using Spearman's rank correlation coefficient (rho). Weak correlations with prior low temperatures were found for upper acute respiratory disease (rho=0.19, p=0.17), pneumonia (rho=0.14, p=0.3) or Influenza A (rho=0.3,p=0.03). Our results do not support the perception of a close temporal relationship between low temperatures and increases in ARI cases. A better understanding of the impact of additional climate variables, such as rainfall and humidity, has the potential to improve clinical perception of ARI in Iquitos.

1132

SURVEILLANCE AND CLINICAL CHARACTERIZATION OF RESPIRATORY INFECTIONS IN WESTERN OF CAMBODIAN TO IMPROVE COMPLIANCE WITH INTERNATIONAL HEALTH REPORTING REQUIREMENTS (IHR)

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Information on respiratory viruses in Western Cambodia is limited, yet these are a leading cause of morbidity and mortality worldwide. Co-infection of viral and bacterial respiratory infections may worsen the severity of illness and increase mortality. We established heightened clinical and laboratory surveillance measures to characterize viral and bacterial co-infections of respiratory tract from influenza-like illness (ILI) patients in Cambodia. A total of 108 nasopharyngeal swabs collected from ILI patients with respiratory symptoms from October 2014 - March 2015 were examined for the presence of influenza A and B virus, non-influenza respiratory viruses and bacterial infections. Positive influenza A virus samples were further subtyped by multiplexed real time PCR. Overall, 25% of subjects (27/108) were positive for influenza virus, with nearly all (26 of 27) of the influenza A/H3N2 subtype, and one case of pandemic A/H1N1 (0.9%). Peak influenza activity occurred in November when 17 of 44 samples collected that month were flu positive. Non-influenza

respiratory tract viruses were detected in 46 subjects with Respiratory Syncytial virus A/B (15%), Rhinovirus (8%) and Adenovirus (5%) the top 3. Bacterial infections were detected in 15 subjects: ***Streptococcus pneumoniae*** (10%), ***Staphylococcus aureus*** (3%), ***Bordetella*** (0.9%). 12% (13/ 108) had infection with more than one virus, and 10% (11/108) had bacterial coinfection. While Influenza A/H3N2 was the most common respiratory pathogen detected in ILI patients in Western Cambodia, 43% had non-influenza etiologies. Viral-bacterial co-infections were identified rarely. Field-expedient multiplexed RT-PCR allowed rapid identification of respiratory pathogen etiologies to guide public health interventions in this previously underserved and under-described area of Cambodia.

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DETECTION AND MOLECULAR CHARACTERIZATION OF HUMAN RHINOVIRUSES IN SEVERE ACUTE RESPIRATORY INFECTIONS IN CAIRO, EGYPT

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Human Rhinoviruses (HRVs) are small, non-enveloped, single-stranded, positive-sense RNA virions which belong to genus Enterovirus and the family Picornaviridae. HRVs are classified into three species HRV-A, -B and -C. In addition to upper respiratory tract infections, recent studies have linked HRV, particularly HRV-C, to severe lower respiratory tract illnesses including pneumonia and chronic obstructive pulmonary disease. Little is known about the circulating types of HRV and their role in respiratory illnesses in Africa and the Middle East, specifically Egypt. In this study we aim to investigate the comparative proportion of severe acute respiratory illness (SARI) cases caused by Rhinovirus and the major species/types involved. To elucidate this we used 1,185 nasopharyngeal/oropharyngeal swab samples collected from SARI patients admitted to two fever hospitals in Cairo, in 2010. Screening samples against HRV and other respiratory viral targets, including respiratory syncytial virus, human metapneumovirus, adenovirus, parainfluenza viruses 1, 2 and 3, influenza B and influenza A and subtypes, by real time polymerase chain reaction resulted in the detection of HRV in 182 (15.4%) samples. Of them, 130 were HRV sole infection and 52 were co-infection with the aforementioned viral targets. Sequencing and phylogenetic analysis of the capsid viral protiens, VP4/VP2, of HRV positive samples revealed the presence of 91 (50%) HRV-A, 20 (11%) HRV-B and 56 (30.8%) HRV- C, which included 36, 7 and 29 types, respectively. A total of 15(8.2%) samples were untypable. All samples were genotypically related with known reference strains, except two HRV-A samples which constituted a novel provisional type. This is the first study in Egypt to demonstrate the genetic diversity found within the circulating Rhinoviruses. HRV-A and HRV-C were more prevalent causes of SARI than HRV-B. Future studies are needed to assess the impact and disease burden of each type.

1134

USE OF PRIMARY HUMAN INTESTINAL EPITHELIUM CULTURES (ENTEROIDS) TO TEST ROLE OF LEPTIN SIGNALING IN REG1-MEDIATED PROTECTION FROM *ENTAMOEBA HISTOLYTICA*

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Regenerating protein 1 (Reg1) and the "satiety hormone" leptin have both been shown to be protective against infection by ***Entamoeba histolytica.*** However, a possible link between these two proteins in mediating resistance to amebiasis has not yet been investigated. We hypothesize that leptin signaling in the gut is protective by inducing Reg1 expression through the STAT3 signaling pathway. Reg1 is expressed during inflammatory conditions in human pancreatic β cells, duodenal Paneth cells, and immature columnar cells of human intestinal crypts, where it promotes cellular proliferation and survival, and is a useful biomarker for detecting gastrointestinal and systemic inflammation. In addition to protecting against ***E. histolytica*** induced killing of intestinal epithelial cells ***in vitro***, Reg1 was found to be the most highly upregulated gene in human colon biopsy samples during ***E. histolytica*** infection. Although leptin signaling is protective against amebiasis, it was found that both humans and mice containing a common polymorphism in the leptin receptor (Q223R) are more susceptible to amebic infection by ***E. histolytica***. This increased susceptibility is due in part to impaired STAT3 signaling. To test the impact leptin has on Reg1 expression, we first generated 3-D enteroids by isolating stem-cell containing duodenal and colonic crypts from both humans and wildtype mice. The crypts were isolated with EDTA and then grown in 24 well plates in Matrigel with media containing Wnt3A, noggin, R-spondin 1, and EGF (among other growth factors) to stimulate the proliferation of the intestinal stem cells. Enteroid formation was observed by light microscopy a few days later. To further test whether the leptin-mediated protection from amebiasis is due to Reg1 expression, we will create enteroids from Reg1 knockout mice, as well as enteroids from Q223 and R223 leptin receptor mice, treat with leptin, and observe Reg1 expression, cellular proliferation, and resistance to killing by ***E.histolytica.*** Successful completion of these studies will further delineate the relationship between leptin and Reg1 mediated resistance in amebic colitis.

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THE ROLE OF AMOEBAPORES AND AMOEBIC LYSOSOMES IN AMOEBIC TROGOCYTOSIS AND CELL KILLING

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Entamoeba histolytica is a protozoan parasite that causes amebiasis, and is an important contributor to the global problem of diarrheal disease. ***E. histolytica*** has the potent ability to kill a variety of host cell types, and tissue destruction is the hallmark of invasive ***E. histolytica*** infection, however, little is known about the mechanisms responsible for these two processes. We have recently discovered that ***E. histolytica*** kills by ingesting fragments of live host cells, which we have termed amoebic trogocytosis. Our data suggest that amoebic trogocytosis is the primary mechanism of cell killing and that amoebae must continue to ingest multiple host cell fragments before the host cell dies. Elevated amoebic lysosomal pH has been shown to decrease cytotoxicity. Pharmacological inhibitors of lysosomal acidification were used to perturb lysosomal function. The rate of trogocytosis and host cell killing, as well as the rate of acidification and turnover of ingested host material were determined using imaging flow cytometry. We found that inhibiting lysosomal acidification significantly decreases ingestion of host material and cell killing, indicating a crucial role of amoebic lysosomes in amoebic trogocytosis. In addition, we are re-examining the role of amoebapores, a family of pore-forming proteins, in cell killing. Amoebapores were long believed to act as secreted effectors. However, based on findings that amoebapores localize to lysosomes and require pH ~5.2 for pore-forming activity, we are investigating the role of amoebapores in the degradation of ingested host fragments within the amoebic lysosome. This work will contribute to a better understanding of the nature of trogocytosis as a fundamental biological process, and may change the current thinking on the role of amoebic lysosomes and amoebapores in cell killing.

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AMEBIASIS IN THE FIRST TWO YEARS OF LIFE: NATURAL HISTORY OF AMEBIASIS IN BANGLADESHI CHILDREN

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Annually one million children die before their fifth birthday from diarrhea. Previous population based surveys of pediatric diarrheal disease identified the protozoan parasite ***Entamoeba histolytica***, the etiological agent of amebiasis, as one of the top causes of diarrhea in the sub-Saharan Africa and South Asia. We examined the impact of this parasite on the health of disadvantaged children in an urban slum in Dhaka Bangladesh during their second year of life. This report is from an ongoing longitudinal study where ***E. histolytica*** colonization was followed by specific qPCR and immunodiagnostic assays of diarrheal and monthly surveillance stools collected during every-other-day home visits. Development of mucosal adaptive immunity was determined by a sIgA ELISA assay and parasite burden and the expansion of ***P. copri*** (an indicator of a pro-inflammatory microbiome) were measured by qPCR. Approximately 80% of infants in the study were infected with ***E. histolytica*** by the end of the second year of life. Consistent with previous results, the development of a fecal sIgA response was correlated with some protection from infection. In contrast to the results obtained in the first year of life, parasite burden in older children was not associated with symptomatic disease but was associated with an expansion in ***P. copri*** levels. Our findings suggest that mucosal defense has an important role in the short-term protection against parasite colonization in endemic regions and that the microbiome could play a significant role in the development of amebiasis in the 1 to 2 year old age group.

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GIARDIA DUODENALIS: CHARACTERIZATION OF A ZOONOTIC STRAIN ISOLATED IN DOG BY PROTEOMICS AND BIOLOGICAL CRITERIA

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Giardiasis is a neglected disease that affects more than 200,000,000 people in the world, mainly children in developing countries.. Symptoms include abdominal pain, diarrhea, steatorrhea and malabsorption syndrome. Giardiasis is considered a zoonotic disease and transmission may occur from animals, including pets, to humans. Canine strains are far less characterized than human isolates of ***Giardia duodenalis.*** In this study, a novel a strain (BHFC1), isolated from a symptomatic dog, was characterized through growth aspects, infectivity for Swiss mice, phylogenetic and ultra-structural analysis, encystment profile, reactivity with canine and human sera, proteomic map and presence of viral genome. The results revealed that BHFC1 is more infective for Swiss mice and grows faster than the human strain Portland-1. Electron microscopy revealed that the flange of the canine strain is larger and the encystment process is slower or incomplete, at 48 hours of growth, when compared to Portland-1. Antibodies in human sera from patients with giardiasis, reacted more strongly with proteins of the canine than the human strain, suggesting a zoonotic potencial for BHCFC1 strain. The proteomic map of soluble and insoluble fractions led to the identification of 212 know and 28 hypothetical proteins of ***G. duodenalis***. Some of these proteins correlated with virulence and pathogenic mechanisms. No amplification of viral genome was detected, suggesting absence of Giardiavirus. These data provide an important source of information for future studies correlating various aspects of the biology of this parasite, such as virulence, protein interaction, metabolomics, ultrastructural characterization, among others. In addition, this well characterized ***G. duodenalis*** strain, virulent in experimental model becomes an useful tool for investigations of antigenicity, immunogenicity, drug resistance and the development of vaccines, diagnosis and effective treatment.

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TOLERANCE FACTORS FOR THE ACQUISITION OF *MYCOBACTERIUM ULCERANS* BY WATER BUGS IN AFRICA ARE ASSOCIATED WITH THEIR ECOLOGICAL TRAITS

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Belostomatidae and Naucoridae families of aquatic Hemiptera which are voracious predators of micro and macro aquatic organisms, may host ***Mycobacterium ulcerans*** the causative agent of Buruli ulcer and transmit them through bites to human. We tested the existence of a correlated evolution between some water bugs' ecological traits and their tolerance to acquire ***M. ulcerans*** using a comparative analysis taking into account the phylogenetic relationships between the different aquatic bugs taxa. These phylogenetic relationships were reconstructed using 171 sequences of the mitochondrial gene coding for the first subunit of cytochrome oxidase (COI). Analyses were performed at water bugs' family level using the BayesTraits program to test first correlated evolution between several ecological traits of water bugs and tolerance to ***M. ulcerans*** acquisition, and secondly to assess the likelihood values of directional and transition rates of MU acquisition with significant Hemiptera ecological traits from the previous analysis. We observed correlated evolution between some ecological traits and tolerance to ***M. ulcerans*** acquisition. Assessing of the likelihood values of directional and transition rates shows that feeding on macro-preys and living near the bottom of water column may facilitate the acquisition of ***M. ulcerans***. However, living on aquatic vegetation does not appear to directly relate to ***M. ulcerans*** acquisition by some water bugs taxa thus challenging the hypothesis of a direct transmission through biofilms developing on the aquatic vegetation. These new findings tend to confirm the possibility of a ***M. ulcerans*** acquisition by some water bugs through their trophic habits with a primary contamination happening at the bottom of the water column where these bacilli might naturally persist in aquatic ecosystems.

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SEROPREVALENCE OF FIVE PARASITIC PATHOGENS IN PREGNANT WOMEN FROM 10 CARIBBEAN COUNTRIES

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Parasitic infections cause considerable suffering, economic losses, and mortality in both developing and developed countries. Epidemiological studies of several important parasitic infections in the Caribbean region to date have been very limited. The objective of this study was to examine the seroprevalence of five parasitic pathogens in pregnant women residing in 10 English-speaking Caribbean countries. From 2009 to 2011, a total of 435 serum samples were collected and separately tested for the presence of IgG antibodies to ***Ascaris lumbricoides***, ***Entamoeba***

histolytica, *Giardia lamblia*, *Schistosoma mansoni,* and *Toxocara canis*. In all tested countries, the seroprevalence values were significantly different among the parasites studied (p<0.001). The most prevalent parasites were *G. lamblia* (40.5%) and *A. lumbricoides* (37.9%), followed by *T. canis* (14.5%), *E. histolytica* (6.7%) and *S. mansoni* (3.0%). *G. lamblia* and *A. lumbricoides* were prevalent in all tested countries, and were the only two parasites positively reported in Bermuda. A comparison of serology results between countries within each parasite showed that with the exception of *G. lamblia* (p=0.09), seroprevalence were significantly different among tested countries for the other four parasites (p<0.05). While overall seroprevalence for *E. histolytica* was 6.7%, 32.6% of participants from Jamaica were serum positive to this parasite. Furthermore, a significant association between *T. canis* and *A. lumbricoides* infections was discovered (Odds Ratio = 13.7, 95% Confidence Interval = 5.4 – 34.4). This study confirms that parasitic infections are occurring in prenatal women in the Caribbean and that the predominant parasites are different among tested countries. Further, this study also highlights the necessity of enhancing existing surveillance programs to evaluate the public health burden of parasitic diseases in the Caribbean.

1140

MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM* AND ZOONOTIC TRANSMISSION OF CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS IN ETHIOPIA

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Cryptosporidiosis is an important cause for chronic diarrhea and death in HIV/AIDS patients. Among common *Cryptosporidium* species in humans, *C. parvum* is responsible for most zoonotic infections in industrialized nations. Nevertheless, the clinical significance of *C. parvum* and role of zoonotic transmission in cryptosporidiosis epidemiology in developing countries remain unclear. In this cross-sectional study, 520 HIV/AIDS patients were examined for *Cryptosporidium* presence in stool samples using genotyping and subtyping techniques. Altogether, 140 (26.9%) patients were positive for *Cryptosporidium* spp. by PCR-RFLP analysis of the small subunit rRNA gene, belonging to *C. parvum* (92 patients), *C. hominis* (25 patients), *C. viatorum* (10 patients), *C. felis* (5 patients), *C. meleagridis* (3 patients), *C. canis* (2 patients), *C. xiaoi* (2 patients), and mixture of *C. parvum* and *C. hominis* (1 patient). Sequence analyses of the 60 kDa glycoprotein gene revealed a high genetic diversity within the 82 *C. parvum* and 19 *C. hominis* specimens subtyped, including *C. parvum* zoonotic subtype families IIa (71) and IId (5) and anthroponotic subtype families IIc (2), IIb (1), IIe (1) and If-like (2), and *C. hominis* subtype families Id (13), Ie (5), and Ib (1). Overall, *Cryptosporidium* infection was associated with the occurrence of diarrhea and vomiting. Diarrhea was attributable mostly to *C. parvum* subtype family IIa and *C. hominis*, whereas vomiting was largely attributable to *C. hominis* and rare *Cryptosporidium* species. Calf contact was identified as a significant risk factor for infection with *Cryptosporidium* spp., especially *C. parvum* subtype family IIa. Results of the study indicate that *C. parvum* is a major cause of cryptosporidiosis in HIV-positive patients and zoonotic transmission is important in cryptosporidiosis epidemiology in Ethiopia. In addition, they confirm that different *Cryptosporidium* species and subtypes are linked to different clinical manifestations.

1141

TOWARDS SOLVING THE MYSTERY OF HOW CHILDREN ACQUIRE *CRYPTOSPORIDIUM* IN AFRICAN HOUSEHOLDS

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Recently *Cryptosporidium* emerged as the second most frequent cause of moderate-to-severe diarrhea in children under two in sub-Saharan Africa and Asia; only slightly behind rotavirus in its frequency. *Cryptosporidium* is also highly associated with mortality in this population. Yet, despite its impact, the source of transmission of *Cryptosporidium* to these vulnerable children is unclear. Previous studies have found that zoonotic transmission is rare in Africa and contaminated water also does not appear to be a primary route of transmission in the resource-limited setting. We are currently enrolling child-caregiver pairs in Western Kenya to test the hypothesis that adults are a reservoir for *Cryptosporidium* and that transmission between caregiver and child occurs frequently. Stool samples from children with acute diarrhea and their caregivers are being tested for *Cryptosporidium* using microscopy, an enzyme-linked immune assay, and PCR. Furthermore, we are genotyping all cases of *Cryptosporidium* to test the hypothesis that more children with acute diarrhea due to *Cryptosporidium* will have a caregiver with a genetically matched isolate than expected by chance alone. Additionally, clinical and household correlates of genetically matched isolates from child and caregiver will be assessed. If we find evidence of household *Cryptosporidium* transmission, then interventions for reducing the morbidity and mortality attributable to this parasite will need to also address infections in household members.

1142

STANDARDIZATION OF SOLID-PHASE FLUORESCENT ASSAY FOR THE SIMULTANEOUS DETECTION OF SPECIFIC ANTI-*TOXOPLASMA GONDII* IGG, IGM AND IGA ANTIBODIES FOR TOXOPLASMOSIS ANTENATAL CARE SCREENING

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Toxoplasma gondii infections are very common, causing treatable central nervous system and eye diseases on the foetus, and pregnant woman must be screened for efficient therapy. Due to lowering toxoplasmosis prevalence in children, an expected high proportion of toxoplasmosis seronegative women would imply costly follow-up additional assays, demanding the development of new, quick and inexpensive assays. New solid-phase fluorescent assays allows direct antibody quantification in microplates as we demonstrated in a previous study the high efficiency, low cost and high throughput of single-well anti-*T. gondii* IgG and IgM detection due to improved fluorescent conjugates. In this work, we standardized a simultaneous detection of IgG, IgM and IgA specific antibodies in solid-phase fluorescent assay for toxoplasmosis antenatal care screening. Affinity-purified conjugates were prepared with different amine-reactive Alexa Fluor® fluorescent dyes and tested in single-well IgG/IgM/IgA detection. We used 34 serum samples from adult volunteers at a large public hospital with external and in house isolated and conjunct anti-*T. gondii* immunoglobulin detection. There are excellent agreement (Kappa index=100%) between isolated and conjunct detection without false-positives and false-negatives results. These initial results suggest that solid-phase fluorescent simultaneous detection of IgG, IgM and IgA could be a promising high throughput serological technique for toxoplasmosis screening. New tests will be perform to evaluate the efficiency of simultaneous fluorescent detection in several numbers of clinical serum samples and then compare with conventional screening immunoassays for validation. The development of new integrated diagnostic approaches should be directed to elimination of interferences found in the current

methods, reducing the number of both: tests and trained staff in multiple techniques, allowing rapid and efficient serological screening for safe patient treatment.

1143

REACTIVATION OF CHRONIC *TRYPANOSOMA CRUZI* INFECTION IN PATIENTS WITH HIV/AIDS

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This study was a retrospective study based on data collected from medical records of patients hospitalized at Infectious Diseases FJ Muñiz Hospital, Buenos Aires, Argentina from 1992 to 2014. Chagas disease reactivation was defined as demonstration of trypomastigotes on cerebrospinal fluid (CSF), blood, pericardial fluid or amastigotes in tissues biopsies in patients with HIV/AIDS. Twenty three patients were included. Twenty-one (91%) patients were male. The media of age was 38 years old. Seven (30%) did not know their HIV status before hospitalization.The media of CD4+ T cell counts was 90 cell/ml (range 1-501) Twenty-one patients were tested for ***Trypanosoma cruzi*** serology: 16 (76%) were reactive, 4(19%) were non-reactive, and 1(5%) was discordant. One patient had vertical transmission as the only risk for both infections. Ten (43%) patients were intravenous drugs users (IDUs) and it was the only risk for Chagas disease in 4 of them. Chagas disease reactivation involved central nervous system (CNS) in 22 patients (18 with cerebral abscesses and 4 with diffuse meningoencephalitis).Two patients had also pericardial effusion. One patient showed fever with trypomastigotes on peripheral blood without CNS damage. The CNS compromise was confirmed by the detection of trypomastigotes in CSF in 17 patients, positive strout in 1 patient, amastigotes in brain biopsy in 1 patient and necropsy in 3 . All patients were treated with benznidazole, 3 had to change to nifurtimox due to adverse drug reactions. Four patients (17%) survived more than one year. One of them died after 8 years due to cirrhosis. The other 3 patients are alive after 8, 5 and 4 years of follow up under antiretroviral treatment compliance. Three out of these 4 received secondary prophylaxis for ***T. cruzi***. In conclusion, Chagas disease reactivation must be considered in the differential diagnosis in HIV/AIDS patients. A correct diagnosis based on epidemiological, clinical and microbiological studies is crucial for this neglected parasitic infection.

1144

PREVALENCE OF PARASITIC INFECTIONS AMONG IMMIGRANTS IN CHICAGO

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In 2013, approximately 41.3 million persons living in the US were immigrants, many of whom migrated from countries where parasitic infections are endemic and highly prevalent. Although extensive testing for infectious diseases is typically performed on refugees, these tests are not provided to immigrant populations, many of whom share similar risk factors and exposure histories as those in the refugee population. In order to assess the prevalence of parasitic infections among the immigrant population in Chicago, we recruited asymptomatic subjects who had moved to the United States within the last five years. Subjects were asked a symptom questionnaire and provided blood and stool samples. Samples were tested for: serum IgE level, complete blood count with differential (CBCD), and ova and parasite exam, with plans to perform polymerase chain reaction (PCR) testing on stool samples for enteric parasites and serologic testing for Chagas disease, neurocysticercosis, toxocara, schistosomiasis, and strongyloides. To date, 40 subjects have been recruited. Enrolled subjects had a mean age of 31 years; 18 of the 40 subjects were female (45%, 22/40 were male, 55%). Subjects had immigrated from Central and South America (13/40, 32.5%), Asia (12/40, 30%), India (10/40, 25%), the Middle East (3/40, 7.5%), and Africa (2/40, 5%). 10/40 (25%) patients were found to have elevated IgE (GM value of those with elevated IgE: 571 IU/mL), while 4/40 (10%) had an elevated absolute eosinophil count (AEC) (GM value of those with elevated AEC: 616 cell/mcL). To-date, one subject (1/40, 2.5%) has been found to have a parasitic infection. The positive subject was a 24 year-old asymptomatic female from Brazil discovered to have ***Giardia lamblia*** cysts and trophozoites on stool O&P; this subject had an elevated IgE level (567 IU/mL), but a normal AEC (100 cell/mcL). Based on our preliminary data, we believe that parasitic infections likely represent a large and as-yet-unidentified burden of disease in the immigrant population. The planned PCR and serologic testing will add additional information about these important health problems affecting our immigrant populations.

1145

ANTI-*TOXOPLASMA GONDII* IGG DETECTION IN SALIVA FOR TOXOPLASMOSIS EPIDEMIOLOGICAL STUDIES

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Worldwide distributed zoonosis affecting about one billion people, toxoplasmosis is mostly asymptomatic, despite ocular disease and severe and lethal disease in fetuses, AIDS patients and transplant recipients. Serology is the main approach for diagnosis allowing prevalence studies but incidence determination is a difficult in most countries. Children are the target in incidence studies based on conventional serology but venipuncture for this age group is an difficult approach. An alternative source of antibodies, non-invasive saliva collection is acceptable for children and it contains small amounts of IgG from mucosal and gingival crevicular fluid. Available commercial antibody detection kits are focused in serum samples, inadequate to alternative biological material, like saliva, due to technical constrains. Here, we standardized immunoassays with high sensitivity for detection of anti-***Toxoplasma gondii*** IgG in paired saliva and serum sample from 20 adult volunteers, which allows both high sensitivity DOT-ELISA and an automated prone IgG capture assay, based on staphylococcal protein A in solid support. The sensitivity and specificity of the saliva DOT-ELISA were similar to sera ELISA. We also tested 100 saliva samples from university graduates in all assays, showing 19% (95%CI 12-28%) frequency of toxoplasmosis in this group, lower than usual prevalence 50% adult prevalence reported for our area. Protein A IgG capture saliva assay was also efficient with similar results, with perfect agreement and high kappa index. Both the DOT-ELISA and protein A capture ELISA for detection of IgG anti-***T. gondii*** in saliva are very promising as tools for Toxoplasmosis incidence studies with cheap, inexpensive and non-invasive collection; high sensitivity, high specificity, low cost and ease of operation in the routine laboratory. The determination of incidence will help health services in the prevention of toxoplasmosis transmission and on-time adequate measures; especially for antenatal care. Immunoassay with saliva IgG for toxoplasmosis is a very promising tool for use for the epidemiology of toxoplasmosis in children or other protected groups.

1146

COMPARISON OF THREE METHODS TO CONCENTRATE *CYCLOSPORA* AND *CRYPTOSPORIDIUM* FROM LARGE VOLUMES OF VEGETABLE WASH WATER

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Foodborne parasites have been isolated in fresh produce worldwide including the U.S. Environmental testing to identify the presence of these parasites has been challenging during outbreak investigations. Produce is usually triple washed and sanitizers are included during these

rinses before packaging. The purpose of this study was to evaluate three methods to concentrate and detect ***Cryptosporidium parvum*** and ***Cyclospora cayetanensis*** from water used for washing and processing vegetables. ***C. cayetanensis*** and ***C. parvum*** oocysts. Tap water, chlorine and peroxyacetic acid based vegetable rinses were used. The water was prepared shredding carrots to reach turbidities of 30 and 60 NTU and shredding red cabbage at 30 NTU. Water was spiked using 10 or 50 oocysts per 10 L and analyzed using three protocols: the EPA 1623 method 2) by flocculation and 3) by filtration using hollow-fiber filter. Filtrates and sediments were tested for the presence of the parasites by nested PCR, immunofluorescence, and autoflorescence. Flocculation was highly effective at concentrating oocysts when using tap and chlorinated rinse water with both spikes (10 and 50 oocysts). The PPA water did not flocculate. Envirocheck and hollow fiber filters could concentrate oocysts, however Envirocheck filters were not easy to work with and the water filtration and processing was longer that the hollow-fiber filters. More samples could be detected in hollow-fiber filtered samples than the Envirocheck filters even at high turbidity levels and low inocula. Results suggest that hollow-fiber filters are effective at concentrating oocysts from all of the rinse waters tested in this study. This system could be implemented for surveillance studies and outbreak investigations particularly in fresh produce rinse water.

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DEVELOPMENT OF BOILING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR SENSITIVE AND RAPID DETECTION OF *TOXOPLASMA GONDII*

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Toxoplasma gondii infections are prevalent in human and warm blooded animals. Felids, including cats, are the natural hosts for this parasite because they can excrete the oocyst into the environment. This study developed a modified loop-mediated isothermal amplification (LAMP) assay for DNA extracted using a simple boiling method. The assay targeted the B1 gene for the sensitive and rapid detection of ***T. gondii*** in experimentally infected cats. The optimal B1-LAMP conditions were a temperature of 60°C and an operating time of 30 min. The B1-LAMP assay was at least 100-fold more sensitive than conventional PCR for detecting ***T. gondii***. The specificity of the ***T. gondii*** B1-LAMP assay was illustrated by the finding that it did not detect genomic DNA from ***Neospora caninum***. The B1-LAMP assay was used to detect DNA extracted from the organs of experimentally infected cats. In addition, the assay yielded positive results when used to test ***T. gondii*** DNA extracted using a crude boiling method. In addition, a positive or negative result of the LAMP products could be clearly visualized by the naked eye using a fluorescent dye (SYBR green or GeneFinder). The advantages of this assay include simplicity, a short analysis time, and low-cost, making it suitable for field laboratory applications.

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COPROLOGICAL DIAGNOSIS OF *BLASTOCYSTIS* SPP. IN HUMANS AND DOGS FROM FIVE BRAZILIAN BIOMES

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Blastocystosis is a parasitic infection caused by the protozoan ***Blastocystis*** spp., and although it has been known for almost a century there are considerable gaps in our knowledge regarding its pathogenicity, transmission and distribution worldwide. This study was undertaken to evaluate the presence of ***Blastocystis*** spp. in five of the seven Brazilian biomes: Caatinga, Savannah, Pantanal, Atlantic Forest and Southern Fields in association with other intestinal parasites. Fecal samples from humans and dogs were collected in 17 municipalities of the five biomes using the spontaneous sedimentation technique for direct microscopic diagnosis. A total of 2528 samples were analyzed, 38% were positive for at least one intestinal parasite and 8.5% were positive for ***Blastocystis*** spp. There was a statistical higher correlation (P<0.005) of ***Blastocystis*** spp. with ***Entamoeba coli, Endolimax nana, Iodamoeba bustchlii***. In conclusion, this is first study in Brazil to cover so many biomes and the chi-square test indicated that the biome influences the percentage of samples positive for ***Blastocystis*** spp, as well as well as a possible zoonotic potential of this parasite in relation dog fecal samples.

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RNA INTERFERENCE COMPLEXES: A NOVEL METHOD TO GENE SILENCING IN *CRYPTOSPORIDIUM*

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The gut protozoan parasite, ***Cryptosporidium***, is a major cause of diarrheal disease worldwide. Diarrheal diseases have been identified as a principal cause for mortality in children under five years old. Due to the parasites' resistance to chemical and mechanical disruption, it has also been identified as a Category B pathogen by the CDC as a potential water source contaminant. The current treatment for cryptosporidiosis, Nitazoxanide, has only moderate efficacy in immunocompetent adults and children, and none in immunocompromised hosts. Development of an improved treatment is urgently needed to control mortality caused by this parasite. Unfortunately, a major impediment in studying potential drug targets is the inability to genetically manipulate ***Cryptosporidium***. RNA interference is a powerful tool that has been developed to study gene function. However, genomic studies determined that ***Cryptosporidium*** lacked enzymes necessary in order to process RNAi constructs. We have proposed a novel method of gene silencing in ***Cryptosporidium*** to identify targets for drug development. We can induce specific degradation of target RNA by transfecting ***Cryptosporidium*** oocysts with the human enzyme Argonaute 2 (hAgo2) preloaded with single-stranded RNA (ssRNA). We have demonstrated the specific slicer activity of hAgo2 in ***Cryptosporidium*** oocysts using this method with subsequent decrease in target protein expression after transfection. In addition, gene silencing using hAgo2-ssRNA has led to a reduction of infection ***in vitro***. Development of a novel gene silencing method in ***Cryptosporidium*** can lead to identification of key proteins which can have a major role in the sustainability of infection.

1150

ACTION OF ATOVAQUONE AND MONENSIN AGAINST *TOXOPLASMA* DEPEND ON THE MITOCHONDRIAL MUTS HOMOLOG, TGMSH1

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The phylum Apicomplexa includes obligate intracellular parasites of great medical importance such as ***Plasmodium falciparum***, ***Cryptosporidium parvum***, and ***Toxoplasma gondii***. Despite significant effort, there are no human vaccines for any of these parasites; thus, drug treatments are the mainstay for reducing morbidity and mortality. Nonetheless, resistance, toxicity and lack of activity against certain parasite life cycle stages severely limit the value of available drugs; consequently there is a dire need for new anti-apicomplexan therapeutics. To fulfill this need we have been exploiting the experimental malleability of ***T. gondii*** to identify novel targets for drug development. We recently showed that the lethal effect of the anti-coccidian monensin on ***T. gondii*** depends on the mitochondrial MutS homolog, TgMSH1. Mitochondrial MSH proteins maintain DNA stability while nuclear MSHs both repair mismatched DNA and directly signal cell cycle arrest and apoptosis in response

to genotoxicity. Interestingly, monensin induces cell cycle arrest and autophagic cell death in ***Toxoplasma*** in a TgMSH1 dependent manner. By monitoring sequence fidelity and copy number of the mitochondrial (mt), nuclear and plastid DNA we have established that TgMSH1 is specifically required for maintenance and repair of the mtDNA. Given that mutations in mitochondrially encoded genes are associated with resistance to atovaquone and myxothiazol we investigated whether TgMSH1 mutant strains were more likely to become resistant to these important anti-parasitic drugs. Through this effort we discovered that TgMSH1 mutant strains are intrinsically tolerant to both atovaquone and myxothiazol without the need for mutations in the actual target proteins. We are currently testing our hypothesis that due to the accumulation of mtDNA damage that occurs without the repair function of TgMSH1 Toxoplasma becomes adapted to the lack of efficient respiration, which leads to a tolerance to anti-mitochondrial drugs. Through our studies we have discovered a novel mechanism by which apicomplexan parasites can become resistant to atovaquone and related drugs.

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IDENTIFICATION OF *BLASTOCYSTIS* SPP. GENOTYPES IN PATIENTS AT SÃO PAULO CITY, BRAZIL

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Blastocystis spp. is an organism in the intestinal tract of humans and other animals, commonly found in stool samples. This organism involves pathogenic and zoonotic aspects unknown. Currently, this organism has been associated with intestinal diseases such as irritable bowel syndrome and clinical manifestations of cutaneous allergic hypersensitivity. This study aims to identify the genotypes of ***Blastocystis*** spp. in patients from the São Paulo, Brazil. A total of 35 stool samples from ***Blastocystis*** spp positive patients of the Section of Parasitology/Division of the Central Laboratory (HC/FMUSP) were used. After collection, stool samples were stored at -20°C for subsequent DNA extraction. Polymerase Chain Reaction assay were performed using ***Blastocystis*** specific primers targeting the small subunit rRNA gene, and sequenced. PCR positivity was of 68.6% (24/35).Three subtypes (STs) were identified: ST1 (17.6%), ST2 (11.8%) and ST3 (70.6%). Phylogenetic analysis was performed in order to identify the origin of human isolates. It was observed that all human isolates were grouped with pig isolates. This study is the first to include molecular characterization and identify the genotypes of ***Blastocystis*** in São Paulo state supporting the zoonotic potential this organism.

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IDENTIFICATION OF FIVE NOVEL *CRYPTOSPORIDIUM* VACCINE CANDIDATES USING REVERSE VACCINOLOGY

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Cryptosporidiosis, caused primarily by ***Cryptosporidium parvum*** and ***C. hominis,*** is a life-threatening disease in immune-compromised individuals worldwide, and a major cause of diarrhea-induced wasting and stunted growth often leading to deaths of young children in developing countries. While vaccination remains one of the most effective methods of preventing infectious diseases, there are no commercially available vaccines against human parasites, including ***Cryptosporidium***. Biological and technical challenges preclude the use of traditional vaccinology approaches for the identification of vaccine targets against human-infecting ***Cryptosporidium***, and the existence of genomic resources for multiple species in the genus make reverse vaccinology a viable alternative. We generated a much improved genome assembly and annotation for ***C. hominis***, increasing average gene length by 500 base pairs (bp), from 1,360 bp to 1,845 bp, bringing it in line with gene length in the close relative ***C. parvum***, which averages 1,785 bp. The improved gene set provides new opportunities to procure and examine novel vaccine candidates. Using bioinformatics approaches we identified close to 400 ***C. hominis*** genes containing properties typical of antigens, such as the presence of glycosylphosphatidylinositol (GPI)-anchor motifs, multiple transmembrane (TMM) domains or targeting to the secretory pathway. We down-selected this set by focusing on potential GPI-anchored proteins lacking human homologs, with at most two TMM domains, homologs in four other ***Cryptosporidium*** species and no polymorphism between two ***C. hominis*** isolates. Further experimental considerations included a minimal number of predicted post-translation modifications and adequate molecular weight of the predicted product. A total of 5 proteins, now undergoing experimental validation, were selected with approach from among all 3,745 proteins in the updated ***C. hominis*** annotation. We provide an online resource, which catalogs all ***C. hominis*** genes and their characteristics mentioned above, including physical attributes, properties related to antigenic potential, and expression data.

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CENTRINS OF APICOMPLEXAN PARASITE - *BABESIA MICROTI* AND THEIR ROLE IN CELL DIVISION

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Intra-erythrocytic apicomplexan protozoans belonging to the genus ***Babesia*** are of great economic and health significance globally. Human babesiosis caused by ***B. microti,*** is prevalent in many parts of United States and about 1250 clinical cases are reported annually with fatality of 5-20%. However, our knowledge on the mechanism of cell division and the molecules associated with this process in ***Babesia*** parasites is very limited. We are investigating the role of centrin, a calcium-binding, cytoskeletal protein involved in cell division in eukaryotes, in ***B. microti***. In ***Plasmodium falciparum***, centrins are associated with nucleus and involved in cell division. Using database searches, we have identified three centrin genes in ***B. microti*** (BmCen) genome (piroplasmadb.org), which we called BmCen2, BmCen3 and BmCen4, based on their homology to the centrins from ***Homo sapiens***, ***P. falciparum*** and ***Toxoplasma gondii***. BmCen2, BmCen3 and BmCen4 encode for polypeptides of 164, 176 and 156 amino acids respectively. BmCen2 and BmCen4 exhibited about 60% homology at amino acid level to the PfCen2 and PfCen4 respectively, whereas BmCen3 was about 25% homologous to PfCen3. Domain search on PROSITE revealed that ***B. microti*** centrins contain the conserved calcium binding EF-hand motifs; BmCen2 and BmCen4 have four EF-hands. Interestingly, BmCen4 has only 2 EF-hands, EF-hand I and EF-hand IV. We have expressed the ***B. microti*** centrins in ***E. coli***. Their purification, biochemical characterization, stage-specific expression and their role in cell division in ***B. microti*** are being investigated. The results of these studies will be presented.

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EVALUATION OF A RAPID POINT-OF-CARE TEST FOR DETECTION OF *CRYPTOSPORIDIUM*

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Diarrheal diseases are a major cause of morbidity and mortality in the developing world. ***Cryptosporidium*** is an enteric protozoan parasite that is a common cause of waterborne diarrheal disease. There is need for a practical point-of-care diagnostic test that is rapid, reliable and feasible for use in the field. We evaluated the performance of a new fecal antigen detection test for ***Cryptosporidium*** (***Cryptosporidium*** EZ VUE, TechLab, Inc) in a cohort of children in Bangladesh. This point-of-care test is a lateral flow dipstick that detects ***Cryptosporidium*** oocyst antigen in stool. Time from sample preparation to visual readout of test results is 10 minutes. The test was conducted on 50 diarrheal stool samples that were known to be Cryptosporidia positive by antigen detection and 50 negative diarrheal stool samples from children living in an urban slum in Dhaka. The test had a sensitivity of 100% and a specificity of 94% when compared to enzyme-linked immunosorbent assay antigen detection. We concluded that the ***Cryptosporidium*** EZ VUE dipstick test offered rapid and accurate point-of-care diagnosis of Cryptosporidiosis.

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HIGH PREVALENCE OF *BLASTOCYSTIS* SPP. AND COST-EFFECTIVENESS ANALYSIS OF THREE PARASITOLOGICAL TECHNIQUES FOR THE DIAGNOSIS OF *BLASTOCYSTIS* SPP. IN PERU

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The role of ***Blastocystis*** spp. as a pathogen is unclear to date. The aim of the present study is to compare three stool parasitological techniques [direct microscopy examination (DMS), spontaneous sedimentation in tube technique (SSTT), and Pavlova Culture (PC)] in the detection of ***Blastocystis*** spp. in stool samples in Peru. Sensitivity was calculated by considering a positive culture for ***Blastocystis*** as the gold standard. A cost-effectiveness analysis was performed in each technique by estimating direct and indirect costs. The cost of a single sample was estimated by the sum of laboratory materials and the time consumed in each technique. A total of 142 stool samples (female:male ratio = 1.7) from people living in Lima city was included in this study. Most of participants (84.51%, n= 120) were under the age of 40 years old (median: 22). More than half of the stool samples were positive for ***Blastocystis.*** Prevalence of ***Blastocystis*** was 55.6% (n=79) by DMS, 61.27% (n= 87) by SSTT and 80.99% (n= 115) by PC. The sensitivity of DMS, SSTT and PC was 68.7%, 75.65% and 100%; respectively. Specificity was 100% in all three techniques. The total cost per a single exam was 0.68$, 0.9$ and 1.2$ for DMS, SSTT and PC, respectively. The cost per case of ***Blastocystis spp.*** detection by each test was 1.22$, 1.46$ and 1.48$ for DMS, SSTT and PC, respectively. In conclusion, the SSTT is a cost-effective technique for the diagnosis of ***Blastocystis*** spp. in low- resource settings where this organism is highly prevalent in a population.

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THE SECRETED CATHEPSIN B PROTEASES ARE ASSOCIATED WITH TISSUE INVASION OF THE CARCINOGENIC LIVER FLUKE, *CLONORCHIS SINENSIS*

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In freshwater fish consumption areas, human clonorchiasis remains an important foodborne zoonosis caused by ***Clonorchis sinensis*** infection. Molecular characterizations of key molecules that are involved into pathogenic processes could speed up the interventions of ***C. sinensis*** infection. In this study, we expressed four ***C. sinensis*** cathepsin B cysteine proteases (CsCB1, CsCB2, CsCB3 and CsCB4) in X-33 yeast cells for biochemical characterization. Eukaryotic expressed CsCBs could hydrolyze substrates Z-Phe-Arg-AMC and Z-Arg-Arg-AMC, and the hydrolyzation could be completely inhibited by specific inhibitors and cysteine protease specific inhibitors at 100%. However, serine protease specific inhibitor PMSF and trypsin specific inhibitor TPCK could only inhibit the activity partially. EDTA has no inhibition on the activity, indicating that yCsCBs belongs to the typical cathepsin B cysteine protease family. yCsCBs exhibited the highest activity at pH value of 5.0-5.5 and temperature of 42°C. Also, yCsCBs could degrade host BSA, HSA, IgG, hemoglobin and fibronectin. Immunohistochemical results showed that CsCBs were located at the intestine of adult worm and excretory vesicle and oral sucker for metacercaria and cercaria stage. In conclusion, we profiled secreted cathepsin B cysteine proteases family for the first time and demonstrated secreted cathepsin B proteases are associated with tissue invasion of the ***C. sinensis*** infection.

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CO-DISPERSAL OF THE BLOOD FLUKE *SCHISTOSOMA JAPONICUM* AND *HOMO SAPIENS* IN THE NEOLITHIC AGE

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The global spread of human infectious diseases is of considerable public health and biomedical interest. Little is known about the relationship between the distribution of ancient parasites and that of their human hosts. ***Schistosoma japonicum*** is one of the three major species of schistosome blood flukes causing the disease of schistosomiasis in humans. The parasite is prevalent in East and Southeast Asia, including the People's Republic of China, the Philippines and Indonesia. We studied the co-expansion of ***S. japonicum*** and its human definitive host. Phylogenetic reconstruction based on complete mitochondrial genome sequences showed that ***S. japonicum*** radiated from the middle and lower reaches of the Yangtze River to the mountainous areas of China, Japan and Southeast Asia. In addition, the parasite experienced two population expansions during the Neolithic agriculture era, coinciding with human migration and population growth. The data indicate that the advent of rice planting likely played a key role in the spread of schistosomiasis in Asia. Moreover, the presence of different subspecies of Oncomelania hupensis intermediate host snails in different localities in Asia allowed ***S. japonicum*** to survive in new rice-planting areas, and concurrently drove the intraspecies divergence of the parasite.

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INVERTEBRATE IMMUNITY: PHENOLOXIDASE ACTIVITY IN THE SCHISTOSOME SNAIL HOST

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To complete its complex lifecycle, ***Schistosoma mansoni*** needs an aquatic snail (***Biomphalaria*** spp.) as intermediate host and a mammalian as definitive host. The immune response of the aquatic snail to parasite infection is poorly understood. Phenoloxidase (PO) enzymes are important in wound healing and tissue pigmentation, and are also considered to be an innate immune defense mechanism against intruding microorganisms and parasites. POs are characterized by their substrate specificity and fall into three groups: tyrosinases, catecholases and laccases. Their activities can be quantified with specific substrates. PO activity has been found in the hemolymph of molluscs such as mussels or oysters, but has not been characterized in Biomphalaria snails. Using spectrophotometric assays with 3 different specific substrates (L-tyrosine (monophenol) for tyrosinase activity, L-DOPA (o-diphenol) for catecholase activity, and p-phenylenediamine (PPD) (p-diphenol) for laccase activity), we first demonstrated the laccase-like activity in hemolymph from uninfected B. glabrata and alexandrina. Then we determined the optimal temperature (45°C) and pH (8.5 which is also the pH of the snail hemolymph) for the PO enzyme and the apparent Michaelis constant (Km) and the apparent Vmax using PPD substrate. After splitting the whole hemolymph into 2 distinct fraction, hemocytes (immune cells) and plasma, we found the highest laccase-like activity in the plasma. Finally, we measured the PO activity on snails parasitized by ***S. mansoni***, and demonstrated a strong reduction of this laccase-like activity starting 7 weeks after the exposure ($p < 0.0001$), most likely because developing parasites eat the snail albumen gland and hepatopancreas. This new spectrophotometric assay with PPD substrate allows accurate measurement of PO activity in the intermediate hosts for S.mansoni.

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A TEGUMENT-SPECIFIC VENOM ALLERGEN-LIKE PROTEIN FROM THE CHINESE LIVER FLUKE *CLONORCHIS SINENSIS*

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The SCP/TAPS (sperm coating protein/Tpx-1/Ag5/PR-1/Sc7) proteins are multifunctional protein found in eukaryotes. Venom allergen-like (VAL) proteins, members of the SCP/TAPS protein superfamily, have been reported from several parasitic helminths. However, little is known about their biological and immunological function. In this study, a VAL protein of the Chinese liver fluke ***Clonorchis sinensis*** was cloned and characterized. A cDNA encoding 25 kDa protein was identified from EST database of ***C. sinensis***. A BLAST search revealed that the protein shares 46% sequence identity with ***Schistosoma mansoni*** VAL 13 protein, and thus, the protein was named CsVAL13. Multiple sequence alignment indicated that SCP/TAPS domain of CsVAL13 shared 39~46% sequence identity with VAL proteins from parasitic helminths. His and Tyr residues, which help to stabilize protein structure, were highly conserved across the VAL protein sequences. Phylogenetic analysis showed that SCP/TAPS domain of CsVAL13 sequence clusters together with other group 2 VAL protein sequences. In the homology-modeled structure of CsVAL13, an α-β-α sandwich structure and residues for a putative active site were highly conserved. PCR-amplified cDNA sequence of CsVAL13 was subcloned into pET32a bacterial expression plasmid vector. Recombinant CsVAL13 protein was produced bacterially and purified by Ni-NTA affinity chromatography. Immune sera were obtained from BALB/c mice immunized with the recombinant CsVAL13 protein. ELISA value of IgG1 in the immune sera against the recombinant CsVAL13 protein was six-times higher than that of IgG2a suggesting that CsVAL13 can induce Th2-polarized immune response. Immunohistochemical localization using the immune sera revealed that CsVAL13 was distributed mainly in the tegument and intrauterine eggs of adult ***C. sinensis***. These findings suggest that CsVAL13 may be involved in host-parasite interactions and immune stimulation on the surrounding host environments.

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THE CHALLENGE OF IMPROVING BOILING: LESSONS LEARNED FROM A RANDOMIZED CONTROLLED TRIAL OF WATER PASTEURIZATION AND SAFE STORAGE IN PERU

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Boiling is the most common method of household water treatment in low- and middle-income countries; however, it is not always effectively practiced. We conducted a randomized controlled trial among 210 households, 93% of which used improved water sources, to assess the effectiveness of water pasteurization and safe storage interventions in reducing Escherichia coli contamination of household drinking water in a water-boiling population in rural Peru. Households were randomized to receive either a safe storage container or a safe storage container plus water pasteurization indicator or to a control group. During a 13-week follow-up period, households that received a safe storage container and water pasteurization indicator had a higher prevalence of stored drinking water contamination relative to the control group, although the difference was not statistically significant (Prevalence Ratio (PR): 1.19, 95% CI: 0.93, 1.52). Receipt of a safe storage container alone had no effect on the contamination of stored drinking water (PR: 1.03, 95% CI: 0.80, 1.32). Although use of low-cost water pasteurization indicators and locally available storage containers did not significantly increase the safety of household drinking water in this study, future research could help illuminate factors that facilitate the effective use of these interventions to improve water quality and reduce the risk of waterborne disease in populations that boil drinking water.

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THE QUALITY OF WATER IS STRAINED: FECAL CONTAMINATION IN RURAL BRAZIL

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Wherever humans concentrate, fecal contamination of surface waters becomes a problem. We previously traced the concentration of human fecal contamination along a river dividing a village in rural Brazil. In this cross-sectional study we found that concentration increased in a downstream direction and diminished as the river left the populated area. This correlated with the prevalence of schistosomiasis. In order to follow longitudinal changes in water quality, we sampled water at 8 points over a km long stretch of the Jiquiriçá River and its tributary, the Brejões, as well water from the local treatment plant. The water is clear in most places, but heavily sedimented after heavy rains. Its temperature was within 1-2 degrees of the ambient temperature and averaged 26.2 °C across all sites. Oxygen saturation was on average good (mean 3.7 mg/d) at most sites. pH decreased as population density increased. For the Jiquiriçá, pH decreased from 6.99 upstream to 6.50 downstream of the last dwelling (mean=6.75). On the Brejões, the same trend was seen (pH 6.96 to 6.63, mean=6.75). The presence of E. coli is better associated with human feces than total coliforms. We found significant correlation between the total coliforms and E. coli (r2=0.81). Over the year, the concentration of

coliforms (337 cfu/ml) and E. coli (184 cfu/ml) was consistently highest at site 8, which corresponded to a point immediately downstream of the major population concentration on the Brejões. On the Jiquiriçá, site 4 had the highest levels of coliforms (190 cfu/mL), but site 3, where the Brejões enters the Jiquiriçá, showed the highest levels of E. coli. When river flow increased we found that levels of coliforms increase statistically (r2=0.99), however, levels of E. coli did not (r2 = 0.05). Samples from the water treatment plant had abundant coliforms at times, but rarely showed E. coli. This suggests that run-off produces the greatest risk of zoonotic contamination, but does not influence the concentration of human sources. qPCR will be used to evaluate more specific markers of human fecal contamination.

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REPRODUCTIVE HISTORY AMONG WOMEN EXPOSED TO INSECTICIDES THROUGH MALARIA CONTROL PROGRAMS IN CENTRAL EAST INDIA

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Indoor residual spraying (IRS) is a cornerstone of malaria vector control; insecticides used for IRS such as DDT, organophosphates, and pyrethroids have been linked in animal and epidemiologic studies with reproductive toxicity. As IRS requires community wide application to more than 80% of homes to be effective, reproductive aged women will be exposed. Our objective was to determine if IRS exposure impacted reproductive outcomes among women in central east India where the government has an established IRS program. Data was available from two similarly conducted cross sectional surveys of pregnant women enrolled at delivery in the states of Jharkhand and Chhattisgarh; 1730 of 1739 subjects had information on IRS exposure (ever sprayed versus never sprayed). We used multivariable logistic regression models to evaluate the association of IRS exposure with reproductive history. A large proportion of women in the cohort (688/1730, 39%) reported having had their homes sprayed by the government. The prevalence of malaria parasitemia (peripheral or placental) was 3.2% and was not affected by history of IRS exposure (p=0.63). Women whose homes had been sprayed during IRS campaigns were more likely to report a history of a stillborn infant (aOR 1.60, 95% CI 1.05-2.44) but not a history of early pregnancy loss (aOR 0.91, 95% CI 0.65-1.30) after adjusting for covariates that differed by IRS exposure group. Our findings are limited as IRS exposure was determined by maternal recall and the timing of exposure in relation to pregnancies was not defined. Further research is warranted to clarify potential toxicity to reproductive aged women from specific insecticides so that policymakers who design IRS programs will be able to identify the safest insecticide(s) for reproductive aged women.

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ASSOCIATION BETWEEN PROXY MEASURES OF HANDWASHING AND PREVALENCE OF CHILD DIARRHEA IN 15 LOW-INCOME COUNTRIES: ANALYSIS OF DEMOGRAPHIC AND HEALTH SURVEYS, 2010-2012

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Children in low-income countries are at increased risk of diarrheal diseases, which are largely preventable. Handwashing (HW) with soap can significantly reduce childhood diarrhoea. We assessed whether a proxy measure of HW with soap, the presence of soap and water at a HW place, was associated with a reduced prevalence of diarrhea among children aged <5 years in low-income countries. We used comparable data from Demographic and Health Surveys (DHS) conducted in 2010-2012 in 15 low-income countries. For each country and pooled across the countries, we estimated crude and adjusted risk ratios (RRs) for 2-week prevalence of diarrhea comparing children from households having water alone, or soap and water, with children from households having no water or soap at a HW place. Compared to children from households with no water or soap at a HW place, children from households with water alone had 3-62% lower prevalence of diarrhea in 6 countries and 6-35% higher prevalence of diarrhea in 9 countries. Children from households with water and soap at a HW place had 6-49% lower prevalence of diarrhea in 9 countries and had 14-45% higher prevalence of diarrhea in 5 countries compared to children from households with no water or soap. There was no difference in diarrhea prevalence between the children in both groups in one country. In pooled analyses, there was a 10% (adjusted RR 0.90, 95% CI 0.74-1.10) lower prevalence of diarrhea among children from households with water alone and an 11% (adjusted RR 0.89, 95% CI 0.76-1.04) lower prevalence of diarrhea among children from households with water and soap compared to children from households with no water or soap. There was substantial heterogeneity among the countries. Availability of HW materials at designated places for washing hands was not significantly associated with diarrhea prevalence. Availability of soap and water may be necessary but not sufficient condition for consistent practice of HW behavior. There is a need to develop indicators of HW determinants that could be used in conjunction with observation of HW materials to better predict true HW behavior of target populations in large-scale surveys such as the DHS.

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OBSERVED HANDWASHING BEHAVIOR DURING INFANT FEEDING, INTERVENTION ASSESSMENT OF A LARGE RANDOMIZED CONTROLLED TRIAL (RCT) IN RURAL BANGLADESH

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We analyzed data from a randomized controlled trial (RCT) to assess if the provision of a supportive environment improved handwashing (HW) practices before child feeding when this key time was not actively promoted during intervention. We studied caregivers' HW practices before feeding different food items. The WASH Benefits study is an ongoing RCT in four districts in Bangladesh. Participants received messages to wash hands with soap after defecation, after cleaning a child's anus and before preparing food, but not before child feeding. Participants also received two HW stations; at the food preparation area and the latrine. We randomly selected 54 households each from the control, HW, combined water, sanitation, HW (WASH), and WASH plus nutrition (WASH, N) arms. At each household we conducted five-hour structured observations from February-July 2014, approximately 1.5 years after initiation of the intervention. During the observation, children were 6-18 months old. HW with soap before breastfeeding was very low (3%) in all three intervention arms and absent among controls. Though the proportion was low HW with soap before feeding water was more common in the HW intervention arm (9%) compared to controls (2%, RR 4.47; 95%CI 1.40, 14.35). This difference was less apparent in the combined arms (6%, RR 3.13, 95%CI 0.99, 9.94). We observed that HW with soap before feeding solid moist food, which maybe particularly conducive to bacterial growth, was more common in the HW intervention arm (26%) compared to the control arms (3%; RR 7.4; 95%CI 2.6, 21.1). This practice was also higher in the WASH (15%; RR 6.4; 95%CI 2.3, 17.8) and WASH, N arms (10%; RR 3.4; 95%CI 1.1, 10.3) compared to controls. HW with soap before feeding dry food (<1%) and fruits (<2%) was rare. Focused promotional messages for HW before child feeding were lacking but a supportive setting was associated with increased HW with soap before feeding solid moist food to children.

Hand cleanliness during complementary feeding may be prompted for habit formation by ensuring a supportive environment for HW. Increased awareness of the impact of HW with soap on child health can foster practices beyond those specific events promoted.

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EXPRESSION OF CYTOKINES IN RESPONSE TO *MYCOBACTERIUM AVIUM* COMPLEX IN ENTEROCYTES AND ITS ASSOCIATION WITH ENVIRONMENTAL ENTEROPATHY

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Environmental enteropathy is a chronic inflammatory condition associated with stunting in children, poor vaccine response as well as malabsorption. Although its aetiology is unknown, it is known to be associated with T-cell activation. Mycobacteria is known to elicit activation of cytokines including tumour necrosis factor, interleukin 6, interferon γ and matrix metalloproteinases. The role played by mycobacteria in the aetiology of enteropathy is unknown. We carried out an investigation to determine the gut cytokine response to ***Mycobacterium avium*** complex. Intestinal biopsy samples were cultured with ***M. avium*** lysate in tissue culture medium in an environment with 95% O2/5% CO2 at 37°C for 24 hrs. The supernatant was collected and we used the cytometric bead array (CBA) human Th1/Th2/Th17 cytokine kit on a Facsverse machine to estimate cytokine expression. Preliminary results from 9 patients show that ***M. avium*** complex increased the expression of interleukin 4 (p=0.01), interlukin-10 (p=0.02), and interferon gamma (p=0.01) in enterocytes compared to negative control samples. There was no difference in the expression of interleukin-2 (p=0.80), interleukin-6 (p=0.23), interleukin-17A (p=1.0) and tumour necrosis factor (p=0.63) in enterocytes compared to the negative controls. Our results have shown that ***M. avium*** induces inflammatory (interferon gamma) as well as anti-inflammatory (il-4 and il-10) cytokines in enterocytes. ***M. avium*** could play a role in environmental enteropathy

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SPATIO-TEMPORAL PATTERN OF HOSPITAL-ACQUIRED *ACINETOBACTER BAUMANNII* INFECTION IN TEACHING HOSPITAL IN SOUTHERN THAILAND

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Molecular and mathematical methods are increasingly used in microbiologic diagnostics, particularly in epidemiologic investigations. Various molecular typing methods are employed to study the molecular epidemiology of ***Acinetobacter baumannii.*** The studied population included 197 ***A. baumannii*** undergone genotypic grouping using pulse field gel electrophoresis (PFGE). Relationship between admission ward, and timing of their occurrence were examined. Randomized representative isolates from each group were further analyzed for multilocus sequence typing (MLST), which were then compared with international database. Fisher test with 2,000 replicate Monte Carlo simulations and dotplot analysis were carried out on the cross-table between PFGE group and Ward Among carbapenem-resistant ***A. baumannii*** (CRAB), multiplex PCR for the bla_{OXA-23}, bla_{OXA-40}, and bla_{OXA-58} genes were done. Ten PFGE groups were identified causing 23 clusters. The average cluster size was 6.09 ± 6.61 cases. The average duration of outbreak was 12.6 ±13 days. The average wards per cluster were 2.41 ± 1.68 wards. Certain PFGE groups tended to be common in certain wards with P value of 0.0005. Dotplot analysis showed those clusters were relatively short and replaced with other successful groups rapidly. The eight sequence typing (STs) were previously described in Thailand, Vietnam and China while 2 STs were novel. Most common gene encoding for carbapenemase is bla_{OXA-23} followed with bla_{OXA-58}. In conclusion, outbreaks of ***A. baumannii*** in this study had a strong spatio-temporal pattern. The etiologic organisms were from different new and endemic sources. Prevention of new incoming organism may be useful in addition to the attempt on prevention of transmission during the outbreak.

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UNDERSTANDING WATER STORAGE PRACTICES OF URBAN RESIDENTS OF AN ENDEMIC DENGUE AREA IN COLOMBIA: PERCEPTIONS, RATIONALE AND SOCIO-DEMOGRAPHIC CHARACTERISTICS

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Household water storage has received special attention in prevention strategies for controlling ***Aedes aegypti*** reproduction, but the evidence about the rationale of this human practice is limited. The objective of this study was to identify and describe water storage practices among residents of an urban area in Colombia (Girardot) and its association with reported perceptions, rationale and socio-demographic characteristics with mixed methods approach. Knowledge, attitudes and practices and entomological surveys from 1,721 household were conducted as well as 26 Semi-structured interviews among residents of Girardot and vector borne technicians. A multiple logistic analysis was performed to identify associations between water storage practice and socio-demographic characteristics, and knowledge, attitudes and practices about dengue and its vector´ immature forms, which were then triangulated with qualitative information obtained from the semi structured interviews. Water storage is a common, and cultural practice in Girardot, 82% of respondents reported this activity. This practice was not associated with socioeconomic characteristics such as years of education (OR 0.97, 95% CI 0.79-1.19), lower income (OR 0.81, 95% CI 0.48-1.36) or knowledge about the vector and it's breeding sites. There are two main reasons for storage: the scarcity concern based on a long history of shortages of water in the region and the perception of high prices in water rates, contrary to what was reported by the local water company. The use of stored water depends on the type of container used, while water stored in large cement basins is mainly used for domestic cleaning chores, water in plastic containers is used for cooking. Despite of the common believe that water storage practices are related to socioeconomic conditions, this practice relays on cultural beliefs. It is essential to understand social practices that can increase or reduce the number of breeding sites of ***Ae. aegypti***. Identification of individuals who store water and the rationale of such storage allow a better understanding of the social dynamics that lead to water accumulation.

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INCREASED ISOLATION FREQUENCY OF TOXIGENIC *VIBRIO CHOLERAE* O1 FROM ENVIRONMENTAL MONITORING SITES IN HAITI

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Since the identification of the first cholera case in 2010, the disease has spread in epidemic form throughout the island nation of Haiti; as of 2014, about 700,000 cholera cases have been reported, with over 8,000 deaths. While case numbers have declined, the more fundamental question of whether the causative bacterium, ***Vibrio cholerae*** has established an environmental reservoir in the surface waters of Haiti remains to be elucidated. In a previous study conducted between April 2012 and March 2013, we reported the isolation of toxigenic ***V. cholerae*** O1 from surface waters in the Ouest Department. After a second year of surveillance (April 2013 to March 2014) using identical methodology, we observed a more than five-fold increase in the number of water samples containing culturable ***V. cholerae*** O1 compared to the previous year (1.7% vs 8.6%),

with double the number of sites having at least one positive sample (58% vs 20%). Both seasonal water temperatures and precipitation were significantly related to the frequency of isolation. Our data suggest that toxigenic ***V. cholerae*** O1 are becoming more common in surface waters in Haiti; while the basis for this increase is uncertain, our findings raise concerns that environmental reservoirs are being established.

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WATER SANITATION AND HYGIENE PRACTICES IN SOME COMMUNITIES IN BAYELSA STATE, NIGERIA

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A study on water sanitation and hygiene practice was carried out in three randomly selected communities in Bayelsa State, South-South geopolitical zone of Nigeria. The study adopted a descriptive cross-sectional design, with the aid of a questionnaire, bacteriological water analysisand sanitary inspection strategies for data collection. The study showed that 57 (33%) out of 169 heads of households studied were aware of improved water supply benefits and 37 (21.9%) were aware of sanitation/hygiene benefits. Eight one percent (81%)of the households sampled did not have access to improved water supply.The meanwater supply coverage was 17.85%, while sanitation was 46.89% in the three communities. From the findings, 45% of the households used private neighborhood borehole and about 20% depended on river and pond water as the main sources of water supply. The bacteriological water analysis showed that 120 out of 160 sampleswere contaminated with ***Escherichia coli, Streptococcus faecalis*** and ***Salmonella typhi***. Based on the above findings, massive health education/promotion is required to minimize water associated infections/ diseases.

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PREVALENCE OF *SALMONELLA* SPP. IN RAW MILK SAMPLES FROM FARM LIVESTOCK DUAL PURPOSE IN CÓRDOBA, COLOMBIA

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Raw milk has not been subjected to any process of thermisation or sterilization and so is considered one of the main transmission routes of pathogens causing food borne diseases (FBD). Salmonellosis is one of the most prevalent zoonosis around the world. Despite advances in technology and educational efforts to improve the food products handling throughout the production and marketing the FBD outbreaks due to ***Salmonella*** spp. are still prevalent in particular those involving dairy foods consumption. This study was undertaken to determine the prevalence of ***Salmonella*** spp. in raw milk samples from livestock dual purpose farms from the department of Córdoba, Colombia. Milk samples obtained during maximum and minimum precipitation periods in 149 farms were collected. ***Salmonella*** spp. were determined by conventional isolation according to protocols established by INVIMA. The suspected strains underwent biochemical tests and confirmed by Polymerase Chain Reaction (PCR) determining the ***InvA*** gene. All data were analyzed using the SAS statistical package. ***Salmonella*** spp. was detected in 1,34% (2/149) and 2,01% (3/149) of the samples collected at minimum and maximum rainfall respectively, which was isolated by conventional bacteriology methods and molecularly confirmed by amplification of a 284 bp region corresponding to ***InvA*** gene. It was established that 60% (3/5) and 40% (2/5) of the isolates corresponded to samples taken at low and high precipitation respectively. In conclusion, the presence of ***Salmonella*** spp. in raw milk can become a risk to human health, so it is clear that the application of best management practices and good sanitary conditions contribute directly in reducing the ***Salmonella*** spp. milk contamination.

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DIAGNOSIS OF INFECTIONS CAUSED BY BACTERIA, FUNGI AND GASTROINTESTINAL PARASITES IN MEAT HANDLERS IN A SLAUGHTERHOUSE IN THE NORTH OF COLOMBIA, 2014

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To determine the presence of infections caused by bacteria, fungi and gastrointestinal parasites in beef handlers in a slaughterhouse in the city of Santa Marta, Magdalena, Colombia 2014. The research corresponded to a cross-sectional descriptive study. The study sample corresponded to the whole of the 36 existing workers in the slaughterhouse. Prior socialization to company officials about the scope of the investigation and achieved the informed consent, the respective throat swab tests, direct examination with 10% KOH for hand and nail fungus and serials stool (three) were performed. Diagnosis of throat swab practiced showed the presence of gram-positive cocci in 36/36 (100%) of the samples, Gram positive in 1/36 (2.8%), Gram negative bacilli in 9/36 (25%) and fungal mycelia in 7/36 (19.4%) of them. The leukocyte reaction, possibly as a result of the presence of cocci, bacilli and fungi, showed a low leukocyte reaction in 23/36 (63.9%) samples, moderate leukocyte reaction in 12/36 (33.3%) and increased leukocyte reaction 2/36 (5.5%) of the samples. The throat swab also showed fusoespirilar association in 6/36 (16.7%) of the patients examined. The stool tests showed a higher proportion of amoeba infections, highlighting ***Endolimax nana*** infection 7/36 (19.4%), followed by ***Entamoeba histolytica*** infections 5/36 (13.9%) and ***E. coli*** 4/36 (11.1%). Protozoa of the genus ***Blastocistis hominis*** were also observed in 4/36 (11.1%) workers infected with ***Giardia lamblia*** and 1/36 (2.8%). Nematode infections were observed only for the genus ***Uncinaria*** spp., 1/36 (2.8%). The presence of yeasts was found in 4/36 (11.1%) handlers. No fungal infections diagnosis with 10% KOH in hands and nails were found. The study showed manipulators throat infections at enteric level compatible with several genera of bacteria and parasites of clinical importance, these infections would also become a source of contamination of manipulated meat, possibility given to consumers. With this study we intend to seek guidance from academic practices by health authorities to ensure the health of handlers and food security.

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A MOLECULAR MECHANISM OF ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA

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Artemisinins are the cornerstone of anti-malarial drugs. Emergence and spread of resistance to them raises risk of wiping out recent gains achieved in reducing worldwide malaria burden and threatens future malaria

control and elimination on a global level. Genome-wide association studies (GWAS) have revealed parasite genetic loci associated with artemisinin resistance. However, there is no consensus on biochemical targets of artemisinin. Whether and how these targets interact with genes identified by GWAS, remains unknown. Here we provide biochemical and cellular evidence that artemisinins are potent inhibitors of ***Plasmodium falciparum*** phosphatidylinositol-3-kinase (PfPI3K), revealing an unexpected mechanism of action. In resistant clinical strains, increased PfPI3K was associated with the C580Y mutation in ***P. falciparum*** Kelch13 (PfKelch13), a primary marker of artemisinin resistance. Polyubiquitination of PfPI3K and its binding to PfKelch13 were reduced by the PfKelch13 mutation, which limited proteolysis of PfPI3K and thus increased levels of the kinase, as well as its lipid product phosphatidylinositol-3-phosphate (PI3P). We find PI3P levels to be predictive of artemisinin resistance in both clinical and engineered laboratory parasites as well as across non-isogenic strains. Elevated PI3P induced artemisinin resistance in absence of PfKelch13 mutations, but remained responsive to regulation by PfKelch13. Evidence is presented for PI3P-dependent signalling in which transgenic expression of an additional kinase confers resistance. Together these data present PI3P as the key mediator of artemisinin resistance and the sole PfPI3K as an important target for malaria elimination.

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IDENTIFYING AND CHARACTERIZING A NOVEL TRAFFICKING COMPONENT OF THE *PLASMODIUM FALCIPARUM* VIRULENCE FACTOR PFEMP1

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Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is the main parasite virulence factor due to its central role in mediating the cytoadherence of infected red blood cells (iRBCs) to the host microvascular endothelium. Directly targeting PfEMP1 as a therapeutic strategy is greatly limited due to the protein's hypervariable nature, which gives rise to ~60 different variants. However, interfering with the trafficking of PfEMP1 to the iRBC surface is an attractive approach, as hypervariability becomes inconsequential and previous studies have demonstrated that reduced surface PfEMP1 levels significantly weaken cytoadherence, likely lessening the severity of malaria symptoms and promoting parasite clearance by the spleen. Interestingly, the ***in vitro*** culture-adapted parasite line 3D7 is inherently defective in exporting PfEMP1 to the iRBC surface. Presuming that PfEMP1 export from the parasite to the iRBC surface is controlled at the genetic level, we hypothesized that 3D7 harbors one or more genetic determinants of impaired PfEMP1 trafficking. To test this possibility, we examined the surface PfEMP1 levels of 16 progeny clones from the genetic cross between 3D7 and the 'trafficking-competent' parasite line HB3. This was assessed using Western blotting and a two-color, three-layer flow cytometry assay with plasma from malaria-immune Malian adults. Normalized to HB3, we found that 3D7 displays 75% less PfEMP1 on the iRBC surface, with progeny phenotypes ranging from 37% more to 88% less PfEMP1. QTL analysis using genome-wide SNP markers identified a near-significant locus with a LOD score of 4.96 on chromosome 12 that explains ~50% of the phenotypic variance. This locus contains a single gene, Pf3D7_1245600, encoding a putative kinesin-6 protein. The role of this gene in the trafficking of PfEMP1 was directly assessed through CRISPR-Cas9-driven parental allele-exchange transfection experiments, and indirectly assessed though ***in vitro*** microtubule manipulation studies. The results of this study strengthen our understanding of malaria pathogenesis and direct attention to a highly understudied parasite protein family.

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FORM AND FUNCTION OF REGULATED MRNP COMPLEXES IN *PLASMODIUM* TRANSMISSION

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Translational repression of specific mRNAs allows control of gene expression by ***Plasmodium*** species, which the parasite uses to prepare for transmission events between a mammalian host and a mosquito vector. Our previous studies demonstrate that Puf2, a RNA-binding translational repressor protein, plays an integral role in maintaining the infectivity of transmitted salivary gland sporozoites, in regulating RNA homeostasis, and in translationally repressing specific transcripts. In the absence of Puf2, the transcript abundances of other RNA-binding proteins are affected, including those that encode for members of the CCR4/Not and DOZI/CITH complexes, which are essential to RNA metabolism and translational repression. To this end, have studied the intersection of these critical mRNA/protein (mRNP) complexes and processes during transmission through genetic manipulation of the rodent-infectious parasite ***Plasmodium yoelii***. First, we have identified that the ALBA4 protein plays an integral role in both gametocytes and sporozoites, and its genetic disruption leads to an enhancement of the number of transmissible gametocytes from host-to-vector, but dampens the wave of sporozoites migrating from the midgut to the salivary gland. Conversely, genetic disruption of CCR4 (a general deadenylase) dampens the wave of gametocytes that can be transmitted from the host, but does not affect sporozoite development. Secondly, we have isolated the mRNP complexes associated with the ALBA family of proteins from transmissible gametocytes and asexual blood stages, and have used high mass accuracy mass spectrometry and RNA-seq to define their composition. Lastly and excitingly, we have for the first time developed the techniques required to isolate mRNP complexes from the ***Plasmodium*** sporozoite. We are extending these approaches to characterize both the ALBA and Puf2 mRNP complexes in developing sporozoites to identify commonalities in their form and function between gametocytes and sporozoites. Taken together, these efforts illuminate the differences between discrete storage granules present during both transmission events.

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EXPERIMENTAL CEREBRAL MALARIA INDUCES CEREBRAL VASCULAR DYSFUNCTION AND COGNITIVE IMPAIRMENT VIA ENDOTHELIN A RECEPTOR SIGNALING

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Cerebral malaria (CM) is a diffuse encephalopathy caused by ***Plasmodium falciparum*** infection, which kills and disables millions of children each year. The potent vasoactive peptide, endothelin-1 (ET-1), which is thought to be involved in CM, mediates blood brain barrier (BBB) permeability, inflammation, and vascular tone. Researchers propose that elevated levels of ET-1 during severe ***P. falciparum*** infection may contribute to cerebrovascular dysfunction ultimately impairing neurocognition. However, the underlying mechanism of ET-1 in the pathogenesis of CM is not fully understood. In the current study, we tested the hypothesis that ET-1 mediates vascular and cognitive dysfunction in the ***P. berghei*** ANKA (PbA) experimental CM (ECM) model. We previously demonstrated that ECM resulted in significant memory loss, even after successful antimalarial treatment. ***P. berghei*** NK65 (PbN) generally does not induce ECM in C57BL/6 mice and has been used as a non-CM negative control for PbA. Here, we demonstrate that exogenous administration of ET-1 induced memory deficits in PbN-infected mice triggering an ECM-like phenotype. Moreover, treatment with an endothelin type A receptor antagonist (ETARA) prevented BBB disruption, cerebral vasoconstriction, and neuroinflammation in PbA-infected mice. ETARA reduced brain endothelial

activation concomitantly diminishing brain microvasculature congestion. Interestingly, ETARA adjunctive therapy prevented neurocognitive impairments induced by PbA-infection. We propose that these effects were mediated by activation of c-Jun N terminal kinase (JNK), a downstream substrate of ET-1 signaling associated with cognitive impairment and is important in the development of ECM. Here, we demonstrate that ET-1 induced the expression of JNK phosphorylation in the brains of PbN-infected mice, while ETARA prevented increased JNK phosphorylation in PbA-infected animals. We therefore conclude that cerebrovascular disturbances and cognitive impairments in ECM are due, in part, to ET-1 signaling. Thus, targeting the ET signaling-axis may serve as a potential adjunct therapy in the treatment of patients with CM.

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A_2MACROGLOBULIN CAN CROSSLINK MULTIPLE *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN 1 (PFEMP1) MOLECULES AND MAY FACILITATE ADHESION OF PARASITIZED ERYTHROCYTES

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Rosetting, the adhesion of ***Plasmodium falciparum***-infected erythrocytes to uninfected erythrocytes, involves clonal variants of the parasite protein ***P. falciparum*** erythrocyte membrane protein 1 (PfEMP1) and soluble serum factors. While rosetting is a well-known phenotypic marker of parasites associated with severe malaria, the reason for this association remains unclear, as do the molecular details of the interaction between the infected erythrocyte (IE) and the adhering erythrocytes. Here, we identify for the first time a single serum factor, the abundant serum protease inhibitor $\alpha_2$macroglobulin (α_2M), which is both required and sufficient for rosetting mediated by the PfEMP1 protein HB3VAR06 and other rosette-mediating PfEMP1 proteins. We map the α_2M binding site to the Cterminal end of HB3VAR06, and demonstrate that α_2M can bind up to four HB3VAR06 proteins, plausibly augmenting their combined avidity for host receptors. IgM has previously been identified as a rosette-facilitating soluble factor that acts in a similar way, but it cannot induce rosetting on its own. This is in contrast to α_2M and probably due to the more limited cross-linking potential of IgM. Nevertheless, we show that IgM works synergistically with α_2M and markedly lowers the concentration of α_2M required for rosetting. Finally, we demonstrate that the capacity to bind α_2M is a common phenotype among ***P. falciparum*** isolates ***in vitro*** as well as ***ex vivo***. Together, our results are evidence that ***P. falciparum*** parasites are able to expand the repertoire of host receptors available for PfEMP1-mediated IE adhesion to include receptors with low affinity for individual PfEMP1 molecules. These receptors likely include carbohydrate moieties that will lead to formation of rosettes when present on erythrocytes. The study opens opportunities for broad-ranging immunological interventions targeting the α_2M- (and IgM-) binding domains of PfEMP1, which would be independent of the host receptor specificity of the clinically important rosette-mediating PfEMP1 antigens.

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ETHICS AND EBOLA: RECOMMENDATIONS OF THE U.S. PRESIDENTIAL COMMISSION FOR THE STUDY OF BIOETHICAL ISSUES

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In February 2015, the Presidential Commission for the Study of Bioethical Issues released Ethics and Ebola: Public Health Planning and Response. The report addresses ethical issues raised by the west African Ebola virus disease (EVD) epidemic, including public health, public, media, and political reactions to U.S. EVD cases, and the actual and perceived likelihood of a U.S. epidemic. After hearing from affected communities, public health leaders, journalists, historians, sociologists, and the public, the Commission focused its deliberations and analysis in three areas: 1) U.S. participation in the global epidemic response; 2) restrictive measures such as travel bans; and 3) research ethics in public health emergencies. The Commission made seven recommendations, including one focused on ethical considerations of implementing liberty-restricting measures during a public health emergency. Public engagement and ethics preparedness can help public health officials to determine when restrictive measures are appropriate and respond to calls for unwarranted quarantine or travel restrictions. Ethical dimensions of restrictive measures include their impact on health care professionals' ability and willingness to participate in epidemic response, which, in turn, can impact the effectiveness of the public health response. Restrictive measures might be ethically implemented in circumstances where the evidence deems them helpful and their public health rationale is effectively communicated to an anxious public. Drawing on examples from EVD and other epidemics such as SARS, the Commission highlighted the importance of restrictive measures being based on scientific evidence and employed only when they will prevent harm to others and have the least possible impact on civil liberties. Moreover, the Commission underscored that decisions about the use of restrictive measures should consider both the immediate burdens they impose on affected individuals and communities (such as loss of income), as well as issues of justice and fairness, including the potential of these measures to exacerbate stigma and social and economic inequalities.

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THE UK'S IMPORTED FEVER SERVICE DURING THE EBOLA EPIDEMIC: A CENTRALIZED ADVICE AND DIAGNOSTIC UNIT FOR IMPORTED INFECTIONS

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The Rare and Imported Pathogens Laboratory (RIPL) is a specialist centre run by Public Health England for advice and diagnosis of a wide range of uncommon infections. In addition to its standard diagnostic activities (24000 samples tested pa.) it operates a telephone hotline (the imported fever service – IFS) for infection specialists seeking advice and access to tests for imported infections, including Ebola. Between April 2014 and March 2015, RIPL received 599 referrals. 316 of these were calls for diagnostics and advice for an imported fever (compared to 187 such calls the previous year), of which 130 required testing for Ebola. Another 243 (46%) were calls about travellers from West Africa specifically related to Ebola testing, of which 101 (42%) were tested. All IFS samples, as well as 83/101 of Ebola requests, were tested using molecular and serological assays for flaviviruses, alphaviruses, phleboviruses and rickettsiae, the exact choice of tests guided by the incidence of infection in each of 10 large geographical areas. In 159 travellers from non-outbreak countries, RIPL diagnoses (including those where IFS advice led to the correct test being performed) were made in 23 and included chikungunya (5), dengue (5), leptospirosis (2), rickettsiosis (3), hantavirus (2), coxiellosis, CCHF, hepatitis E, malaria genus (species determined by MRL), scrub typhus, West Nile (one each). In travellers from the outbreak area (231 individuals) RIPL diagnoses were made in 32 (14%): undiagnosed malaria (18), rickettsiosis (5), Ebola (3), dengue (3), leptospirosis, Rift Valley and Toscana virus (one each). IFS experience shows the value of a one-stop specialist diagnostic network to support clinicians in local hospitals. Malaria is overrepresented and chikungunya is absent in our travellers from West Africa. A significant number of infections, some of serious clinical or public health importance,

have been identified in cases referred to RIPL. The centralisation of specialised testing facilitates communication and quality control and a unit such as RIPL is well placed to respond to the need for testing returnees from outbreak areas who are unwell.

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NIGERIA'S RESPONSE TO THE EBOLA OUTBREAK: CRITICAL LESSONS FOR HEALTH SYSTEMS STRENGTHENING IN AFRICA

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The Ebola virus spread from Liberia to Lagos, Nigeria in July 2014 and an uncontrolled Ebola outbreak would have been catastrophic in Nigeria, a country of 170 million people. Nigeria succeeded in containing Ebola through a "quick and forceful" rapid public health response. 20 people were affected and 8 died. While there has been extensive international aid to help Ebola affected countries, few of these efforts have been specifically targeted to building up their health systems. The Ebola outbreak is a huge health crisis but also a wake up call for immediate sustainable and scalable health systems strengthening. From a focused literature review, and on site visit to Nigeria this paper uses Nigerias experience as a trajectory and suggests methods for health systems strengthening by seeking answers to critical questions 1) Why was the response from African countries to Ebola slow and uncoordinated ? 2) Was Nigeria prepared or lucky? 3) What steps had Nigeria taken in the past to inadvertently prepare for Ebola and what can other countries learn? 4) What did Nigeria do differently during the crisis 5) How can African countries move from short term public health measures to long term initiatives and disaster preparedness and what are the next steps in developing a people centred health system? African countries with fragile health systems need a more robust approach in responding to health challenges including : improved health governance, research funding, quality improvement, effective health worker training and retention, and widespread public health education. Conclusions, countries must embrace strategic and pragmatic thinking as innovative solutions are sought for challenging and emerging public health problems.

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FORGING GLOBAL HEALTH PARTNERSHIPS THROUGH EBOLA PREPAREDNESS: US NAVAL MEDICAL RESEARCH UNIT-3 ASSISTS IN REGIONAL PUBLIC HEALTH RESPONSE, INFECTION CONTROL TRAINING AND SAMPLE MANAGEMENT IN CAIRO, EGYPT

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The recent outbreak of Ebola Virus Disease (EVD) has prompted many nations outside of West Africa to strengthen their public health response to prepare for regional epidemics. In Egypt, US Naval Medical Research Unit No. 3 (NAMRU-3) assisted local and regional partners in EVD preparedness thereby fostering global health engagement. NAMRU-3 acquired the capability to test EBOV-Zaire strain by rtPCR and as a World Health Organization (WHO) Collaborating Center for Emerging Infectious Diseases, responded to WHO's request for training of laboratory and public health representatives in the region. Scientific seminars covering the virology of EBV, sample inactivation, handling and transport, and infection control precautions were offered through "just-in-time assessments", workshops and training for the host nation and Eastern Mediterranean Region (EMRO) partner nations. As a regional asset, NAMRU-3 also contributed 3 microbiologists to the US DoD Operation United Assistance to build lab capacity in Liberia. After the initial seminar on 2 September, the local authorities assessed their Ebola Treatment Unit (ETU). They requested assistance with assessing the ETU and their Personal Protective Equipment (PPE) protocols for infection control. The training team assessed the ETU hospital site, provided hands-on training on PPE, and infection control. Additional training was provided to the Mobile Response Team. A three-day workshop for 13 participants from Jordan, Morocco, Tunisia, Lebanon, and Egypt was held from 28-30 October. Hands-on rtPCR laboratory and PPE training followed two days of educational seminars. Pre- and post- assessment quizzes showed an increase in the participants understanding of EBV from 60% to 73%. Specifically clarification was needed to distinguish between the PPE of avian influenza and EBV and exact route of transmission. The longstanding partnership between NARMU-3, Egypt, WHO and the EMRO region will allow for more agile response and preparedness to an evolving disease epidemic. During this session we will present how these relationships serve as a model for international global public health cooperation.

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DOCUMENTING THE RESPONSE TO THE EBOLA EPIDEMIC IN LIBERIA THROUGH THE PERSPECTIVE OF THE LOCAL PRESS

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Mass media plays an important role in documenting national responses to crises like Ebola. Reviewing media documentation helps a country better prepare for current and future public health challenges. Ebola articles first appeared in the Liberian press in March 2014. Our objectives were to determine the frequency of newspaper accounts and the major issues covered. We conducted content analysis of Ebola coverage in three Liberian newspapers from March through December 2014. We reviewed electronic publications of three main newspapers by searching for the term "Ebola". Data collected for each article included date of publication, and topic. Data were compiled in Microsoft Excel. After reading the first 50 articles, we inductively generated codes to capture the news content and compiled these into a codebook. The codebook was constantly refined as additional articles were read. Codes were organized into major themes. A total of 1,793 articles were published across the 3 newspapers over the 10-month period. The frequency of publications on Ebola ranged from 27 in March 2014, but increased to 95 April. Coverage dropped to only 15 in June, but began to rise sharply in August (227), reaching its peak in October (345). News reports frequency paralleled the incidence pattern of the disease. Major themes included the state of the epidemic, health care, psychosocial issues, international aid, political response, prevention, and local support. Overall political response to Ebola and the impact of Ebola on health workers received the most attention. In the early days common themes were border security and requests for aid. At the peak key themes were health worker problems and political responses. A review of the national press during a crisis like Ebola provides a valuable overview of the response of the different players ranging from health services and NGOs to international partners and government. It reflects political will and conflicts and can help a central operations team better coordinate resources and responses of partners.

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PRECIOUS....LIFESAVING, BUT NOT WITHOUT PROBLEMS; A MIXED-METHODS STUDY EXAMINING BARRIERS AND FACILITATORS TO INFECTION PREVENTION AND CONTROL IN HEALTH FACILITIES DURING THE EBOLA VIRUS DISEASE EPIDEMIC IN SIERRA LEONE

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During the Ebola virus disease (EVD) epidemic in Sierra Leone, health care workers (HCW) were frequently infected due to poor infection prevention and control (IPC). IPC was initially prioritized in Ebola treatment units (ETUs) though cases continued to present to primary health facilities. By December 2014, 64% of HCW infections had occurred outside of ETUs. Following a national scale-up of IPC in primary health facilities, we investigated gaps in IPC uptake. We conducted a mixed-methods study in eight facilities in Bo and Kenema Districts. Interviews with HCWs and focus groups with community members explored attitudes and experiences with IPC. Workshops for HCWs and health authorities were conducted to examine preliminary findings and develop IPC improvement plans. Quantitative measures of knowledge, attitudes and practice, including a survey and structured observations of HCWs, were administered before and after the workshops. Community members and HCWs described a similar series of emotions relating to EVD: from incredulity and disbelief to fear and sadness. HCWs described IPC practices as "precious" and "life-saving," but personal protective equipment (PPE) as uncomfortable and inducing fear, panic and "bad scenes" among patients. Endline measures of acceptance of glove use with patients (90.2%), PPE use with family members (87.8%) and HCWs (70.7%), and self-efficacy in screening (90.2%) were high. Structured observations showed consistent gaps in practice. Gloves and PPE items were inappropriately reused between screenings and multiple HCWs were present in screening areas. Screening behaviors, including standing the correct distance [RR 1.1, 95% CI 1.0-1.2] and facing patients from the side [RR 2.5, 95% CI 1.6-3.7], improved. Although PPE is available and attitudes are favorable, critical practice gaps merit consideration. HCWs may make informed judgments about IPC by valuing highly self-protection but valuing less inter-patient transmission. They may lack a sense of urgency given that fewer patients screen positive. As EVD wanes in Sierra Leone, IPC in routine practice requires refinement and reinforcement.

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THE MOBILE EBOLA LABORATORY EXPERIENCE: PERSPECTIVES ON FUTURE DISEASE SURVEILLANCE AND RESPONSE IN SIERRA LEONE

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As part of the global Ebola response effort in West Africa, MRIGlobal deployed a DTRA-funded mobile laboratory to the Moyamba District of Sierra Leone in late December, 2014. Operating in association with the Ebola Treatment Center (ETC) managed by Médicos del Mundo España and Solidarités International in the village of Moyamba, the Moyamba Ebola Diagnostic Center (MEDaC) processed clinical samples from the ETC and the local government-run hospital, and buccal swabs collected by burial teams throughout the district as part of corpse disposal procedures. As the global response effort increasingly focuses on the outbreak's end, questions regarding the transition of laboratory capabilities to future disease surveillance and response efforts are being raised. Based on our lessons learned on the ground in Sierra Leone, we have identified challenges that should be considered for laboratory sustainment and capacity building in-country. Discussion of the future must include impending challenges related to maintaining the laboratories themselves, such as operating costs, utilities, material availability and replacement, and staffing, as well as challenges related more directly to laboratory operations, including laboratory location, laboratory diagnostic capabilities, in-country communications, and sample logistics.

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REFERRAL FROM COMMUNITY HEALTH WORKERS: EVIDENCE FROM CLUSTER RANDOMIZED TRIALS OF MRDTS IN TWO AREAS OF HIGH AND LOW MALARIA TRANSMISSION IN UGANDA

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Integrated community case management (iCCM) is being scaled up in Sub-Saharan Africa to increase access to prompt and effective treatment. The global research agenda on iCCM has prioritised the need for evidence on the referral processes from community health workers (CHWs). We evaluated the impact of training in community case management of malaria and referral [with and without malaria rapid diagnostic tests (mRDTs)] on referral practices by CHWs and caretaker compliance to CHW's referral advice, as part of a cluster randomised trial in which villages were randomised to either mRDT or presumptive diagnosis (control) arms. CHWs in both arms were trained how to prescribe ACTs and recognise illnesses requiring referral to a health centre. Data from CHWs' treatment registers were linked to health centre records and analysed using logistic regression. Over 18 months CHWs in the high transmission area saw 18,497 children. Compared to control arm, CHWs using mRDTs referred more children (35% vs. 1% control) and referral of mRDT negative children was higher (61% vs. 3% mRDT positive). CHWs only referred 60% of children that met the eligibility criteria for referral, and were less likely to refer children at the weekend or during the rainy season. There was no difference with distance to the nearest health centre. Only 10% of all referred children complied with CHW's referral advice. Compliance with referral advice was higher amongst children who had received an mRDT test (odds ratio (OR) 8.0, 95% confidence interval (CI) 1.6-40.8); and was lower during the rainy season (OR 0.6, 95%CI 0.4-0.8); weekend (OR 0.6, 95%CI 0.4-0.8) and as distance to the health centre increased (OR 0.7, 95%CI 0.5-0.9). In conclusion, mRDT testing was associated with both increased referral making by CHWs and with increased caretaker compliance, compared to a presumptive malaria diagnosis. Independent factors that negatively affected CHW's decision to refer and caretaker's decision to comply included age, day and season of consultation and distance to the health centre. Data on referral in the 3651 children consulting CHWs in the low transmission trial will also be presented.

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A SYSTEMATIC REVIEW ON THE EFFECTIVENESS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES: PRELIMINARY RESULTS ON HEALTH OUTCOMES

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Health workers (HWs) play essential roles in delivering health care. In low- and middle-income countries (LMICs), HW performance is often inadequate. To characterize the effectiveness of strategies to improve HW performance in LMICs, we conducted a systematic review of 15 electronic databases, 30 document inventories of international organizations, and bibliographies of 510 articles. We included studies meeting accepted criteria for methodological adequacy (e.g., trials with adequate comparison groups) of any strategy on any health topic in any language, published or not. After screening, data from relevant reports were double-abstracted and entered into a database. This analysis focuses on studies that measured health outcomes (morbidity and mortality rates). Effect sizes were calculated as percent change over time in the intervention group minus percent change over time among controls. We screened >105,000 citations, 822 reports met inclusion criteria, and 63 studies measured health outcomes as a rate (29 on morbidity only, 27 on mortality only, and 7 with both). Many intervention strategies have been tested, usually with multiple intervention components. However, most strategies were tested by only one study. The median effect size (MES) across all studies was an improvement of 10 percentage-points (%-points) (interquartile range [IQR]: 0, 40). We examined strategies tested by at least 3 comparisons between an intervention arm and control arm for better generalizability. Among 45 studies focused on facility-based HWs, the strategy with the greatest health impact was HW training + group problem solving (MES = 31 %-points across 5 comparisons, IQR: 5, 67). Among 18 studies focused predominantly on lay HWs, the strategy with the greatest health impact was patient or community education + HW training (MES = 53 %-points across 18 comparisons, IQR: 6, 64). Contextual and methodological heterogeneity made comparisons difficult. Results from this review, which will be finalized by mid-2015, should inform decision-making on how best to improve performance of both facility-based and lay HWs and health outcomes in LMICs.

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ENSURING HEALTHCARE DELIVERY TO NOMADS: ENUMERATION OF SETTLEMENTS AND MONITORING FOR POLIO VACCINATION CAMPAIGNS. SEPTEMBER 2012-MARCH 2015. MARU, ZAMFARA STATE- NIGERIA

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Nigeria maintains wild polio virus transmission. States in the north of the country are the main source of polio infection for other regions and neighboring countries. Nomadic communities common in the north are completely missed or partially covered during immunization plus days (IPDs) due to frequent mobility and remoteness of settlements. Most cases of wild polio virus (WPV) identified in 2012 were among nomadic children. Existing local government and ward micro planning was inadequate to identify and reach nomads. We revised ward micro plans, conducted a census of nomadic populations and subsequently monitored IPDs.Secondary data abstractions of existing ward micro plans and key informant interviews with traditional leaders were conducted. Indigenous nomadic guides and data collectors were trained in the use of GPS devices and data entry tools. Missed children, unlisted settlements and unreported acute flaccid paralysis (AFP) cases were identified.14 teams dispatched and supervised to 10 wards gathered baseline data on nomadic settlements, assessed vaccination coverage, under-five population and obtained GPS co-ordinates. Data were entered and analyzed with Epi-info software. out of 283 nomadic settlements 172(60.8%) were listed in the micro plan, 111(39.2%) unlisted settlements were identified. 63 (22%) of total settlements had never been visited by vaccination teams, 47(17%) of settlements were missed during the previous IPDs. 1 case of unreported acute flaccid paralysis (AFP) was identified. The total under-five population was 1272, 525(41.3%) were recorded in the micro plan. Among under-fives in unlisted settlements 345 (46.2%) were never vaccinated. During monitoring of subsequent IPDs 747(58.7%) under-fives in enumerated unlisted settlements were vaccinated by special teams. Ward level micro plans were adequately updated.Special nomadic teams providing added personnel are essential to ensuring effective vaccination coverage of nomadic communities.Partnership between traditional rulers, religious leaders and immunization teams established and maintained ensured success.

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TARGETING ANTIMALARIAL SUBSIDIES TO CONFIRMED CASES IN THE RETAIL SECTOR - TESTING A DIAGNOSIS-DEPENDENT VOUCHER SCHEME IN WESTERN KENYA

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In an era of emerging resistance to artemisinin, diagnosis of malaria infection before treatment with ACTs is critical and has been recommended by the WHO since 2010. Significant improvements in malaria testing and ACT targeting have been reported in the formal health sector of several countries. However, a large proportion of suspected malaria patients purchase medicines over the counter in the informal retail sector. These patients generally do not have a diagnostic test and targeting of ACTs to true malaria cases is poor. We designed a randomized experiment to test the effect of community-based malaria testing coupled with a diagnosis-dependent voucher on testing and treatment decisions of participants. Individuals experiencing an acute malaria-like illness were randomized to one of four groups; 1) free malaria testing, 2) free malaria testing plus an ACT voucher if the test is positive, 3) malaria testing at a cost of 0.45 USD, 4) malaria testing for 0.45 USD plus an ACT voucher for a positive test. Participants who chose to be tested visited their local CHW for a malaria RDT. The voucher was provided to patients with a positive test in groups 2&4 and could be redeemed at a drug retailer for a discount of 0.50 USD towards a quality-assured ACT. The study was designed to measure whether a diagnosis-dependent voucher created an incentive to be tested before purchasing a drug. We will report the main effect of the conditional voucher on uptake of testing and estimate whether the effect is attenuated by charging a fee for testing. Preliminary results from the first 320 of 500 enrolled show that across all groups 60% of participants were tested for malaria. 65% of positive participants bought AL compared to only 16.5% of negative and 21% of untested participants. We will describe treatment choices for RDT-negative clients and RDT-positive clients in all four groups in order to understand whether adherence to the test results was influenced by the voucher or by the cost of the RDT. This approach could target retail sector antimalarial subsidies to individuals with confirmed infection, thus enhancing the sustainability of government and donor sponsored subsidies.

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IMPROVING THE EXCHANGE OF INFORMATION OF CHWS AND HEALTH WORKERS IN BENIN THROUGH MHEALTH INITIATIVES

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Medical Care Development International (MCDI), through the United States Agency for International Development/PMI-funded ARM3 project, implemented an mHealth initiative utilizing Dimagi's CommCare platform to facilitate the exchange of real-time health information between community and facility-level health workers in two health zones (HZ) in northern Benin. Over 12 months in 2014, 140 community and facility health workers used Android-enabled smartphones to report data electronically (replacing traditional paper-based forms). The reporting information included referrals for malaria, diarrhea, and pneumonia cases in children <5; counter-referrals; and drug stock outs. MCDI conducted an assessment in Nov. 2014 to identify lessons learned, best practices, and assess the feasibility of scale up to other HZs. A cross-sectional study using quantitative/qualitative metrics and an LQAS sampling method was conducted from a sample of 38 CHWs, 10 health facility chiefs, and 2 statisticians and completeness was assessed through a review of data sent by CHWs to the CommCare HQ database. 92% (35/38) of CHWs felt the electronic reporting system was useful in their daily work and 98% of all users preferred electronic to paper reporting. 84% of CHWs explained the usefulness of the system as allowing for real time data exchange, improving patient referral, and enhancing monitoring of CHW activities. The monthly average of completed reports submitted was 18% (Tchaourou) and 32% (Bassila). Reporting rates peaked when users had training/retraining on the "Case Sharing" feature (53% in Tchaourou and 60% in Bassila). Low reporting rates were explained by various technical challenges, including internet outages, malfunctioning solar chargers, and interruptions in phone credit allocation (due to strikes by health workers). Findings from the pilot show that leveraging mHealth initiatives may be an effective way to improve reporting and the exchange of information between CHWs and health facility workers. However, regular supervisory visits and refresher trainings are required to enhance the CHW motivation to complete reports and technical challenges need to be considered before scale up.

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ADHERENCE TO ANTIMALARIALS AND ANTIBIOTICS PURCHASED AT PRIVATE SECTOR DRUG SHOPS IN EASTERN UGANDA

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Little is known about adherence to medication purchased in the private sector in low income countries. There is particularly no evidence on adherence to antibiotics purchased for children under-5-years of age from private sector drug shops in these settings. Low adherence to first line medication can lead to microbial resistance, higher expenditure on second or third line drugs and increased morbidity. This study was undertaken to determine the level of adherence to anti-malaria drugs (Artemisinin Combination Therapy – ACTs) and antibiotics (amoxicillin) purchased for children less than 5 years of age from drug shops in Eastern Uganda. Twenty drug shop were randomly selected from all registered drug shop (N=44) in one district in Eastern Uganda. Sample size was estimated for cross sectional studies based on 95% CI, 5% error margin and 10% non-response. The outcome was adherence to drugs bought at the drug shop, measured using both pill count and caregiver reports on day 4 for ACTs and day 6 for Amoxicillin. Patients were classified as non-adherent if they have any leftover tablet(s) in the blister pack. All drugs sold were pre-packaged, age-specific, single dose, and sold in blister packets with pictures demonstrating how and when the medication should be taken. The study was conducted between in May and June 2012. A total of 499 children were recruited into the study. Adherence to ACTs was assessed in 259 children and adherence to Amoxicillin was assessed in 240 children. 85% of the children still had the blister packet that was dispensed with the medication and showed this to the data collectors. Adherence to both ACTs and amoxicillin was similarly low, 54% and 53% respectively. The main reasons for non-adherence were improvement in symptoms of the child (38%) and caretaker forgetfulness (35%). Predictors of non-adherence will be presented. In conclusion, we found low adherence to ACTs and amoxicillin purchased at drug shops for children under-5 years of age. Community awareness on importance of completing doses by children is recommended. Further, drug sellers dispensing drug should emphasize completion of doses to caretakers of children.

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HEALTH SYSTEM BARRIERS TO THE UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY IN MALI

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Auxiliary midwives and community health volunteers provide most antenatal care (ANC) to women in rural Mali. The country recommends at least three doses of sulfadoxine-pyrimethamine (SP) during pregnancy as intermittent preventive treatment of malaria (IPTp). Nevertheless, only 20% of pregnant women receive at least two doses of SP. This study aimed to identify health system barriers to adequate IPTp uptake among pregnant women in the Sikasso and Koulikoro regions of Mali. We conducted in-depth interviews and focus groups with pregnant women, members of the community health associations, community health workers and community health volunteers. We also conducted key informant interviews and structured observations of antenatal care (ANC) visits at health clinics. Our results show four major facility-level barriers to SP uptake: i) inadequate community health worker training, ii) stock-outs of SP iii) poor counselling by health workers and iv) failure to administer SP as directly observed therapy (DOT). The management cited poor training of health care workers as a principal barrier. Stock outs are reportedly not a major issue, but when they occur, women are referred to private pharmacies which charge for SP. Cost is a major deterrent to SP acquisition. The use and distribution of SP was compared with that of the long-lasting insecticidal nets (LLINs) in the same study population. There is a lower demand for and less knowledge about SP use compared to that of LLINs. Pregnant women have incorrect or low knowledge about SP, pointing to inadequate counseling by healthcare workers. Health workers also allow women to take SP home rather than adhering to DOT guidelines. Our preliminary analysis suggest that interventions targeting training of health care workers on SP and improved counselling practices will improve IPTp uptake. We realize that an increase in the proportion of women attending two or more ANC visits is imperative to increase IPTp uptake. Further analysis of how these facility-level barriers inform the broader health system in Mali is needed.

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THE MALPIGHIAN TUBULE TRANSCRIPTOME OF THE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*: NEW INSIGHTS INTO BLOOD MEAL PROCESSING BY MOSQUITO 'KIDNEYS'

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The renal (Malpighian) tubules of mosquitoes play critical roles in salt and water balance. For example, in adult females, which ingest the equivalent of their body mass in blood when feeding on vertebrates, , the Malpighian tubules mediate a pronounced diuresis that begins while still on the host and lasts for 1-2 h after feeding to excrete the excess salt and water of the

ingested blood. However, the physiological roles of the Malpighian tubules during the chronic processing of blood meals (3-24 h after ingestion)_ when the ingested blood cells are digested and metabolized_are not well known. Here we attempt to fill this gap of knowledge by using RNA-Seq to characterize the transcriptome of Malpighian tubules in adult female Asian tiger mosquitoes (***Aedes albopictus***) under non-blood fed and blood fed (3-24 h after ingestion) conditions. The Asian tiger mosquito is one of the most invasive mosquito species in the world and a vector of important arboviruses, such dengue and chikungunya fevers. We found that the transcriptome of Malpighian tubules in non-blood fed mosquitoes is enriched with transcripts encoding proteins associated with 1) the active transport of ions and water, and 2) redox/detoxification mechanisms. After blood feeding, we find a shift in the transcriptome, where 1) transcripts encoding proteins associated with redox and detoxification mechanisms are up-regulated, such as glutathione S-transferases (GSTs), thioredoxin, xanthine dehydrogenase, and ABC transporters; and 2) transcripts encoding proteins associated with the active transport of ions and water are down-regulated, such as subunits of the V-type H^+-ATPase, aquaporins, and ion channels/transporters. Assays to measure urine excretory capacity in mosquitoes, GST activity in Malpighian tubules, and uric acid content in Malpighian tubules confirm that the changes in transcript expression after a blood meal manifest as functional changes in the mosquito and Malpighian tubules. Our results indicate that the Malpighian tubules play a previously unrecognized role in the chronic processing of blood meals by mosquitoes.

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GENOMIC ISLANDS, REPRODUCTIVE ISOLATION AND ASYMMETRIC INTROGRESSION BETWEEN *ANOPHELES COLUZZII* AND *AN. GAMBIAE*

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Within the ***Anopheles gambiae*** complex, the sibling species ***An. coluzzii*** and ***An. gambiae*** s.s. are major vectors of malaria thought to be undergoing a process of sympatric speciation with gene flow. In the absence of intrinsic post-zygotic isolation between the two cryptic taxa, speciation is thought possible through the association of assortative mating and ecological adaptation genes with genomic regions protected from recombination. These regions of genetic divergence are located within the pericentric regions of chromosomes and known as speciation islands. Spatial swarm segregation has been shown to play a major part in assortative mating in sympatric populations of the two species. Given its role in the process of speciation, the genetic determinants of spatial segregation are expected to be associated with one or several of the known islands of speciation described in the pericentric regions of the X, 2L and 3L chromosomes. In order to demonstrate the association between spatial swarm segregation and speciation islands, we sampled 2063 male and 266 female ***An. coluzzii*** and ***An. gambiae*** individuals from swarms in an area of western Burkina Faso where hybridization is known to occur. Individuals were genotyped at 16 SNPs located within the X, 2L, 3L and assigned to either species based on a majority rule. Despite evidence from extensive introgression between the two sibling species, the overall majority of swarms where still found to be monospecific. Whether non-hybrid, first generation, or backcrossed individuals male and females were associate with ***An. coluzzii*** or ***An. gambiae*** swarms was strictly determined by their genotype at the X chromosome island of speciation. The association between swarm type and the 3L chromosome island was significantly weaker than that with the X island. The association with the 2L island was broken down by the recent adaptive introgression of pesticide resistance loci located within this genomic region. These findings are important for our understanding of the ecological processes and genomic structures associated with the process of sympatric speciation in this important vector species complex.

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APPLICATIONS FROM A FAIRE-SEQ OPEN CHROMATIN PROFILING STUDY IN *AEDES AEGYPTI*

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Despite substantial progress in mosquito genomic research, few cis-regulatory elements (CREs), DNA sequences that control gene expression, have been identified in vector mosquitoes. The resulting deficiency, a significant gap in the basic knowledge of mosquito genetics, has resulted in a lack of drivers to manipulate gene expression in selected tissues at specific times. Such tools, which have revolutionized research in genetic model organisms, would benefit all avenues of mosquito research. FAIRE-seq, formaldehyde-assisted isolation of regulatory elements paired with DNA sequencing, is emerging as a powerful new high-throughput tool for global CRE discovery. During the FAIRE process, chromatin is cross-linked with formaldehyde, sheared, and then phenol-chloroform extracted, allowing for preferential recovery of open chromatin DNA fragments, an evolutionarily conserved indicator of regulatory activity. We recently performed a FAIRE-seq study in the dengue vector mosquito ***Aedes aegypti***. These efforts resulted in identification of thousands of CREs throughout the ***A. aegypti*** genome. We will present results from a high throughput screen in transgenic insects that examines the ability of these elements to promote tissue-specific gene expression ***in vivo***. In addition to validating the FAIRE-seq data set, the screen was designed to select for elements that will drive gene expression in tissues of vector importance in multiple vector insect species. The FAIRE-seq data set has facilitated our analysis of gene regulatory networks in the developing mosquito nervous system. Genome-wide CRE identification has also allowed us to explore how DNA polymorphisms in regulatory regions have contributed to the changes in gene expression observed in dengue susceptible and refractory strains. This investigation, which is generating a toolkit of gene drivers for mosquito research, facilitating the study of mosquito gene regulatory networks, and promoting an understanding of how enhancer evolution contributes to pathogen susceptibility, will promote the use of FAIRE-seq in additional vector mosquito species.

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COMPLEX AND EVOLVING INSECTICIDE RESISTANCE SPANS SPECIES BARRIERS IN *ANOPHELES COLUZZII*

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In 2006, the insecticide resistance allele (kdr) introgressed from ***Anopheles gambiae*** s.s. into ***A. coluzzii*** in parts of West Africa, coincident with the start of an insecticide treated bed net campaign. Here, we demonstrate that no other major genomic regions were introgressed, but we describe a selective sweep on putative cis-regulatory variation in the P450 insecticide resistance candidate gene CYP9K1. The modern ***A. coluzzii*** with kdr and a nearly fixed haplotype at CYP9K1 (cyp-I) is highly resistant to permethrin compared to ***A. gambiae*** (KD50=54min(N=86) vs 21min(N=55)) and has increased in relative frequency in the population from ~65% prior to 2006 to 88% in 2014 (N=179). Interestingly, preliminary bioassay results suggest that cyp-I is not more resistant than another ***A. coluzzii*** haplotype (cyp-II) in the absence of kdr (KD50=32min(N=40) vs 38min(N=7), respectively), potentially indicative of an allele-specific interaction between kdr and cyp-I. To functionally validate putative regulatory mutations associated with the highly selected cyp-I haplotype in ***A. coluzzii*** we have estimated allele-specific expression in F1 hybrids between ***A. coluzzii*** and ***A. gambaie***

(cyp-lll) individuals in the context of insecticide exposure. We have also explored the role of cis-regulatory variation in species divergence between A. gambaie and ***A. coluzzii***.

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BUBBLY GENOMES OF POLYMORPHIC VECTOR SPECIES: ASSEMBLY QUALITY AND PRESERVED VARIATION

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The existence of a reference genome assembly for a vector species facilitates analysis of the physiology, behavior, and population genomics of that species, all of which are critical to vector control efforts. However, creating a reference is often a technically challenging process requiring costly inputs; the high levels of polymorphism found in many insect vectors can complicate assembly even more. New assembly algorithms offer a chance for relatively simple and low-cost genome assembly, but have not been stringently evaluated in highly polymorphic insect vectors. Using 250 bp paired-end reads sequenced from a single library of the major malaria vector ***Anopheles arabiensis***, we show that DISCOVAR ***de novo*** can generate an assembly with contig N50 of 20,645, exceeding the contiguity of recently released assemblies for some closely related vectors. This DISCOVAR assembly of ***An. arabiensis*** contains nearly all the sequence present in an assembly for the same species made from multiple libraries of three insert sizes. The new assembly also recovers a set of universal benchmarking genes with completeness approaching that of the existing assembly, and with better completeness than an existing transcriptome assembly, which represents one of the most common low-cost alternatives to whole genome assembly. Additionally, DISCOVAR ***de novo*** preserves polymorphism information often lost during the assembly process. Using this information, we find that more than a third of the genome comprises polymorphic haplotypes in our sample, despite inbreeding by isofemale founding to reduce heterozygosity. We categorize the observed variants, and approximate their physical location based on alignment to the PEST reference genome for ***An. gambiae***, a major malaria vector closely related to ***An. arabiensis.*** We also analyze haplotype information present in the output. We discuss the potential functional consequences of these genomic variants, both for their direct relevance to a major malaria vector and as a demonstration of the kinds of information that could be gleaned for other vector species.

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WEST AFRICAN *AEDES AEGYPTI FORMOSUS* COLLECTIONS SHOW DIFFERENTIATION AT SEX CHROMOSOMES

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Sex in the yellow fever mosquito, ***Aedes aegypti***, is known to be controlled by an autosomal sex determination locus (SDL) on chromosome 1. Attempts to construct F_1 intercross families between West African and New World ***Ae. aegypti*** parents produced post-zygotic reproductive isolation patterns. Additional crosses indicated patterns consistent with Haldane's rule wherein families arising from male hybrids had significantly lower egg to pupal survival as compared to offspring of female hybrids. Because Haldane's rule applies to species with heterogametic sex chromosomes, we further examined the linkage of sex and ***white-eye*** which are known to be 14 cM apart on chromosome 1. Ninety-four families from four West African collections and 11 families from a Mexican collection were tested. ***White-eye*** and sex co-segregated at 14 cM in Mexico families but segregated independently of sex in 16-60% of West Africa families. Genetic diversity on the three autosomes was compared using high throughput sequencing (HTS) of exon-enriched libraries from pooled males or females from a rural Thailand collection and a sylvatic collection from southeastern Senegal (PK10). Genetic differences (F_{ST}) between marker frequencies in males and females were uniform across all chromosomes in the Thailand collection but were significantly different among sexes in PK10, with the largest differences occurring on chromosome 1; these differences were uniformly distributed across chromosome 1. These observations are consistent with the presence of two subspecies of ***Ae. aegypti*** in West Africa; one with and the other without heterogametic sex chromosomes.

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ANOPHELES GAMBIAE PREFOLDIN AS A *PLASMODIUM* AGONIST INTERACTING WITH LRRD7

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In the malaria vector ***Anopheles***, the IMD pathway is a major player in regulating the mosquito's innate immune defense against ***Plasmodium falciparum***. IMD pathway exerts its anti ***P. falciparum*** response through effector molecules. Previously, we and others have identified LRRD7 (also known as APL2 or LRIM17) is involved in the defense against both human and rodent ***Plasmodium*** parasites. In order to elucidate the mechanisms of anti-malaria function of LRRD7, we used yeast two-hybrid system to fish out the putative interaction partners of LRRD7 and identified prefoldin subunit 6. Subsequently, RNAi-mediated gene silencing assays showed that depletion of prefoldin resulted in a significant decrease in the number of oocysts per midgut, suggesting prefoldin as a ***Plasmodium*** agonist that can interact with LRRD7. The role of prefoldin as a ***Plasmodium*** agonist suggested that it could serve as a target for transmission blocking antibodies. We fed mosquitoes on ***P. falciparum*** gametocyte cultures containing different concentrations of the purified prefoldin antibody, and determined infection intensity. These experiments showed a dosage-dependent transmission-blocking activity, suggesting prefoldin as a putative blocking target to inhibit ***Plasmodium***. Prefoldin, a heterohexameric chaperone, is associated with other molecules to promote proper protein folding. Here we describe the characterization of prefoldin and its potential function as a ***Plasmodium*** agonist that interact with, and presumably inhibit, ***Plasmodium*** antagonists such as LRRD7, or Tep1 in the anti-***Plasmodium*** protein complex. We also discuss ***Plasmodium*** transmission-blocking strategies in mosquitoes using small molecules that inhibit prefoldin.

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HIGH RATES OF ENTERIC FEVER DIAGNOSIS AND LOW BURDEN OF CULTURE-CONFIRMED DISEASE IN RURAL NEPAL

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Kathmandu has been described as the "enteric fever capital of the world", but there are few data on the burden of enteric fever in rural areas of Nepal. We reviewed data on reported enteric fever cases from the Health Management Information System from 1995-2014. We performed a prospective study at four facilities (3 rural, 1 peri-urban), recruiting patients >1 year of age presenting for care who reported fever for >3 days. We

administered a structured questionnaire and obtained blood for culture. Annual enteric fever cases reported in the public sector increased from 85,137 cases in 1995-1996 to 499,761 cases in 2013-2014 (587% increase), when 2.0% of the country's population was diagnosed with enteric fever in the formal sector alone, and enteric fever was the most common diagnosis cited for hospitalization. Total outpatient visits rose significantly during this period, but enteric fever cases rose 40% more. From July 2013-March, 2015, we enrolled 1,776 patients with acute febrile illnesses; enteric fever was the most common clinical diagnosis at all sites. Overall, 1.9% (34) of participants had typhoidal ***Salmonella*** recovered from blood (rural: 0.4%; peri-urban: 2.4%). All but one of the ***Salmonella*** isolates had intermediate susceptibility or resistance to Ciprofloxacin. Enteric fever is commonly diagnosed in rural Nepal, with annual diagnosis rates that exceed incidence rates reported from high burden, urban settings. By contrast, the prevalence of culture-confirmed typhoid among febrile patients at rural sites was <1%. Even assuming a low sensitivity of blood cultures of 40%, our results suggest that the vast majority of individuals in rural Nepal who are clinically diagnosed and treated for enteric fever do not actually have that illness. Additional analyses of collected samples is on-going. Geographically representative laboratory surveillance for typhoid and other acute febrile illness are needed to inform antibiotic use in rural areas.

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TYPHOID FEVER IN YOUNG CHILDREN IN BANGLADESH: CLINICAL FINDINGS, ANTIBIOTIC SUSCEPTIBILITY PATTERN AND IMMUNE RESPONSES

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Typhoid fever caused by Salmonella enterica serotype Typhi (S. Typhi) is a potentially life-threatening systemic disease. High prevalence rates of typhoid fever have been reported in resource-limited regions of the world, with children under 5 years of age bearing a large burden. However, immune responses and clinical findings in children are not well defined. We describe clinical and immunological characteristics of young children with typhoid fever and antimicrobial susceptibility patterns. Previously we characterized antibody-in-lymphocyte secretion responses (TPTest) during acute typhoid fever in adults and also measured the membrane preparation (MP) IgA responses in young children at day of enrolment, and then 7-10 days and 21-28 days later. We also assessed plasma IgA, IgG and IgM responses, and T cell proliferation responses to MP antigen. We compared the responses in young children (1-5 years) with those seen in older children (6-17 years), adults (18-59 years), and age-matched healthy controls. We found that, the patients in all age cohorts had significantly higher MP-IgA responses in lymphocyte secretion at clinical presentation compared to age-matched controls, and the values fell in all groups by late convalescent stage. Similarly, plasma IgA responses in patients were elevated at presentation compared to controls, with acute and convalescent IgA and IgG responses being highest in adults. T cell proliferative responses were increased in all age cohorts by late convalescence. In all age cohorts clinical characteristics were similar, although younger children were more likely to present with loss of appetite, less likely to complain of headache compared to older cohorts, and adults were more likely to have ingested antibiotics. Multi-drug resistant strains were present in approximately 15% of each age cohort, and 97% strains had resistance to nalidixic acid. This study demonstrates that S. Typhi bacteremia is associated with comparable clinical courses, immunologic responses in various age cohorts, including in young children, and that TPTest can be used as marker of recent typhoid fever, even in young children.

1200

COMPARISON OF THE PERFORMANCE OF THE TPTEST, TYPHIDOT AND TUBEX IMMUNODIAGNOSTIC ASSAYS IN DETECTING PATIENTS WITH ENTERIC FEVER IN BANGLADESH, INCLUDING LATENT CLASS MODELING

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Enteric fever caused by Salmonella enterica serotype Typhi (S. Typhi) and serotype Paratyphi are important public health problems in Bangladesh. Previously, we developed and evaluated a diagnostic method for typhoid and paratyphoid fever (TPTest) and found highly sensitive and specific. In this study, we compare the TPTest with Tubex and Typhidot in our setting. For analysis, we categorized 92 patients into four groups: S. Typhi bactermic patients (n=28); patients with fourfold change in Widal test from day 0 to convalescent period (n=7); patients with Widal titer ≥1:320 (n=13); patients suspected enteric fever, but with negative blood culture and Widal titer (n=44). We also tested healthy controls (n=20) and other febrile illness patients (n=15) (kala-azar and tuberculosis). Out of 28 S. Typhi bacteremic patients, 28 (100%), 21 (75%) and 18 (64%) patients were positive by TPTest, Tubex and Typhidot respectively. For the four-fold change of Widal titre, the TPTest, tubex and typhidot were positive in 7 (100%), 6 (86%), 5 (71%) respectively. For patients with Widal ≥1:320, the TPTest, Tubex and Typhidot were positive for 9 (69%), 5 (38%), 3 (23%). The TPTest method was negative for all healthy controls whereas Tubex and Typhidot were positive for 3 (15%) and 5 (25%) respectively. For other febrile illnesses, TPTest, tubex and typhidot were negative for 14 (93.3%), 14 (93.3%), and 13 (86.7%) respectively. Among 44 patients who were negative by blood culture and Widal test, but clinically diagnosed as enteric fever, 24, 09 and 15 were positive by TPTest, Tubex and Typhidot respectively. In latent class model in which sensitivity and specificity of all diagnostics were estimated simultaneously, the sensitivity of TPTest was estimated at 96.0% (95% CI: 87.1%-99.8%), Tubex was 60.2% (95% CI: 49.3%-71.2%), and Typhidot was 59.6% (95% CI: 50.1%-69.3%). Specificity was estimated at 96.6% (90.7%-99.2%) for TPTest, 89.9% (79.6%-96.8%) for Tubex, and 80.0% (67.7%-89.7%) for Typhidot. These results suggest that the TPTest is highly sensitive and specific in diagnosis of enteric fever in endemic regions like Bangladesh compared to tubex and typhidot assays.

1201

A NOVEL HUMAN MODEL OF SALMONELLA ENTERICA SEROVAR PARATYPHI A CHALLENGE IN HEALTHY ADULT VOLUNTEERS

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A new human paratyphoid challenge model provides an opportunity to gain insight into the immunobiology of this little studied cause of enteric

fever . A dose (de-)escalation oral challenge study with S. Paratyphi A (NVGH308 strain) was conducted in 40 healthy adult volunteers. Following ingestion of bicarbonate buffer solution to neutralise gastric acid, participants ingested either 103 (n=20) or 800 (n=20) CFU of S. Paratyphi A. Successful infection was defined by positive blood culture and/or fever ≥ 38°C for at least 12 hours. Antimicrobial treatment was commenced on diagnosis or 14 days after challenge. The 103 CFU oral challenge dose was associated with a higher attack rate (60% vs. 40%), shorter incubation period (median 6 vs. 8 days), longer duration of bacteraemia (mean, 4 vs. 3 days; range, 1-7 vs 1-6 days) and higher fever rate amongst those developing enteric fever (7/12 vs. 2/8). Fever correlated with symptoms (mean, 32 solicited symptoms per participant with fever ≥38°C vs. 8 in those who were bacteraemic without fever), with headache and general malaise most commonly reported. Preliminary serological data after challenge with the 103 CFU dose confirms that those participants who developed paratyphoid have raised IgM, IgA and IgG antibody responses against LPS by day 28 after challenge. An attack rate between 60% and 75% rate in an antigen-naïve cohort is attained at a dose of 103 CFU of S. Paratyphi A, providing a model for study of novel paratyphoid vaccines.

1202

A MAGNETO-DNA NANOPARTICLE SYSTEM FOR THE RAPID DIAGNOSIS OF ENTERIC FEVER

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There is currently no optimal assay for diagnosing patients with acute typhoid or paratyphoid fever. Amplification techniques that target bacterial genomic nucleic acids have been limited due to the low bacterial burden present in peripheral blood. Alternative approaches for typhoid diagnostic assays are needed. We have previously described the gene expression profile of Salmonella enterica serovars Typhi and Paratyphi A in the blood of infected humans in Bangladesh. Using this information, we designed primer pairs for S. Typhi and S. Paratyphi A genes expressed at high levels in the blood of infected humans. We selected genes that were specific for S. Typhi (staG) and S. Paratyphi A (hsdR and SPA2472), and we designed primer pairs for conserved genes specific to Salmonella spp. (sopB and sipC). To evaluate this approach, we first produced cDNA from mid-log ***in vitro*** cultures of S. Typhi, S. Paratyphi A, S. Typhimurium, and E. coli. Using asymmetric amplification, we detected target products for all 3 Salmonella serovars, but no amplification product was observed from E. coli samples. We detected amplified target cDNA using novel magneto-DNA probes and a miniaturized nuclear magnetic resonance device. The detection limit of this assay was 0.01-1.0 CFU/ml. For proof of principle, we extracted total RNA from a 500 mcl blood sample from 3 patients bacteremic with S. Typhi in Bangladesh, and we were able to detect amplified target cDNA in 3 out of 3 samples using the hand-held nuclear magnetic resonance device. Our results suggest that a magneto-DNA nanoparticle system may be a promising platform for the rapid and culture-free diagnosis of enteric fever and non-typhoidal Salmonella bacteremia.

1203

DIARRHEA ASSOCIATED WITH *SHIGELLA* IN CHILDREN UNDER FIVE YEARS AND SUSCEPTIBILITY TO ANTIMICROBIALS IN RURAL WESTERN KENYA, 2008-2012: THE GLOBAL ENTERIC MULTICENTER STUDY (GEMS)

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We examined clinical features, risk factors and antimicrobial susceptibility of ***Shigella*** among children with moderate-to-severe diarrhea (MSD) in rural western Kenya using prospective, hospital-based surveillance. MSD case was defined as a child aged 0-59 months, passing ≥3 loose stools in the previous 24 hrs. with ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization within 7 days of diarrhea onset. Bacterial pathogens identified in stool were tested against commonly used antibiotics by Kirby-Bauer disk diffusion. Characteristics of ***Shigella***- infected vs. non-infected MSD cases were compared using unconditional logistic regression. From January 2008-September 2012, 130 (7.3%) of 1,778 specimens collected from MSD cases tested positive for ***Shigella***, including 80 ***S. flexneri*** (61.5%), 24 S. sonnei (18.5%), 20 S.dysenteriae (15.4%), and 6 S. boydii (4.6%). The median age of ***Shigella***- infected vs. non-infected patients was 19.5 (IQR: 10.0-34.0) versus 12.0 (IQR: 7.0-23.0) months (P <0.0001). Compared to non-infected patients, those with ***Shigella*** were more likely to be 24-59 months old (40.8% vs. 24.6%, %) (odds ratio [OR] 2.45; 95% confidence interval [CI] 1.61-3.74]) and experience blood in stool (39.5% vs. 8.8%, ([OR 6.77, 95% CI 4.58-10.02]); they were less likely to vomit ≥ 3 times/24hrs (31.5% vs. 49.6%; OR 0.47, 95% CI 0.32-0.69). Isolates were susceptible to in decreasing order: ceftriaxone (99.2%), gentamicin (98.4%), ciprofloxacin (98.4%), chloramphenicol (56.9%) 51.2%, amoxicillin-clavulanic acid (51.2%), ampicillin (37.4%) and tetracycline (14.6%). In conclusion, ***Shigella*** was most prevalent among the older range of under-five children with MSD, and was significantly associated with bloody diarrhea; ***S. flexneri*** was the most prevalent species. Although ***Shigella*** isolates were largely susceptible to ciprofloxacin and ceftriaxone, reduced susceptibility to many of the inexpensive and commonly used antimicrobials in this setting limits therapeutic options, increasing the economic burden and risk of treatment failure for shigellosis.

1204

DEVELOPMENT OF A LIVE ATTENUATED ORAL VACCINE AGAINST SHIGELLOSIS AND TYPHOID FEVER

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There are more than 125 million cases of shigellosis globally; with an estimated ~450,000 cases in United States every year. 72% of the US cases are caused by ***Shigella sonnei***, and antibiotic-resistant ***S. sonnei***

is on the rise. Our goal is to address several significant vaccine challenges simultaneously: 1) The lack of a licensed vaccine for prevention of morbidity and mortality due to shigellosis; 2) The need for a multivalent vaccine that will simultaneously protect against multiple disease agents, and 3) The need for an easy-to-administer, child-friendly, safe, oral vaccine vector platform for administration of multiple foreign immunogens that generates long term efficacy following a rapid immunization regimen and can be distributed without the need for refrigeration. We have exploited the extensive safety record of the live, oral, attenuated ***Salmonella*** Typhi vaccine (Ty21a) by utilizing it as a vector to develop a safe, stable, easily administered combination oral vaccine that will simultaneously protect against shigellosis and typhoid fever. As a first step, we have recombineered the ***S. sonnei*** (Ss) form 1 O antigen gene cluster into the Ty21a chromosome to create Ty21a-Ss, which stably expresses ***S. sonnei*** O antigen for up to 100 generations. A fully characterized seedbank was used to immunize mice either intraperitoneally or through mucosal route. Mice immunized with Ty21a-Ss produced high levels of serum IgG antibodies against both ***S. sonnei*** (53G) and ***S.*** Typhi. In contrast, mice immunized with Ty21a only produced comparable antibodies against S. Typhi but not ***S. sonnei***. The titers against ***S. sonnei*** are higher than that from a previously published ***S. sonnei*** LPS-based vaccine that protected guinea pigs from ***S. sonnei*** infection. We are currently assessing the protective efficacy of Ty21a-Ss by a mucosal challenge with lethal ***S. sonnei*** infection. Our work provides the foundation for construction of a multivalent anti-shigellosis vaccine that will protect against more than 85% of shigellosis worldwide and against typhoid fever.

1205

RAPID REORGANIZATION OF THE PARASITE PLASMA MEMBRANE INDUCED BY NEW CLASSES OF ANTIMALARIAL DRUGS THAT DISRUPT NA+ HOMEOSTASIS

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An international collaborative effort has identified pyrazoleamide (PA) class of compounds with highly potent activity against malaria parasites, leading to the designation of PA21A092 as a preclinical antimalarial candidate. Our investigations suggest the PA compounds and another recently developed antimalarial, spiroindolone (KAE609), target a common vulnerable pathway. These compounds rapidly and potently disrupted Na+ homeostasis in ***Plasmodium falciparum***. We are investigating consequences of Na+ homeostasis disruptions by these compounds using a variety of approaches. Live cell imaging revealed significant increase in intraerythrocytic parasite volume within a 2 h exposure to PA and other compounds. Furthermore, exposures to several compounds with distinct chemical structures (but all with the ability to disrupt Na+ homeostasis) result in rapid parasite plasma membrane reorganization as indicated by the acquisition of saponin-sensitivity assessed as leakage of cytosolic proteins following mild saponin treatment. Experiments involving methyl-beta-cyclodextrin mediated cholesterol extraction of freed parasites suggest that the treatment with PA compounds results in rapid cholesterol incorporation into the (usually cholesterol-poor) plasma membrane of the trophozoite stages. Electron microscopy also suggests that these compounds cause a rapid reorganization of the parasite plasma membrane. These and other observations suggest that this series of compounds are prematurely initiating a cascade of events generally associated with the egress of parasites at the last phase of schizogony. We hypotheize that such changes contribute to a rapid clearance of infected RBCs from the circulation ***in vivo***

1206

THE PHENYLALANYL-TRNA SYNTHETASE IS A DRUGGABLE TARGET IN *PLASMODIUM FALCIPARUM*

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Sustained availability of efficacious drugs is essential for worldwide efforts to eradicate malaria. The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern with reports of artemisinin-based treatment failures escalating the need for novel antimalarial chemotherapies. Thus the discovery of novel druggable targets and pathways including those that are critical for multiple life stages is a major challenge for the development of next-generation therapeutics. Using an integrated chemogenomic approach combining screening efforts of a Diversity Oriented Synthesis (DOS) library, drug-resistance selection, and whole genome sequencing, we have identified the ***Plasmodium falciparum*** cytoplasmic phenylalanine-tRNA synthetase (***Pf***cPheRS) as the target of a novel antimalarial candidate, BRD3444. The DOS library aims to cover chemical space extending beyond the common confinements set by "drug-like" parameters, which is characteristic for traditional pharmaceutical libraries and limits the diversity of represented compounds. The additional structural complexity exploited by the DOS library allows us to probe novel pathways and targets in the parasite, as exemplified by BRD3444. Aminoacyl-tRNA synthetases (aaRSs) are validated targets in several microorganisms and have more recently been proposed as attractive targets for chemotherapeutic intervention in malaria. Our work identifies PheRS as a druggable target in ***Plasmodium*** and provides a path forward to next-generation antimalarials.

1207

A DOSE RANGING CLINICAL TRIAL TO EVALUATE THE PHARMACOKINETICS AND PHARMACODYNAMICS OF THE COMBINATION OF DSM265 AND OZ439 IN A *PLASMODIUM FALCIPARUM* INDUCED BLOOD STAGE MALARIA SYSTEM

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WHO recommends that ***Plasmodium falciparum*** malaria be treated using a combination of two drugs with complementary pharmacokinetic and pharmacodynamics properties as well as targeting different biochemical pathways in the parasite to optimise cure and prevent resistance. In the past, drug and dose selection of the combination therapy has been empirical and, in general, formal dose finding was not conducted. As a consequence, therapies were not necessarily optimized, thereby risking suboptimal clinical efficacy, selection of resistance, unexpected toxicities, promotion of gametocytemia, and un-necessary cost of goods. For the development of novel antimalarial combination therapies, new approaches for dose finding and selection of partners are therefore required. Recently, the Induced Blood Stage Malaria (IBSM) human challenge model has been validated using approved antimalarials, and used to characterise new chemical entities (NCE) as single agents. We have investigated for the first time the combination of two NCE, OZ439 and DSM265 in the IBSM model. The study was designed as adaptive approach, using sequential cohorts each of 8 healthy subjects. Participants in the first cohort were treated with OZ439 and DSM265, administered as single oral doses of 200 mg and 100 mg respectively. No significant pharmacokinetic interactions or adverse effects were observed. In contrast to the outcome of a previous

study where OZ439 was used in IBSM in an identical dose, clearance of parasitemia was observed. However, 4/8 subjects experienced recrudescent parasitemia between 11 and 21 days after treatment. The mean Minimal Inhibitory Concentrations at this dose level were 0.3 ng/ml and 573 ng/ml for OZ439 and DSM265 respectively. The study is still ongoing, with data from subsequent cohorts to be presented. However, these preliminary results show that the OZ439 / DSM265 combination shows promise for future development, and suggest that the IBSM model is suitable for documenting the pharmacokinetics and pharmacodynamics of the combination of antimalarials under development.

1208

A PHASE 2, RANDOMIZED, CONTROLLED, DOSE-ADAPTIVE TRIAL OF PRIMAQUINE IN COMBINATION WITH DIHYDROARTEMISININ-PIPERAQUINE TO REDUCE TRANSMISSION OF *PLASMODIUM FALCIPARUM* MALARIA IN MALI

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Despite considerable progress in malaria control, tools that specifically prevent malaria transmission are needed to achieve malaria elimination in the majority of African settings. Primaquine (PQ), when added to artemisinin combination therapy, may radically prevent malaria transmission to mosquitoes. High doses of PQ (0.75 mg/kg) have been associated with hemolysis. We determined the safety and efficacy of single-low dose (SLD) PQ. Between September 2013-December 2015, we conducted a phase 2, randomized, double-blind, controlled, dose-adaptive study of SLD PQ among 81 G6PD non-deficient males in Mali infected with uncomplicated ***Plasmodium falciparum*** malaria. Microscopy positive gametocyte carriers were treated with dihydroartemisinin-piperaquine (DP) and allocated 0.5, 0.25, 0.125, 0.0625 and 0 mg/kg primaquine. The primary outcome was percent change in mosquito infectivity at Day 2 following primaquine treatment. Mosquito infectivity was assessed through membrane-feeding assays at enrolment and days 2 and 7 following treatment; gametocyte carriage was quantified by Pfs25 mRNA quantitative reverse transcriptase PCR (qRT-PCR). Safety endpoints included the average within-person change in hemoglobin concentration and adverse events (AE) during follow-up. We found large and statistically significant reductions in mosquito infectivity following treatment with PQ doses ranging from 0.25 mg/kg to 0.5 mg/kg primaquine, compared to controls. Participants taking 0.25 mg/kg PQ experienced a 93% reduction in infectivity at Day 2 (vs. 11% reduction in controls, $P<0.01$) and a 93% reduction in infectivity at Day 7 (vs. 45% reduction in controls, $P=0.01$). Compared to DP, qRT-PCR gametocyte carriage was significantly reduced in all PQ arms and lowest in the 0.5mg/kg PQ arm. We did not find meaningful or statistically significant drops in hemoglobin in any group during follow-up. AE occurrence was not associated with PQ treatment group ($P=0.34$). SLD PQ, given in conjunction with DP, appears to be safe and efficacious for the prevention of malaria transmission in G6PD non-deficient populations.

1209

DRUG-DRUG INTERACTION STUDY OF TAFENOQUINE AND THE ACTS DIHYDROARTEMISININ-PIPERAQUINE (DHA-PQP) AND ARTEMETHER-LUMEFANTRINE (AL)

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Tafenoquine (TQ) is an 8-aminoquinoline (8-AQ) in development as a single dose treatment for the radical cure of ***Plasmodium vivax*** malaria. Tafenoquine is being co-administered with chloroquine to kill the blood stages of ***P. vivax*** during phase 3 registrational studies. However, artemisinin combination therapies (ACTs) may also be used in combination with 8-AQs to achieve radical cure. Recognizing the recommendation in some national treatment guidelines to use ACTs as first line treatment for ***P. vivax*** malaria, a better understanding of potential drug-drug interactions (DDI) is required. We have therefore undertaken a DDI study (200951, NCT02184637) of TQ co-dosed with dihydroartemisinin-piperaquine (DHA-PQP) or artemether-lumefantrine (AL). This 5-cohort, randomized, open-label, parallel-group study recruited a total of 120 glucose-6-phosphate dehydrogenase (G6PD) phenotypically non-deficient healthy volunteers equally into each cohort. The co-primary objectives of this study were to characterize both the effects of a 300mg single dose of TQ on the pharmacokinetics (PK; using changes in AUC(0-t), AUC(0-∞), and Cmax as endpoints) of each of the two ACTs and their major metabolites when co-administered according to their prescribed dose as well as the effects of the ACTs on the PK of TQ. Key secondary objectives included documenting frequency of adverse events, changes in haemoglobin (in particular related to genetic G6PD deficiency status) and characterizing QTcF changes by analysing maximum change from baseline in all cohorts and for subjects who receive DHA-PQP, a measure of the change from baseline versus 4-hours post-third dose. Headline PK and safety data from this study will be presented and implications for co-administration of ACTs and TQ will be discussed.

1210

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN HIV-UNINFECTED PREGNANT WOMEN WITH DIHYDROARTEMISININ-PIPERAQUINE: A DOUBLE BLINDED RANDOMIZED CONTROLLED TRIAL

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Intermittent preventive treatment with sulfadoxine pyrimethamine (SP) remains one of the primary interventions for the prevention of malaria in HIV-uninfected pregnant women in Africa. However with the spread of antifolate resistance, new interventions for the prevention of malaria in pregnancy are urgently needed. We are conducting a double blinded randomized controlled trial comparing 3 dose SP vs. 3 dose dihydroartemisinin (DP) vs. monthly DP in HIV-uninfected pregnant women living in Tororo district, Uganda, a high malaria transmission setting. Between June-October 2014, 300 women were enrolled at 12-20 weeks of gestation based on ultrasound dating, given a long lasting insecticide treated net, and randomized to therapy. Participants are being followed up in a dedicated study clinic for all their medical care and encouraged to

deliver at the hospital adjacent to the study clinic. The primary outcome is risk of placental malaria by histopathology. Secondary outcomes included other measures of malaria during pregnancy and delivery, adverse events and birth outcomes. As of 28th February 2015, 195 women had delivered and 9 women were prematurely withdrawn before delivery. Malaria incidence during pregnancy was 1.88 and 0.45 episodes per person year before and after starting study drugs, respectively. Of the 195 deliveries, 3 (1.5%) were spontaneous abortions 2 (1.0%) were stillbirths, 4 (2.1%) had congenital anomalies, 22 (11.2%) were preterm deliveries, and 31 (16.3%) were low birth weight. The risk of placental malaria was 40.7% by histopathology and 3.7% by placental blood smear. During follow-up, a total of 27 grade 3 or 4 adverse events occurred, of which 15 were due to anemia. It is anticipated that all women will have delivered by May 2015 and that the final un-blinded results of the trial will be presented at the meeting.

1211

IMPACT OF THE NUTRITIONAL STATUS ON ARTEMETHER-LUMEFANTRINE EFFICACY IN UNCOMPLICATED FALCIPARUM MALARIA IN CHILDREN IN MALI

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The aim of this study was to evaluate the impact of nutritional status on the Artemether-Lumefantrine (AL) efficacy in children under 5 years on 42 days. From July 2014 to December 2014 during a prospective longitudinal study in children with uncomplicated malaria aged 6 to 59 months, we measured the efficacy of AL on falciparum malaria in Faladje in Mali. Anthropometric measures (malnourished, z-score < -2 SD, and non-malnourished, z-score:-1 and -2 SD) and body mass index (BMI) based on NIH WHO Guidelines for girls and boys were used and AL efficacy was compared between the various nutritional statuses. Deuterium was used as a tracer to estimate body composition using three saliva samples collected pre-dose (H0 before treatment), post-dose1 (at 3 hours) and post-dose 2 (at 4 hours). The percentage of body fat was determined using a Fourier transform infrared spectrophotometer (FTIR). Cases of severe malnutrition were not included in this study. 151 children were included with a rate of lost to follow-up of 1%. Without molecular correction we found that rates of early treatment failures were 0%, late clinical failures were 18.% and late parasitological failures were 36.1% and raw adequate clinical and parasitological response were 45.8%. Anthropometric measures showed 11% (n=17) of emaciation, 18% (n=27) of underweight and 14% (n=21) of chronic malnutrition. AL efficacy by 42 days was comparable in malnourished and non-malnourished children. Salives analysis on 104 subjects revealed that 16.35% (n = 17) were malnourished against 83.65% (n = 87) for non malnourished. According to body composition, the efficacy of A L by 42 days of follow-up was comparable in the two groups. Molecular correction of treatment efficacy is underway. Conclusion : Without molecular correction, the AL efficacy was not impacted by mild-malnutrition status.

1212

HAITI 2012 NATIONAL COMMUNITY SURVEY: MALARIA PREVALENCE IS LOW WITH A NOTABLE PROPORTION OF ASYMPTOMATIC CASES

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Haiti intends to eliminate malaria by 2020 and its low level of transmission is most recently evidenced from a community survey conducted in 2011 which showed that the national parasitemia prevalence was <1%, even by sensitive polymerase chain reaction (PCR). In December 2012, during the expected high transmission season, the survey was repeated after implementation of new treatment guidelines and a bed net campaign earlier that year. This survey employed a cross-sectional, two-stage cluster survey design, where enumeration areas (EAs) were selected with a probability proportional to size and 20 households (HHs) within EAs were systematically selected. Questionnaires were conducted and HH members of all ages were tested for malaria by rapid diagnostic tests (RDTs), expert microscopy, PCR, and serology (presented separately). Sixty-two EAs were sampled and 7,607 persons surveyed. Some participants did not consent to provide specimens for all procedures. RDTs were performed for 6,365 persons, and 84 (1.3%) were positive. A total of 5,001 blood slides were read by a reference lab, and 3 (0.06%) were positive. Dried blood spots (DBS) were provided by 5,511 persons; photo-induced electron transfer (PET)-PCR found 17 (0.3%) positive for ***Plasmodium*** genus. Nested PCR confirmed 14 ***Plasmodium falciparum (Pf)***, two ***P. malariae*** (***Pm***), and one mixed ***Pf*** and ***Pm*** infections. PCR confirmed two of three positive microscopy results and one RDT, although 32 positive RDTs could not be tested by PCR. The median age of those with a positive test was 13.5 years; range <1 to 71 years. For those with any positive test, fever was reported in the prior two weeks for four of 100 persons. In low transmission settings, discordance among tests is likely. These results confirm the low-transmission setting for Haiti and demonstrate that almost all parasitemia was asymptomatic. Additionally, inclusion of more sensitive serological methods and applying a sub-national, sampling strategy will be essential for characterizing transmission hotspots.

1213

CHARACTERIZING TYPES OF HUMAN MOBILITY TO INFORM DIFFERENTIAL AND TARGETED MALARIA ELIMINATION STRATEGIES IN NORTHEAST CAMBODIA

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Population mobility has been identified as a major challenge for malaria elimination in the Greater Mekong Subregion. Given the large sociocultural differences between various types of mobile populations, the aim of this study was to characterize different types of mobility in relation to malaria vulnerability. The study was conducted in Ratanakiri Province, Cambodia.

A parallel mixed-methods study design was used, combining qualitative ethnographic research (n=410 interviews, participant observation) and quantitative surveys. Different surveys were used to quantify malaria vulnerability among different types of mobile populations and in relation to malaria control measures (n=824 Indigenous Population Survey; n=704 Khmer Migrant Population Survey; n=4996 Indigenous Malariometric Survey). Different structural types of human mobility were found with differential risk and vulnerability towards malaria. Among the indigenous population, although access to malaria testing and treatment through village malaria workers and LLIN coverage was high, control strategies fail to take into account farmers' residence system, entailing overnights and prolonged stays at forest farms/fields (61% during rainy season), increasing their malaria risk (OR 1.66, 95% CI 1.21-2.28, p=0.002). Additionally, various types of irregular mobility were identified including cross-border mobility, hunting and logging. Khmer migrants are active on rubber plantations and mines (82%), representing a fundamentally different social group that was not reached by LLIN-distribution campaigns (67%) since most were not registered (79%) and (95%) unaware of the village malaria worker system due to poor social integration. Furthermore, most purchased non-treated bed nets (75%), the majority of which (55%) were damaged. In conclusion, different mobility patterns co-exist and are associated with differential malaria risk. This study demonstrates the need for differential malaria control strategies among groups that are, nevertheless, usually jointly categorized as "mobile populations".

1214

RE-ORIENTING TOWARDS ELIMINATION IN MATABELELAND SOUTH, ZIMBABWE: PERFORMANCE OF AN ENHANCED DIGITIZED CASE-BASED SURVEILLANCE SYSTEM

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Matabeleland south province in Zimbabwe plans to reach malaria elimination by 2017. Currently, cases are reported in two surveillance systems: a routine passive system where data are aggregated monthly, and an individual case-based system. To support elimination efforts, the individual case-based system was re-oriented from a paper-based to digitized system in September 2014. The transition involved questionnaire and documentation revision, and district and health facility level capacity assessments. The new system provides real-time case data, enabling identification and targeting of high risk areas and populations, and generating evidence for policy change. Data was extracted from the paper-based system database at baseline (January to July 2013), the current digitized system database (September to December 2014), and routine surveillance database for both time periods. Proportion of cases reported, proportion of cases classified as local or imported, and proportion of cases investigated were estimated and compared over time. At baseline, discrepancies were found between case numbers reported in the paper-based reporting system (276) and the routine passive system (910). With the new digitized system, 295 confirmed malaria cases were reported compared to 390 confirmed cases from the routine surveillance system, revealing a 48% reduction in discrepancy. At baseline, the case investigation rate was low (28%), travel history was recorded for 5% of cases, but none were classified. Significant improvements were noted using the new digitized system: 54% of cases were investigated, and 59% of cases were classified. Improved performance can be attributed to simplification of data entry with a reduction from 300 to 30 fields in data collection forms and availability of real-time case data (one month maximum delay). The new, digitized case-based surveillance system has shown significant improvements in epidemiological and efficiency indicators, and results have already been used to improve targeting and efficiency of interventions.

1215

THE GAMETOCYTOCIDAL EFFICACY OF PRIMAQUINE IN MALARIA ASYMPTOMATIC *PLASMODIUM FALCIPARUM* CARRIERS IN THE GAMBIA: RESULTS OF A RANDOMIZED CONTROLLED TRIAL

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Primaquine is recommended for treatment campaigns against ***Plasmodium falciparum*** gametocytes in (pre-) elimination settings. Although the recommended dose is believed to be safe, there is weak evidence on its efficacy. Studies are needed on the efficacy of primaquine, particularly in asymptomatic parasite carriers as they can maintain transmission. Asymptomatic, malaria infected, G6PD-normal individuals determined through community screening were randomised to receive either dihydroartemisinin-piperaquine (DHAPQ) alone (control arm) or with a single dose of primaquine (PQ) at three different dosages, i.e. 0.75mg/kg, 0.40mg/kg and 0.20mg/kg. The primary endpoint was the prevalence of ***Plasmodium falciparum*** gametocyte on Day 7 of a 42-day follow-up determined by quantitative nucleic acid sequence based amplification assay. A total of 667 participants were enrolled in the trial. Preliminary analysis shows significant reduction in Day-7 gametocyte prevalence between the control and the 0.75mg/kg PQ and the 0.40mg/kg arms. Adverse events were few with 8 (2.4%) reports of anaemia (Hb <8.0g/dl) which resolved on iron supplements. DHAPQ plus PQ; 0.75mg/kg and 0.40mg/kg PQ seem to be able to reduce gametocyte carriage at Day-7. The safety profile of primaquine was overall good.

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TOWARDS MALARIA ELIMINATION IN ZANZIBAR: DEVELOPMENT OF EARLY EPIDEMIC DETECTION THRESHOLDS IN AN INCREASINGLY LOW TRANSMISSION SETTING

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The Malaria Early Epidemic Detection System (MEEDS) was implemented in Zanzibar in 2008 to monitor malaria incidence. We analysed MEEDS data from 2008 to 2014 to assess trends in malaria in Zanzibar, and to develop thresholds for monitoring at district and heath facility levels. We conducted mixed effects negative binomial regression modelling at district and health facility levels using weekly malaria cases as the primary dependent variable. Specification of the predictor variables involved 5 key features: fixed effects for districts; flexible smooth characterisation of seasonal variations by inclusion of restricted cubic spline transformations of epidemiologic week number; interaction of district dummy variables

and the week spline terms; yearly random effects to model deviations from typical mean; and random effects for health facilities. Alert and alarm thresholds were respectively defined as the 95th and 99th percentile of a Poisson distribution with corresponding smoothed mean function. We compared our results to the cumulative sum and the 75th percentile methods, with allowance for different lengths of the baseline reference period. Across the 10 districts of Zanzibar, the number of health facilities participating in the MEEDS surveillance programme increased from 137 facilities in 2008 to 160 in 2014. Our thresholds conformed with apparent seasonal variations, and in contrast to thresholds based on conventional methods, were less susceptible to erratic variations when underlying data were sparse. The thresholds identified all known historical excesses of malaria cases in some districts for some particular years, and the frequency of alerts and alarms raised per year were acceptably few when considering only threshold breaches lasting for at least two consecutive weeks. Zanzibar has made tremendous progress in achieving millennium development goal #6, reducing malaria incidence by 70% in children and about 50% in adults over a 7-year period. These thresholds should facilitate greater efficiency in continued monitoring and delivery of interventions, eventually progressing to malaria elimination in Zanzibar.

1217

POTENTIAL IMPACT OF RAPID DIAGNOSTIC TEST DIAGNOSTIC ERROR ON INCREASED MALARIA TRANSMISSION OF EMERGING UNTREATABLE MALARIA

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Emerging untreatable malaria in the Greater Mekong Subregion could result in a public health disaster when it reaches Africa. Malaria diagnostic tests are recommended by the World Health Organization to obtain a specific malaria diagnosis before; however, all diagnostic test have errors associated with use. We examined diagnostic quality in Vietnam, where microscopy is the standard of care and RDTs are recommended only where or when microscopy is not available. In remote areas of Vietnam, RDTs are now being distributed where presumptive treatment was previously used. While microscopy appears to be of a very high quality, both diagnostic tests will have errors resulting in patients with undiagnosed, and hence, untreated malaria. This has the potential for malaria will mortality, as well as persistent parasitemia, and gametocytemia leading to on-going malaria transmission. In two provinces in central Vietnam, a project was established to capture diagnostics data to allow assessment diagnostics error in the real-world setting. Mathematical modeling tools will be employed to investigate the benefit of malaria microscopy and RDTs, as well as estimate the potential danger of false negative diagnostic results in an elimination setting. This evaluation began in January 2015 and will complete in August 2015. In the two provinces, 2.14% of 654 negative where reread by expert microscopists as positive (3 ***Plasmodium falciparum***, 10 ***P. vivax***, 1 both species). The sensitivity of front-line microscopists (commune-based) was 85.6% with a species accuracy of 68.5%. Real world RDT assessment is pending. While the manufacturer's package insert reports xx, for both species, some field studies (in South America) suggest 92.0% for Pv and 88.1% for Pf. In Vietnam, approximately XX cases were diagnosed with malaria microscopy and XX with RDT in 2014 ((confirm)). With this number of cases and the documented performance of microscopy and RDTs, we initially estimate XX ***P. falciparum*** and YY ***P. vivax*** cases are going initially untreated. These results will be used to model the potential impact of these diagnostic error within a malaria elimination program.

1218

CHANGES IN MALARIA PARASITE POPULATION GENETICS WITH APPLICATION OF MASS DRUG ADMINISTRATION IN ZAMBIA

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We have detected dramatic changes in parasite population genetic signals over time in Senegal under conditions of major intervention application. These signals indicate both decline in transmission and rebound of transmission using epidemiological modeling frameworks. Based on these data, we hypothesize changes in parasite population structure in Zambia under conditions where Mass Drug Administration (MDA) has been applied in the Southern Province. These predicted signals include decreases in complexity of infection (COI) and increases in the proportion of monogenomic infections (PMI) as well the increase in relatedness among parasites as detected using a molecular barcode approach. We applied genotyping approaches to samples obtained from a nested cohort within a larger community randomized trial testing use of MDA with dihydroartemisinin piperaquine (DHAp) to reduce malaria burden. The trial contains three arms: 1) community-wide MDA; 2) focal MDA (fMDA), where everyone in a household receives treatment if anyone in that household tests positive by malaria rapid diagnostic test (RDT); and 3) control, where current standard malaria control practice is carried out without mass treatment campaigns. The study was stratified by malaria transmission above and below 10% prevalence, and after one round of intervention parasite prevalence decreased from 8.3% to 4.6% at a time when transmission normally rises dramatically. Genotyping data from the first 6 months of this infection incidence cohort study will be presented to ask if genetic signals track with clinical or epidemiological indicators anticipated to decline under MDA; and if these differ between the two populations with high or low transmission intensity. Specifically we will share data showing whether COI or PMI changed, and whether there was evidence of increasing clonality or relatedness among parasites as interventions were deployed; whether these indicators track with clinical or epidemiological indicators; the relatedness between parasites within household clusters; and, any changes in drug resistance markers as a consequence of MDA application in these settings.

1219

PHASE 1 STUDY OF DIFFERENT FORMULATIONS OF A TETRAVALENT DENGUE PURIFIED INACTIVATED VACCINE (DPIV): SAFETY AND IMMUNOGENICITY DATA IN HEALTHY ADULTS FROM PUERTO RICO THROUGH MONTH 13

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The safety and immunogenicity of 2 doses of an investigational tetravalent DPIV were studied in Puerto Rico. We report results up to study month (M) 13. In this ongoing Phase 1, observer-blind study (NCT01702857), 100 healthy adults were randomized 1:1:1:1:1 to receive saline placebo or 1 of 4 DPIV formulations (1µg per dengue virus [DENV] type adjuvanted with aluminum hydroxide [Alum], $AS01_E$ or $AS03_B$, or 4µg per DENV type adjuvanted with Alum) at Days (D) 0 and D28. Subjects were followed up for serious adverse events (SAEs), potential immune-mediated diseases (pIMDs) and medically-attended AEs (MAEs) up to M13. Hematological and biochemical laboratory parameters were assessed up to M13. Neutralizing antibody titers were determined by microneutralization assay (MN50). Two subjects in the 4µg+Alum group reported 4 SAEs, and 2 in the placebo group reported 5 SAEs; all were considered unrelated to vaccination. Worsening of a pre-existing pIMD (rheumatoid arthritis) was reported for 1 subject in the 1µg+$AS03_B$ group and 1 case of auto-immune thyroiditis in the placebo group. Fifty-two MAEs were reported, without notable differences across groups. Three subjects had grade 3 anemia (2 in the 4µg+Alum group at M7 and M10, and 1 in the 1µg+$AS03_B$ group at M7, M10, and M13). Iron deficiency was considered as the most likely etiology. The M13 per-protocol immunogenicity cohort included 83 participants (78 seropositive for ≥1 DENV type). M13 geometric mean titers (GMTs) against DENV-1, -2, -3, -4 were 1122, 793, 447, 1142, respectively, in the 1µg+Alum group; 2291, 1272, 1092, 3133 in the 4µg+Alum group; 1309, 1566, 1139, 1751 in the 1µg+$AS01_E$ group; 2051, 1847, 1389, 3267 in the 1µg+$AS03_B$ group; 983, 603, 348, 1441 in the placebo group. M13/D0 GMT ratios were highest in the 1µg+$AS03_B$ group and ranged from 3.2 to 3.7 for the 4 DENV types (1.5 to 2.1 in the 4µg+Alum group; 0.9 to 1.3 in the placebo group). D56/M13 GMT ratios ranged from 1.2 to 1.7 in the 1µg+$AS03_B$ group (1.0 to 1.7 in the 4µg+Alum group; 0.7 to 1.1 in the placebo group). All DPIV formulations were well tolerated and no safety signals were identified up to M13; MN50 GMTs remained high up to M13.

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A SINGLE DOSE OF TV003 ELICITS PROTECTIVE EFFICACY AGAINST CHALLENGE WITH A HETEROTYPIC DENGUE VIRUS TYPE 2

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Dengue is the most important arboviral infection in the world. Several candidate dengue vaccines are in clinical trial; however, variable efficacy of the lead candidate CYD due to DENV serotype and dengue-immune status at vaccination have made it difficult for vaccine manufacturers to determine which candidates should be evaluated in efficacy trials in endemic areas where vaccine failure or partial immunity could predispose subjects to more severe disease upon subsequent DENV infection. A dengue human infection model (DHIM) would be useful in down-selecting candidate vaccines prior to testing in endemic areas as well as identifying putative correlates of protection. We have developed a DHIM to evaluate the protective efficacy of the live attenuated tetravalent dengue vaccine TV003 developed by the NIAID. In Phase I clinical trials, a single dose of TV003 induced neutralizing antibody to DENV-1, DENV-2, DENV3, and DENV-4 in 92%, 76%, 97%, and 100% of vaccinees, respectively. It induced a tetravalent response in 74% of vaccinated subjects and a trivalent or better response in 98%. Because the frequency of seroconversion to DENV-2 was lowest, we sought to evaluate protection against a heterotypic strain of DENV-2. We vaccinated 24 flavivirus-naïve subjects with a single dose of TV003; an additional 24 subjects received a placebo. Six months later all recipients received 1,000 PFU of rDEN2Δ30, an under-attenuated DENV-2 strain. TV003 elicited 100% protection against viremia, rash, and neutropenia induced by rDEN2Δ30. In contrast, 100% of placebo recipients who received rDEN2Δ30 at challenge were viremic (mean peak titer = 2.3 $\log_{10}$ PFU/mL, mean duration of viremia = 6.1 days); 80% developed rash (38% graded as moderate); and 20% developed neutropenia (75% graded as moderate). Only 6 vaccinees had > 4-fold increase in $PRNT_{50}$ to DENV-2 following challenge, indicating that TV003 induced sterilizing immunity in 71% of vaccinees. Therefore, a single dose of TV003 induces strong protective efficacy against challenge to at least 6 months and should be further evaluated in dengue-endemic areas.

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SAFETY EVALUATION AND IMMUNOGENICITY OF A SINGLE-DOSE LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE IN DENGUE ENDEMIC POPULATIONS

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Dengue virus (DENV) continues to be the most important arbovirus worldwide with millions of cases of dengue fever and severe dengue occurring annually. Because a secondary DENV infection with a serotype different from that which caused the primary infection is a significant risk factor for severe disease, a safe and effective DENV vaccine must induce a long-lived, protective immune response to all four DENV serotypes. The goal of the NIAID intramural DENV vaccine program remains focused on the development of a single-dose, live-attenuated tetravalent dengue vaccine that is minimally reactogenic, highly immunogenic in both dengue virus primed and naïve recipients, and is cost-effective and safe for the community. Fifteen years of research has produced an optimal tetravalent mixture of viruses with a suitable level of infectivity and balance and has yielded clinical results in naïve adult subjects that support its continued development and progress into Phase 2/3 evaluation. Adult cohort studies in Brazil and Thailand were undertaken to evaluate the properties of the vaccine among populations with previous exposure to some DENV serotypes due to the endemic nature of the virus in these areas. To date, the favorable safety profile of the vaccine remains unchanged compared to that observed in DENV-naïve adult subjects. The vaccine has been evaluated in progressively younger subjects in Bangkok, including adolescents (13 - 17 years old), children (5 - 12 years old), and eventually in younger children (1 - 4 years old). A Phase 2 study under the direction of the Butantan Institute in Sao Paulo has been conducted and supports

the further evaluation of the vaccine in its first Phase 3 efficacy trial. Summary data from these two international studies will be presented and discussed.

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SAFETY AND IMMUNOGENICITY OF A TETRAVALENT RECOMBINANT SUBUNIT VACCINE FOR DENGUE: RESULTS OF A PHASE I CLINICAL TRIAL

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A recombinant subunit vaccine is being developed to prevent dengue. The vaccine candidate comprises truncated dengue envelope proteins (DEN-80E) from all 4 serotypes produced in Drosophila S2 cells. Nine active vaccine formulations were assessed in a randomized, placebo-controlled, Phase I trial in healthy, flavivirus-naïve adults in Australia. Three dosage levels of the tetravalent DEN-80E antigens were evaluated in a dose-escalation design. The 9 vaccine formulations either included ISCOMATRIX™ adjuvant (2 different dosage levels), aluminum-hydroxide adjuvant, or were unadjuvanted, and were compared to phosphate-buffered saline placebo. Volunteers received 3 injections of the assigned product on a 0, 1, 2 month schedule, and were followed for safety through 1 year after the last injection. Antibody levels were assessed at 6 time points: enrollment, 1 month after each dose, and 6 and 12 months Postdose 3 (PD3). The primary immunogenicity endpoint was the seroconversion rate (SCR) of virus-neutralizing antibody, measured by a qualified Focus Reduction Neutralization Test with 50% neutralization cutoff. The protocol-specified criterion for a positive immune response, applied to each formulation, was that at 28 days PD3, at least 3 serotypes will have a seroconversion rate of ≥75%. The trial has been completed. Of the 98 randomized participants, 90 received all 3 injections. All 9 active vaccine formulations were generally well tolerated; the safety profiles varied by the adjuvant component, but not by dose level of antigen or adjuvant. All 6 formulations with ISCOMATRIX™ adjuvant met the criterion for a positive immune response; per-protocol serotype-specific SCRs at 28 days PD3 ranged from 85.7% to 100%. Among the 3 formulations that were either aluminum-adjuvanted or unadjuvanted, 2 showed evidence of immunogenicity but did not meet the pre-specified criterion; per-protocol serotype-specific SCRs at 28 days PD3 ranged from 14.3% to 62.5%. These data suggest that a tetravalent DEN-80E vaccine may be generally well tolerated and immunogenic in flavivirus-naïve individuals. Complete unblinded study results will be presented.

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RECENT SCIENTIFIC AND CLINICAL ADVANCES IN SANOFI PASTEUR'S DENGUE VACCINE PROGRAM

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In 2014, results from the first phase 3 efficacy studies of a dengue vaccine, across ten endemic countries in more than 30,000 children and adolescents in Asia (CYD14, aged 2-14) and in Latin America and the Caribbean (CYD15, aged 9-16) were reported, and the clinical safety database now includes over 28,000 subjects who have received at least one dose of vaccine. The pre-protocol primary endpoints were successfully achieved in both trials, showing a reduction in overall disease by 56.5% (CYD14) and 60.8% (CYD15) against virologically-confirmed symptomatic disease, regardless of severity, against any serotype, as reported previously. In the active phase of surveillance (from first vaccination through to 25 months), a favourable safety profile was observed consistent with prior trials and additional results demonstrated efficacy against hospitalized and severe disease, serotype-specific efficacy and the importance of the dengue baseline status and age on subsequent efficacy. Pertinent meta-analysis for point estimates of serotype-specific efficacy during the active phase, regardless of severity, show the consistence of efficacy against individual serotypes; the hierarchy was >70% for serotype 3 and 4, 50.2% for serotype 1 and 39.6% for serotype 2. Long-term surveillance of hospitalized dengue cases in both trials continues with a high level of sustained subject participation and available data will be presented. Post-phase 3 investigations are addressing ***post-hoc*** analyses based on the unprecedented level of accrued cases and related clinical data, including the sequencing of viruses and the application of new immunological assays. Mathematical modelling demonstrates the ability of the dengue vaccine to significantly reduce the burden of disease in endemic regions. In totality, a vaccination strategy targeting high disease burden age ranges and combines routine vaccination with several catch-up cohorts at introduction, would substantially reduce the burden of dengue disease in endemic regions.

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PERSISTENCE OF NEUTRALIZING ANTIBODIES ONE YEAR AFTER TWO DOSES OF A CANDIDATE RECOMBINANT TETRAVALENT DENGUE VACCINE IN SUBJECTS AGED FROM 1.5 TO 45 YEARS

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Takeda's live attenuated tetravalent dengue vaccine candidate (TDV) contains a molecularly characterized dengue serotype 2 virus (TDV-2) and three recombinant viruses expressing the pre-membrane (prM) and envelope (E) structural genes for serotypes 1, 3, and 4 in the TDV-2 genetic backbone. In a previously reported phase II placebo-controlled, multi-center, age-descending trial (NCT 01511250) we assessed the safety, reactogenicity and immunogenicity of two doses of TDV or placebo, 90 days apart, in subjects living in dengue endemic areas (Puerto Rico, Colombia, Singapore or Thailand). Subjects in four age-cohorts: 21-45 year-old adults (n = 38), 12-20 year-old adolescents (n = 36), and 6-11 (n = 38) and 1.5-5 year-old (n = 36) children, were randomized 2:1 to receive TDV or placebo. Vaccination was well tolerated with no safety signals in any age group. When neutralizing antibodies against each of the four serotypes were measured after one dose at Day 28, 94.4-96.7% of vaccinees were seropositive to DENV-1, DENV-2 or DENV-3, while responses to DENV-4 were between 59.1% and 85.7% according to age group. A second dose at Day 90 increased responses to DENV-4 to 87.5% of vaccinees, but had little impact on other serotypes. By Day 120, 98% of vaccinees were seropositive to multiple serotypes compared with 4% of placebo recipients. When assessed at Days 180 and 360 there was little or no waning of antibodies in those vaccinees initially seropositive at baseline. Subjects who were seronegative at baseline did display some waning of antibodies, but all vaccine-groups remained seropositive compared with placebo recipients. These patterns of antibody response were similar across the four age groups. One dose of the candidate TDV vaccine elicited immune responses against the four DENV serotypes in initially seropositive and seronegative subjects, from 1.5 to 45 years of age, with little impact of a second dose, and persistence of neutralizing activity through 360 days irrespective of age or initial immune status.

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A NOVEL PROTEIN SCAFFOLD-BASED RECOMBINANT DENGUE 2 CANDIDATE VACCINE INDUCES ROBUST NEUTRALIZATION AND CONFERS PROTECTION AGAINST DENGUE INFECTION IN NON-HUMAN PRIMATES

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Dengue virus (DENV) is the most prevalent arthropod-borne virus worldwide causing approximately 200 million infections each year, and currently there is no licensed vaccine. Here we describe construction and characterization of a novel protein-scaffold based DENV-2 vaccine candidate. The acyltransferase component (E2) of the pyruvate dehydrogenase complex of Geobacillus stearothermophilus self-assembles into a 60-mer core with icosahedral symmetry and a 24 nm diameter. E2 can be modified to express E2 fused with exogenous proteins that subsequently self-assemble into 60-mer virus like particles (VLPs). For this candidate vaccine, DENV-2 envelope protein domain III (EDIII)-E2 recombinant proteins were generated in E.coli and purified by gel filtration. EDIII specific mAb were used to confirm correct presentation of the DENV-2 EDIII antigen. Six DENV-naïve rhesus macaques (RMs) were vaccinated with intramuscular delivery of EDIII-E2 VLPs and a DNA plasmid encoding DENV-2 EDIII at wk 0, 4, and 12, achieving 50% neutralization titers (FRNT50) ranging from 1:3,000 to 1:100,000 by 14 wks post vaccination. The vaccinated RMs and 3 naïve controls were subsequently challenged with DENV-2, with complete protection (6/6) against detectable DENV-2 viremia by infectious virus assays and partial protection (3/6) by PCR. Interestingly, days of PCR detected viremia was negatively correlated with pre-infection FRNT titers (r=-0.88, p=0.01), suggesting a threshold neutralizing titer was necessary to fully control detectable viremia. These preliminary studies demonstrate proof of principle for protein scaffold based dengue vaccines and lay the groundwork for developing formulations of EDIII-E2 fusion proteins of each serotype that could self-assemble into tetravalent VLPs.

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EFFECTS OF THE EBOLA VIRUS EPIDEMIC ON NTD INTERVENTIONS IN LOFA COUNTY, LIBERIA AND PLANS FOR RESUMPTION OF MASS DRUG ADMINISTRATION

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We initiated a study in 2012 to compare the impact of annual and semiannual mass drug administration (MDA) for the control and elimination of neglected tropical diseases (NTDs, lymphatic filariasis, onchocerciasis, soil-transmitted helminths, schistosomiasis) in Foya and Kolahun Districts in Lofa County, NW Liberia. MDA compliance rates by village ranged from 70-83% in 2013. Emergence of Ebola virus disease (EVD) in Lofa interrupted a follow-up parasitological survey in April 2014. By February 2015, 705 EVD cases (332 confirmed) and 433 deaths were recorded in the county. Although EVD cases were highly clustered in a small number of villages, MDA was suspended in the county and across the country for more than 1 year. This study was conducted to assess the willingness of our study communities to resume MDA for NTDs and to regain trust required for high compliance. Anecdotal reports indicated that people were suspicious of international health workers and researchers because of the EVD outbreak, and some thought that these outsiders were responsible for the epidemic. We conducted questionnaire survey in March 2015 of 140 community leaders in 32 villages (3 to 7 participants per village). Despite a continued fear of EVD, participants reported high levels of enthusiasm for MDA and a willingness to participate in parasitological surveys. Participants reported 92% compliance with MDA in 2013, and a similar percentage said they were willing to take the medications now. Community leaders consistently said that it was important to widely publicize the plan to resume MDA prior to drug delivery and to make it clear that the medicines were the same ones that they had received earlier to control worm infections. In this way, resumption of MDA provided by the MOH may be reassuring to some as evidence that the country's health system is capable of resuming general health services and moving past the Ebola crisis. Social mobilization will start soon, and the next round of MDA will be distributed in in May 2015. This study has provided new information on the indirect impact of events such as the EVD outbreak on NTD programs and on factors to consider prior to restarting MDA.

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EVALUATING TREATMENT COVERAGE FOR MASS DRUG ADMINISTRATION: A MULTI-COUNTRY COMPARISON OF THREE SURVEY METHODS

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Coverage surveys are an important tool for evaluating the performance of mass drug administration (MDA) for neglected tropical diseases (NTDs). Unfortunately they are seldom implemented by national programs, which may be attributed, in part, to a lack of standardized guidelines for conducting coverage surveys across the NTDs. When coverage surveys are conducted a commonly used approach is the Expanded Program on Immunization's 30-cluster survey (EPI), widely known for its practicality but criticized for falling short of probability sampling. We compared the feasibility of the EPI sampling design to two alternative sampling designs for measuring MDA coverage in Burkina Faso, Uganda and Malawi. The first of these alternatives, Lot Quality Assurance Sampling (LQAS), divided a district into five programmatically relevant supervisory areas (SAs) from which 19 persons were selected and the SA coverage was classified as good/poor. SA results were weighted and combined at the district level to estimate overall coverage with a precision of ± 10%. The second method, Probability Sampling with Segmentation (PSS), used segmentation within each selected cluster to simplify and expedite the selection of individuals while maintaining probability sampling. The EPI and PSS sample sizes were calculated for a precision of ± 5%, considered more useful for MDA evaluation. In Burkina Faso and Malawi, each method was conducted in a separate district; in Uganda, all three were used in the same district. When averaged across the three countries, LQAS took the least days to complete (16), followed by EPI (18) and PSS (20). Cost was closely tied to duration, with LQAS being the least expensive and PSS the most; however, all three methods were under $5,000 to implement per district. When asked about ease of use, survey teams generally scored the three methods similarly. Since PSS required only a relatively small increase in time and cost and was the only method of the three that generated unbiased coverage estimates with the desired precision, we conclude that it may be the best tool for conducting coverage evaluations to improve the performance of NTD programs.

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COMMUNITY DRUG DISTRIBUTOR PERFORMANCE IN THE CONTROL OF NEGLECTED TROPICAL DISEASES: ARE WE ASKING TOO MUCH?

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Trusted literate, or semi-literate, community drug distributors (CDDs) are the primary implementers in integrated preventive chemotherapy (IPCT) programmes for Neglected Tropical Disease (NTD) control. The CDDs are responsible for safely distributing drugs and for galvanising communities to repeatedly, often over many years, receive annual treatment, create and update treatment registers, monitor for side-effects and compile treatment coverage reports. These individuals are 'volunteers' for the programmes and receive, no, or minimal, remuneration for their annual work commitment. A mixed methods approach, which included pictorial diaries to prospectively record CDD use of time, structured interviews and focus group discussions, triangulated data on how 58 CDDs allocated their time towards their routine family activities and to NTD control activities in Uganda. The opportunity costs of CDD time were estimated using salary values; performance assessed by determining the relationship between time and programme coverage; and CDD motivation for participating in the programme was explored. Key findings showed approximately 2.5 working weeks (range, 0.6 to 11.4 working weeks) were spent on NTD control activities per year. The amount of time on NTD control activities significantly increased with each additional drug delivery that was required within an IPCT campaign. CDD time spent on NTD control activities had an impact of time available for subsistence and income generating engagements. As CDDs took more time to complete NTD control activities their treatment performance decreased. Motivation for the programme was low and CDDs felt undervalued.CDDs contribute a considerable amount of opportunity cost to the overall economic cost of the NTDCP in Uganda due to the commitment of their time. Nevertheless, programme coverage of at least 75%, as required by World Health Organization, is not being achieved and vulnerable individuals may not have access to treatment. The presenter will make recommendations for improvements in programme implementation and alternative support systems for the CDDs in order to utilise resources effectively and efficiently.

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PARTNERING FOR SUCCESS: INTEGRATED TRANSMISSION ASSESSMENT SURVEYS FOR LYMPHATIC FILARIASIS, SOIL-TRANSMITTED HELMINTHS AND MALARIA IN HAITI

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The island of Hispaniola is the only remaining Caribbean island that is endemic for malaria and lymphatic filariasis (LF), with Haiti bearing the greater burden of both diseases. Haiti's National Program for the Elimination of LF (NPELF) reached full national coverage with mass drug administration (MDA) for LF in 2012. Several areas have reduced antigenemia prevalences to <2%, and in 2014 NPELF began conducting transmission assessment surveys (TAS) to determine eligibility to stop LF MDA. Haiti plans to conduct 48 TAS by the end of 2017. National malaria prevalence is low (0.4% in 2011), and with 16,872 confirmed cases reported in 2014, elimination of this disease is also considered feasible. Elimination goals for both diseases revealed opportunities to explore potential programmatic synergies, particularly as both programs must establish surveillance methods to identify potential remaining ***foci*** of transmission. In 2014, the World Health Organization (WHO) was finalizing guidelines for using LF TAS as a platform for soil transmitted helminth (STH) assessments, prompting NPELF to develop its own integrated protocol that also included malaria ('TAS-STH-malaria'). Haiti's TAS-STH-malaria survey addressed the need to determine if LF transmission had been interrupted, while also responding to WHO's call for integration of programs with common features of control and elimination strategies. In November 2014, a TAS-STH-malaria survey was piloted in Nippes department, Haiti. Teams enrolled 1632 children in 45 schools. Children were tested for LF, malaria, and STH. In all, three (0.2%) of 1632 children were positive for LF (immunochromatographic card test), zero (0%) of 1632 were positive for malaria (rapid diagnostic test), and 47 (17%) of 284 were positive for STH (Kato Katz). Blood spots are currently being analyzed for antibody responses to LF (Wb123, Bm14, and Bm33) and malaria (LSA-1, MSP-1-19, MSP-1-42(D), MSP-1-42(F), AMA-1, NS MSP-1-19) antigens using a multiplex platform. This approach demonstrated feasibility of an integrated TAS-STH-malaria survey, which is presently being implementing in four additional evaluation units.

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EVALUATING NEGLECTED TROPICAL DISEASE DATA REPORTING SYSTEMS: RESULTS FROM 6 DATA QUALITY ASSESSMENTS

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With the WHO target date of 2020 for NTD elimination fast approaching, it is essential that national program managers understand their program performance so that they can take any necessary corrective actions in a timely manner. However, in order to use evidence for decision-making

appropriately, data must be available, complete, timely, and accurate. Data Quality Assessments (DQAs) are an effective tool to measure the quality of NTD data and the data management system, in order to identify areas that need improvement and develop actions to strengthen the reporting system. Six countries in Africa and Asia implemented DQAs in 2013-14 to evaluate the quality of their NTD data and reporting system. Available data were recounted and compared to reported values to assess data accuracy, and focused assessments were conducted with key stakeholders at every level of the reporting system in each country, totaling 165 sites. We found the following: overall, 77% of reports were available for recounting, 65% being complete with all the indicators assessed; data were submitted on time in only 12% of instances; approximately 50% of reported data were verified through recounting, with overreporting occurring in almost 1/3 of instances. Nearly all (91%) respondents indicated that data flow was through the national system, and 71.7% reported that there were designated staff responsible for reviewing quality of data received. However, many respondents noted that the data collection tools (e.g., treatment registers) did not have sufficient precision to measure indicators accurately. These findings suggest that the quality of data and reporting systems need to be strengthened for national program managers to make optimally informed decisions. As a result of these DQA exercises, countries have revised their data collection tools to better capture data, including sex- and age-disaggregated results, and strengthened training to include a larger focus on data management. Further effort and innovative approaches are needed to improve timeliness of data submission. Follow-up DQAs should be conducted as part of overall program evaluation activities.

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ARE WE REACHING WOMEN AND MEN EQUALLY WITH MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES? DATA FROM TWELVE COUNTRIES

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Preventive chemotherapy (PC) is a key strategy for control and elimination of a group of Neglected Tropical Diseases (NTDs): Lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthes and trachoma. With over 800 million persons treated globally in 2012, it is essential to understand whether there is evidence of gender equity in the availability and utilization of the benefits of PC programs. Datasets created by national NTD programs supported by United States Agency for International Development recorded persons treated betweenOctober 2012 and September 2013. Records included nearly 200 million treatments, delivered to around 90 million people in 12 countries from 3 world regions. Gender-disaggregated data were available in all 12 countries for at least 1 NTD and for all targeted districts and diseases in 7 countries. Treatment ('epidemiologic') coverage was determined by dividing total (or sex-disaggregated) persons treated by the at-risk population, with gender-specific proportions of the at-risk population estimated from values of the most recent national census. Analysis showed that treatment coverage among females was overall similar to that of males, averaging 60.4% for females and 59.5% for males. This apparent gender equity held true when analyzed both by disease and by world region, though differences could sometimes be seen in countries with mass drug administration that targeted schistosomiasis or trachoma. No evidence of lower utilization of PC services by women was found. This suggests that the strategies used to take NTD treatment to communities, many living beyond the reach of routine health services, is providing good access to health for women. On the other hand, if women not eligible for treatment (for certain PC-NTD drugs, this may include women who are pregnant and/or in the first trimester of pregnancy, or lactating in the first week after birth) were to be removed from the denominator, treatment coverage would be consistently lower for men - a finding that warrants further exploration.

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GOING PAPERLESS? A MULTI-COUNTRY EXPERIENCE ON THE USE OF SMART PHONES IN THE CONTROL OF NEGLECTED TROPICAL DISEASES

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Up-to-date disease distribution maps coupled with routine disease monitoring and evaluation data are crucial to design, plan and guide neglected tropical disease control programs (NTD). Data collection using mobile phones has increasingly been suggested as a favourable alternative to the paper based system. Country wide integrated NTD mapping in Rwanda, Burundi and Ethiopia and NTD treatment coverage survey validation in Uganda, Zanzibar and Malawi was conducted using the Task Force for Global Health's LINKS and EpiCollectPlus SMART phone data collection systems respectively. The tools allowed for simultaneous data collection and entry, quick uploading to a central database and real time data visualisation, analysis and storage. No extra data entry staff were needed and any errors in the uploaded data were promptly spotted and corrected. The progress of the teams, and the project itself, could be easily be monitored using the time stamps captured at each upload. However, requiring internet connectivity to upload data was a considerable limitation in the rural areas. Additionally sample processing necessitated the full time use of gloves making physical use of mobile phone difficult. Entries requiring open ended answers could not be incorporated using LINKS and significantly slowed down data entry using EpiCollectPlus due to slow typing speeds. The LINKS tool could only be modified in Atlanta, removing the flexibility of fixing any errors even when caught early during the field survey. The server also being stored in Atlanta, raises important questions over ownership of data/sovereignty of the programme. These challenges, in addition to the inevitable loss or damage of phones, resulted in the continued use of paper forms on site as backups, possibly introducing errors. Training time was prolonged as training on the use of a SMART phone was needed first before training on using the tool itself. In our experience, SMART phones were useful for household coverage surveys which have a limited number of questions however they are not yet robust and/or versatile enough to do away with the need to collect information on paper forms.

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COMPARABILITY BETWEEN INSECTICIDE RESISTANCE BIOASSAYS FOR MOSQUITO VECTORS: TIME TO REVIEW CURRENT METHODOLOGY?

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Insecticides play an integral role in the control of mosquito-borne diseases. With resistance to insecticides on the rise, surveillance of the target population for optimal choice of insecticides is a necessity. The Centers for Disease Control and Prevention (CDC) bottle assay and the World Health Organization (WHO) susceptibility test are the most frequently used methods in insecticide resistance monitoring. However, the two bioassays differ in terms of insecticide delivery and how resistance is measured. To evaluate how equivalent data from the two assays are, we compared them side-by-side. We did a literature search from 1998 to December 2014 to identify publications that performed both assays on the same mosquito population. We then tested the two bioassays on laboratory strains of ***Aedes aegypti***, ***Anopheles stephensi***, ***An. gambiae*** and ***An. arabiensis*** with different resistance levels against permethrin, λ-cyhalothrin, DDT, bendiocarb and malathion. We also measured the relationship between time-to-knockdown and 24 hours mortality. Both published data and results from the present laboratory experiments showed heterogeneity

in the comparability of the two bioassays. Following their standard procedures the two assays showed poor agreement in detecting resistance at the WHO cut-off of 90% (Cohen's $\kappa = 0.06$). There was better agreement when 24 hour mortality was recorded in the CDC bottle assay and compared with that of the WHO susceptibility test (Cohen's $\kappa = 0.5148$). Although statistically significant, time-to-knockdown was a weak predictor of 24 hour mortality (OR = 0.97, $p < 0.001$). In conclusion, though the two assays can detect insecticide resistance, they may not be used interchangeably. While the diagnostic dose in the WHO susceptibility test does not allow for detecting shifts at low or extreme resistance levels, time-to-knockdown measured in the CDC bottle assay is a poor predictor of 24 hours mortality. Therefore, dose-response assays could provide the most flexibility. New standardized bioassays are needed that produce consistent dose-response measurements with a minimal number of mosquitoes.

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ENVIRONMENTALLY FRIENDLY USE TOOL TO CONTROL MOSQUITO POPULATIONS WITHOUT RISK OF INSECTICIDE RESISTANCE: THE LEHMANN'S FUNNEL ENTRY TRAP

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Malaria vector control needs ground-breaking approaches to complement existing tools and accelerate our global efforts toward the disease elimination. For decades malaria vector control in Africa has relied on IRS and LLINs, both of which operate within houses. The emergence and spread of insecticide resistance and changes in vector biting behaviour have seriously challenged these conventional tools/strategy and there is an urgent call for innovation. Malaria vectors present several vulnerable points during their life cycle that can be exploited to tackle them, but they have seldom been investigated. Here we exploit the inherent feeding behaviour of malaria vectors inside houses to design an environmentally friendly use trap to cut down the high reproductive rate of mosquitoes. The Lehmann's trap needs no insecticide and it combines the function of killing and blocking entry into houses. Preliminary studies conducted in two ecological settings in Burkina Faso has indicated that the trap could reduce mosquito density by up to 80% in houses and has the potential to swift population structure by cumulatively killing old females that are epidemiologically dangerous. Here we focus on improving the trap design for improved performance and end user acceptability and standardized product specifications to enable broad manufacturing. There is a global consensus that new intervention tools are needed to cross the last miles in malaria elimination and the Lehmann's trap can contribute to fill this gap. It showed an excellent promise in suppressing mosquito densities even in area of high insecticide resistance. It requires no chemicals, is self-operational and it stands from its simplicity.

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ENTOMOLOGICAL SURVEILLANCE SYSTEM IN ZANZIBAR: CONTRIBUTIONS TO MALARIA ELIMINATION EFFORTS ON THE ISLANDS

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Malaria vector surveillance is important for planning and implementation of evidence-based vector control interventions. In Zanzibar transition from malaria control to elimination stage has been complicated by the changing ecology and behaviour of local mosquito populations. Studies conducted by the Zanzibar malaria elimination program (ZAMEP) in 22 sentinel surveillance sites, (10 in Pemba and 12 in Unguja) from 2007 to 2014 show that malaria transmission is probably mediated by ***Anopheles arabiensis***. This vector feeds more on the bovine 72% rather than on human (8%) in Pemba. The behavioral patterns of different species (***An. fanestus***, ***An. arabiensis*** and ***An. gambiae***) has maintained ongoing malaria transmission in hotspot sites in Unguja Island. Historically, malaria transmission has been dominated by vector species that primarily feed and rest indoors, where they can be efficiently targeted with long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Following universal coverage of vector control through LLINs and IRS, majority of bites are now out-doors (91% and 55% in Pemba and Unguja, respectively). There is growing evidence across the islands that the widespread use of LLINs and IRS is driving vector species composition toward those with more flexible behaviour such as ***An. arabiensis***. This could not only prevent the achievement of elimination, but also undermine the continued effectiveness of interventions. Therefore LLINs and IRS will need to be supplemented by other vector control interventions to reduce mosquito-human contact. In addition, enhanced entomological surveillance will be important to target appropriate vector control interventions. Monitoring vectors to identify ***foci*** of transmission during the elimination phase can be challenging as estimate of most factors of vectorial capacity become extremely difficult due to the very small number of infected vectors, as a result of control interventions. Nonetheless, at least monitoring of larval and adult vector densities is important and should be an integral part of all elimination efforts.

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THE USE OF ENDECTOCIDES IN CATTLE TO CONTROL OUTDOOR RESIDUAL MALARIA TRANSMISSION IN WESTERN KENYA

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The opportunistic blood feeding behavior of malaria vector species such as ***Anopheles arabiensis*** Patton complicates vector control by conventional methods such as indoor residual spraying and insecticide treated nets. The objective of this research was to evaluate the efficacy of the insecticidal active ingredients eprinomectin and fipronil, at reducing malaria vector abundance when applied to cattle (***Bos indicus***) in a small scale field study. Pilot studies were carried out in the Samia District of western Kenya during 2014-2015. Treatment and control areas were allocated by a randomized block design comprised of 50 homes per area. Prior to cattle treatments, baseline mosquito collections were performed by pyrethrum spray catch or backpack aspiration. For the eprinomectin trial, cattle in the treatment area were administered topical applications of eprinomectin at 0.5 mg/kg once per week for two consecutive weeks. For the fipronil trial, cattle were dosed once orally with fipronil at 0.5 mg/kg body weight. Post-treatment mosquito collections were performed weekly for two weeks following the eprinomectin treatments, and for four weeks following the fipronil treatment. Mosquitoes were identified morphologically, and screened for molecular species identification, sporozoite presence, and blood host by PCR-based methods. For the eprinomectin trial, the indoor resting density of ***An. arabiensis*** was significantly lower (95% probability) in the treatment area compared to the control area one week post-treatment, representing a significant reduction in the indoor resting population of this species by 32%. Two weeks post-treatment with eprinomectin, the number of mosquitoes per hut was not significantly different between the treatment and control areas. Additional entomological parameters will be presented, as well as the results of the fipronil field trial. Endectocidal treatment of cattle has the potential to significantly impact residual, outdoor malaria transmission driven by ***An. arabiensis*** and is an important tool to supplement existing vector control interventions which target more anthropophilic species.

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DEPLOYMENT OF OPTIMAL-DOSE, VOLATILE PYRETHROIDS FOR THE PREVENTION OF BITING AND DISEASE TRANSMISSION BY INSECTS

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Existing vector control tools are compromised by coverage, cost, insecticide resistance and human compliance. Outdoor biting insects are particularly hard to target. Fast, flexible, safe methods of insecticide delivery, offering dramatic decreases in biting, across a range of environments, are highly desirable. An under-exploited group of volatile pyrethroids offers considerable potential in this regard. Passive formulations of odourless, polyfluorinated compounds, applied at optimal doses, can stop mosquitoes biting and may therefore have profound effects on disease transmission. We demonstrate the potential of metofluthrin, transfluthrin and heptafluthrin to deliver dramatic protection from bites via a mixture of lethal and confusant effects on knock down, mortality, host-seeking and probing. In real-world, well-ventilated rooms, >40% of ***Aedes aegypti*** mosquitoes were knocked down and > 80% of bites were prevented 3 metres from a passive device that used neither heat nor power and that remained effective for >20 days (10% metofluthrin w/w). In salt marsh habitats, 3m3 tents baited with humans and containing the same device contained just 5% of the mosquitoes (Aedes vigilax) that were collected from unprotected baited tents. None of those remaining mosquitoes attempted to bite. In the same salt marshes, simple candle formulations of another volatile, heptafluthrin (5% w/w), reduced light-trap and human landing catches by >93% in close proximity (1.5m) downwind of the candles. Importantly, these compounds may be largely unaffected by conventional pyrethroid-resistant mechanisms. Strongly permethrin and lambdacyhalothrin resistant ***Aedes aegypti*** were as behaviourally affected as fully susceptible insects in the presence of sublethal metofluthrin doses. Neither have we observed any evidence of true repellency (orientation away from the volatile point source) from the formulations that we tested (3-10% w/w). This suggests that treatment of one area by these compounds does not simply redistribute vectors to unprotected zones.

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SUB-LETHAL EFFECTS OF INSECTICIDES: CONSEQUENCES FOR MALARIA VECTOR CONTROL

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Insecticides have been the most effective and widespread control strategy for vector-borne diseases. Increasing levels of insecticide resistance in malaria vectors across Africa and Asia have potential to threaten the effectiveness of this control strategy. These fears stem from observed declines in the proportion of mosquitoes killed on immediate contact with treated surfaces. However, the epidemiological consequences of this resistance may be lower than expected if mosquitoes that survive direct contact with insecticides suffer a reduction in longer-term fitness (sub-lethal effects). Sub-lethal effects could manifest either as reduced long-term survival, and/or reproduction through changes to their egg production or larval development. Here we investigate the potential epidemiological impact of sub-lethal effects of insecticides as expected to arise from long lasting insecticide treated bednets. Using data from laboratory experiments in which the survival of African malaria vectors that survived initial contact with insecticide was measured, we extend traditional survival and transmission models to include age structure and investigate immediate and age-specific long lasting effects of the interventions. Our aim is to illustrate if and by what extent current estimates of the detrimental impacts of insecticide resistance on malaria vector control may be overestimated by the existence of sub-lethal effects.

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DO HOLES IN LONG LASTING INSECTICIDAL NETS COMPROMISE THEIR EFFICACY AGAINST PYRETHROID RESISTANT *ANOPHELES GAMBIAE* AND *CULEX QUINQUEFASCIATUS*? RESULTS FROM A RELEASE-RECAPTURE STUDY IN EXPERIMENTAL HUTS

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Experimental hut and observational studies in Benin show that pyrethroid resistance reduces the insecticidal effect and personal protection of long lasting insecticidal nets (LLINs) especially when they become torn. The World Health Organization has proposed a threshold for when nets are "too torn" at 1,000 cm2. This study examines whether there is a threshold above which LLINs don't reduce malaria transmission by resistant mosquitoes. Intact and artificially holed LLINs and untreated nets were tested by releasing mosquitoes from a susceptible ***Anopheles gambiae*** colony, a local pyrethroid-resistant ***An. gambiae*** population and a local resistant ***Culex quinquefasciatus*** population in closed experimental huts in Benin. The efficacy of LLINs and untreated nets was evaluated in terms of protection against blood feeding, insecticidal effect and potential effect on malaria transmission. Personal protection by both LLINs and untreated nets decreased exponentially with increasing holed surface area, without evidence for a specific threshold beyond which LLINs could be considered as ineffective. The insecticidal effect of LLINs was lower in resistant mosquitoes than in susceptible mosquitoes, but holed surface area had little or no impact on the insecticidal effect of LLINs. LLINs with 22,500 cm2 holed surface area and target insecticide content provided a personal protection of 0.60 (95% CI: 0.44-0.73) and a low insecticidal effect of 0.20 (95% CI: 0.12-0.30) against resistant ***An. gambiae***. Nevertheless, mathematical models suggested that if 80% of the population uses such nets, they could still prevent 94% (95% CI: 89-97%) of transmission by pyrethroid-resistant ***An. gambiae***. Even though personal protection by LLINs against feeding mosquitoes is strongly reduced by holes, the insecticidal effect of LLINs is independent of the holed surface area but strongly dependent on insecticide resistance. Badly torn nets that still contain insecticide have potential to reduce malaria transmission. The relationship between LLIN integrity and efficacy needs to be understood in order to guide LLIN distribution policy.

1240

GENETIC MODIFICATION OF PARASITE ANTIGEN RESCUES HOST DENDRITIC CELL ACTIVATION

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Filarial nematodes establish chronic infections by suppressing the host's immune system. Successful DNA vaccination using the ***Litomosoides sigmodontis*** model demonstrated that immunisation targeting suppressive molecules such as the abundant larval transcript-1 (Ls-ALT) and

the cysteine protease inhibitor-2 (Ls-CPI), can increase immune responses during subsequent infection and consistently reduce the production of microfilariae two months post first exposure to the parasite. However, because protective effects of these vaccines are only detected during late stages of the infection, it remains unclear how these vaccines push the immune system into a more protective phenotype before exposure to the parasites. Since dendritic cells (DC) play a key role in initiating adaptive immune responses, and as homologues of Ls-CPI have previously been shown to interfere directly with antigen presentation and DC activation, we decided to examine the effects of vaccines containing either Ls-CPI or the modified, functionally impaired, Ls-CPIm on DC activation ***in vitro*** and ***in vivo***. Surface activation marker CD86 expression was increased on DC from Ls-CPIm- but not in Ls-CPI-immunised mice. The functional significance of these effects was then assessed by allowing bone-marrow derived DC (BMDC) to prime T cells ***in vitro***: prior to the addition of T cells, BMDC loaded with Ls-CPIm produced more IL-12 and IL-6, and less IL-10, than BMDC loaded with Ls-CPI. Co-culture with naive T cells showed increased CD86 and MHC-II expression on BMDC, and increased IL-12 and IL-6 production with Ls-CPIm as compared to Ls-CPI. We are now investigating if the increased DC activation also corresponds to an increase in T cell activation, and whether it has an effect on Th2 polarization. So far we demonstrate the importance of DC activation before the onset of infection and provide an insight on how anti-filarial vaccines can achieve protection.

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CHRONIC *LITOMOSOIDES SIGMODONTIS* INFECTION IMPROVES BACTERIAL SEPSIS OUTCOME VIA TLR2 DEPENDENT MACROPHAGE MODULATION

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Immunomodulation by the filarial nematode ***Litomosoides sigmodontis*** (L.s.) has been demonstrated to improve various inflammatory disorders including autoimmune diseases and allergy. In the current study we investigated whether chronic L.s. infection reduces E. coli-induced acute, systemic inflammatory immune responses as they occur during sepsis. Chronic L.s.-infected BALB/c mice had a milder E. coli-induced hypothermia, diminished systemic pro-inflammatory cytokine concentrations and improved bacterial clearance compared to E. coli-only challenged controls and led to an increased sepsis survival. Analysis of peritoneal macrophages from E. coli-challenged L.s.-infected mice further revealed a reduced macrophage activation and apoptosis as well as an improved phagocytosis. Peritoneal macrophages were essential for the improved sepsis outcome, as L.s.-mediated protection was lost in clodronate liposome treated mice. Wolbachia endosymbionts of L.s. express lipoproteins that activate mononuclear cells via TLR2. ***In vitro*** priming of macrophages with L.s. extract reduced macrophage activation in a TLR2 and Wolbachia dependent manner and PCR array analysis further showed that negative regulators of TLR/NFkB signaling were upregulated in macrophages of E. coli-challenged, L.s.-infected mice. Transfer of ***in vivo*** and vitro modulated macrophages improved sepsis outcome in a TLR2 and Wolbachia-dependent manner and L.s. infection of TLR2 deficient mice did not improve macrophage phagocytosis and E. coli challenge resulted in an increased bacterial load, exacerbated systemic inflammation and sepsis caused death. Taken together this study suggests that macrophage modulation by L.s. infection has a dual beneficial effect on bacterial sepsis, reducing systemic inflammatory immune responses and improving bacterial clearance.

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MATERNAL PARASITIC INFECTIONS DURING PREGNANCY AND SPECIFIC ANTI-PARASITE CYTOKINE RESPONSES IN CORD BLOOD ARE ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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We have shown that antenatal malaria and helminth infections impair the IgG antibody response to Haemophilus influenzae (Hib) and diphtheria toxoid (DT) among infants. We have also shown that soluble products of these chronic parasitic infections can cross the placenta and induce a spectrum of immune responses in the fetus, which persist into early childhood and may impair early childhood response to vaccines. To examine this possibility we investigated whether prenatal exposure to helminth and malaria infections and subsequent T cell response in cord blood lymphocytes (CBL) affect the infant immune response to Hib and DT. 450 Kenyan mother/infant pairs were enrolled in the study and tested for lymphatic filariasis (LF), urogenital schistosomiasis and malaria. The newborns were followed every 6 months from birth to 3 years of age and plasma was tested for PRP-specific (induced by Hib) and DT-specific IgG at each time point. IFN-Υ, IL5, IL13, IL10, IL6 and TNF-α recall responses were evaluated in CBL to blood stage malaria antigens (***Plasmodium falciparum***), ***Schistosoma*** antigen (SWAP), and filarial antigen (BMA). Overall, 79% of the mothers were infected with LF, urogenital schistosomiasis and/or malaria. There was a spectrum of immune responses in cord blood lymphocytes that included pro-inflammatory responses characterized by predominantly IL-6 and TNFa production, a Th2-type phenotype (predominantly IL5 and/or IL13) or regulatory responses (IL10). Using a generalized estimating equation analysis, parasite antigen-induced pro-inflammatory responses were associated with markedly reduced DT-specific IgG levels (p=0.02 to 0.0003), and regulatory responses were associated with reduced DT-specific IgG, p=0.0007. By contrast, parasite-specific Th2-type responses were associated with enhanced DT-specific IgG (0.02 to 0.0007) and PRP-specific IgG (p=0.027 to 0.046). Thus, the type of fetal immune response acquired to parasite antigens ***in utero*** may persist into early infancy and modulate IgG levels to childhood vaccines.

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MASS DRUG ADMINISTRATION WITH PRAZIQUANTEL BOOSTS ANTI-SCHISTOSOME PROTECTIVE ANTIBODY RESPONSES IN SCHOOL CHILDREN FROM WESTERN KENYA

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Most countries where schistosomiasis is prevalent have intensified efforts to control the disease through annual mass drug administration (MDA) using praziquantel. The main target of MDA programs are school-age children as recommended by the World Health Organization (WHO). However, the effect of annual MDA on schistosome-specific immune responses in this age group has not been investigated. About thirty children per class (total of 314, age range 6-18) were recruited from 4 primary and secondary schools in Rarieda, Siaya County, western Kenya.

Venous blood was collected, and plasmas were prepared. Thereafter, MDA was carried out in the four schools. One year later, follow-up samples were collected from 98 children (ages 7 to 13) who had received treatment. Total IgG, IgG4 and IgE antibody responses to schistosome crude egg (SEA) and adult (SWAP) antigens as well as the recombinant ***Schistosoma mansoni*** Tegument-Allergen-Like (SmTAL) proteins, SmTAL-1, SmTAL-2, and SmTAL-5 were assayed by ELISA. Treatment increased the number of responders to SEA and mean anti-SEA IgE levels while decreasing mean anti-SWAP and anti-SEA IgG4. Moreover, there was a more robust IgE response to TAL-1 (a surrogate for resistance) in older children at baseline and a general increase in the percentage of children with IgE responses to TAL-1 after treatment. Additionally, following MDA there was a striking decrease in mean anti-SmTAL 5 IgE, which is expressed in cercariae and worms but not eggs. However, there was a significant boost in mean IgG4 levels against anti-SmTAL 2 (an egg-specific antigen), in all age groups after treatment. The most notable finding from this study is that MDA boosts IgE responses and tends to increase the number of responders in younger age-groups. IgE is associated with resistance to reinfection in schistosomiasis; thus, our data suggest that MDA, in addition to treating current infections, may help protect children from subsequent infection.

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IMMUNE RESPONSE OF INNATE LYMPHOID CELLS IN HUMAN FILARIAL INFECTIONS

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The CD4+ T cell response in acute filarial infection is characterized by an expansion of IL-4-producing (Th2-associated) cells that gradually contracts with longstanding infection. The factors involved in initiating this Th2 expansion are still not completely understood, but certainly involve innate lymphoid cells (ILCs). Previously, we have shown that a subset of human ILCs that express CD117 (cKit) were expanded during relatively acute filarial infections. With better definition of human lineage negative (Lin-) ILCs consisting of 3 distinct subsets: ILC1s (Lin-CD45+CD127+cKit-NKp44-), ILC2s (Lin-CD45+CD127+CRTH2+) and ILC3s (Lin-CD45+CD127+cKit+NKp44-/+), we have established standardized protocols using multiparameter flow cytometry and analyzed the frequency and composition of ILCs in human whole blood (n=45) and human skin (n=6) from healthy donors. Under homeostatic conditions, human blood is dominated by ILC1 (Geometric Mean (GM) 59%) whereas the remainder is split almost equally between ILC2 (GM=22%) and ILC3s (GM=19%). In contrast, human skin has a quite different ILC composition with more than 29% ILC2s, 26% ILC3s and 13%ILC1s; the remaining 32% of the ILCs in the skin were "unclassifiable" in that they were Lin-CD45+CD127+ but CRTH2-NKp44-ckit-. When these more clearly defined definitions of ILC populations were examined in relatively acutely infected patients with filarial infections (microfilariae [mf] positive) and uninfected controls we found that the filarial-infected individuals had a marked (up to 5-fold) increased frequency of ILCs compared to uninfected individuals. Surprisingly, the composition of expanded ILC subsets in filarial infected individuals were largely ILC3s (63%) with ILC1s (22%), ILC2s (9%) and "unclassifiable" (6%) making up the rest. Studies are currently underway to determine if ***ex vivo*** exposure to live mf (found in the blood) or L3 parasites (that transit through the skin) directly (or indirectly) drive CD4+ T cell subset differentiation through parasite-induced ILC activation.

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A NEWLY IDENTIFIED ROLE FOR IL-22 IN BLADDER IMMUNITY

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In contrast to mechanisms demonstrated in the gut and lung, it is currently unclear how inflammatory pathways in the urinary tract contribute to host protection and pathology during parasitic or bacterial infection. Recent reports indicate that interleukin-22 (IL-22) is a key cytokine supporting epithelial immunity while its improper regulation may contribute to pathologies such as fibrosis and cancer. We hypothesized that IL-22 may have a role in bladder immunity during ***Schistosoma haematobium*** and E.coli infections. IL-22-null (KO) and wild type (wt) female mice underwent bladder wall injection with S. ***haematobium*** eggs or transurethral infection with type 1 piliated uropathogenic E. coli UTI89. Levels of IL-22 and its soluble binding protein, IL-22BP, were increased in the bladder after exposure to S. ***haematobium*** eggs. Genes typically induced by IL-22 were expressed at higher levels after S. ***haematobium*** egg injection. IL-22 receptor α1 expression was detectable in the urothelium by immunofluorescence and qPCR. Injection of S. ***haematobium*** eggs into IL-22-KO vs wt mice resulted in differential expression of genes related to glutathione transferase activity, transferase activity, and epithelial cell development. Numerous genes for the uroplakins and Wnt/Hedgehog pathways were downregulated in egg-injected, IL-22-KO mice relative to their wt counterparts. These changes suggest that, as in the gut, IL22 is important for replenishing the bladder epithelial lining during infection-related injury. IL-22-KO and wild-type littermate mice were transurethrally infected with UTI89, which uses the adhesin FimH to adhere to mature urothelial cells. IL-22-KO mice had lower bacterial counts in their urine, bladder, and kidneys. Giving stabilized IL-22 cytokine (IL-22-Fc) to UTI89-infected mice led to higher kidney bacterial counts and increased morbidity. Our data suggest that IL-22 is indeed important in urinary tract immunity and may interfere with clearance of bacteria from the urinary tract, potentially through its role in maintenance of mature urothelium.

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PAN-AMERICAN MIGRATION PROMOTES THE SPREAD OF PATHOGENIC *TRYPANOSOMA CRUZI* HYBRID STRAINS

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The principal reproduction strategy of ***Trypanosoma cruzi***, the aetiological agent of Chagas disease, is the subject of an intense, decades-old debate. Despite the existence of two recent natural hybrid lineages (TcV and TcVI), which are sympatric with severe disease in southern endemic areas, a pervasive view is that recombination has been 'restrained' at an evolutionary scale and is of little epidemiological relevance to contemporary parasite populations. With improved sampling strategies, the geographical distribution of TcV and TcVI appears to be expanding. High resolution nuclear and mitochondrial genotyping of potential hybrid isolates from domestic vectors and human infections in Colombia was undertaken, in comparison to representative strains from across South America, to resolve their putative status as novel recombinants. All suspected Colombian hybrids were highly heterozygous, minimally diverse and possessed intact parental alleles at each loci. Compared to local Colombian isolates, hybrids were distinct from, but more closely related to, those identified in southern reference TcVI strains. Based on independent inheritance patterns of microsatellite loci, our data support the hypothesis that two recombination events led to the formation of TcV and TcVI. However, more private alleles among Colombian hybrids and the sharing of mitochondrial haplotypes between southern TcV isolates and a Colombian TcVI strain, suggests the evolution of these recombinant lineages may be more complicated than previously assumed. The origin of these Colombian hybrids is unclear; they are unlikely to be predecessors of southern TcVI strains, but were also not clear descendants, and may instead represent a sibling group, which diverged and anthroponotically dispersed northwards, following a single hybridization event between heterozygous southern TcII and TcIII isolates. We discuss the important implications the geographical range expansion of TcVI has for emergent

human Chagas disease in Colombia, considering the successful, epidemic establishment of this low-diversity genotype among domestic transmission cycles in the South.

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GENOTYPING OF *TRYPANOSOMA CRUZI* DNA IN BLOOD SAMPLES FROM AUTOCHTHONOUS CHAGAS DISEASE PATIENTS FROM SOUTHEASTERN AND CENTRAL TEXAS

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The parasitic protozoa ***Trypanosoma cruzi***, the causative agent of Chagas disease, represents a major public health problem in Latin America. Although the United States was initially defined as non-endemic for Chagas disease due to the rarity of human cases, ***T. cruzi*** has now been amply demonstrated as enzootic in different regions of the Southern United States. Recently our group described the first newly identified geographic clustering of locally-acquired human infections in the Southern US. This study focused on the molecular identification and genotyping of ***T. cruzi*** in human blood samples collected from autochthonous Chagas disease patients in Southeastern and Central Texas. ***T. cruzi*** infection was determined by standard PCR using primers specific for the minicircle variable region of the kinetoplastid DNA with the primers 121-122 and the highly repetitive genomic satellite DNA with the primers TcZF-R. Genotyping of discrete typing units (DTUs) was performed by amplification of mini-exon using a set of three primers, reported by different authors, to evaluate the sensitivity in the DTUs detection. We found a higher than expected percentage of blood samples positive by PCR for ***T. cruzi*** using two different DNA markers. The kDNA marker was more sensitive than the satDNA marker. Of the PCR positive samples, we were able to genotype approximately half. Single infection with TcI DTU and mixed infection with TcI and non-TcI DTUs were detected in a minority of the samples. Single infection with the non-TcI DTU was detected in the majority of the samples. Sequencing is currently underway to classify the non-TcI DTUs. Cardiac findings were measured by electrocardiogram (ECG), and classified using current clinical guidelines for Chagas cardiac related ECG abnormalities. Cardiac morbidity was prevalent and associated with both TcI and non-TcI DTU types. The novel results of this study provide a unique insight into the distribution of locally circulating DTUs for enhanced diagnosis of Chagas disease in the Southern US. Additionally, our findings provide supporting evidence that the circulating DTUs are competent causes of cardiac morbidity.

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ESTIMATING THE BURDEN OF CHAGAS DISEASE IN THE UNITED STATES

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With changing human migration patterns, the burden of Chagas disease in the United States has increased. The most recent prevalence estimate for Chagas disease in the US was created in 2009. The objective of this study is to provide an update of the current national case estimate for Chagas disease in the US and, for the first time, to estimate the number of cases at the state level and analyze the epidemiologic data on cases diagnosed and treated from 2007-2013. State-level estimates of Chagas disease cases were computed using State-Level Foreign-Born Hispanic Population Estimates by single Country of Origin group from the 2012 American Community Survey for a five-year period (2008-2012) and country prevalence estimates from the World Health Organization. Geographic Information Systems was used to compare the distribution of estimated cases in each state to the number of infections identified in the donated blood supply from 2007-2013 per data from the AABB (formerly American Association of Blood Banks) and treatment releases of benznidazole and nifurtimox over the same period per data from the US Centers for Disease Control and Prevention. The results of this analysis show an updated national estimate of 243,048 cases of ***T. cruzi*** infection in the United States. The state level results show four states with over 10,000 cases and an additional two states with over 5,000 cases. Moreover, since 2007, the AABB has confirmed 1,908 cases of ***T. cruzi*** infection in the donated blood supply and the CDC has released treatment for 422 infected patients over this same period. In conclusion, this study demonstrates a decreased but still substantial burden of Chagas disease in the US at the national level and offers the first state level prevalence estimates for Chagas disease. We show a geographically focal burden and one that is largely but not completely congruent with confirmed cases in the US blood supply. Finally, this study not only lends important new insight into the distribution of this disease in the US but also suggests the need for greater study and resources to support both screening and treatment.

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BUILDING AN INVENTORY OF THE HEALTH FACILITY INFRASTRUCTURE IN COUNTRIES WITH ENDEMIC SLEEPING SICKNESS FOR TARGETING DIAGNOSTIC RESOURCES

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Human African Trypanosomiasis (HAT) is a highly debilitating vector born disease that is endemic in a number of Sub-Saharan African countries. It is important to identify cases early in infection when clinical signs can be mild but the highly toxic and onerous treatment makes it vital to obtain the correct diagnosis. Being forced to travel long distances can form a barrier to reporting and diagnosis, whilst poorly targeted resources can result in an infrastructure that is difficult to maintain and support. Therefore, in order to correctly target HAT diagnostics, it is necessary to have up to date information on the location and capacity of diagnostic facilities. To collect these data, we worked with HAT control programmes in 8 African nations to compile databases of health infrastructure in their HAT ***foci***. Data collected include the location, equipment and staffing resources and history of diagnosing HAT cases. Facilities in ***foci*** in Uganda, Tanzania, Nigeria and Guinea have been mapped entirely, whilst in South Sudan, Chad and the DRC, the ***foci*** with the greatest prevalence have been characterised. In total, detailed data has been collected on 4803 facilities. The data have been used to inform HAT elimination programs in all countries except Tanzania through the delivery of enhanced diagnostics in an integrated hierarchical structure in the passive surveillance systems. As part of these programs, 1310 facilities have been equipped with new rapid diagnostic tests, 53 with new fluorescence microscopes and 13 large facilities with loop mediated isothermal amplification of DNA (LAMP) incubators. This required the selection of appropriate facilities for improvement of diagnostics to ensure full coverage of the population at risk and on-going monitoring of diagnostic activities at the facilities. By improving HAT screening infrastructure in all levels of health systems, new cases have been identified through passive surveillance in participating countries. Crucial to this surveillance model is the detailed characterisation of health facilities. These data are owned by the countries and can be used for general management of healthcare provision.

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FEASIBILITY OF ELIMINATING VISCERAL LEISHMANIASIS FROM THE INDIAN SUBCONTINENT

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The neglected tropical disease visceral leishmaniasis (VL), transmitted by sand flies, is set for elimination as a public health problem on the Indian subcontinent (ISC) by 2017 (incidence of symptomatic cases <1/10,000 at (sub)district level). ISC-countries are committed to reaching this ambitious target and have set different intervention strategies, mainly focusing on early detection and treatment of symptomatic VL cases as well as vector control. We developed a mathematical model to investigate whether elimination may be achieved with current strategies, and what additional interventions might be required. Based on an existing model we created a deterministic compartmental model as well as a stochastic individual-based (IBM) variant. The IBM includes individual heterogeneities regarding exposure to the sand fly and health seeking behavior. Also, it allows for estimating elimination probabilities under the selected strategies. The model was fitted to the KALANET dataset from the highly endemic Bihar region (India) and was tuned to predict different transmission scenarios (degree of endemicity, and equilibrium or outbreak situation). Our model predictions suggest that the target incidence of <1/10,000 can be achieved with the selected strategies for districts that currently experience endemicity levels at or below 10/10,000. Settings with higher baseline endemicities require additional efforts such as increased screening. Further, our simulations suggest that patients with post kala-azar dermal leishmaniasis, which occurs months to years after symptomatic VL, may serve as a reservoir of infection when nearing the elimination target. Our findings are robust against alternative assumptions about duration of immunity and infectiousness of different disease states (e.g. asymptomatic infection). We conclude that elimination of VL on the ISC is feasible for most regions with current strategies, but the duration of control and monitoring and evaluation of transmission will be pivotal to prevent recrudescence of infection.

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DYNAMICS OF AMERICAN TEGUMENTARY LEISHMANIASIS IN A HIGHLY ENDEMIC REGION FOR *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION IN NORTHEAST BRAZIL

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Intimate knowledge of the geographic distribution of leishmaniasis and how it propagates within ***foci*** of active transmission may guide new approaches to disease control measures. We explored incidence and geographic location data of three clinical types of American tegumentary leishmaniasis (ATL) cases, diagnosed in three different time periods, contained within a two decades time span. These patients lived in a focal region of northeast Brazil that is highly endemic for ***Leishmania (Viannia) braziliensis***. The study revealed that cutaneous (CL), mucosal (ML) and disseminated leishmaniasis (DL) actively spread within the affected region, but in different patterns. Whereas CL and DL seemed to propagate in clusters, ML was characterized by sporadic appearance of cases. The incidence of ML also fluctuated over time at a rate that was distinct from those of CL and DL. These findings might be used to improve the efficacy of control measures, for example exploring the combination of early detection of sentinel patients with focused active surveillance for surrounding cases of disease.

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NATURAL INFECTION AND THE DYNAMICS TRANSMISSION OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* IN WILD AND SYNANTHROPIC RODENTS INVOLVED IN THE ZOONOTIC CYCLE OF CUTANEOUS LEISHMANIASIS IN ENDEMIC AREA OF ATLANTIC RAIN FOREST IN NORTHEAST BRAZIL

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In northeast Brazil the predominant ***Leishmania*** species causing American cutaneous leishmaniasis (ACL) is ***L.*** (***Viannia***) ***braziliensis***. One of the principle difficulties in understanding the eco-epidemiology of ACL associated with ***L.*** (***V.***) ***braziliensis*** is identifying the relative importance of its reservoir hosts and sand fly vectors. The Amaraji municipality is located in the Zona da Mata biome of Pernambuco state, and is an important ACL focus. The objective of this study was the detection of natural infections of ***Leishmania*** (***Viannia***) spp in wild and synanthropic animals responsible for in maintaining zoonotic cycle in this region. Wild and synanthropic mammals were captured in Amaraji between May 2012 and July 2014 Captured animals were anesthetized, marked with microchip and skin samples (right ear) and blood were collected. They were then returned to their capture site. DNA was extracted and purified from all the tissue samples, and the ***Leishmania*** parasite load was determined by real-time PCR. A total of 637 animals were collected that belonged to 11 different species. 602 specimens were taken from 327 males and 275 females each being marked with a microchip. The predominant species were ***Nectomys squamipes*** (244/637) and ***Rattus rattus*** (148/637), corresponding to respectively 38.3% and 23.2%. Based on DNA blood and tissue analysis 106 (16.6%) animals were positive, the average parasite load being 3528fg/uL (9,26-50060 fg/µL). We observed that the highest infection rates as well as highest parasite loads occurred between the months of October and November 2012 (55 positive animals - 8266fg/uL); June 2013 (33 positive animals - 1503fg/ µL) and January 2014 (25 positive animals - 505fg/ µL). In conclusion, the results suggest that the potential for transmission was greatest during these periods and that accordingly the risk of human infections is greater. On going studies are in progress to correlate this data with sand fly xenodiagnosis infectivity assays to support the hypothesis that the intensity of wild and synanthropic rodent ***Leishmania*** infections modulates zoonotic ACL.

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DENGUE VIRUS NON-STRUCTURAL PROTEIN 1 INDUCES ACTIVATION OF HEPARANASE/CATHEPSIN-L AND DEGRADATION OF THE ENDOTHELIAL GLYCOCALYX, LEADING TO ENDOTHELIAL PERMEABILITY

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Dengue is the most prevalent arboviral disease in humans and a major public health problem worldwide. Systemic plasma leakage leading to shock is a critical determinant of dengue severity. Increased vascular permeability without morphological damage to the capillary endothelium in severe dengue suggests the shock syndrome may be due to endothelial dysfunction. In the endothelium, dynamic structures, including intercellular junctional proteins and the endothelial glycocalyx (EG), a network of membrane-bound proteoglycans and glycoproteins, control the barrier function critical for vascular homeostasis. However, the mechanism of vascular dysfunction in dengue is still unclear. We previously described the role of soluble NS1 in inducing endothelial permeability by measuring trans-endothelial electrical resistance (TEER) in human pulmonary

microvascular endothelial cells (HPMEC) grown on a transwell permeable membrane system. Here, we demonstrate that DENV NS1-induced endothelial permeability is initially triggered by the increased expression/activation of heparanase, an endo-β-d-glucuronidase that degrades heparan sulfate (HS) in the extracellular matrix of HPMEC, resulting in altered distribution of EG components such as sialic acid, syndecan-1 and perlecan. This effect is specific to DENV NS1 and is not observed with NS1 from the related flavivirus West Nile Virus. Specific inhibitors of heparanase and its activator, cathepsin-L, prevent NS1-mediated endothelial permeability associated with altered distribution of HPMEC EG components. Further, conditioned supernatant from NS1-treated HPMEC monolayers increases endothelial permeability when transferred to naïve HPMEC, even in the presence of anti-NS1 monoclonal antibodies. Finally, recombinant syndecan decreases TEER in a dose-dependent manner. Our findings propose a new mechanism of NS1 directly triggering endothelial vascular dysfunction through the early enzymatic activation and cleavage of HS proteoglycans by heparanase that leads to disassembly of the extracellular matrix, contributing to increased plasma leakage that occurs in severe dengue.

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FLUID MANAGEMENT OF DENGUE FEVER PATIENTS - EVIDENCE FROM AN OBSERVATIONAL MULTICENTER PROSPECTIVE STUDY IN FOUR SOUTHEAST-ASIAN AND THREE LATIN AMERICAN COUNTRIES

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Effective case management of patients with severe dengue relies on frequent monitoring and judicious IV fluid therapy. However, in patients with vascular leakage early use of IV fluids may aggravate fluid accumulation and result in respiratory distress. Using data from a major multi-centre observational study, we describe the clinical status of confirmed dengue patients at first severe event, and analyse the subsequent development of complications. Trained physicians followed suspected dengue cases prospectively at seven hospitals across Asia and Latin America, using a single comprehensive report form that included daily clinical assessment for fluid accumulation. A CXR/USS assessment was also performed around defervescence. Applying univariable and multivariable Cox regression, we evaluated risk factors for the development of shock with and without fluid accumulation, as well as for respiratory distress. Among 1734 confirmed dengue patients, the majority (88%) never experienced DSS. Fluid accumulation was identified in 45% of cases without shock and in almost twice that proportion with shock: 176/210 (84%) cases. Respiratory distress was diagnosed in 78 patients, including 44 cases with shock. In the univariable model, referral from another facility and IV fluid use before enrolment were protective for shock without fluid accumulation, but increased the risk for shock with fluid accumulation. In the multivariable model, the risk for respiratory distress with fluid accumulation increased significantly as the infused volume increased (AHR 1.15 [95% CI 1.08-1.23)] per 10 ml/kg over the preceding 24h period; p<0.001), irrespective of age group, respiratory rate, or presence of serosal effusions or shock in the preceding 24h. Age below 15 years, increased respiratory rate, and presence of ascites in the preceding 24h were independent risk factors for respiratory distress with fluid accumulation. Fluid accumulation, either assessed clinically or potentially quantified by radiological methods, could be a useful intermediate severity endpoint for therapeutic intervention trials and/or pathogenesis studies.

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INHIBITION OF MAMMALIAN HOST GLUCOSIDASE ENZYMES USING THE IMINOSUGAR UV-4B DOES NOT SELECT FOR RESISTANT INFLUENZA AND DENGUE VIRUSES

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Unither's iminosugar platform targets host αglucosidases I and II, enzymes that contribute to glycosylation and folding of proteins in the endoplasmic reticulum. Blockade of host αglucosidases impairs glycosylation resulting in improperly folded viral envelope glycoproteins and impaired functionality, stability, orientation and immune stealth. Glucosidase inhibition results both in reduced virus numbers and production of defective viruses that are replication incompetent. The iminosugar UV-4B has ***in vitro*** activity against a diverse set of glycosylated, enveloped RNA and DNA viruses including dengue, influenza, Ebola, and vaccinia and promotes survival in lethal dengue and influenza mouse models. An important benefit of host-targeted antivirals is reduced selection for resistant viruses. Antiviral drugs directed against genetic sequences or viral encoded proteins quickly select for resistant viruses within a few replication cycles especially in the case of RNA viruses that have high mutation rates and exist as quasi-species. Targeting a host enzyme does not expose the virus to direct selective pressure, but rather denies the virus a critical substrate for which there is no biochemical or viral gene-encoded alternative and minimizes selection of resistance. Absence of viral resistance to glucosidase inhibitors was confirmed against multiple mutation-prone viruses. Dengue viruses exposed to UV-4B ***in vitro*** did not generate resistant variants after 38 serial passages. In addition, no change in efficacy was detected in dengue or influenza after the five serial passages performed in mice treated with UV-4B. Deep sequencing was performed to examine the appearance of resistance-conferring mutations in the drug passaged dengue and influenza viruses. The absence of viral escape mutants elicited from sustained exposure to UV-4B over multiple passages ***in vivo***, provides additional evidence that there is a high genetic barrier to the generation and selection of escape mutants exposed to host-targeted iminosugar antivirals.

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EFFECT OF IMINOSUGARS ON DENGUE VIRUS STRUCTURAL PROTEINS

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Dengue virus (DENV) is among the most rapidly emerging human pathogens and can cause severe illness and death. Currently, no effective therapeutics are available. Unither Virology (UV) has identified multiple iminosugar analogs, UV-1, UV-4B, and UV-12 that show ***in vitro*** activity against DENV serotype 2. We recently demonstrated the efficacy of UV-4B (Perry et al. 2013) ***in vivo*** against a lethal DENV challenge in mice even when administered 24-48 hours post-infection (PI). Iminosugars inhibit resident ER α-glucosidases involved in processing of N-glycans on nascent glycoproteins found on DENV envelope (E) protein. Inhibition prevents proper folding of E, affecting critical steps in the viral life cycle. Targeting a host protein, decreases the likelihood of developing viral resistance. We investigated the effects of iminosugar analogs (UV-1, UV-12) on modifications of E and their effect on virus entry and maturation.

Comparisons of viral counts in the supernatant of cells treated with UV-1 or UV-12 (100 µM) collected every 24 hours PI reveal the number of virus particles released (RNA molecules/ml) remains the same over time compared to untreated cells. However, the number of infectious virus particles released (PFU/ml) is decreased compared to untreated cells suggesting treatment with UV-1 and UV-12 affects infectivity. Cryo-EM reconstruction of particles prepared in the presence of UV-1 failed to converge on a reference structure of native DENV-2 particle. Additionally, quantification of purified virus particle types (smooth, mosaic, spiky, and bumpy) indicates structural heterogeneity between particles within subpopulations. Preliminary mass spectrometry analysis of purified particle maturation (cleavage efficiency (M/(prM+M)) showed a decrease in percent maturation of treated virus relative to untreated virus. Live imaging of single virus particles revealed that particles treated with UV-1 and UV-12 were delayed in attachment/adsorption. Our results indicate that UV-1 and UV-12 affect proper E protein folding, preventing maturation and infectivity of the virus.

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EXPLORING THE ROLE OF DNA SENSORS DURING DENGUE VIRUS INFECTION

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Dengue virus (DENV) is an enveloped (+) strand RNA virus transmitted by mosquitos, which infect near to 390 million humans per year. Upon infection, DENV can replicate in immune cells like macrophages and dendritic cells (DCs). DCs are professional immune cells that contain complex networks of sensing macromolecules that can quickly detect and signal the control of invading intracellular pathogens through the activation of the IFN system and the subsequent establishment of the antiviral state. We have described in our laboratory that DENV can counteract the induction of type I IFNs by cleaving the adaptor STING in human cells. In this work we explored the partner molecules that collaborate with STING in detection of DENV replication. Using a qRT-PCR approach, we observed activation of genes involved in DNA sensing, independently of the IFN production in primary human MDDCs. Preliminary results suggest that the novel DNA sensor cGAS is involved in the detection of DENV infection by recognizing illicit cytoplasmic DNA generated during the replication. Using ***in vitro*** and ***ex vivo*** models we perform a systematic study to characterize the PAMP sources that can be stimulating the cGAS/STING/IRF3 pathway during DENV infection. Also, by pull down experiments of cGAS in DENV infected cells, we analyzed the nature of the DNA molecules that were bound to it. Finally we propose a new mechanism of DENV detection by the cellular DNA sensing machinery.

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DENGUE VIRUS INFECTIONS OF HUMAN TONSIL HISTOCULTURES AND PRIMARY CELLS REVEAL SEROTYPE-SPECIFIC PROFILES

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Dengue virus (DENV) is the most prevalent mosquito-borne virus causing disease in humans, with an estimated 390 million infections per year worldwide. There are currently no licensed vaccines or specific therapeutics, and the death rate for severe dengue without proper supportive care can be over 20%. Of the four circulating DENV serotypes, DENV-2 infection has been correlated with increased disease severity while DENV-4 infections have been associated with a milder clinical presentation. In this study, we have investigated the infection kinetics and immune responses of DENV-2 versus DENV-4 during primary infections of human monocyte-derived dendritic cells (DCs) and in a newly developed, human tonsil histoculture (HC) model system. We hypothesize that DENV-4 will be attenuated compared to DENV-2 and that these relevant human models will yield new insights into immune responses against these viruses, which will help elucidate DENV immunopathogenesis in patients. In preliminary studies in human DCs, DENV-4 shows faster replication kinetics that peak at 24 hours post infection but drastically diminish by 72 hours post infection, while DENV2 exhibits slower but longer lasting replication kinetics. Similarly, DENV-4 replication dramatically decreases after 72 hours post infection compared to DENV-2 in tonsil HCs. Furthermore, while our laboratory has previously demonstrated that DENV-2 abrogates type I interferon (IFN) production in human primary DCs, our current data shows that DENV-4 infection induces both type I IFN and the IFN-stimulated gene IP-10 in both DCs and tonsil HCs. Moreover, DENV-4 infection of tonsil HCs induces higher levels of IFN-γ, TNF-α, IL-10, and RANTES during a 12-day time course compared to DENV-2 infection. This suggests that DENV-4 may be more immunogenic than DENV-2 in humans and can be more efficiently inhibited by antiviral responses, which could correlate with its diminished replication capacity in our human systems.

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EFFICACY OF RUPATADINE IN ACUTE DENGUE INFECTION

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Vascular leak is the hall mark of severe dengue infection. Our previous studies showed that platelet activating factor (PAF) was a potent mediator of vascular leak. Therefore, we proceeded to investigate the efficacy of rupatadine which is a PAF receptor blocker in patients with acute dengue infection. We are conducting a phase II, open labeled, randomized control trial to determine the efficacy of rupatadine in preventing or reducing vascular leak in patients with acute dengue and also to determine its efficacy in reducing complications.The study was designed to be conducted in three arms, which were 80 patients for ruptadine 10mg, 80 patients for rupatadine 40mg and 80 patients for the placebo arm. The tablets were given orally once a day to the participants. Apart from receiving the drug, all patients were managed according to the 2011 World Health Organization guidelines. The patients were examined at least twice a day and the haematocrit and platelet counts were measured at least twice a day to detect any complications and fluid leakage. Daily ultra sound scans were done in all patients from the time they were admitted to hospital to determine the presence and the quantity of fluid leakage. Our primary end point was a reduction in fluid leakage in the rupatadine treated group. The secondary end points were reduction in the incidence of liver failure; reduction in the development of shock; reduction in the need for blood transfusion or colloids; or the duration of illness. The efficacy and safety findings of this clinical trial will be presented at the conference.

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A FOUR-YEAR PROSPECTIVE STUDY OF THE IMPACT OF RED BLOOD CELL VARIANTS ON CHILDHOOD FALCIPARUM MALARIA IN SOUTHERN MALI

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Red blood cell (RBC) variants protect African children from severe ***Plasmodium falciparum*** malaria. Their individual and interactive impacts on mild disease and parasite density, and their modification by age-dependent immunity, are poorly understood. We conducted a 4-year, prospective cohort study of children aged 6 months-17 years in Mali in 2008-2011. RBC variants were hemoglobins S (HbS) and C (HbC), α-thalassemia, ABO blood groups, and glucose-6-phosphate dehydrogenase (G6PD) deficiency encoded by the X-linked A- allele. The primary outcome was malaria incidence, which was modeled as incidence rate ratios (IRRs) with quasi-Poisson regression; the secondary outcome was ***P. falciparum*** density, which was analyzed with Generalized Estimating Equations. We diagnosed 4,091 malaria episodes in 1,543 children over 2,656 child-years of follow-up. RBC variants were common, with prevalences of: HbAS 14%, HbAC 7%, α-thalassemia 28%, type O blood group 40%, and G6PD deficiency 9% in boys and 20% in girls. The overall malaria incidence was 1.54 episodes per child-years of follow-up, and ranged from 2.78 at age 3 years to 0.4 at age 17 years. Malaria incidence was reduced 34% in HbAS compared with HbAA children (adjusted IRR [aIRR] 0.66; 95% CI 0.59-0.75) and 49% in G6PD A-/A- compared with A+/A+ girls (aIRR 0.51; 95% CI 0.29-0.90), but was increased 15% in HbAC compared with HbAA children (aIRR 1.15; 95% CI 1.01-1.32). Parasite density was reduced in HbAS (median 10,550 parasites/μL) compared with HbAA children (15,150 parasites/μL; p=0.0004). Age significantly modified the effects of HbAS on both malaria risk (p=0.022) and parasite densities (p<0.0001); both outcomes were reduced more substantially in the youngest children. There was no statistically-significant interaction between HbAS or HbAC and α-thalassemia for either malaria risk or parasite density. Individual and interactive impacts of HbAS, HbAC, and G6PD A-/A- on malaria risk and parasite density define clinical and cellular correlates of protection. Further identification of the molecular mechanisms of these protective effects may uncover novel targets for intervention.

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EVALUATION OF THE NATURAL HISTORY OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* PARASITEMIA IN A HIGHLY ENDEMIC AREA OF UGANDA

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Understanding the epidemiology of asymptomatic ***Plasmodium falciparum*** infection in the era of bednets (ITNs) and artemisinin-based combination therapy (ACT) remains an important goal. We enrolled a dynamic cohort of children 0.5-10 years of age (n=333) from 100 randomly selected houses from an area in Eastern Uganda, where transmission is holoendemic with 2 seasonal peaks and an aEIR of 310 infective bites PPY. Participants were provided an ITN at enrollment and received all care, including passive surveillance for malaria and active surveillance for parasitemia by microscopy every 3 months between Aug 2011-Sep 2014. Associations between the probability of asymptomatic parasitemia during active surveillance and risk factors of interest were made using generalized estimating equations, and associations between risk factors and the cumulative risk of developing malaria following asymptomatic infection were estimated using a cox proportional hazards model adjusted for repeated measures. The overall prevalence of parasitemia at routine surveillance was 24%, and 82% of these episodes were asymptomatic. Compared to children < 3 years of age, the odds of being asymptomatic if parasitemic were significantly higher in children 3-7 (aOR=1.97, P=0.008) and 7-10 years of age (aOR=3.09, P<0.001). Increasing parasite densities were associated with significantly lower odds of being asymptomatic (aOR 0.28 per log10 increase in parasite density, P<0.001), with no significant associations with calendar year or season. Following asymptomatic infection, children 3-7 years and 7-10 years of age were significantly less likely to develop malaria within 30 days compared with children < 3 years (aHR 0.38 and 0.21, P<0.001, respectively). Both the high season (aHR 1.31, P=0.04) and calendar year (aHR 1.42 yr 3 compared to yr 1, P=0.047) were associated with an increased hazard of developing malaria following asymptomatic parasitemia, although parasite densities had no association. A better understanding of the epidemiology of asymptomatic parasitemia will inform efforts for malaria control in high transmission settings.

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DETERMINANTS OF HETEROGENEITY IN MALARIA TRANSMISSION IN PAPUA NEW GUINEA

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A renewed emphasis on malaria control in Papua New Guinea has resulted in a significant overall reduction in the prevalence and incidence of malaria. However the reduction observed at the national/provincial level masks substantial heterogeneity of transmission at the local district and village level. To investigate this, repeated community cross-sectional surveys and longitudinal child cohorts were undertaken in two hyper-/holoendemic areas of PNG, combining sensitive molecular diagnosis of infections (multiplicity of infection (MOI), molecular force of blood-stage infection ($_{mol}$FOB) and gametocyte-specific RT-qPCR), with demographic and household GPS data. ***Plasmodium falciparum*/*P. vivax*** prevalence (PCR) decreased from 71.6% (2005) to 10.9% (2013) in cross-sectional community surveys. Cluster analysis for high rates of infection identified 4 spatial clusters for infections of any species, 6 ***P. falciparum*** and 7 ***P. vivax*** clusters. Risk factors for households to be in a cluster were a lower proportion of the household reporting LLIN use for all infections, distance to health centre for ***P. vivax*** infections and altitude for infections with any species. Many hotspots identified in 2013 were areas of the highest prevalence in 2005. In a 2009 cohort of 5-10 year old children, the prevalence of ***P. falciparum*** was 24%, making ***P. vivax*** the most common species (48% by PCR). Prevalence at enrolment and $_{mol}$FOB varied significantly between neighbouring villages for both species with up to 40-fold differences in $_{mol}$FOB (***p***<0.001). More recently, the prevalence of

malaria infections at enrolment in longitudinal cohorts of 1-5 and 5-10 year old children was 24% and 37% respectively. The vast majority of these infections are asymptomatic and ongoing analysis of demographic, molecular and spatial data will provide an in-depth perspective on malaria transmission at the village and household level. Understanding the extent of local heterogeneity in malaria transmission and the driving factors is critical to be able to identify and implement targeted control strategies to ensure the ongoing success of malaria control in PNG and make progress towards elimination.

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INFECTION DYNAMICS OF NATURAL *PLASMODIUM* INFECTIONS IN PAPUA NEW GUINEAN CHILDREN

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Measuring key parameters of malaria infection dynamics such as acquisition and clearance rates of individual parasite clones is challenging in semi-immune individuals because of multi-clone infections, frequent super-infections and generally low parasite densities. Investigating the persistence of individual clones in longitudinal studies is complicated by imperfect detectability causing apparent loss and reappearance. Little data exists on the dynamics of natural malaria infections particularly in non-African settings where ***Plasmodium*** species co-exist. We combined high-resolution genotyping with mathematical modelling to assess detectability, acquisition and clearance rates in a longitudinal study in Papua New Guinean (PNG) children aged 5-10 years from an area with ***P. vivax*** as predominating species. Mean detectability of ***P. falciparum*** clones was 57% and age-independent while ***P. vivax*** detectability decreased from 48% in the youngest to 18% in the oldest children corresponding to age patterns in parasite density. Adjusting for imperfect detectability increased clone acquisition rates by 25% for ***P. falciparum*** and 16% for ***P. vivax***. Acquisition rates were 3.5-fold higher for ***P. vivax*** than ***P. falciparum*** and correlated well with prevalence by village for both species (R2 = 0.83 and 0.97), while no association with age was observed. Naturally cleared infections with ***P. falciparum*** persisted longer than with ***P. vivax*** (77 and 48 days). An increase in infection duration with age was observed for ***P. vivax***, reaching 154 days in the oldest children, but not for ***P. falciparum***. Unlike ***P. vivax***, ***P. falciparum*** infection duration was moderately correlated with endemicity (R2 = 0.54) with maximum of 114 days in a highly endemic village. In endemic settings, age serves as a proxy of exposure and thus of natural immunity. Our data support the hypothesis that acquired immunity, present against ***P. vivax*** but not ***P. falciparum*** in PNG children of this age, controls infection mainly by limiting parasite densities thus allowing infections to persist at low level for extended periods in semi-immune individuals and potentially contributing to transmission.

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PLACENTAL MALARIA EXPOSURE AND SEVERE MALARIAL ANEMIA IN AFRICAN CHILDREN

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Previous studies suggest offspring of mothers with placental malaria (PM) may be at higher risk of clinical malaria infection and reduced hemoglobin in early childhood. However, in a region with high drug resistance, intermittent preventative treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) has been associated with increased risk of severe malaria. Using multi-center, birth cohort data from 880 participants in the MOMS Project Study in Tanzania, 2002-2006, and 1502 participants in the MRTC-Laboratory of Malaria Immunology and Vaccinology Immuno-Epi Observational Study in Mali, 2010-2015, the authors investigated the relations of PM and IPTp-SP with severe malarial anemia (SMA), defined as a positive bloodsmear for ***Plasmodium falciparum*** and hemoglobin (Hb) <5g/dL, in children under four years. Children exposed to PM were at increased risk of incident SMA (adjusted hazard ratio (AHR) = 2.17, 95% confidence interval (CI): 1.16, 4.08), an effect which was stronger in children of younger mothers (***P*** for interaction with mother's age = 0.002), and also experienced malaria infections with elevated parasite density (***P*** = 0.019) and reduced levels of interferon-γ (***P*** = 0.044) and erythropoietin (***P*** = 0.011) relative to their unexposed peers. Further investigation revealed that the relation with PM was specific to malaria-related anemia events: Whereas there was no evidence of an association with non-malarial severe anemia (Hb<7g/dL) risk (AHR = 0.85, 95% CI: 0.61, 1.20), PM was positively associated with malarial severe anemia (Hb<7g/dL) risk (AHR = 1.68, 95% CI: 1.24, 2.26). Furthermore, in Mali where drug resistance remains low, maternal IPTp-SP had an inverse, dose-response association with SMA (AHR per dose = 0.59, 95% CI: 0.30, 1.14). In conclusion, these findings support the hypothesis that prenatal exposure to PM can alter the pathogenicity of subsequent pediatric malarial infections and suggest the prevention of malaria in pregnancy may have substantive ancillary benefits for children's health.

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THE EFFECT OF MALARIA DURING PREGNANCY ON INFANT SUSCEPTIBILITY TO MALARIA

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Malaria during pregnancy threatens the health of mothers and newborns and may have long-lasting consequences on infant health. Observational studies suggest that placental malaria is associated with increased risk of malaria in the infant. However, previous studies are limited by the inability to control for shared exposures and to detect episodes of malaria that occurred prior to delivery. We hypothesize that placental malaria will be associated with elevated risk of malaria during infancy and that maternal peripheral malaria in the absence of placental infection will be protective as compared to no maternal malaria. We conducted a randomized clinical trial of three chemo-prevention strategies resulting in three distinct malaria exposure groups during pregnancy: placental malaria; peripheral malaria without placental infection; and no malaria during pregnancy. We are following the infants through active and passive molecular surveillance

for malaria; 412 infants have been followed for a median duration of 197 days after birth. Of these, 308 infants were born to mothers with no evidence of malaria during pregnancy, 55 to mothers with placental malaria and 49 to mothers with only peripheral infection. Malaria infection was detected in 10.5%, 14.3% and 16.7% of those born to mothers with no malaria, placental malaria and peripheral malaria during the first nine months of life, respectively. The cumulative incidence of malaria per person year was highest among infants born to mothers with placental malaria, 0.29 compared to 0.15 and 0.12 per person year of follow up among infants born to mother with no malaria and peripheral malaria respectively. These differences were not statistically significant (p=0.2). Follow up is underway to collect more data from the infants as they get older and experience more malaria episodes. Results of this study will inform the design and implementation of innovative and highly effective prenatal interventions to protect the health of pregnant women, newborns and infants from malaria.

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MORE THAN FOUR ANTENATAL MALARIA SCREENS REDUCES THE EFFECTS OF MALARIA ON ADVERSE PREGNANCY OUTCOMES

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125 million pregnancies are at risk of malaria each year, and malaria in pregnancy causes preterm birth and intrauterine growth restriction (IUGR). Preterm birth and small for gestational age (SGA), a proxy for IUGR, increase the risk of neonatal death 15-fold. Women are often infected multiple times, but the effect of the number and gestational age at the time of ***Plasmodium*** spp. infections on adverse pregnancy outcomes, especially in first trimester, has not been fully elucidated. We analysed prospective data on 43,664 pregnancies screened frequently for malaria by microscopy at antenatal visits on the Thai-Myanmar border between 1986 and 2014. Cox regression with time-varying exposures was used to estimate the effect of the number and gestational age of malaria episodes on preterm birth and SGA, including an interaction with the number of malaria screens. 35,717 pregnancies had no malaria and 7,947 pregnancies had between one and eleven episodes. 8% of newborns were preterm, and 10% were SGA. In those who had four malaria screens or less, the hazard of preterm birth and SGA increased with each malaria episode by 1.34 fold (95% confidence interval (CI): 1.13, 1.61) and 1.66 fold (95% CI: 1.09, 2.52), respectively. Compared to no malaria, both second- and third-trimester episodes were equally harmful, increasing the hazard of preterm birth by 2.11 fold (95% CI: 1.59, 2.80) and SGA by 2.92 fold (95% CI: 1.28, 6.66), while first-trimester episodes had minimal effect on either preterm birth (HR: 1.24; 95% CI: 0.89, 1.74) or SGA (HR: 0.66; 95% CI: 0.29, 1.48). Notably, malaria was either not associated or weakly associated with preterm birth and SGA in women who had more than four screens, regardless of the number or gestational age of episodes. These patterns, albeit weaker, held true when analyses were restricted to ***P. vivax*** infections only. Screening and treatment can help to counter the cumulative adverse effects of both ***vivax*** and falciparum malaria throughout pregnancy. However, the WHO recommends four antenatal visits, whereas our data suggest that the optimum number of antenatal malaria screens for preventing adverse outcomes is likely greater than four.

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EMERGENCY EVALUATION OF CONVALESCENT PLASMA FOR EBOLA VIRUS DISEASE (EVD) IN GUINEA

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Evaluation of convalescent plasma (CP) for the treatment of Ebola Virus Disease (EVD) has been prioritized by the WHO in the current epidemic. The Ebola_Tx trial is designed to assess the feasibility, safety and efficacy of CP against EVD in Conakry, Guinea. Pathogen-reduced CP is administered as two units (200-250ml each) given consecutively on the same day, from two different donors. Survival 14 days after intervention is the primary outcome measure. The survival of patients treated with CP + supportive care will be compared to that of patients receiving supportive care alone, in an open-label, non-randomised comparative study. A 20% lower case fatality rate in patients treated with CP will be considered proof of clinical efficacy. All consecutive eligible and consenting patients of any age (including pregnant women) with confirmed EVD will be enrolled; exclusion criteria are limited to contra-indications for CP or patients very close to death. Available ABO compatible plasma is given, within 48 hours after diagnosis, and patients with no compatible plasma available will be enrolled as concurrent controls. The control group will be complemented with historical controls if needed. A minimum of 130 patients will be treated with CP. Secondary outcomes include 1) 30 day all-cause mortality; 2) transfusion-related serious adverse reactions; 3) change in viral load and association with titres of neutralising antibodies in the donor plasma; 4) safety risks in health workers administering CP; 5) risk factors for mortality. The first plasma collection started on February 9, 2015 and the first CP administration was done February 19. As of April 7, a total of 94 donors have presented for plasma donation, of which 81 have donated at least once. Qualified plasma (no transfusion transmissible infections detected) was obtained for 69. A total of 83 patients have been enrolled, of which 81 received CP. Of these, 9 additionally received favipiravir via another trial, leaving 72 patients for inclusion in primary analysis. The main analysis will be done after 130 CP-treated patients have reached day 14 (planned early June). This is the largest trial ever conducted of a convalescent blood product against EVD. If found to be effective, this intervention can then be scaled-up.

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LARGEST DOCUMENTED CLUSTER OF EBOLA VIRUS DISEASE AMONG HEALTH WORKERS

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The 2014-15 Ebola virus outbreak in West Africa is the largest in history. Health workers (HWs) are among those at highest risk of infection because of exposure during patient care. As of April 1, 2015, 861 HW infections and 495 deaths have been reported in Guinea, Liberia, and Sierra Leone. An unusually high number of Ebola virus infections and deaths were noted among HWs in the Kenema District of Sierra Leone. To explore the nature of virus exposures in this high-risk population, we conducted a descriptive analysis using combined data sources, including the national Viral Hemorrhagic Fever database, contact tracing records, hospital staff rosters, Ebola Treatment Unit (ETU) rosters, and burial logs. A HW was defined as anyone who worked in a healthcare facility or engaged in healing practices. All suspected and confirmed HW cases were included in the analysis. A total of 94 Ebola cases in HWs were identified, of which 63 died (case fatality ratio: 67%). The most common self-reported symptoms at the time of presentation included fever (67/78, 86%), vomiting (29/76,

38%), and diarrhea (29/76, 38%). Sixty-six (70%) HWs worked at Kenema Government Hospital (KGH) and 19 (20%) in other health facilities (no data were available for the remaining 9). Less than half (32, 48%) of HWs at KGH were assigned to the ETU and only 30 (32%) reported having contact with a known Ebola case, including sick family members (43%), colleagues (30%), patients (10%), and friends (10%). It was widely suspected that many HWs also saw patients privately after normal working hours, often without full personal protective equipment. In conclusion, most Ebola-infected HWs had numerous risk factors for exposure both in ETUs, other areas of the hospital, and in the community. Specific infecting events were rarely identifiable. Infection prevention and control measures to protect HWs must address a wide array of risk factors both in formal and informal care settings as well as in the community.

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MINIMALLY INVASIVE AUTOPSY: AN ACCURATE TOOL FOR CAUSE OF DEATH DETERMINATION IN DEVELOPING COUNTRIES

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There is a need for accurate estimates of cause of death (CoD) in low income regions. Verbal autopsies and clinical records have shown to be inaccurate. Complete diagnostic autopsies (CDA), the gold standard for CoD determination are challenging due to poor acceptability and limited facilities and human resources. Minimally invasive autopsy (MIA), a simplified procedure involving body fluid and organ-directed sampling followed by pathology and microbiology analyses could become an alternative to CDA. However, there is very limited information on its performance. We aimed to analyze the concordance between MIA and CDA. A standardized procedure for the MIA was tested in a series of 50 autopsies from adults performed at the Maputo Central Hospital, in Mozambique. The MIA was performed by a single pathologist and was followed by a CDA done by a different pathologist. The histological examination of the MIA was done blindly, without any knowledge of the clinical data or the results of the CDA. The microbiological evaluation of the MIA included culture of organs and body fluids as well as routine analyses for HIV, HBV, malaria and tuberculosis, plus specific analyses guided by the histological results. The final CoD obtained in the MIA was compared with the results obtained in the gold standard CDA. Final CoD, as determined in the CDA, were infectious diseases in 30/50 (60%), malignant tumors in 10 (20%) and other causes in 10 patients (20%). A complete agreement between the MIA and the CDA results was observed in 44/50 procedures (88%). The MIA identified 29/30 (97%) of the infectious diseases and in 27 of them the specific etiologic agent was identified in the microbiological analyses. 8/10 malignant tumors and 7/10 other causes were also correctly identified by the MIA. In conclusion, a simple MIA procedure identifies the specific CoD in a high proportion of cases and shows a very high concordance with the results of CDA, the gold standard for CoD attribution.

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GENETIC RISK FACTORS ASSOCIATED WITH DENGUE SEVERITY: A CASE-CONTROL STUDY

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Dengue disease results in a wide spectrum of disease manifestations ranging from subclinical infection to severe and fatal disease. Severe dengue is characterized by an increase in vascular permeability that leads to life-threatening hypovolemic shock (dengue shock syndrome-DSS). Furthermore, there is an increasing trend of hospitalized dengue adult patients with warning signs over the last decades. Genetics risk factors that may have predisposed individuals to the differential dengue severity are still lacking as most studies are limited by the candidate gene approach and relatively small sample size. From our previous genome-wide association analysis (GWAS) involving about 6000 subjects, we have identified single-nucleotide polymorphisms (SNPs) in the gene MICB, which is a heavily glycosated ligands for the NKG2D type II receptor that activates the cytolytic response of natural killer cells. Using targeted-next generation sequencing, we further explored the potential causal variant of dengue severity. We identified a nonsynonymous substitution due a rare SNP (minor allele frequency=0.01) that converts an arginine amino acid to a stop codon, that was postulated to generate a truncated MICB ligand for activation of cytolytic response of immune cells. This SNP was validated in an independent cohort with 4000 DSS cases and 6000 controls with per-allele risk effect of 1.82 (P-value=0.00026). In addition, GWAS was also performed using 600 blood samples collected from hospitalised adult patients with signifcant dengue severity and 600 non-hospitalised mild patients to identify SNPs that may have predisposed these individuals. We identified SNPs from TPO (per-allele OR=1.54;P-value<0.000001) gene and noncoding regions near SNX16 gene (per-allele OR=0.65;P-value<0.000001). In conclusion, these studies have identified potential genetic risk factors in a genome-wide approach as baseline risk, which may be useful in an early triage tool for implementation of dengue prevention initiatives such as the future dengue vaccine to a more targeted population.

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REGULATORY T CELL RESPONSES IN ENDEMIC BURKITT LYMPHOMA PATIENTS ARE ASSOCIATED WITH POOR OUTCOME

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Endemic Burkitt lymphoma (eBL) is a B cell lymphoma uniquely associated with both Epstein-Barr virus (EBV) and malaria, and accounts for the majority of pediatric cancers in sub-Saharan Africa. Unlike other EBV+ tumors for which novel T cell modulating therapies are being developed, T cell contribution to eBL disease progression and survival has not been characterized. Previous studies suggest that tumor development is associated with a loss of EBV-specific pro-inflammatory responses, and a role for regulatory T (Treg) cells has been shown in other EBV-associated cancers. We hypothesized that T cells from eBL patients have higher Treg cell frequencies and higher EBV-specific regulatory responses that decrease with successful disease resolution. By flow cytometry, we examined peripheral blood mononuclear cells collected in a longitudinal eBL cohort study and found that at the time of diagnosis, patients who died had significantly higher frequencies and absolute cell counts of CD25+Foxp3+ Treg cells, with higher counts of CD45RA+Foxp3lo naïve and CD45RA-Foxp3hi effector Treg subsets. Examination of longitudinal time points showed that Treg frequencies remain low in event-free survivors, while patients who relapsed had peaks of elevated Treg frequencies. CD4+ T cells in non-survivors secreted higher levels IL-10 in response to both EBV and malaria antigens. Both CD4+ and CD8+ T cells in eBL patients exhibit a more exhausted phenotype with higher levels of PD-1, and patients who died were deficient in CD8+ IFN-γ responses to EBV antigen. These

data suggest that poor outcomes in eBL patients are associated with a predominantly immunoregulatory environment. Treg frequencies are a potential biomarker of disease progression, and suppression of Treg activity is a potential therapeutic target to improve eBL survival.

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HIGH THROUGHPUT MULTIPLEX DNA EXTRACTION PCR IN THE EVALUATION OF PATIENTS WITH INFECTIOUS DIARRHEA

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In the majority of cases of infectious diarrhea no specific microbial pathogen is identified. Culture independent diagnostic techniques including high throughput multiplex DNA extraction PCR should increase our diagnostic capabilities with increased sensitivity and by allowing the simultaneous detection of a variety of enteric pathogens including bacteria, protozoans and viruses. Such a multiplex approach is well-suited to the syndromic nature of infectious diarrhea. We describe the use of one such multiplex platform, the BioFire FilmArray GI panel which tests for 22 enteric pathogens. Stool specimens were collected in Cary Blair transport media from patients who presented to our clinic with either travelers' diarrhea, community acquired diarrhea or from specimens obtained in patients undergoing colonoscopy for screening or for evaluation of non-diarrheal gastrointestinal complaints. 394 patient specimens were collected from May 14, 2014 to March 5, 2015. Of these, 104 specimens were from patients who had recently traveled, 214 were from patients with community-acquired diarrhea and 76 were obtained from patients during colonoscopy. A total of 134 specimens (34%) were positive for enteric pathogens. Of these 42 specimens (31%) were positive for multiple pathogens. 47 (45%) travel patients tested positive for enteric pathogens. Pathogens most often identified in this group were EPEC (17), EAEC (16), Giardia (6) and Sapovirus (6). 71 (33%) patients with community acquired diarrhea tested positive for enteric pathogens. Most common in this group were EPEC (33), EAEC (20), and C. difficile. 16 (21%) specimens obtained from patients during colonoscopy were positive for enteric pathogens. Most common in this group were C. difficile (9), EPEC (9), and EAEC (5). Our results confirm an increased detection rate in patients with diarrhea compared with conventional methods such as bacterial culture and microscopy. Multiplex testing appears to be associated with a higher rate of mixed infections. In addition asymptomatic carriage of enteric pathogens appears to occur in patients undergoing colonoscopy for non-diarrheal indications.

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FIELD EVALUATION OF PORTABLE MICROSCOPES FOR THE DIAGNOSIS OF SCHISTOSOMIASIS, INTESTINAL PROTOZOA, AND MALARIA IN RURAL CÔTE D'IVOIRE

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Basic microscopy services are lacking in many resource-constrained settings where the greatest need exists. Portable and mobile phone-based microscopes are being developed to provide quality diagnostic support and may be valuable tools in resource-constrained settings. Virtually all studies evaluating this new technology are in ideal laboratory environments or with expert microscopists, and more work is required to validate these devices in real-world settings. We evaluated laboratory technicians' performance of two novel microscope devices - the Newton Nm1 Portable Field Microscope and the reversed-lens CellScope for the diagnosis of schistosomiasis, intestinal protozoa and malaria infections in a community-based screening program in rural Côte d'Ivoire. One Kato-Katz prepared stool samples from 228 individuals was first evaluated by conventional light microscopy (gold standard) and subsequently by experimental microscopes. For ***Schistosoma mansoni*** and S. ***haematobium*** diagnoses, laboratory technicians using the Newton Nm1 microscope demonstrated sensitivities of 91.7% and 81.1% respectively, and specificities of 99.5% and 97.1% respectively. The sensitivity and specificity for S. ***mansoni*** and S. ***haematobium*** diagnosis using the reversed-lens CellScope was 50.0% and 35.6% respectfully, and 99.5% and 100% respectively. 121 separate slides were examined by both conventional microscopy and the Newton Nm1 microscope for intestinal protozoa infections. The Newton Nm1 microscope demonstrated a sensitivity of 80.2% for Entamoeba histolytica/dispar and Giardia intestinalis diagnoses, and specificity was 100% for both pathogens. Finally, 223 malaria thin smears were examined by both conventional microscopy and the Newton Nm1 microscopy. The Nm1 microscope demonstrated a sensitivity of 80.2% and a specificity of 100% for the diagnosis of ***Plasmodium falciparum***, and quantitative agreement of parasitemia was excellent (Pearson's r: 0.997). Portable and mobile phone microscopy have the potential to deliver quality diagnostics in resource-constrained settings and facilitate clinical and public health initiatives.

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A PHASE 1 TRIAL TO ASSESS THE SAFETY, ATTENUATION AND IMMUNOGENICITY OF GENETICALLY-ATTENUATED P52-/ P36-/SAP1- *PLASMODIUM FALCIPARUM* PARASITES VIA THE BITES OF INFECTED *ANOPHELES STEPHENSI* MOSQUITOES

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Attenuated ***Plasmodium*** sporozoite vaccines achieve unprecedented levels of protection against infectious challenge. Both irradiated sporozoites and genetically-attenuated parasites (GAPs) are under consideration for product development. In contrast to genetic heterogeneity observed with irradiated sporozoites, GAPs undergo definitive gene deletion with permanent genetically-defined attenuation and may provide a more rational approach for inducing consistently protective immune responses. Multiple rodent GAPs have been produced, and the cumulative data show that the GAP approach can confer sterile protective immunity. A double gene deletion ***P. falciparum*** GAP lacking ***p52*** and ***p36*** (GAP2KO, Pf ***p52***$^{-}$/***p36***$^{-}$) was generated. A Phase 1 clinical trial of GAP2KO was conducted and at the high 200-bite dose, one of six subjects developed a breakthrough blood-stage infection, indicating conspicuous but incomplete attenuation. To ensure further attenuation, a third deletion was made to the critical liver stage gene encoding Sporozoite Asparagine-rich Protein 1 (GAP3KO, p52^{-}/p36^{-}/sap1^{-}). We conducted a single arm, open-label, phase 1 experimental medicine study designed to evaluate the safety and tolerability of GAP3KO. The study was designed to confirm attenuation of GAP3KO using peripheral blood smears and to evaluate cellular and humoral immune responses. A total of 10 healthy, malaria-naïve adult subjects were enrolled and received GAP3KO via the bites of 150-200 GAP3KO-infected A. stephensi mosquitoes. To confirm attenuation, subjects were evaluated for safety, reactogenicity, and signs and symptoms of malaria infection for 28 days, including monitoring in a hotel setting on Days 8-18 post-GAP3KO administration. All 10 subjects that received GAP3KO did so without incident and completed the 28 day study period. Local and systemic solicited adverse events reported as primary endpoints were classified as Grade 1 (mild) and 2 (moderate). All subjects remained negative for patent parasitemia as demonstrated by evaluation of peripheral blood smears. GAP3KO elicited significant immune responses and will proceed in development.

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EFFICACY AND SAFETY OF A PHASE 1 TRIAL WITH CHALLENGE TO ASSESS THE SAFETY AND BIOMARKERS OF PROTECTION IN MALARIA-NAÏVE ADULTS OF IMMUNIZATION VIA MOSQUITO BITE WITH RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES (IMRAS)

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The "IMRAS" (immunization via mosquito bite with radiation-attenuated sporozoites) study is a comprehensive, systems biology-based effort to identify and validate biomarkers of protection with ***Pf***RAS immunization, comparing sterilely protected to non-protected study subjects. The primary objective of the clinical trial is to achieve approximately 50% sterile protection. Twenty-four malaria-naïve adults will receive five doses of approximately 200 infectious bites (200-400 bites total) from ***Pf***RAS-infected mosquitoes (true-immunization) and 8 additional subjects will receive the same from irradiated, uninfected mosquitoes (mock-immunization). Three weeks after the fifth immunization, all immunized subjects (true-immunized and mock-immunized) plus non-immunized infectivity controls will undergo controlled human malaria infection (CHMI). To increase sample size and enable flexibility toward the protection goal, the study was designed with two cohorts and an adjustable dosing schedule for Cohort 2 in the event protection in Cohort 2 was <40% or >60%. During 2014, 95 subjects were screened; 28 were enrolled and assigned to study groups in Cohort 1. Seventeen subjects received at least one true (n = 13) or mock (n = 4) immunization, and six subjects were retained as infectivity controls. Fourteen subjects received the complete series of five true (n = 11) or mock (n = 3) immunizations. The immunizations were generally well tolerated although two subjects (one true-immunized and one mock-immunized) developed large, local reactions with significant proximal extension that became generalized, but confined to the skin. In both cases the reaction resolved uneventfully over one or two days without sequelae, however both subjects were withdrawn from participation. Six of the 11 (55%) true-immunized subjects were sterilely protected, thus achieving the goal of approximately 50% protection. All mock-immunized and infectivity controls were non-protected. Enrollment and immunization of Cohort 2 will mirror that of Cohort 1 and efficacy data is expected late 2015.

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LONG TERM EFFICACY, SAFETY, REACTOGENICITY, AND IMMUNOGENICITY OF PHASE IIA, OPEN-LABEL, CONTROLLED STUDY FOR THE RTS,S/AS01B MALARIA VACCINE CANDIDATE ADMINISTERED AS STANDARD DOSES AT 0 AND 1 MONTHS AND 1/5TH STANDARD DOSE AT 7 MONTHS (DELAYED FRACTIONAL DOSE GROUP) AND ADMINISTERED AS THREE STANDARD DOSES ONE MONTH APART (0, 1, 2-MONTH GROUP) IN HEALTHY MALARIA-NAÏVE VOLUNTEERS AGED 18-50 YEARS

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Plasmodium falciparum malaria continues to be a leading cause of global morbidity and mortality. Although the RTS,S/AS01 malaria vaccine has been submitted to the European Medicines Agency for regulatory review, efforts continue to improve its efficacy and durability. In one of the first ***P. falciparum*** controlled human malaria infection (CHMI) trials evaluating early formulations of RTS,S, six of seven subjects vaccinated with RTS,S/AS02$_A$ were protected against sporozoite challenge. After systemic reactogenicity was observed following the second dose, and given the limited experience with this adjuvant formulation at the time, the third dose was reduced to one-fifth of the standard dose and administered 6 months after the previous dose (0, 1, and 7 months). Despite the high level of protection, this delayed fractional dose (DFD) regimen has never been repeated in subsequent studies. It is biologically plausible that a DFD regimen may have clinically important qualitative and quantitative impacts on vaccine immunogenicity and consequently on vaccine efficacy. Considering this, we conducted a phase 2a study (NCT01857869) of RTS,S/AS01$_B$ with CHMI to re-investigate the DFD regimen in a larger trial of healthy malaria-naïve adult volunteers. The study enrolled 34 volunteers in the DFD group (received standard doses of RTS,S/AS01$_B$ at 0 and 1 months and 1/5th standard dose at 7 months) and 17 in the active control group (received standard doses RTS,S/AS01$_B$ at 0,1, and 2 months). ***P. falciparum***-infected mosquito CHMI, including infectivity controls, was performed 21 days after the third vaccine dose. Re-challenge was also conducted 7 months after the third vaccine dose, with or without a fractional booster dose in a subset of volunteers. Trial safety, reactogenicity, immunogenicity, and efficacy after CHMI will be reported.

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SAFETY, IMMUNOGENICITY AND EFFICACY OF THE COMBINATION MALARIA VACCINE REGIMEN OF RTS,S/AS01B CONCOMITANTLY ADMINISTERED WITH CHAD-MVA VIRAL VECTORS EXPRESSING ME-TRAP

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The global healthcare burden of ***Plasmodium falciparum*** malaria remains high. Both RTS,S/AS01 and a prime-boost regimen using viral vectors ChAd63 and MVA expressing the ME-TRAP antigen have shown partial efficacy. In an attempt to increase vaccine efficacy an initial trial was conducted in 2013/2014 in which RTS,S/AS01$_B$ and the viral vectors were administered in a staggered fashion, and subsequently evaluated for protection by controlled human malaria infection (CHMI). A high level of protective efficacy was seen in both the RTS,S/AS01$_B$ plus viral vectors group, and RTS,S/AS01$_B$ alone groups (82.4% [95% CI 64%-100%] and 75% [95% CI 54%-96%] respectively). To further investigate this initial finding, we designed this phase I/IIa, open-label, CHMI study (NCT no. NCT02252640) to assess the safety, immunogenicity and efficacy of concomitant administration of viral vectors with RTS,S/AS01$_B$. Furthermore, to evaluate the finding of high efficacy (86% [95% CI 12%-98%]) in a CHMI trial conducted in 1997, where subjects had received 2 standard doses of RTS,S/AS02$_A$ and a reduced (1/5th standard dose) third dose, RTS,S/AS01$_B$ was administered in this current trial as a standard dose regimen or in a regimen where the third dose was reduced to 1/5th of the standard dose. We enrolled healthy malaria naïve adults into 1 of 4 groups using a factorial design. All groups received 3 doses of RTS,S/AS01$_B$. Groups 1 and 3 received 3 standard doses of RTS,S/AS01$_B$ at weeks 0, 4 and 8, whilst groups 2 and 4 received 2 standard doses at weeks 0 and 4 and a fractional (1/5th) dose at week 8. In addition, groups 3 and 4 received concomitant vaccinations at the same site with ChAd63 ME-TRAP at week 0, and MVA ME-TRAP at weeks 4 and 8. Efficacy was assessed by CHMI delivered by mosquito bites approximately 3 weeks after the last dose. Safety, immunogenicity of the vaccine regimen, and efficacy results from the CHMI will be presented.

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PHASE 1 TRIAL WITH CONTROLLED HUMAN MALARIA INFECTION: AN OPEN LABEL DOSE-ESCALATION SAFETY, REACTOGENICITY, IMMUNOGENICITY, AND EFFICACY STUDY OF THE CANDIDATE VACCINE *PLASMODIUM FALCIPARUM* MALARIA PROTEIN (FMP012), AN *E. COLI*-EXPRESSED CELL-TRAVERSAL PROTEIN FOR OOKINETES AND SPOROZOITES (*PF*CELTOS), ADMINISTERED INTRAMUSCULARLY WITH THE ADJUVANT SYSTEM AS01$_B$ IN HEALTHY, MALARIA-NAÏVE ADULTS

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A highly efficacious malaria vaccine against ***Plasmodium falciparum*** is a key element in the effort to prevent infection and eradicate malaria. The pre-erythrocytic antigen named cell-traversal protein for ookinetes and sporozoites (CelTOS) appears to be critical in order for the sporozoite to traverse host cells in vertebrates and successfully initiate an infection. CelTOS is highly conserved among ***Plasmodia***, suggesting an important functional role. Falciparum malaria protein 12 (FMP012) is a recombinant subunit protein based on CelTOS from the 3D7 clone of ***P. falciparum*** expressed in and purified from ***E. coli*** using a unique synthetic "codon harmonized" gene construct resulting in optimal folding and expression. In an earlier clinical trial at Walter Reed Army Institute of Research, FMP012 in combination with an alternate adjuvant was well tolerated but did not induce sterile immunity. One subject had a significant delay in the pre-patent period and had both the highest antibody titer and highest IFN-γ ELISpot response to FMP012 on the day of challenge compared to the other subjects (NCT01540474). To further assess potential efficacy, we performed a phase 1 vaccine safety and immunogenicity study of FMP012 formulated with the Adjuvant System AS01$_B$ followed by controlled human malaria infection (CHMI) challenge (NCT02174978). The study incorporated dose-escalation of the antigen in combination with a fixed adjuvant dose. A total of 30 volunteers were divided into 2 dosage groups (15 subjects per group), each receiving 4 intramuscular injections at 0, 1, 2, and 6 months. A ***P. falciparum***-infected mosquito CHMI was performed in 6 infectivity control volunteers and both vaccine groups 21 days following the fourth vaccination. The safety, reactogenicity, immunogenicity, and efficacy results of the FMP012/AS01$_B$ vaccine are reported.

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A TRANSMISSION BLOCKING VACCINE AGAINST *PLASMODIUM FALCIPARUM*, PFS25H-EPA/ALHYDROGEL®: RESULTS FROM THE FIELD

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Transmission blocking vaccines (TBV) are a critical strategy for malaria elimination and eradication in Sub-Saharan Africa. A double blind, randomized, controlled Phase 1 clinical trial was completed to assess the safety and immunogenicity of Pfs25H-EPA/Alhydrogel® in malaria exposed Malian adults. One hundred and twenty healthy adult volunteers aged 18-45 years old living in the village of Bancoumana, Mali were enrolled into the study. Among the 120 participants, 20 (low dose of 16µg Pfs25H-EPA/Alhydrogel® or control) received 2 doses (Days 0 and 56) and 100 (high dose of 47µg Pfs25H-EPA/Alhydrogel® or control) received 4 doses (Days 0, 56, 112, and 480). The clinical study was recently unblinded in March 2015. Overall, Pfs25H-EPA/Alhydrogel® TBV has been well tolerated and produced significant antibody responses in a malaria exposed adult population. Unblinded results of safety, immunogenicity, and functional transmission blocking activity (measured by standard membrane feeds, direct skin feeds, and experimental hut studies) will be presented.

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ASSESSING EFFICACY OF THE PFSPZ VACCINE BY CONTROLLED HUMAN MALARIA INFECTION IN BAGAMOYO TANZANIA

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Assessing efficacy of the PfSPZ Vaccine by controlled human malaria infection in Bagamoyo Tanzania Seif Shekalaghe, Said Jongo, Elena Moreno, Omar Lweno, Ali Hamad, Kamaka Kassim, Omar Juma, Conrad Gwandu, William Kato, Caroline Mavere, Ramla Rashid, Jackson Mollel, Maximillian Mpina, Anneth Tumbo, Catherine Mkindi, Selina Joseph, Mohammed Mgeni, Claudia Daubenberger, Peter Billingsley, Thomas L Richie, Marcel Tanner, Salim Abdullah, Stephen L Hoffman African scientists are now spearheading phase 1 or proof-of-concept studies that have a controlled protection component. Recently, Sanaria Inc., has developed aseptic, purified, cryopreserved infectious ***Plasmodium falciparum*** (Pf) sporozoites (SPZ) (PfSPZ Challenge), suitable for parenteral injection and shippable to any location able to conduct GCP studies. PfSPZ Challenge has now been tested in the US, Europe, Africa. 3200 infectious PfSPZ administered by direct venous inoculation (DVI) reproduces the near 100% infection rate and 11-12 day pre-patent period of the traditional five-mosquito bite CHMI. For the first, we used PfSPZ Challenge to test the efficacy of a candidate malaria vaccine against CHMI administered by DVI. PfSPZ Vaccine are non-replicating due to radiation attenuation. PfSPZ Vaccine has shown 100% efficacy in a study at The Vaccine Research Center, National Institute of Allergy and Infectious Diseases, in the group received the highest (135,000 PfSPZ by DVI at wks 0, 4, 8, 12 and 20). Ifakara Health Institute at Bagamoyo has compared the same regimen, but delivered twice (270,000 PfSPZ) at each of the five time points, in adults. Vaccine was assessed for protection against CHMI delivered by DVI at 3 and 24 weeks after the fifth dose. We will present safety, tolerability and efficacy data for this unique study that is opening an exciting pathway for malaria vaccine testing and development in Africa and the world.

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THE SOIL INHABITING *METARHIZIUM* FUNGI AND THEIR VIRULENCE AGAINST MALARIA MOSQUITOES *ANOPHELES GAMBIAE* S.L. IN BURKINA FASO

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Fungi can infect mosquitoes through direct contact with the cuticle, so lend themselves to strategies currently used for delivery of chemical insecticides. In addition, they are environmentally friendly alternatives to pesticides. Much attention has focused on the ascomycetes ***Metarhizium spp***. and ***Beauveria spp***. These species are soil-inhabiting, insect-pathogenic fungi and are associated with the rhizosphere. However, not much is known about the distribution of these fungi in the rhizosphere and their potential virulence against adult malaria mosquitoes in the field in Burkina Faso. Here, we monthly explored a preferred habitat of these fungi during the dry and wet season from 2014-2015 in five different sites. Fungi were isolated and cultured in a sterile environment. Potato Dextrose Agar (PDA), a common medium for isolating fungi, and PDA + CTAB+ Chloramphenicol, a selective medium were used for isolation. ***Aspergillus spp***., ***Trichoderma spp***. and ***Metarhizium spp***. were the main genera isolated from all the sites and throughout the year. ***Metarhizium spp***. represented between 34 and 75% of isolated samples according to the sites and the season. Eleven different ***Metarhizium spp***. isolates from the rhizosphere at the concentration 10^8 conidia/mL were used for bioassays to infect adult mosquitoes ***Anopheles gambiae*** s.l. Overall, the corrected mortality percentage by the Abbott's formula was between 79% and 100% after 2 weeks infection. In addition, the LT50 of these isolates globally varied from 6 to 10 days. To follow up, the stress tolerance experiments to determine the fitness and PCR analysis for species identification will be carried out on the virulent species. These results are extremely encouraging for using locally isolated ***Metarhizium*** isolates in biological and transgenic biocontrol methods against malaria.

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ACTIVITY OF THE PYRROLE INSECTICIDE CHLORFENAPYR ON LLIN IN MOSQUITO BIOASSAY AND SEMI FIELD TRIALS: IMPLICATIONS FOR FUTURE INSECTICIDE TESTING

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The rapid selection of pyrethroid resistance in Africa is a serious threat to malaria vector control. Chlorfenapyr is a pyrrole insecticide which shows no cross resistance to conventional insecticide classes and is effective on mosquito nets in semi field conditions. Unlike neurotoxic insecticides, chlorfenapyr owes its toxicity to disruption of metabolic pathways in mitochondria. A series of experiments explored whether standard World Health Organization guidelines for evaluation of long-lasting insecticidal nets are adequate for evaluation of non-neurotoxic insecticides. The efficacy of WHO recommended test methods were compared for pyrethroids and chlorfenapyr. Mosquito behaviour and response to chlorfenapyr ITN in bioassays conducted at night was compared to day and across a range of temperatures representative of highland and lowland transmission. Standard bioassay tests on chlorfenapyr produced extremely low levels of mortality which was not at all predictive of the levels of mortality achieved under field conditions. The endogenous circadian activity rhythm of anophelines results in inactivity by day and

raised metabolism and flight activity by night. A model which explains improved toxicity of chlorfenapyr ITN when tested at night, and during the day at higher ambient temperature, is that activation of chlorfenapyr and disruption of respiratory pathways is enhanced when the insect is more metabolically and behaviourally active. Testing according to accepted guidelines is not suitable for certain types of non-neurotoxic insecticide which, though highly effective in field trials, would be overlooked at the screening stage of evaluation through bioassay. WHO testing methods must be tailored to the characteristics and mode of action of each insecticide class. The WHO tunnel test or experimental hut trial on night active anophelines is only reliable bioassay for identifying the toxicity of novel insecticides on nets.

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IVERMECTIN INHIBITS THE DEVELOPMENT OF *PLASMODIUM VIVAX* IN *ANOPHELES DIRUS*

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Novel vector control interventions are urgently needed to assist malaria drug resistance containment and elimination efforts in the Greater Mekong Subregion (GMS). In West Africa, ivermectin MDAs have been shown to significantly reduce the survivorship of ***Anopheles gambiae*** up to six days and the proportion of ***Plasmodium falciparum***-infectious ***An. gambiae*** up to fifteen days post MDA in Senegal, Liberia, and Burkina Faso. Previous laboratory work demonstrated that ivermectin inhibits the development of ***P. falciparum*** in ***An. gambiae*** (***ie***. sporontocidal). Ivermectin is lethal to numerous important GMS malaria vectors including: ***An. dirus***, ***An. minimus***, ***An. campestris***, and ***An. sawadwongporni***. Here we present that ivermectin is sporontocidal to ***P. vivax*** in ***An. dirus*** when ivermectin is co-ingested with gametocytes at the lethal concentration that kills 25 and 5 percent of mosquitoes (***ie***. LC_{25} and LC_5). Briefly, gametocytemic blood was drawn from ***P. vivax***-infected persons reporting to malaria clinics in Thailand, the blood was mixed with dilutions of ivermectin, and then fed to ***An. dirus*** in membrane feeders with and without ivermectin. Oocyst prevalence was reduced at the ivermectin LC_{25} by 34% ($\chi^2 = 22.75$, $P < 0.0001$) and LC_5 by 29% ($\chi^2 = 16.9$, $P < 0.0001$). In ***An. dirus*** that were ***P. vivax***-infected, oocyst intensity was reduced at the ivermectin LC_{25} by 42% ($P = 0.0096$) and LC_5 by 48% ($P = 0.0123$). Interestingly, ***An. dirus*** that ingested ***P. vivax*** were substantially more susceptible to the ivermectin LC_{25} compared to control blood meals with ivermectin LC_{25} with a 48% reduction in survivorship to day 7 ($\chi^2 = 64.83$, $P < 0.0001$). The combined mosquito-lethal and sporontocidal effects of ivermectin suggest that ivermectin MDAs to humans could be a powerful new tool to aid elimination efforts in the GMS. Future directions include repeating these trials with ***P. falciparum*** using malaria-naïve serum from ivermectin-treated individuals, and a clinical trial to assess the safety, tolerability, pharmacokinetic interaction, and mosquito lethal efficacy of ivermectin with artemisinin combination therapies, and primaquine.

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THE CYTOCHROME P450 CYP6Z1 GENE AND THE N485I ACE-1 MUTATION CONFER CARBAMATE RESISTANCE IN THE MAJOR AFRICAN MALARIA VECTOR *ANOPHELES FUNESTUS*

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Despite increasing reports of resistance to carbamate insecticides in the major African malaria vector ***Anopheles funestus***, its molecular basis remains uncharacterized preventing the design of suitable resistance management strategies. Here, we dissected the molecular basis of bendiocarb resistance showing that carbamate resistance is driven by both metabolic and target-site resistance and designed a diagnostic tool to detect and track this resistance. Using a microarray-based genome-wide transcription profilling and qRT-PCR, we detected candidate carbamate resistance genes in Malawian ***An. funestus***. The cytochrome P450 genes CYP6P9a, CYP6P9b and CYP6Z1 were the most up-regulated in both bendiocarb and pyrethroid resistant mosquitoes suggesting possible cross-resistance. Using In silico modelling/docking and both ***in vivo*** transgenic expression in Drosophila and ***in vitro*** recombinant enzyme metabolism assays, we demonstrated that specific CYP6Z1 confers cross-resistance to both insecticide classes whereas CYP6P9a and CYP6P9b are only pyrethroid metabolisers. Polymorphism analysis further confirmed that CYP6P9a and CYP6P9b were only associated with pyrethroid resistance. Investigation of the target resistance through the cloning of the full 2208 bp of the acetylcholinesterase 1 gene did not detect the common G119S but 4 other mutations. A Taqman genotyping of the N485I mutation revealed that it significantly correlates with bendiocarb resistance (Odds ratio 7.3; P<0.0001). Furthermore, detection of multiple haplotypes in single mosquitoes after cloning suggests the duplication of the Ace-1 gene in ***An. funestus*** and further supported by the absence of any homozygote (RR) resistant mosquitoes in the field. Genotyping of the N485I mutation in 10 countries reveals that it is only located in Southern Africa (Malawi, Mozambique and Zambia) with frequency of 10-15% suggesting a recent occurrence of this mutation. These findings will help monitor the spread and evolution of carbamate resistance and improve the design of effective resistance management strategies to control this major malaria vector.

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ENDECTOCIDE-TREATED BIRD FEED FOR THE CONTROL OF WEST NILE VIRUS TRANSMISSION

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Currently there are limited options for controlling West Nile virus (WNV) transmission, including the use of larvicides and adulticides to target the mosquito vector. However, these applications are poorly-targeted, restricted to wealthy semi-urban and urban areas that are able to fund the efforts, and face opposition due to toxicity concerns. This study evaluated the use of endectocide-treated bird feed to control WNV transmission by targeting the primary vector in Colorado, ***Culex tarsalis***, through its blood feeding behavior. Ivermectin (IVM) susceptibility in ***C. tarsalis*** was measured through IVM-spiked bloodmeals with membrane feeders, and the LC_{50} was determined to be 49.94 ng/ml ([39.71-59.93], n = 988). Chickens were then fed IVM-treated feed to examine its safety and palatability, and mosquitoes were blood fed directly on the chickens to assess ***in vivo*** effects. Finally, IVM pharmokinetics were analyzed using vein blood from chickens as well the ***C. tarsalis*** that bloodfed on the chickens. A mixture of 200 mg IVM/kg of bird feed was determined to be a palatable and safe dose that chickens would consume while also being effective in killing ***C. tarsalis*** in bioassays with 95% mortality in IVM fed mosquitoes compared to 100% survival in unmedicated controls. Pharmacokinetic data from the ***in vivo*** tests produced conflicting results compared to ***in vitro*** blood feeds but IVM was detected in chicken blood at concentrations that may be expected to affect ***C. tarsalis***. Dosing, safety, and bioassays were also conducted in doves, where 200 mg/kg was safe and palatable, while killing 88% of IVM-fed mosquitoes compared to 15% control mortality. Additional studies are currently determining the effect of IVM on mortality in WNV-infected mosquitoes, as well as if sub-lethal doses of ivermectin reduces WNV replication and transmission. Preliminary data showed that ***C. tarsalis*** mortality is significantly increased when given a blood meal including 73.66 ng/ml of IVM and $5x10^5$ PFU/ml WNV as compared to a blood meal containing only IVM. Our study indicates that the use of IVM-treated bird feed could be a novel strategy for control of WNV transmission.

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SUCCESSFUL APPLICATION OF A SEQUENCE-BASED GENOMEWIDE ASSOCIATION STUDY (GWAS) TO IDENTIFY THE GENETIC BASIS OF PYRETHROID RESISTANCE IN *ANOPHELES ARABIENSIS*

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The genomic resources available for major vectors within the ***Anopheles gambiae*** complex create opportunities to apply genomewide association (GWAS) approaches to medically-important phenotypes such as insecticide resistance. However, in nature the genomes and population genetics of mosquitoes present extreme challenges for GWAS, at least using standard designs from human studies. We developed a novel hybrid-GWAS design founded on advances in ***Anopheles gambiae*** genome sequencing. Focussing on recent and spreading pyrethroid resistance in the increasingly-important vector ***An. arabiensis***, we sequenced and compared multiple pools of partially or fully resistant and susceptible females from wild populations in northern Tanzania and Zanzibar. Following application of a stringent analysis pipeline we identify very strong, replicated, association signals around gene clusters with known involvement in metabolic resistance. Our results suggest that with pragmatic experimental design, GWAS in ***Anopheles*** is both a feasible and powerful option to identify the genetic basis of medically-relevant phenotypes and discover diagnostic markers.

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STUDY OF CUTICULAR RESISTANCE IN *ANOPHELES GAMBIAE*, FROM PHENOTYPE TO PROTEIN FUNCTION

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Cuticular resistance reduce the penetration of insecticide through a change in the cuticle composition (lipids and proteins). This resistance mechanism has often been described in insects of agricultural interest but less characterized in mosquitoes. Cuticle thickness was associated with Pyrethroids (Py) resistance in ***Anopheles funestus***. The aim of this study is to determine if there is a resistance to Py through cuticular proteins in ***An. gambiae*** and to characterize their involvement in the process. To do this, we selected a resistant strain without kdr mutation to avoid interaction between resistance mechanisms. The resistant strain selection of ***An. gambiae*** without kdr allele was performed through successive cross-breeding between a wild resistant strain and the susceptible reference strain. Insecticide pressure has been applied on this strain on adult stage. Biochemical and susceptibility tests were performed for resistance monitoring level. Candidate cuticle and metabolic genes were been taken on resistant An transcriptomic data of previous studies. RT-qPCR will be performed on these candidate genes and the expressions compared between resistant and susceptible. The overexpressed genes in resistant will be confirmed by RNAi. To measure the penetration of insecticides through cuticle, we will proceed a topical application of Py. The insecticide and his metabolite within mosquitoes will be determined by GC-MS/MS or GC-ESD. The resistant mosquitoes group will be compared to the susceptible one. Microscopic study will be conducted on the ultrastructure of the resistant cuticle versus susceptible as well as proteins involved by proteomic approach. The resistant strain has been selected and the pressure selection based on insecticide is in progress. Biochemical tests already showed an overexpression of non-specific esterase alpha, beta and Glutathione S-Transferase. Currently the strain selected with deltamethrin recorded 60.93% of mortality after 30 minutes of exposure and that selected with permethrin, 84% of mortality after 40 minutes of exposure. The study is in progress and interesting results are expected in next months.

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GENETIC MODIFICATION OF THE DIARRHEAL PATHOGEN *CRYPTOSPORIDIUM PARVUM*

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Cryptosporidium is the second most important pathogen after rotavirus to cause moderate to severe diarrhea in children below two years of age. ***Cryptosporidium*** is also an opportunistic pathogen for immunocompromised individuals such as HIV/AIDS patients and organ transplant recipients. Currently, there are no fully effective drugs or vaccines to treat or prevent cryptosporidiosis. The main roadblock in the development of drugs and vaccines is the overall poor tractability of ***Cryptosporidium*** due to lack of continuous culture system, poor animal models and lack of genetic tools. We report here a powerful approach to genetically modify this pathogen. We demonstrate the transfection of ***Cryptosporidium parvum*** sporozoites in tissue culture and optimization of regulatory sequences and electroporation conditions to drive expression of a luciferase reporter gene. We developed a mouse model for ***C. parvum*** infection to inject electroporated sporozoites directly into the small intestine of IFN-γ knockout mice. We used the CRISPR-Cas9 system to knockout the parasite's thymidine kinase (tk) gene and replace it with a cassette expressing the luciferase reporter fused to a neomycin resistance gene that conferred paromomycin resistance ***in vivo***. Quantitative 18S ribosomal RNA PCR and luminescence measurements were used to monitor the course of parasite infection in mice and development of paromomycin-resistant transgenic parasites. We tested the stable transgenic parasites for the loss of the tk gene, and evaluated the use of transgenic oocysts for performing drug assays. Our ability to genetically engineer ***C. parvum*** will help to answer key questions related to parasite biology and accelerate the development of novel therapeutics for disease intervention.

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MOLECULAR DIAGNOSTICS FOR GASTROINTESTINAL PARASITES AND IMPACT ON INTESTINAL MICROBIOTA IN RURAL ARGENTINIAN CHILDREN

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Worldwide, there are over 2 billion people infected with gastrointestinal (GI) parasites. Depending on the species, these parasites can disrupt intestinal bacterial flora affecting nutritional status. We implemented a multi-parallel quantitative real-time PCR (qPCR) and have begun NextGen sequencing analysis for microbiota and 8 common GI parasites including the helminths, ***Ascaris lumbricoides (Al), Ancylostoma duodenale (Ad), Necator americanus (Na), Strongyloides stercoralis (Ss), Trichuris trichiura (Tt)*** and protozoa, ***Cryptosporidium parvum (Cp), Entamoeba histolytica (Eh)*** and ***Giardia lamblia (Gl).*** Stool samples were collected from 116 asymptomatic children (under 10 years old) from rural Argentina. qPCR and next generation sequencing was performed on microbiota enriched stool sample DNA using shotgun sequencing. Among samples, qPCR identified the helminths ***Al*** (54.3%) and 37.4% for two hookworm species (***Na*** and ***Ad***). For ***Ss*** and ***Tt***, 63.6% and

0.8% were positive, respectively. For the protozoa, ***Gl*** (65.5%), ***Eh*** (1.7%), and ***Cp*** (0%). qPCR was also able to detect polyparasitism of 2 or more parasites in 70.6% of patients. For ***Gl*** samples, microbiome analysis data shows decrease in microbiota biodiversity in the parasite infected group compared to those non-infected, with a shift away from ***Firmicutes*** toward increased ***Bacteroidetes***, with the degree of shift related to intensity of infection (qPCR data). The abundant bacteria within the ***Bacteroidetes*** were due to increased ***Prevotella,*** compared to the non-infected group with increased ***Ruminococcus*** ($p < 0.05$***)***. Clustering between the groups was examined using PCoA ordination and Shannon alpha diversity (parasite group 1.65; non-infected group 2.7, $p = 0.033$). This first use of multi-parallel qPCR and NextGen microbiota sequencing in Argentina, shows an association of GI parasite infections and decreased microbiome biodiversity in asymptomatic children. The results will enable refinement of treatment options on a public health scale, leading to better health outcomes in endemic settings.

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ELUCIDATING MECHANISMS OF DIARRHEA AND ENTEROPATHY DURING MALNUTRITION AND CRYPTOSPORIDIOSIS

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Cryptosporidium is a major cause of moderate to severe diarrhea, persistent diarrhea, and stunting in children living in resource-limited countries. Malnutrition increases risk of cryptosporidiosis, and experimental models demonstrate compounded deleterious effects of infection during nutrient deficient states. The pathogenesis of diarrhea due to ***Cryptosporidium***, however, is incompletely understood. Using a murine model of protein malnutrition (PEM), we interrogated epithelial barrier disruption ***in vivo***. We identified that PEM leads to up-regulation of claudin-1 but reduced expression of claudin-2. The combination of PEM with ***C. parvum*** enhanced disease concurrent with increased numbers of claudin-2 antibody stained cytoplasmic vesicles and led to a generalized reduction in occludin expression in the ileum, but zonula occludins (ZO)-1 was not significantly affected. IL-13, a known inducer of claudin-2 was elevated in protein malnourished mice following primary ***C. parvum*** infection, but mice re-challenged after ***C. parvum*** priming neither lost weight nor demonstrated elevated IL-13 (113.30 vs 0 pg/ml; $P<0.05$). In comparison, infection with the non-invasive protozoan G. lamblia, led to no appreciable change in claudin-2 expression, but marginal increases in ZO-1 and claudin-15. A progressive increase in lamina propria lymphocytes occurred through 21 days after ***C. parvum*** challenge, concurrent with stunted growth relative to uninfected controls. These studies demonstrate differential and pathogen-specific influences on tight-junction protein expression at the epithelial barrier during conditions of malnutrition, and in the case of ***Cryptosporidium***, raises the potential for a mechanism of diarrhea involving a unique and specific disruption in the epithelial barrier that may be in part driven by the host immune response.

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IL-25 MEDIATED PROTECTION FROM AMOEBIC COLITIS

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Entamoeba histolytica is an enteric parasite that causes diarrhea. ***E. histolytica*** adheres to host cells by Gal/GalNAc lectin and disrupts the mucosal barrier, penetrates underlying tissue and destroys cells. Host responses at the site of infection are critical for resistance, thus the mucosal response against ***E. histolytica*** infection is a significant contributor to the immune response. Intestinal epithelial cells (IECs) serve as effectors of the mucosal immune system and produce inflammatory mediators including IL-1β during amebiasis. IL-1β is known to suppress the production of IL-25 and is increased during ***E .histolytica*** infection, which is also produced by IECs. We have observed that IL-25 expression is suppressed in a time course of ***E. histolytica*** infection in CBA mice. We hypothesize that during ***E. histolytica*** infection IL-25 has a protective role and to test this hypothesis we intraperitonealy administrated rIL-25 in CBA mice. We have found that rIL-25 treated mice had a significantly lower infection rate through culture, lower antigen load by ELISA and also lower numbers of ***E. histolytica*** through QPCR. Histologically there was significantly less epithelial disruption in rIL-25 treated mice. Therefore we have concluded IL-25 plays a protective role during amebiasis. We have found that Reg3gamma (Reg3g) is increased with rIL-25 administration during ***E. histolytica*** infection. Therefore we hypothesized Reg3g could play a role in IL-25 mediated protection by maintaining the epithelial barrier and ***E. histolytica*** killing. To test the importance of Reg3g, we will block Reg3g activity in rIL-25 treated mice. We have also found that IL-25 can induce the Th2 cytokine IL-13 at the acute stage of infection. IL-13 is known to induce paneth cell degranulation to release antimicrobial peptides. To confirm the role of IL-13 in IL-25 mediated protection through Reg3g, we will block IL-13 in IL-25 treated mice and hypothesize that there will be decreased level of Reg3g to make mice susceptible. This work will inform our knowledge on the role of IL-25 in the mucosal defense against amebic infection, ultimately improving treatment and vaccine strategies.

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A 5-AMINOPYRAZOLE-4-CARBOXAMIDE *CRYPTOSPORIDIUM* THERAPEUTIC ALLEVIATES DIARRHEA IN THE CALF-CHALLENGE MODEL

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New therapies to treat ***Cryptosporidium*** infection are urgently needed to address severe and life-threatening diarrhea in <2 yo children in the developing world. It was previously shown that targeting calcium-dependent protein kinase 1 (CDPK1) of ***Cryptosporidium parvum*** (Cp) with pyrazolo-pyrimidine bumped-kinase inhibitors leads to reduction of parasite host cell invasion ***in vitro*** and a reduction of infection in the immunosuppressed mouse model of Cp (JID 2013;208:1342). We have now tested a 5-aminopyrazole-4-carboxamide (AC) bumped-kinase inhibitor (1517), that also inhibits CpCDPK1 (IC50 = 1nM) and blocks Cp invasion and proliferation ***in vitro*** (EC50 = 50nM). We tested the therapeutic efficacy of 1517 in the newborn-calf-Cp challenge model. Calves were challenged with Cp and subsequently treated with 1517 (10mg/kg given orally every 12 hrs for 5 days) or vehicle-alone (control) starting on day 2 after challenge. Calves began to have diarrhea on day 2 after challenge. Compared with control calves, calves treated with 1517 had a significantly improved clinical score, less days of diarrhea, and reduced fecal volume. Oocyst excretion levels are currently being evaluated and will be reported at the meeting. These data demonstrate that bumped kinase inhibitors improve clinical outcomes in calf-Cp infection, even after diarrhea is established. This suggests that these promising therapeutics could help children in the developing world with ***Cryptosporidium*** diarrhea.

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DEFINING THE EGRESS SIGNALING NETWORK IN THE APICOMPLEXAN *TOXOPLASMA GONDII*

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Egress from the host cell is a crucial and highly regulated step in the biology of obligate intracellular parasites such as ***Plasmodium falciparum*** and ***Toxoplasma gondii***. We recently established that the Toxoplasma calcium dependent protein kinase 3 (TgCDPK3) is required for efficient egress. Interestingly, we showed that the closest homolog from ***P. falciparum***, PfCDPK1, complements the lack of TgCDPK3 in Toxoplasma and we have used this transgenic parasite strain to identify novel anti-malarials. To elucidate the mechanisms by which TgCDPK3 regulates Toxoplasma egress we have successfully implemented the proximity-based protein interaction trap BioID to identify potential substrates. For this purpose we fused TgCDPK3 to a mutant version of the biotin ligase, BirA*, which in the presence of biotin modifies any nearby or interacting protein. When parasites expressing TgCDPK3-BirA* are grown in the presence of biotin, 18 unique proteins appear to be biotinylated based on Western analysis. Through mass spectroscopic analysis we have determine the identity of these proteins, which include Myosin-A, a critical component of the parasite motor system. Interestingly, Myosin-A was among the 156 proteins shown to be less phosphorylated in Tgcdpk3 mutant strains in a phosphoproteome study. We subjected Myosin-A to a non-biased peptide array analysis using purified recombinant TgCDPK3 and found that TgCDPK3 can specifically phosphorylate S21, and S743 of Myosin-A ***in vitro***. When we complemented Myosin-A null mutants with Myosin-A in which either S21 or S743 is mutated to non-phosphorylable alanine, parasites showed an egress defect suggesting that phosphorylation of S21 and S743 of Myosin-A is important for egress ***in vivo***. Importantly, TgCDPK3 mutant parasites expressing Myosin-A in which serines 21 and 743 were changed to aspartic acid exhibited normal levels of iiegress indicating that phosphomimetic mutations in the motor protein overcome the lack of the TgCDPK3 kinase function. Thus, our studies establish that phosphorylation of Myosin-A by TgCDPK3 is responsible for initiation of motility and parasite egress from host-cell.

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IDENTIFICATION OF PARASITE-INDUCED HOST CELL TRANSFORMATION FACTORS BASED ON THE RE-ANNOTATION OF THE *THEILERIA PARVA* GENOME AND ON COMPARATIVE GENOMICS

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The discovery of mechanisms of virulence is critical for rational vaccine and chemotherapeutic innovation targeting apicomplexan parasites. The parasite ***Theileria parva*** has a unique mode of virulence among apicomplexans: it transforms host lymphocytes by inducing a variety of cancer-like phenotypes, including uncontrolled cellular proliferation. While comparative genomics can be leveraged to generate novel hypotheses of these mechanisms, it is highly dependent on the accurate gene structure annotation of the genome. Using the first RNA-seq dataset for the ***T. parva***, we re-annotated the parasite genome, improving the annotation of 48% of all protein-coding genes. Then, in order to identify potential virulence factors associated with host cell transformation, we used comparative genomics to identify genes, peptide domains, short linear motifs, or subcellular localizations specific to ***T. parva*** and ***T. annulata***, the latter also a host-transforming parasite, but absent in other apicomplexans including non-transforming ***Theileria*** species. We used the improved ***T. parva*** annotation as well as 55,520 publicly available protein sequences from complete proteomes of 12 apicomplexans, to generate jaccard-filtered clusters of orthologous genes. We identified hundreds of specific ***Theileria***-encoded factors potentially involved in host-cell transformation, which we are categorizing by gene ID, motif, domain, and description. In one such case, this search revealed 15 pairs of ***T. parva-T. annulata*** genes with a motif for binding host retinoblastoma protein (RB). RB is a tumor suppressor in mammalian cells and the target of some adenovirus proteins that induce host proliferation. Under homeostatic conditions, RB binds E2F transcription factors in the cytosol. Adenovirus protein E1A then uses two motifs to bind the RB pocket domain and compete off the E2F transcription factors, inducing host proliferation. In order to determine if the ***T. parva*** proteins with these motifs induce host proliferation in the same way, we are currently performing surface plasmon resonance experiments to test the binding of ***T. parva*** short peptides to the RB pocket domain.

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ULTRASTRUCTURAL AND BIOLOGICAL CORRELATES OF ATTENUATION IN INTRAHEPATIC *PLASMODIUM FALCIPARUM* PARASITES INDUCED BY GAMMA-IRRADIATION

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Radiation-attenuated malaria sporozoites, while capable of invading and growing inside hepatic cells, produce non-infectious merozoites that fail to cause blood stage infection. Interestingly, radiation-attenuated sporozoites are highly effective in inducing sterilizing immunity in experimental challenge studies including in humans. In spite of their vaccine value, the structural and antigenic changes in developing liver stage parasites following infection with radiation-attenuated sporozoites are poorly understood. We performed immunofluorescence and electron microscopy and genome-wide transcriptional profiling of untreated verses radiation-treated intrahepatic parasites on days 3 and 6 post-invasion of human HepG2 hepatic cells by ***Plasmodium falciparum*** sporozoites of the 3D7 strain to study the structural alterations and identify parasite gene targets of growth attenuation and enhanced immune protection induced by radiation. While the structural changes were subtle and difficult to decipher, we found that 180 parasite genes had significantly altered transcriptional expression in response to radiation on day 3 post-cultivation in hepatic cells. We observed a marked upregulation of 37 genes coding for proteins expressed on the cell surface or exported into the host cell; at least 4 membrane associated transporters were found to be upregulated upon radiation. We think that this radiation induced expression of surface and exported proteins might be a contributing factor to sterilizing immunity by increasing the number of parasite antigens available as processed epitopes on the hepatocyte surface or as released antigens from apoptosis of infected hepatocytes. At least 10 proteins related to protein misfolding and stress-related protein processing responses and 3 members of the FAST domain family that may be involved in RNA processing are induced. The molecules identified under this study may be novel targets for the generation of genetically attenuated sporozoite candidate vaccines and biomarkers of attenuation of radiation-attenuated malaria sporozoites.

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A NOVEL SECRETED PROTEIN FROM *PLASMODIUM FALCIPARUM* OOKINETES MEDIATES TRANSMISSION TO MOSQUITOES

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Interventions that interrupt the transmission of malaria will be critical in the renewed efforts to eliminate and eradicate this deadly disease. Transmission-blocking interventions (TBIs) that target sexual, sporogonic, or mosquito stages and inhibit ***Plasmodium*** parasite development within the vector will be essential in achieving this goal. Identifying novel proteins that are involved in the mosquito-parasite interactions that facilitate parasite transmission, and are conserved across ***Plasmodium*** species, may potentially provide new TBI targets. Here, we report on a conserved, hypothetical protein, designated PFE0360c, that is crucial for ***P. falciparum*** transmission. Other than a signal sequence and putative PEXEL, the 107 kDa protein was not predicted to contain any other known domains, but its expression was shown to be upregulated in both sporozoites and ookinetes. Genetic deletion of PFE0360c in ***P. falciparum*** NF54 and its ***P. berghei*** orthologue, PBANKA_110690, revealed a severe midgut invasion defect in ***Anopheles stephensi***, with significant reduction in the number of oocysts, and subsequently sporozoites, per mosquito as compared to wild type controls. This reduction in transmission was not found to result from a defect in ookinete development or motility, suggesting a role in invasion or traversal of mosquito midgut epithelial cells. Infection of C57BL/6 mice with ***P. berghei*** PBANKA_110690 deficient sporozoites revealed no defect in liver stage development, time to blood stage infection, or parasitemia as compared to controls. Although the PEXEL motif of PFE0360c was cleaved by plasmepsin V ***in vitro***, attempts to confirm its export have been unsuccessful. These findings provide new insight into our understanding of parasite-mosquito interactions and indicate a novel target for investigation of future transmission-blocking interventions.

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MOLECULAR DISSECTION OF *PLASMODIUM* SPOROZOITES AND KUPFFER CELL INTERACTIONS USING A PHAGE DISPLAY LIBRARY

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Malaria infection stars when an infected mosquito releases ***Plasmodium*** sporozoites into the skin of a person. Next, sporozoites enter a blood vessel and circulate through the whole body. To generate a productive infection, sporozoites must exit the circulation in the liver to subsequently infect hepatocytes. A large body of evidence suggests that sporozoites leave the circulation in the liver by preferentially traversing Kupffer cells, a macrophage-like component of the liver blood vessel (sinusoid) lining. The molecular basis for this preferential Kupffer cell traversal is unknown. By use of a phage display library we identified a peptide - P39 - which strongly binds to Kupffer cells and by doing so, inhibits sporozoite entry. We hypothesize that P39 competitively binds to a Kupffer cell receptor that is required for sporozoite entry. In agreement with this hypothesis, an anti-P39 antibody recognizes a sporozoite surface protein(s) and inhibits sporozoite mouse liver invasion. Moreover, we found that P39 binds to CD68, a Kupffer cell surface protein and putative sporozoite invasion receptor. CD68 knockout mice showed 70 % reduced sporozoite liver burden compared to wild type mice. Molecular dissection of the ***Plasmodium*** sporozoite-Kupffer cell interactions may provide insights for the development of novel malaria control strategies.

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A *PLASMODIUM* RHOPTRY PROTEIN IS INVOLVED IN SPOROZOITE INVASION OF HEPATOCYTES

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It has been demonstrated that some of ***Plasmodium*** rhoptry proteins are released to the tight junction and involved in merozoite invasion of erythrocytes. We recently proposed that at least 9 known rhoptry proteins are also expressed in spozozoites, salivary gland and hepatocyte invasive forms. To elucidate the functions of rhoptry proteins in sporozoites, we have developed the sporozoite stage specific gene silencing system and demonstrated that RON2 is important for sporozoite invasion of the salivary gland. Here, we intended to elucidate the comprehensive functions of all possible sporozoite rhoptry proteins, and found that one protein (RON) plays important roles in sporozoite transmission to mammalian hosts. Unlike RON2-cKD sporozoites, RON repressed sporozoites (RON-cKD) can invade the salivary gland normally. On the other hand, intravenous injection of RON-cKD sporozoites into mice showed a >100-fold reduction in the infectivity ***in vivo***, compared to control sporozoites. Efficiency of the liver infection of sporozoites was measured by real time PCR using the perfused livers 24h after sporozoite inoculation. It was demonstrated that RON-cKD liver stage parasite amount was approximately 500 times lower than control parasites, indicating that RON is required for the early step for parasite infection of mammalian hosts. Furthermore, ***in vitro*** infection system using hepatoma cell line, HepG2, revealed that that the invasion ability of RON-cKD sporozoites was reduced to 25% of control. These results demonstrate that RON is involved in sporozoite invasion of hepatocytes.

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INHIBITION OF RETICULOCYTE MATURATION TO INCREASE *PLASMODIUM* PROLIFERATION *IN VITRO*

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Human malaria is caused by five different species of ***Plasmodium*** parasites. Research has largely focused on ***P. falciparum*** because a robust ***in vitro*** culture system is available. However, ***P. vivax*** is more geographically widespread, causes a relapsing disease and transmission stages develop prior to clinical symptoms; making disease control extremely challenging. One of the major obstacles to the development of an ***in vitro*** culture is the restriction of ***P. vivax*** to reticulocytes. Reticulocytes rapidly mature to erythrocytes ***in vitro***. Consequently, a persistent replenishment of reticulocytes is essential for sustained parasite propagation, which effectively dilutes the culture and masks appreciable growth. We hypothesize that inhibition of reticulocyte maturation would increase the number of viable cells for invasion and would enhance ***Plasmodium*** proliferation overall. Reticulocyte maturation involves hallmark cellular processes: (i) membrane remodeling (including loss of specific surface receptors) and (ii) autophagy of organelles. We targeted these processes with a panel of small molecules. We were able to inhibit reticulocyte maturation as evidenced by decreased surface remodeling and decreased mitophagy. We determied IC_{50} and IC_{90} values of the small molecules for the reticulocyte, ***P. falciparum*** in normocytes, ***P.***

falcipcarum in reticulocytes and *P. knowlesi* H in Rhesus normocytes to determine the top candidates. The top candidates had low IC_{90}s in the reticulocyte and relatively high IC_{50}s in the parasite, i.e. a low concentration is needed to inhibit reticulocyte maturation without harming the parasite. Significantly, the top candidates were shown to enhance the adaptation of the macquae parasite, ***P. knowlesi,*** to growth in human reticulocytes. The top candidates are currently being tested on clinical isolates of ***P. vivax*** to determine their promise in the development of a sustained and robust ***in vitro*** culture.

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ECONOMIC COSTS AND BENEFITS OF MORBIDITY MANAGEMENT AND DISABILITY PREVENTION FOR LYMPHATIC FILARIASIS

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Lymphatic filariasis (LF) is endemic in 73 countries, with 120 million people infected of whom 40 million suffer serious disability (15 million with lymphedema, 25 million with hydrocele). Repeated acute attacks of fever and disabling pain (adenolymphangitis or ADL) aggravate lymphedema and prevent work for 4-7 days per attack. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has two goals: interrupting LF transmission by 2020 and caring for people already infected through morbidity management and disability prevention (MMDP). By 2014, 60 countries had ongoing mass drug administration, but only 27 had begun MMDP, in part due to its perceived high cost and low return. Simple, low-cost interventions at the community level, including instruction in limb washing and provision of soap, topical antibiotics, and antifungals can reduce ADL and slow progression of lymphedema. MMDP programs attenuate disability and productivity loss. Research to date suggests societal cost of untreated LF far exceeds cost of MMDP. In India, patients enrolled in community-based care (CBC) MMDP averaged 29 fewer lost work days per year than prior to enrolment. We estimate costs and benefits of MMDP over the lifetime of the population affected and at risk in Odisha State. Actual program costs are based on CBC delivered to 21,497 people in 2008. In the Odisha CBC-MMDP program, present value of start-up costs plus annual maintenance costs, estimated to grow 5% p.a. (discounted at 3%), is US$97 per person for the 25-year program. We calculate the potential benefit of MMDP by comparing direct and indirect costs of two scenarios, with and without MMDP. Without MMDP, per-patient cost of untreated morbidity due to acute attacks and chronic lymphedema, including lost earnings and out-of-pocket medical costs over 50 years, is US$1748. With MMDP, the lifetime per-person cost is US$828, or US$920 less. The benefit of savings in direct costs and productivity loss of $920 per person compares favorably with a lifetime program cost of $97 per person (benefit-cost ratio of 9.5) and provides an economic rationale in addition to the ethical mandate for MMDP, the second pillar of GPELF.

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COMBINING NUTRITIONAL SUPPLEMENTATION WITH SEASONAL MALARIA CHEMOPREVENTION IN NIGERIA DECREASES THE ODDS OF CONFIRMED CLINICAL MALARIA: FINDINGS FROM A CASE-CONTROL STUDY

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In 2012, the World Health Organization recommended seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine (SP-AQ) to prevent malaria in children in the Sahel region. As malnourished children are twice as likely to die from malaria, the delivery of SP-AQ was combined with Lipid-based Nutrient Supplements (LNS) for children aged 6-24 months in Kano state in northern Nigeria during the 2014 malaria season. The commodities were delivered door-to-door to approximately 4,000 children each month from August to November. We conducted an unmatched case-control study to assess the impact of combining SP-AQ and LNS on uncomplicated and severe malaria. All children aged 6-24 months consulting for fever in one of the 13 primary health care facilities in the pilot study area between August 28 and December 1, 2014 were recruited. A case was defined as any child with a fever ≥37.5 C within the past 48 hours and a positive malaria rapid diagnostic test. Controls had fever but a negative malaria test. Information was collected on demographics (age, gender, residence), disease history, bednet use, and intervention coverage. The relationship between exposure to SP-AQ and LNS and confirmed malaria was estimated using multivariable logistic regression, adjusting for demographics, facility, month of consultation, and bednet use. A total of 96 cases and 285 controls were recruited. Cases had on average half the odds of controls of having taken SP-AQ in the last 30 days, but the result was not statistically significant (OR= 0.47; 95% CI: 0.18-1.23, p=0.13). Cases had one-fifth the odds of having taken both SP-AQ and LNS (OR=0.17; 95% CI: 0.05-0.51, p=0.002). Receiving both SP-AQ and LNS was associated with significantly lower odds of confirmed malaria compared to receiving SP-AQ alone (p=0.02). Adding LNS to the delivery of SP-AQ during an SMC campaign was associated with decreased odds of malaria in children visiting health facilities. The absence of statistical significance of SP-AQ alone may be due to the small sample size. Larger studies are needed to confirm these findings and estimate the cost-effectiveness in this and other settings.

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INEQUALITIES IN CHILD SURVIVAL IN A RURAL AREA OF SENEGAL WHERE MALARIA HAS DECLINED

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A sharp decline in under-5 mortality rates has been observed in Senegal, as in some other parts of Africa, over the last 10 years, associated most obviously with a reduction in malaria transmission that occurred in the same time period. Since 2008 we have maintained demographic surveillance in 725 villages in central Senegal with a population of about 600,000 people. We have analyzed variations in under-5 mortality in order to understand the reasons for the recent decline, and the extent of inequalities and the factors associated with them. Malaria incidence was

determined by collecting data from all health facilities and from villages with community case management for malaria, details of all malaria cases confirmed by Rapid Diagnostic Test. Household occupancy, births and deaths were recorded in household visits every ten months. In addition, in 123 villages, village reporters were used to cross-check completeness of reporting of deaths, and in these villages verbal autopsies were performed on all deaths in children. Data for 95,000 children under 5 years of age have been included. Random effects Poisson regression was used to assess area, household and individual risk factors for child mortality. The leading causes of death were diarrhoea (26.6%), perinatal infection (22.2%) and malaria (10.8%). Children living more than 5km from a health facility had a 2-fold (95%CI 1.6, 2.5) higher risk of mortality than those living nearer to health facilities. Mortality rates were associated with the level of malaria transmission, an increase in the malaria incidence rate in children of 1 per 1000 per year was associated with a mortality rate ratio of 1.04 (95%CI 1.02, 1.05). Children born to mothers under 16 years of age had a 1.6-fold increased risk of mortality (95%CI 1.1, 2.3) compared to children of older mothers. Senegal is close to achieving its MDG4 target, but national average figures conceal substantial inequalities. Higher rates of mortality are associated with areas of persisting malaria transmission, poverty, and poorer access to health care. Strategies targeting these communities are required to improve child survival and reduce inequalities.

1303

EVALUATION OF THE NATIONAL CASE-BASED MEASLES SURVEILLANCE SYSTEM IN SOUTH AFRICA; JANUARY 2009 TO DECEMBER 2013

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Measles still remains the leading cause of death among children under five years worldwide. In South Africa the case-based measles surveillance was initiated in 1998 in line with the recommendation from World Health Organization (WHO) to monitor progress towards measles elimination. Blood specimens are collected from suspected measles cases in all health facilities. This study described the epidemiology of measles in South Africa from 2009-2013 and evaluated specific performance indicators for measles surveillance recommended by WHO.A retrospective descriptive analysis was conducted on secondary measles surveillance data collected from 2009-2013 in all age groups. The blood samples were tested at the measles serology laboratory at the NICD. The percentage for each of the indicators was calculated and compared with the WHO minimum standard values for measles surveillance. In 2009-2011, there were 18,000 confirmed measles cases reported, which included cases identified during the measles outbreak from June 2009 to July 2010 in all nine provinces. The number of cases decreased to 335 between 2011-2013. The age groups most affected were those >14years, while the least affected were those between the ages of 2-4 years. Out of the seven performance indicators evaluated, five met WHO specified targets, including all districts reporting at least one measles case per year (WHO target ≥ 80% of the districts) and blood specimens collected in more than 80%of the suspected measles cases (WHO target of ≥ 80%).Two laboratory indicators did not meet the WHO targets; between 2009 and 2013, 32%, 28%, 46%, 49% and 52% of blood specimens respectively reached the laboratory within three days of being sent (WHO target ≥ 80%) and between 2009 to 2013,14%,15%,65%, 68% and 66% of blood samples respectively were tested within seven days (WHO target≥ 80%). The case-based measles surveillance met most of WHO-specified performance indicators. Addressing timeliness of specimen delivery to the laboratory, timeliness of laboratory testing and subsequent feedback of the results to the health facility will ensure proper management of cases and rapid response to outbreaks.

1304

ECONOMIC ANALYSIS OF GENETICALLY MODIFIED MOSQUITO STRATEGIES TO CONTROL DENGUE

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Vector control is currently the only prevention strategy to reduce dengue virus (DENV) transmission. Traditional vector control methods have not halted the spread of ***Aedes aegypti***, the main dengue vector, and of dengue outbreaks. Despite millions of dollars spent annually in vector control, there are few effectiveness or economic evaluations to inform policy decisions. High costs and seemingly low effectiveness have encouraged the search for new vector control technologies. While still under development, genetically modified (GM) mosquitoes are a potentially promising approach to control dengue. GM mosquitoes have been developed with the goal of producing mosquito offspring that die at an early developmental stage. We develop a framework and present a preliminary application to assess the economic impact of this technology. Combining data from various dengue-endemic countries, we generated a mathematical model of costs and benefits and calibrated it with available evidence. We assumed a mosquito suppression phase of 1 year followed by a maintenance phase of 4 years, with annual costs per capita of $25-$75 and $10-$20, respectively. Combining the highest cost and most favorable effectiveness in dengue control (50% suppression and 100% maintenance), we obtained a benefit/cost ratio of 1.1 in an area with high DENV transmission, ongoing intensive vector programs, and high income. Under these same assumptions, if the technology were applied in areas with dengue incidence, vector control programs, and income similar to Brazil, the cost-effectiveness of the program would range from $30,000 to $163,000 per disability adjusted life year (DALY). In a lower incidence area like Mexico, the cost-effectiveness would range from $180,000 to $457,000 per DALY. Without major cost reductions, GM vector control is unlikely to be cost-effective. The most salient need for more data is clarifying the relation between vector population and DENV transmission. If effective, new and improved technologies of vector control could save billions of dollars annually in averted medical costs, productivity losses, and premature deaths from dengue.

1305

ASSESSING THE IMPACT OF HOUSEHOLD AIR POLLUTION ON HEALTH: FEASIBILITY OF AMBULATORY BLOOD PRESSURE MONITORING AND REPEAT-ASSESSMENT "HOME" BLOOD PRESSURE MONITORING IN A RURAL GHANAIAN SETTING

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Over half the global disease burden of Household Air Pollution (HAP) from biomass burning is attributable to cardiovascular disease (CVD), yet few studies have examined HAP in relation to CVD risk factors and the feasibility of collecting out-of-clinic blood pressure (BP) assessments, especially in rural settings. In a pilot study, we assessed the feasibility of collecting 24-hr Ambulatory Blood Pressure (ABP) and home-based blood pressure assessments to evaluate BP before and after delivery of a cleaner cookstove intervention to a subset of participants in Kintampo, Ghana as part of the Ghana Randomized Air Pollution and Health Study (GRAPHS). Two BP methods were tested. In the ABP group, 24-hour ABP monitoring was conducted with SpaceLabs 90207 monitors (Snoqualmie, WA). BP was recorded every 20 minutes (daytime) and 30 minutes (nighttime). In

the Home BP group, trained field workers measured BP twice daily for 5 consecutive days using Omron (Lake Forest, IL) digital automatic BP monitors. Enrollment: 44 women (27 in the ABP group; 17 in the Home BP group). 53 BP recordings out of an expected 54 were completed in the ABP group; and 33 out of 34 in the Home BP group. According to the validity criteria of the International Database of Ambulatory Blood Pressure in relation to Cardiovascular Outcome (IDACO) project, 89% of the completed ABP sessions were valid. Within valid ABP sessions, the mean [SD] number of BP readings was 51.7 [11.8] (daytime: 30.1 [9.4]; nighttime: 20.9 [4.1]). Within completed Home BP sessions, 99% of targeted measurements were obtained, with a mean [SD] number of readings per 5-day session of 9.9 [0.3] (mornings: 4.9 [0.2]; evenings: 4.9 [0.2]). Mean [SD] BP levels were as follows. ABP group: SBP 107 [6.4]; DBP 62.6 [4.8]. Home BP group: SBP 100 [8.6], DBP 65.5 [5.5]. These results indicate that both 24-hour ABP monitoring and repeat-assessment "Home" BP monitoring are well-tolerated and feasible methods of BP assessment in this predominately rural West African setting. Results with regard to the hypothesis that switching to a cleaner cookstove is associated with a reduction in post-intervention BP will be available in 3-4 months.

1306

A DOUBLE BURDEN OF CHILD MALNUTRITION IN COMMUNITIES WITH DIFFERENTIAL ACCESS TO ROADS: ANALYSIS IN RURAL ECUADOR, 2004-2013

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Nutritional trends towards both over and under nutrition are occurring globally throughout low and middle-income countries. Access to roads can influence both protective and risk factors associated with nutrition by affecting a number of social and biological processes. In northern coastal Ecuador the construction of a new road created a gradient of remoteness among villages, providing a unique opportunity to examine the impact of roads on the double burden of over and under nutrition. Within the study, anthropometric and hemoglobin measurements were collected on 2,369 children < 5 years in Esmeraldas, Ecuador from 2004-2013 across 24 villages with differing road access. Logistic general estimating equations assessed the relationship between village remoteness and prevalence of stunting, wasting, underweight, overweight, obesity, and anemia, accounting for longitudinal dependencies. Race was tested as a modifier of the remoteness-malnutrition relationship. Overall prevalence of stunting was 13%, underweight 6%, wasting 5.7%, overweight 5.6%, obesity 1.9%, and anemia 55%. Stunting, while still high, decreased as obesity increased over time and other nutritional outcomes remained stable. Remoteness relative to a road was found to be significantly associated with stunting (OR=0.46, 95% CI=[0.31, 0.68]) and anemia (OR = 0.53, 95% CI=[0.41, 0.68]). Over time, greater remoteness becomes less associated with lower stunting (OR=0.36, 95% CI=[0.20, 0.66] early versus OR=0.57, 95% CI=[0.35, 0.94] late). Indigenous Chachi children were found to have a higher prevalence of all malnutrition outcomes compared to Afro-Ecuadorian and Mestizo children. These results suggest an emerging double burden of child malnutrition in the study site, which is likely is the result of how changes in remoteness influence food availability and consumption. Within rural areas, heterogeneous nutritional outcomes are observed; however, as road development continues, this heterogeneity diminishes. This suggests that the influence of roads on health outcomes, such as nutrition, unfold over multiple time scales.

1307

GENOME ANALYSES REVEAL INSIGHTS INTO THE UNIQUE PARASITIC LIFESTYLE OF THE LYME DISEASE TICK, *IXODES SCAPULARIS*

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Ticks (subphylum Chelicerata: subclass Acari; suborder Ixodida) are notorious ectoparasites and the most important arthropods affecting human and animal health globally. We describe the 2.1 Gbp nuclear genome of the tick, ***Ixodes scapularis*** (Say), the vector of the pathogens that cause Lyme disease, granulocytic anaplasmosis, and babesiosis. The genome sequence, the first for a medically important chelicerate, offers insights into the molecular processes that underpin the remarkable parasitic life-style of the tick and its success as a vector. The large genome reflects extensive accumulation of repetitive DNA, including multiple families of low complexity tandem repeats, new lineages of retro-transposons, and gene architecture patterns resembling ancient eukaryotes rather than pancrustaceans. Annotation revealed 20,486 protein-coding genes and expansions of gene families associated with tick-host interactions. Genome analyses informed on parasitic processes unique to ticks, including host "questing", prolonged feeding, blood meal concentration, novel methods of hemoglobin digestion, heme detoxification, reproduction and prolonged survival off-host. Proteins associated with transmission of anaplasmosis, an emerging disease, and the encephalitis causing Langat virus, were identified. Analyses of ~ 34,000 SNP markers identified from individual ***I. scapularis*** using the Restriction-site Associated DNA sequencing (RADseq) approach support a single species classification for the tick across North America and revealed a North-South population structure correlated to life-history traits and Lyme disease transmission. Foundational studies of genome organization and population genomics, coupled with the development of genomics resources, will advance research to determine the genetic basis of tick phenotypes. Efforts are also underway to discover novel chemistries that selectively disrupt molecular targets identified from the genome and deliver new, selective interventions for the control of ticks and tick-borne diseases.

1308

DEVELOPMENT AND IMPLEMENTATION OF ODORANT BAITED TRAPS TO MONITOR AND CONTROL *TRIATOMA DIMIDIATA* IN TOLEDO, BELIZE

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Triatoma dimidiata (Hemiptera: Reduviidae:) is one of the three most epidemiologically significant vectors for Chagas' disease in the Americas. Although Chagas' disease has been eliminated in many regions from the domestic habitats, there are emerging threats of re-infestation by sylvatic species. ***T. dimidiata*** inhabits domestic, peri-domestic, and sylvatic niches, thus posing a serious threat to the continued interruption in Chagas transmission. This study aimed to develop a novel odor baited trap (OBT) employing odors derived from hosts and natural habitats to monitor ***T. dimidiata*** populations in Toledo, Belize. Odorants were isolated using electrophysiological methods linked to gas chromatography and identified by mass spectrometry. The identified chemostimuli were tested under different regimes to determine their behavioral effects. The odorants or blends that induced attraction were incorporated into OBTs that were tested in the field. Captures from the OBTs were compared against manual searches in the Toledo district of Belize. Verbal questionnaires were utilized to determine end user acceptability of trap design as well as identify gaps in knowledge regarding vectors and Chagas transmission. Our efforts are

aimed at providing recommendations to the Ministry of Health (MOH) regarding the utility of OBTs and development of educational material targeted towards Triatomine surveillance and vector control.

1309

RHODNIUS PROLIXUS SURVIVAL WHEN INFECTED WITH *TRYPANOSOMA CRUZI* AND *T. RANGELI*: THE EFFECT OF INFECTIVE DOSE AND CO-INFECTION

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All parasite-host interactions are not created equal; they begin under an array of possible conditions. This is especially true for interactions between Chagas disease agent ***Trypanosoma cruzi*** and its triatomine bug vectors. ***T. cruzi*** infects 100s of mammal species, all of which present different parasitemias and disease courses, resulting in the triatomine bug vectors receiving highly variable infective doses from the different host species they feed upon. Additionally, in many areas ***T. cruzi*** circulates with a sister parasite species called ***T. rangeli***. ***T. rangeli*** is of interest, as it presents the opposite pathogenicity as ***T. cruzi***, being pathogenic to triatomine bugs, but apathogenic to its mammal hosts. Thus, all ***T. cruzi*** infections of its vector begin not only with varying infectious doses but also various co-infection statuses. However, does this variation matter for Chagas disease transmission? We investigated this question in the triatomine bug species ***Rhodnius prolixus*** when infected with ***T. cruzi*** and ***T. rangeli***, both in single and co-infections. We measured the effect of ***T. rangeli*** infective dose, ***T. cruzi-T. rangeli*** co-infection order (***T. cruzi*** first vs. ***T. rangeli*** first), and co-infection timing (simultaneous vs. staggered) on insect survival in the 100 days post-infection. We observed that insects almost always had higher survival when co-infected at lower ***T. rangeli*** doses, regardless of infection order or timing, suggesting that ***T. cruzi***-T. rangeli co-infection is beneficial to the insects at low ***T. rangeli*** doses. At high ***T. rangeli*** doses, we found that co-infected insects had decreased survival, unless they were infected with ***T. cruzi*** first, suggesting that infective dose, order and timing interact in their effect on ***R. prolixus*** survival. Our results indicate that vector-borne ***T. cruzi*** transmission dynamics may be influenced by the parasite load and parasite species composition of the blood meal taken up by the vector, and these factors may be important to consider when assessing local ***T. cruzi*** transmission and designing vector control strategies.

1310

RAPID AND NON-DESTRUCTIVE NEAR-INFRARED SPECTROSCOPY CAN PREDICT THE AGE AND *WOLBACHIA* INFECTION IN *AEDES AEGYPTI* MOSQUITOES

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Regular monitoring of infection and survival rates of the Wolbachia based vector control strategy is a prerequisite for its sustainability. Currently, molecular techniques such as polymerase chain reaction (PCR) are employed to detect Wolbachia infections in mosquitoes whereas the use of transcriptional profiles is used to monitor their survival. However, these techniques are not amenable to a large scale intervention. We have investigated the potential of using a non-destructive and instantaneous near-infrared spectroscopy (NIRS) as a high throughput technique for detecting the age and the presence or absence of two strains of Wolbachia infection in male and female laboratory reared ***Aedes aegypti*** mosquitoes. Calibrations were developed using spectra collected from heads and thoraces of laboratory reared mosquitoes using partial least squares (PLS) regression and were used to predict the age and infection of samples that were excluded from the calibration model. A highly significant correlation was found between the true and predicted ages of mosquitoes. The correlation coefficients for ***Ae. aegypti*** females and ***Ae. aegypti*** males were R2=0.83 and 0.78 respectively (P< 0.001 in both instances). The age of female and male ***Ae. aegypti*** could be identified as < or ≥ 8 d old with an accuracy of 91% and 76%, respectively. The mean predicted age was generally within ±3 d of the actual age. NIRS differentiated females and males infected with wMelPop from uninfected samples with an accuracy of 97% and 93%, respectively and females and males infected with wMel from uninfected samples with accuracies of 93% and 88%, respectively. Moreover, NIRS differentiated females infected with wMelPop from wMel infected females with 98% accuracy. This non-destructive technique is at least 35 times faster and over 10 times cheaper than conventional age prediction and polymerase chain reaction techniques. While robust calibrations using mosquitoes reared from different geographical regions are required to validate NIRS as an age/infection prediction tool for wild caught ***Ae. aegypti***, our results strongly indicate the potential of the NIRS for this purpose.

1311

IDENTIFYING OVIPOSITION ATTRACTANTS FROM THE LARVAL REARING MEDIUM OF *PHLEBOTOMUS PAPATASI*, THE VECTOR OF OLD-WORLD ZOONOTIC CUTANEOUS LEISHMANIASIS

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Phlebotomine sandflies are the vectors of the protozoan parasites of the genus ***Leishmania***. The control of sand flies is currently limited to personal protection and residual spraying. An attract-and-kill approach has been shown to be effective in a variety of other vector by which an attractive blend is used to attract the insect to a lethal trap. Targeting control efforts on gravid females could affect disease transmission by reducing the abundance of blood-fed females and by suppressing the vector's population growth rate. The study presented here describes results from a multidisciplinary project in which we applied a systematic integrated approach to elucidate the cues that drive the oviposition behavior of the sand fly ***Phlebotomus papatasi***. Since sand fly larvae feed on decomposing organic material of predominantly fecal origin, we hypothesized that gravid female sand flies would be attracted to volatile olfactory cues indicating the presence of such material. We have used a multiple-choice oviposition bioassay in which gravid ***P. papatasi*** females were presented with five candidate source materials from their larval rearing habitat on filter paper disks placed on a layer of plaster at the bottom of oviposition cups. To evaluate whether the total oviposition response measured in the oviposition cup bioassays also indicates attraction, we have conducted Olfactometer bioassays using a 3-chamber linear Olfactometer in which we tested all five candidate materials. Our results from the behavioral bioassays and the Olfactometer experiments suggest that the total oviposition response and highest attractance occurred when the treatment was frass from the 2nd-3rd instar larval medium. We have cultured the 2nd-3rd instar larval material and isolated its constituent bacterial community. Bacterial isolates were tested individually and in mixture in olfactometer bioassays for behavioral evaluation. Several candidate bacterial strains have demonstrated high attractance at specific concentrations, which indicates potential applications in constructing the optimal attractive bait.

1312

MOSQUITO HEARING: OPENING THE DOOR TO A NEW MOSQUITO SENSORY MODALITY

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For over a century, vector biologists have focused on sensory ecology of mosquito sensation as they relate to host finding such as olfaction and heat seeking, which confirms the primacy of these modalities. However acoustic sensation has been largely ignored. Recent work by our lab and others has demonstrated the important role of acoustics in the pre-copulatory mating interaction. Here we present new neurophysiological and behavior findings on the acoustic sensitivity of male mosquitoes that demonstrate their ability to hear sounds in the far auditory field at lower intensity and over greater distances than ever imagined. Collectively, this work opens the possibility for hearing as a significant modality in the mosquito sensory world.

1313

HOW DO BITING DISEASE VECTORS FUNCTIONALLY RESPOND TO HOST AVAILABILITY?

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Advanced molecular methods in biological fingerprinting are uncovering new opportunities in disease vector incrimination and behavioral ecology through the rapid and sensitive analysis of arthropod bloodmeals. However, for this potential to be realized, the interpretation of the species composition of vector meals must be guided by sound ecological theory. Even modern, complex simulation models of vector-borne diseases assume a simplistic, linear relationship between host species blood source and host availability. In ecological terminology, this constitutes a Type I Holling's functional response - a response type that is atypical of invertebrates in relation to their resource consumption. A new model is proposed that expands upon the classic functional responses to accommodate more qualitatively distinct disease vector behaviors. The substantial consequences of non-linear responses of biting disease vectors to human host availability are described for pathogens with three distinct transmission routes: falciparum malaria (strict anthroponotic competence), Chagas disease (anthroponotic and zoonotic competence) and Lyme disease (strict zoonotic competence).

1314

TARGETING *TRYPANOSOMA BRUCEI* METHIONYL-TRNA SYNTHETASE FOR NOVEL TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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Human African trypanosomiasis (HAT) threatens populations in 36 countries of sub-Saharan Africa. New drugs with improved safety, efficacy, and ease of use are needed to replace the antiquated drugs that are currently available. A validated drug target in ***Trypanosoma brucei*** (the etiologic agent of HAT) is the methionyl-tRNA synthetase (MetRS). Inhibitors of this target were identified with potency as low as 4 nM (EC50) on bloodstream trypanosomes, but lacked the necessary permeability properties for oral uptake and brain permeability, the latter necessary for treating late-stage HAT. Guided by structure-based drug design, over 500 analogs to the original aminoquinolone scaffold were synthesized including a new series of fluorinated imidazo-pyridines and additional variants. The new compounds demonstrate potent inhibitory activity against ***T. brucei*** MetRS (IC50s <2 nM), ***T. brucei*** EC50s as low as 0.4 nM, improved blood-brain barrier permeability, and good oral bioavailability (88% in rats). A compound in the optimization pathway cures an acute ***T. brucei*** rhodesiense mouse model with an oral dose as low as 8 mg/kg (>20 days parasite free) and partially cures chronic ***T. brucei*** mouse model (>70 days parasite free). These studies serve as proof of concept that methionyl-tRNA synthetase inhibitors are viable drug candidates for HAT.

1315

RNAI OF *TRYPANOSOMA BRUCEI* CYTOSOLIC HSP83 AND MITOCHONDRIAL TRAP-1 REVEALS ROLES FOR THESE HSP90 HOMOLOGUES IN CYTOKINESIS AND KINETOPLAST DIVISION

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17-AAG, an Hsp90 inhibitor, has potent activity against ***Trypanosoma brucei***. We found 17-AAG causes rapid and severe cell cycle defects, including delayed cleavage furrow ingression and delayed kinetoplast segregation. To compare the cell cycle effects of 17-AAG treatment with knock-down of Hsp83, the trypanosomal Hsp90, or TRAP-1, the mitochondrial Hsp90, we generated separate RNA interference (RNAi) cell lines with a tetracycline inducible stem-loop hairpin construct for Hsp83 and tbTRAP-1. For Hsp83 RNAi nine independent clones were obtained, and all displayed a severe growth defect by 24 h after addition of tetracycline (tet). Northern blot analysis demonstrated a decrease in Hsp83 RNA levels, and western blot analysis with anti-hHsp90 antibody showed a decrease in an 83kDa band, the expected size of Hsp83. Microscopy examination of the tet-treated cells revealed an accumulation of cells containing two nuclei and partially ingressed cleavage furrows, a cytokinesis defect. For tbTRAP-1 RNAi, ten independent clones were obtained, and all displayed normal growth until 62 h after addition of tet, when growth stopped and populations began to decline. Northern blot analysis demonstrated a loss of tbTRAP-1 RNA. Cell cycle analysis by microscopy revealed a loss of cells with two kinetoplasts from 62 h onwards, and an increase in cells with no kinetoplasts. Southern blot analysis of kinetoplast free-minicircle DNA revealed defects in minicircle replication, with the appearance of abnormal catenated forms. This work provides genetic evidence for functions of Hsp83 and TRAP-1 in cytokinesis and kinetoplast replication respectively. The cell cycle defects seen with 17-AAG are a combination of those seen by RNAi of Hsp83 and TRAP-1, suggesting 17-AAG may be inhibiting both proteins in ***T. brucei***, consistent with its specificity for the unique ATP binding pocket of Hsp90s. Overall we have confirmed that ***T. brucei*** Hsp83 is essential and a promising drug target.

1316

COMPARATIVE GENOMIC ANALYSIS BETWEEN *LEISHMANIA (VIANNIA) PERUVIANA* AND *LEISHMANIA (V.) BRAZILIENSIS*

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The ***Leishmania (Viannia) braziliensis*** complex is responsible of many cases of New World tegumentary leishmaniasis. This complex includes two closely related species, ***Leishmania (Viannia) peruviana*** (***LVP***) and ***L. (V.) braziliensis*** (***LVB***), that present different geographic distribution and disease phenotypes including the risk of mucocutaneous disease. However, the genetic basis of these differences is not well understood and the status of ***LVP*** as distinct species has been questioned by some. We

sequenced the genomes of two ***LVP*** isolates (LEM-1537 and PAB-4377) using Illumina high throughput sequencing at approximately 40x coverage. ***LVP*** is sufficiently close to ***LVB*** that we were able to map more than 90% ***LVP*** reads on the ***LVB*** M2904 reference genome. Comparative analyses were focused on the detection of Single Nucleotide Polymorphisms (SNPs), insertions and deletions (INDELs), aneuploidy and gene copy number variations. We found 92,698 SNPs between ***LVP*** and ***LVB*** M2904 reference genome while only 26,853 variants were detected between the two ***LVPs***. Analysis in coding sequences revealed 26,750 SNPs and 1,513 INDELS in both ***LVP*** isolates and two ***LVB*** pseudogenes that appear to have coding function in ***LVP***. Allele frequency profiling showed a highly heterogeneous pattern of chromosome ploidies with an overall disomic tendency in both ***LVP*** isolates in contrast with a trisomic pattern in the ***LVB*** M2904 reference. Read depth analysis allowed us to detect more than 368 gene expansions and 14 expanded gene arrays in ***LVP*** that are not present in ***LVB***. The presence of extensive variation in chromosome and gene copy numbers as well as high SNPs and INDELs between ***LVP*** and ***LVB*** support maintaining their classification as distinct species. The absence of expanded amastin gene arrays in ***LVP***, the presence of different genes in array expansions in ***LVP*** and extensive polymorphism in coding sequences could result in different expression profiles potentially influencing parasite development and host interactions possibly related to its differences in phenotype versus ***LVB***.

1317

IMPLICATION OF APOL1 EXPRESSION AND CODING POLYMORPHISMS IN DETERMINING RESISTANCE/ SUSCEPTIBILITY TO *TRYPANOSOMA BRUCEI GAMBIENSE* INFECTION IN GUINEA

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Most African trypanosome species are sensitive to trypanolytic factors present in human serum. Trypanosome lysis was demonstrated to be associated with apolipoprotein L-1 (APOL1). ***Trypanosoma brucei*** gambiense (***T. b. gambiense***) and ***T. b. rhodesiense*** have evolved distinct mechanisms to escape lysis from APOL1 and are able to infect humans. In T. b. gambiense, resistance seems to be in part controlled by a reduced capacity to internalize APOL1, thus limiting the lytic effects. Interestingly, APOL1 variants (G1 and G2) were shown to be associated with the development of kidney disease in African Americans and it was suggested that they may have been positively selected in African population because they provide some degree of protection against human African trypanosomiasis. In this study led in Guinea, the most affected country in West Africa, we have studied APOL1 expression levels as well as APOL1 coding sequence genetic variant in (i) unaffected endemic controls, (ii) HAT patients and (iii) seropositive subjects that are apparently able to control infection. We show that APOL1 expression appears to be induced by T. b. gambiense infection (in both seropositive and HAT patients) and that significant genetic association (with G1 and G2 but not others) were observed in seropositive subjects but not in HAT patients when compared to controls. These results suggest that these APOL1 polymorphisms may indeed provide a certain degree of resistance against T. b. gambiense infections but protection is far from complete.

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GENETIC DIVERSITY IN THE NATURAL *TRYPANOSOMA CONGOLENSE* POPULATION

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Trypanosoma congolense is one of the major pathogens responsible for animal African Trypanosomiasis (AAT), a disease affecting about 10 million km^2 of the sub-Saharan region on the African continent. Isometamidium Chloride (ISM) is the principal drug used to counteract ***T. congolense*** infection in livestock, both as a prophylactic as well as curative treatment. Here, numerous cases of ISM resistance in different African regions have been reported, representing a serious problem in the battle against AAT. An important step in the fight against AAT is to monitor the level and distribution of genetic diversity in ***T. congolense*** populations, to reveal the biologically relevant structural variations present in their genomes that influence pathogenesis, virulence, and mechanisms leading to drug resistance. Using Next-Generation Sequencing technology, we sequenced 54 ISM-resistant and -sensitive field-isolated strains sampled between 1971 and 2010 in 9 countries where the disease is endemic. A total of 614859 SNPs were identified and long deletions were detected in both coding and non-coding regions, revealing a high genetic diversity. Interestingly, analyses of linkage disequilibrium decay suggested that genetic recombination and complex genetic exchanges are frequent between ***T. congolense*** parasites. Additional phylogenetic and structure analysis highlighted a geographically asymmetric distribution of genetic diversity across Africa. Parasites collected within a short period of time in Zambia showed a higher diversity compared to the strains sampled over a period of 30 years in the other countries. The factors resulting in this asymmetric diversity remain speculative but we suggest that the close proximity to wildlife observed in this region as well as the presence of specific tsetse fly vectors are playing a key role. This last hypothesis is currently being tested by studying the capability of several tsetse species to develop a mature ***T. congolense*** infection.

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TRACHOMA ELIMINATION IN AN ENDEMIC ISLAND SETTING IN WEST AFRICA: ARE TWO DOSES OF ORAL AZITHROMYCIN BETTER THAN ONE?

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Trachoma is caused by ocular ***Chlamydia trachomatis*** (Ct) infection. The remote Bijagós Archipelago is trachoma-endemic. The WHO recommends community mass drug administration (MDA) of azithromycin if follicular trachoma (TF) prevalence in 1-9 year olds > 10% to achieve elimination (TF<5%). Single dose oral azithromycin MDA has significantly reduced TF prevalence and in some populations eliminated Ct infection. In trachoma-endemic communities, despite high coverage MDA, infection and disease persist. Optimal MDA frequency is unknown and may vary between settings. We evaluated the efficacy of single versus dual dose azithromycin MDA in this setting to evaluate options for effective trachoma control on the islands. A census of all 1-9 year olds was conducted in all communities on 3 study islands. Each of 39 settlements was randomized to a study

arm. MDA was given to all community members. Arm 1 received the 'intervention' (single observed height-based dose of oral azithromycin on day 1 and day 7) and Arm 2 was the control arm (single observed height-based dose of oral azithromycin on day 1 (WHO policy)). Study teams and participants were masked to trial arm assignment until after the first dose of treatment. Prior to MDA, all enrolled children were examined for TF and a conjunctival swab was taken for Ct DNA PCR. The census was updated at one year and children aged 1-9 years were re-examined for TF and Ct infection. 1714 1-9 year olds were enrolled into the study at baseline. 1482 were examined and had conjunctival swabs taken. TF prevalence was 10% (147/1482)(95% CI 8.5-11.5%). Ct infection prevalence was 13% (193/1482). At one year, 1270 1-9 year olds were followed up. 1152 had repeat examinations and swabs taken. TF prevalence was reduced to 2.3% (27/1152) (95% CI 1.43-3.17%). Preliminary cluster-summarized TF prevalence at follow-up suggests a difference between treatment arms. We will present cluster-summarised mean TF and infection prevalence between study arms. Results from this study may inform trachoma elimination activities on these islands, where infrastructure is lacking and trachoma is prevalent.

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CAN ONE ROUND OF MASS DRUG ADMINISTRATION WITH AZITHROMYCIN IN A HYPOENDEMIC DISTRICT REDUCE THE PREVALENCE OF TRACHOMA TO LESS THAN 5%? RESULTS FROM THREE DISTRICTS IN UGANDA

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The Uganda Ministry of Health Neglected Tropical Disease Program is working towards the elimination of blinding trachoma in Uganda by 2020 using the SAFE strategy (S= surgery; A= antibiotics given during Mass Drug Administration (MDA) with azithromycin; F=Facial cleanliness promotion; E= environmental sanitation improvement). In 2006, baseline prevalence of trachoma follicular (TF) in children 1-9 years in Buyende, Kamuli and Iganga districts of Uganda was found to be above 10% (33.6%, 33.6% and 20.1%, respectively), the intervention threshold for MDA with azithromycin. All three districts have carried out 3-5 rounds of MDA as recommended by World Health Organization, followed by impact assessments in 2013. Impact assessment results showed a substantial decrease in prevalence of active trachoma (5.9%, 6.4%, and 5.8% respectively). The new WHO treatment guidelines recommend a single round of MDA for districts under the treatment threshold of 10%, yet above the elimination goal of 5% TF in children aged 1-9 years. One round of mass drug administration with azithromycin was implemented in Buyende, Kamuli and Iganga districts in 2014 with varying levels of coverage (83.8%, 49.7%, and 87.4%, respectively). A second round of district-level impact assessments will be carried out in all three districts in May 2015. Results from these prevalence assessments are expected in June 2015, and will be used to guide programmatic implementation in Uganda and to provide valuable information to the global trachoma community about the validity of the new treatment guidelines for achieving elimination of blinding trachoma.

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THE ITI FLYING GENEXPERT: BRINGING NAAT DIAGNOSTICS FOR TRACHOMA TO THE END OF THE ROAD TO ACCELERATE RESEARCH, REDUCE COSTS, AND IMPROVE TRACHOMA PROGRAMS

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Studies are ongoing to evaluate Nucleic Acid Amplification Techniques (NAAT) as an indicator of infection in trachoma control programs, in addition to clinical signs of trachoma, and antibody serology. NAAT tests cannot always be done in the countries where the eye swab samples are collected. International transport of samples is costly and time consuming, requires material transfer agreements, and prohibits the building of laboratory and research capacity in trachoma endemic countries. To address these issues, we purchased a GeneXpert IV 4-module portable PCR machine and GeneXpert CT tests for the high-burden developing countries (HBDC) reduced price (Cepheid, Sunnyvale, CA, USA). The GeneXpert is a cartridge based test with minimal sample processing. Partners can borrow the "ITI Flying GeneXpert" to test eye swab samples for ***C. trachomatis***, in settings where there is no access to NAAT, for whatever reason. The GeneXpert IV can be checked-in as luggage on commercial flights, and is exempt of tax in customs, with appropriate documentation. To further reduce costs, 4-5 samples are usually pooled per test. The ITI Flying GeneXpert went from Atlanta to two remote settings in Nepal for a trachoma surveillance study. In 36 days, we tested 1543 samples in 310 PCRs (5 samples pooled per test). We tested samples within 5 days of collection. While survey teams collected samples, a technician processed samples at a make-shift laboratory. The ability to test in the field meant that we had results within days rather than weeks or months. In addition, we could identify positive clinical cases that were truly infected with ***C. trachomatis***, and discuss the results with the local authorities. The ITI Flying GeneXpert reduces the cost of research because 1) partners need not purchase a machine for temporary use, 2) there is no international transport of samples and associated regulatory procedures, and 3) laboratory staff salaries in developed countries are higher. The ITI Flying GeneXpert will increase the use of the GeneXpert platform for trachoma research, and will facilitate and accelerate the generation of data to inform and improve trachoma control programs.

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ACANTHAMOEBA SPP. AS POSSIBLE RESERVOIR AND VECTOR FOR THE TRANSMISSION OF *MYCOBACTERIUM ULCERANS* IN CAUSING BURULI ULCER (BU) DISEASE: PROOF OF PRINCIPLE

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The route of ***Mycobacterium ulcerans*** (MU) transmission in causing Buruli ulcer (BU) disease in humans (mammal) remains poorly understood although a number of possible routes have been proposed. Since ***Acanthamoeba*** species is capable of harboring the closely related ***Mycobacterium leprae*** and ***Mycobacterium marinum***, we hypothesized that ***Acanthamoeba*** spp. may be natural reservoirs and possible vectors for ***M. ulcerans*** transmission. To test this hypothesis, intact or shaved-pinpricked rumps of ICR mice were treated with either: (i) ***M. ulcerans alone;*** (ii) a co-culture of ***A. polyphaga*** and ***M. ulcerans***

or (iii) ***A. polyphaga*** only. To investigate differences in virulence, two additional groups of mice were injected in their footpads with identical concentrations of either ***M. ulcerans alone*** or ***M. ulcerans*****-**infected ***A. polyphaga.*** All groups were observed daily for development of lesions. Fine needle aspirations were performed on lesions from live animals, while infected parts of dead animals were excised and homogenized, decontaminated, Zeihl-Neelsen acid-fast (ZN) stained for acid fast bacilli and then inoculated on Lowenstein-Jensen (LJ) plates for growth of ***M. ulcerans*** for six weeks incubation at a temperature of 32°C. Both topically applied ***M. ulcerans*** only and ***A. polyphaga:M. ulcerans*** elicited inflammation (Day 31), edema (Day 45 and Day 44 respectively) and ulcers (Day 49) at infected sites for pinpricked but not intact skin groups. Also, for the same concentrations of ***M. ulcerans, M. ulcerans*****:*****A. polyphaga** caused early inflammation in mice footpads by day 3 compared to day 14 of ***M. ulcerans*** only. The aspirates and homogenized tissues of ulcers were all positive for acid-fast bacilli (MU) and showed growth of ***M. ulcerans*** when cultured. Evidence provided by this study supports the hypothesis that ***A. polyphaga*** may be a possible reservoir and a vector in BU transmission and also suggests that ***Acanthamoeba*** may enhance the virulence of ***M. ulcerans*** in the environment. This study also confirms the possibility of passive infection by ***M. ulcerans*** as a likely transmission route and also provides another mouse model, other than BALB/c, for BU studies.

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GLOBAL COMPARATIVE STUDY OF BACTERIAL MENINGITIS DYNAMICS

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Bacterial meningitis, caused primarily by invasive infection with ***Neisseria meningitidis***, ***Haemophilus influenzae*** type b, and ***Streptococcus pneumoniae***, inflicts a significant disease burden in numerous countries around the world, often resulting in mortality rates of as high as 15-20% of cases. In the African meningitis belt, a group of countries in sub-Saharan Africa that experiences some of the highest bacterial meningitis incidence rates in the world, cases of bacterial meningitis exhibit a distinct seasonality wherein outbreaks only occur during the dry season. However, little is known about the seasonal dynamics of bacterial meningitis outside of this region. To generate the data necessary to investigate these trends, we created a global database of weekly and monthly bacterial meningitis incidence data from 63 countries, including 44 countries outside the African meningitis belt, 27 of which possessed meningococcal meningitis incidence data for the 10-year time period between 2003 and 2012. Of these 27, Brazil, Ireland, and Iceland exhibited the highest average annual meningococcal meningitis incidence whereas Japan, Thailand, and Hong Kong exhibited the lowest rates. Using wavelet analysis methods, we analyzed the time series data from these 27 countries to assess periodicity and seasonality, and compared pathogen-specific time series to determine if synchronicity could be observed between the relative frequencies of cases caused by these pathogens. We found that meningococcal meningitis exhibited significant seasonality in 14 of the 27 countries tested, but we observed no significant synchronicity between the three pathogens. In general, meningitis incidence was frequently the highest during the winter months and the lowest during the summer months. These results provide some of the first key steps toward evaluating possible environmental, demographic, social, and immunological factors driving these patterns.

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GENOMIC ANALYSIS OF CARRIED AND INVASIVE SEROGROUP A *NEISSERIA MENINGITIDIS* FROM THE 2011 EPIDEMIC IN CHAD

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Serogroup A ***Neisseria meningitidis*** (NmA) was the most common cause of meningitis in the African meningitis belt before the introduction of the TT-PsA vaccine (MenAfriVac). This bacterium, often carried asymptomatically is considered to be an 'accidental pathogen'. The mechanisms driving the transition from carriage to disease remain poorly understood. This study examined possible roles of bacterial genome diversity in this transition by comparing the genomes of geographically and temporally matched invasive and carried isolates. Purified DNA was obtained from 10 carried NmA collected by MenAfriCar and 14 invasive NmA identified during the Chadian meningitis surveillance in 2011. Whole genome sequence (WGS) data were collected, de novo assembled and submitted to the PubMLST/Neisseria website for automated annotation and analysis. Genomes were compared at 3 different levels: 7 MLST genes, 53 ribosomal MLST (rMLST) genes and 2070 whole genome MLST (wgMLST) using the Genome Comparator module. Phylogenetic networks were generated using the NeighborNet algorithm. One serogroup X isolate was found and not analyzed further. Of the 23 remaining isolates, 21 were ST7 and one was ST9021. One isolate had no ST assigned due to a deletion including gdh, one of the MLST loci. There were 6 distinct rSTs. 242 variable genes and 1542 identical genes were identified among all isolates with wgMLST; the isolates clustered into three distinct groups, but no systematic clustering by disease or carriage source was observed. wgMLST provided a high-resolution view of the genetic diversity of these NmA isolates, which were indistinguishable at lower resolution. The invasive meningococcus population circulating during the epidemic was not homogeneous. Instead our results show that a variety of closely related but distinct clones were circulating in the human population and no systematic genetic differences were found between carriage and disease isolates. This supports the idea that it is a change in the host-pathogen interaction and/or the nasopharynx environment that drives the bacteria invasive phenotype, rather than solely bacterial factors.

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IMMUNOGENICITY FOLLOWING THE INTRODUCTION OF A NOVEL MENINGOCOCCAL B VACCINE TO CONTROL AN OUTBREAK AT A U.S. UNIVERSITY IN 2013

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An outbreak at a New Jersey University in 2013 caused by ***Neisseria meningitidis serogroup B*** (MenB) ST-409 from the 41/44 clonal complex led to eight cases and one death. After months of sustained transmission, the FDA approved the use of a novel meningococcal B vaccine 4CMenB (Novartis), which was not licensed in the US. Overall,

98% of undergraduates received the first dose and 93% received the second dose. We aimed to understand the impact of 4CMenB on population-level immunity against the outbreak strain, immunity against the vaccine strains, and reasons for vaccine refusal. To address these questions, we conducted a cross-sectional seroprevalence survey in April 2014. Six hundred and seven students enrolled. Sixty-six percent (95% CI: 62-70%) of students who received two doses of vaccine in December and February, 59% (33-82%) of those who received one dose only in December, and 21% (6-46%) of those who remained unvaccinated had serum bactericidal antibody (hSBA) titers using human complement ≥4 against the outbreak strain. Students vaccinated with two doses had statistically significantly higher GMTs against the outbreak strain compared to unvaccinated students (7.6 (6.7-8.5) versus 2.8 (2.3-3.5), p=0.0015). The 7% of participants who remained unvaccinated or did not complete the series cited concerns about possible side effects, a lack of concern about MenB, and a lack of time as reasons. In the fall of 2014, we conducted a pre/post-vaccination immunogenicity study among incoming students who were also offered 4CMenB. The pre-vaccination survey enrolled 140 students; 75% returned for the post-vaccination survey four weeks after the second dose, of which 61 had received two doses and 33 remained unvaccinated. An additional 88 students who had received two doses were enrolled post-vaccinated. Preliminary analyses are underway. Our investigation of immunity following the introduction of 4CMenB is the first evaluation of this vaccine in the context of an outbreak and the first study among young adults in the US. Our results are key to informing public health policy and strategies for better preventing and controlling future outbreaks.

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THE CHALLENGES OF MAINTAINING GOOD CLINICAL LABORATORY PRACTICES IN LOW-RESOURCE SETTINGS: A HEALTH PROGRAM EVALUATION FRAMEWORK CASE STUDY FROM EAST AFRICA

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Limited capacity and quality of laboratory services remain critical barriers to healthcare improvements in low-resource settings. The objective of this study was to characterize challenges faced by clinical laboratories in East Africa. Using the US Centers for Disease Control and Prevention framework for program evaluation in public health, we evaluated the laboratory sections of the Kilimanjaro Clinical Research Institute managed by the Kilimanjaro Christian Medical Centre-Duke University Health Collaboration in Tanzania, a full-service clinical laboratory that supports clinical research studies and abides by Good Clinical Laboratory Practice standards. Review of records from November 2012 through October 2014 revealed that the laboratory experienced 23 serious malfunctions among 8 instruments, of which 7 (30.4%) precluded testing of study protocol-required analytes. The median (range) time elapsed until vendor-contracted engineers arrived on-site for instrument repair was 9 (1-55) days. Of the laboratory's 21 reagent and consumable suppliers, 16 (76.1%) were based outside Tanzania. International reagent and consumable shipments were held in Tanzanian customs for a median (range) of 9 (1-51) days, and customs clearance charges accounted for US$22,980.11 (7.1%) of all reagent and consumable expenditures (US$321,801.82) over a two-year period. Monthly test throughput among laboratory sections was a median (range) of 0.6% (0.2%-2.7%) of instrument capacity. Of 9 laboratory technologists employed during the review period, 5 (55.6%) left during this period. This evaluation highlights key challenges for maintaining high quality clinical laboratory services in low-resource settings. Improved timeliness of instrument repair services, expanded local supply of reagents and consumables, reduction of customs barriers, and workforce retention represent necessary steps towards establishing quality-assured laboratory capacity in East Africa. Progress in these areas will require coordinated, goal-directed partnerships between national and international stakeholders, donor agencies, and biomedical industries.

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IMPROVING EFFICIENCY AND QUALITY IN CLINICAL TRIALS IN SUB-SAHARAN AFRICA

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Clinical research is indispensable for efficient health systems. Research centers in sub-Saharan Africa face particular constraints through increasing trial related workload and administration paired with capacity limitations. We therefore investigated the main challenges in the conduct of clinical trials to optimize processes for resource-effective and high quality trials in low-resource settings. The working hypothesis was that the main difficulty was that existing regulations are not adapted to these particular situations and that the possible leeway for interpretation was not sufficiently exploited. Semi-structured individual interviews were performed in 2014 and 2015 in three English- and two French-speaking African countries (Kenya, Tanzania, Ghana, Burkina Faso and Senegal). The interviews were conducted in two clinical research centers per country with a total of 69 clinical trial staff. For data triangulation, various staff levels were interviewed and additional informal information was gathered. The interview guide consisted of general questions about quality, guidelines, challenges, and perceived inefficiencies in clinical trials. The interviews were transcribed verbatim. Thematic analysis was then performed to identify themes relevant both across settings and positions. Poorly developed protocols, patient management and ineffective trial management are the themes that emerged: Protocols are often poorly adapted to the respective research settings ignoring capacity limitations and local culture and values. This together with unrealistic deadlines contributes to time loss in trials. Unclear delegation was another frequently described challenge. In turn, unexpectedly, the administrative burden resulting from the guidelines has never been mentioned as a difficulty; rather, researchers were grateful for having guidance in their daily work. To avoid protocols unsuitable for the context, we suggest better and earlier involvement of local staff. We also encourage careful and realistic planning with regular exchange and a test run before the enrolment of the first patient.

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THE FEASIBILITY USING OF HOSPITAL LOGBOOKS TO MEASURE BASELINE PERIOPERATIVE MORTALITY RATES IN ETHIOPIA

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Ethiopia is a low-income country on the Horn of Africa with a developing healthcare infrastructure. Currently, there is no benchmark for the safety of surgery and anesthesia in Ethiopia that can be used to drive and track quality improvement. A recent consensus meeting held between representatives of Surgical and Anaesthetic Colleges and Societies supported the use of perioperative mortality rates (POMR) as a regional and national health indicator. The POMR is defined as total number of surgical deaths divided by total number of operations in a given year. POMR is measured at two time periods: death within 24 hours of surgery (or on the day of surgery) or within 7 days of surgery or prior to

hospital discharge. We examined the feasibility of measuring the baseline perioperative mortality rate (POMR) in Ethiopia using in a combination of intraoperative, anesthesia, and surgical ward logbooks at two hospital sites in Addis Ababa. A total of 7162 operations and 7 deaths were recorded, with 1 death occurring at the time of surgery and the remaining 6 in the surgical ward or ICU. The POMR was measured at .097%. We encountered limitations in the form of missing logbook pages, illegible entries, and misplaced logbooks that may serve to underestimate the POMR. Furthermore, only the anesthesia logbooks recorded ASA status and cause of death, which was not kept at one of the sites. In conclusion, the methods used to establish the baseline POMR through logbook analysis will vary across hospital sites until a standardized system of surgical record keeping is established in Ethiopia.

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ONE ENVIRONMENT, MANY ETHNIC HEALTH DISPARITIES

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Members of many different ethnic groups including Thai citizens and trans-border migrants live in close proximity in relatively homogeneous ethnic communities in rural northwestern Thailand, near the border with Myanmar. Most of the people in all communities farm crops such as maize and peanuts for the international market. All Thai citizens and many trans-border migrants have government-provided health insurance and all share a common government health care system with low cost to patients. All pregnant women and their husbands are eligible for low cost antenatal care including HIV counseling and testing. Despite living in the same natural and health care environment, Hmong, Lahu, Tai Yai, and especially Chinese ethnic minorities compared with Northern Thai ethnic majority have significantly less knowledge of HIV transmission, prevention and treatment, greater stigmatization of HIV/AIDS, report more constraints that delay or prevent use of health facilities and make less use of health facilities. Statistical controls for citizenship, Thai language ability, education, health insurance, economic status and presence of a motor vehicle in household reduce but do not eliminate highly significant disparities in these variables. Ethnic-specific tailoring to reduce disparities in health knowledge, HIV stigmatization, and effective use of health service includes training and using bi-lingual community members as translator-interpreters in clinics and as community health educators.

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USE OF COMMUNITY HEALTH VOLUNTEERS TO INCREASE COVERAGE FOR INTEGRATED COMMUNITY CASE MANAGEMENT IN BONDO, KENYA

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Bondo County is located in the Western region of Kenya. It has an IMR of 110 and an U5MR of 208 per 1,000 live births which is thrice the national U5MR of 74/1000. There continues to be limited access to and use of health services in some rural areas that are underserved by health facilities. This provided the impetus for advocating for the implementation of integrated Community Case Management (iCCM) as a way to address these health disparities. An 18-month study is underway in Bondo to test whether community health volunteers (CHVs) can effectively deliver an iCCM package in the context of the existing community health strategy platform. The study is a quasi-experimental design with intervention and comparison groups of four community units each. Fifty-eight intervention group CHVs were trained on iCCM and health promotion, provided with iCCM commodities, and a monthly stipend of $23. In the comparison group CHVs were only trained in health promotion and receive a similar stipend. Baseline survey was done in October 2013 and midline in July 2014; the latter was limited to the intervention group only. An endline survey is planned for June 2015. Overall introduction of iCCM resulted in over 100% increase in iCCM cases managed from baseline compared to midline (2,367 vs. 4,868), with the CHVs' share being 56%. In terms of performance, the CHVs demonstrated good ability to follow the iCCM algorithm from the identification of signs to the classification of illness, and deciding whether to treat at home or refer to the health facility. The greatest improvement was in the ability to examine or "look" for signs of illness (average of 3% at baseline vs. 74% at midline), $p < 0.05$. Key stakeholders reported that there were various benefits of iCCM in Bondo such as improved access to health services, improved health behaviors at individual and community level, community empowerment, and increased trust of the CHVs by the community. Based on these results so far, CHVs can effectively provide iCCM services and thus contribute to reducing childhood morbidity deaths in Bondo, Kenya.

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THE ROLE OF SOCIAL NETWORKS IN INTERVENTION STRATEGIES TO CONTROL TUBERCULOSIS: A CASE STUDY OF TWO COMMUNITIES IN LUSAKA, ZAMBIA

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The global burden of tuberculosis (TB) currently is estimated to be 30-50% and is on the rise. In Zambia, as well as throughout the developing world, persons infected with TB often are stigmatized within their communities. Additionally, information about the disease is transmitted via informal networks. This research explores the structural mechanics of social networks in two low-resource communities in Lusaka, Zambia to understand the role of these networks in creating sustainable health behavior change after the implementation of intervention strategies that targeted tuberculosis-infected persons and their families. The project uses social network analysis to study how interpersonal networks in these communities operate with the goal of measuring the efficacy of intervention strategies aimed at reducing tuberculosis-related stigma. Social network analysis is an effective tool in which to examine the role of relationships within communities since relationships reflect emergent dimensions of complex social systems that cannot be captured in statistical models of health and demographic data. Analyzing network structures can highlight the flow of information among its members and the likelihood of subsequent behavior change. In low-resource settings, sustainable cost-effective intervention strategies are necessary to encourage TB-infected persons to seek treatment. Therefore, understanding these social networks potentially may allow for more effective program planning by harnessing the processes of information exchange within similar communities worldwide.

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DETERMINATION OF WEANING FOODS USED IN THE DOMINICAN REPUBLIC, EL SALVADOR AND HONDURAS AND THE IMPACT THEY HAVE UPON GASTROINTESTINAL PARASITIC INFECTIONS IN CHILDREN

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Contaminated weaning foods account for a substantial portion of parasite-induced diarrheal diseases among infants and young children in developing countries. The sources of food contamination are numerous and without appropriate food safety measures and hygienic quality control of infant foods including drinking water, parasitic infections will remain

high in both infants and children. In a published study by Childers and Palmieri (2014) on the prevalence of gastrointestinal parasites in children from Verón, a rural city of the Dominican Republic, they examined 128 fecal samples of children ranging from 2-15 years and found 127 positive for one or more parasites. Percent infection rates were 43.8% for Ascaris lumbricoides, 8.5% for ***Enterobius vermicularis***, 21.1% for ***Entamoeba histolytica***, and 22.7% for ***Giardia duodenalis***, and with 7.8% of the children examined having double infections. The extremely high infection rate of gastrointestinal parasitic diseases in the Punta Cana-Bávaro-Veron-Macao municipal district in young children may be attributed to what the local population use for weaning foods and how the weaning foods are prepared. Presently, there are not published reports on weaning foods in this area. In this study approximately 250 women with children in Honduras, El Salvador, and the Dominican Republic will be interviewed to determine breastfeeding practices, age at which weaning took place from breast milk or formula, what the weaning foods are, and how the weaning foods were prepared, and to correlate this information with transmission cycles for the known parasitic diseases in children in the area.

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ADVANCEMENTS AND CHALLENGES IN DESIGNING ACCURATE, POINT-OF-CONTACT, INFRASTRUCTURE-FREE MOLECULAR RAPID DIAGNOSTIC TESTS

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Currently, most clinically relevant nucleic acid amplification tests (NAATs) require laboratory or electrical infrastructure, preventing accurate infectious disease diagnostics in low-resource settings (LRS). Although simple and ubiquitous at the point-of-contact (POC), antigen based rapid diagnostic tests (RDTs) may lack the sensitivity required for diagnosing acute or low density infections. In an effort to extend the reach of molecular diagnostic tools, PATH has previously demonstrated the versatile, non-instrumented nucleic-acid amplification (NINA) reusable platform across HIV-1, Buruli ulcer, and ***Plasmodium*** spp. isothermal amplification assays. NINA provides precise temperatures required for isothermal NAATs by coupling an exothermic reaction to a phase change material (PCM) to create an energy dense, low-cost, consistent heat source. Furthering this technology, PATH has developed a single-use disposable miniature format, enabling low-cost diagnosis free from reusable components or infrastructure. This hardware advancement enables molecular diagnostics in an easy-to-use form factor, taking accurate diagnostics further into LRS. Significant engineering and manufacturing challenges encountered in device miniaturization include thermal management, performance repeatability, ambient operating range, and temperature precision. We have met these challenges through PCM composite engineering, paper microfluidic reagent delivery, computational modeling, and batch manufacturing methods. Here, we discuss design strategies and challenges in the manufacture of 150 miniature alpha prototypes to support evaluation by the US Center for Disease Control and Prevention (CDC) of a point-of-contact RT-LAMP HIV-1 NAAT. These recent developments show progress toward our vision of simple, sample-to-results molecular RDTs, capable of being used anywhere in the world.

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THE ANALYSIS OF CO-MORBIDITIES AND THE PROMISE OF IMPROVED DIAGNOSTICS FOR A HIGH DISEASE-BURDEN COMMUNITY

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The most effective path to substantial improvements in public health and medicine in high-disease burden areas is unclear in part because the effect of changes in diagnostics or treatment options are difficult to anticipate. Co-morbidities radically alter the progression and outcome of disease relative to solitary infection and play a major role complicating medicine and public health, making the necessary analysis difficult. Well-documented examples of altered progression and outcome include HIV disease, tuberculosis, and malaria, which jointly influence progression and the outcome of concomitant acute bacterial infections. Several mechanisms tie changes in disease progression to epidemic progression and endemic state, including the maintenance of subclinical infections, and modulation of immunity. While disease progression and contagion are moderately well-characterized for these diseases in isolation, mathematical epidemiology of co-morbidities is not well-established. Relying on extensive clinical data from Siaya, Kenya, we analyze the model sensitivities for simultaneous disease progression and contagion models in the context of holoendemic malaria and co-morbidities in Siaya. The problem of constraining several simultaneous disease progression and contagion models (two acute bacterial infections, malaria, tuberculosis) including their co-morbidities is challenging and we report the co-varying uncertainties in the parametrization of our model's interactions between co-morbidites, and in the parametrization of contagion. Models such as the one reported here promise to improve our ability to understand co-morbidities and their complex interactions with medicine and public health.

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KEY SUCCESS INDICATORS OF A COMMUNITY ENGAGEMENT STRATEGY: THE CASE OF A TROPICAL MEDICINE RESEARCH UNIT ON THE THAI-MYANMAR BORDER

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Community engagement is increasingly promoted to strengthen the ethics of medical research in low-income countries. One strategy is to use community advisory boards (CABs), however there is a paucity of data on how to evaluate it. The Shoklo Malaria Research Unit (SMRU) has been conducting research in tropical medicine e.g. malaria in a population of refugees, migrant workers, and displaced people on the Thai-Myanmar border for nearly 30 years. In 2009 SMRU facilitated the establishment of the Tak Province Community Ethics Advisory Board which consists of members from the border community in an effort to formally engage with the local people both to obtain advice and to establish a participatory framework within which studies and the provision of health care can take place (https://www.youtube.com/watch?v=HFYJWfGoVeQ). Six years on, we wonder if the CAB been successful, which begs the question "what are the key success indicators of a CAB and how do we evaluate it?" Based on our experience facilitating the CAB, attending its meetings and conducting formal interviews with relevant stakeholders, we have

identified the following indicators, both qualitative and quantitative: (1) key factors that motivate members who serve on the CAB (2) the level of trust among the members, and between researchers and members (3) the nature and depth of discussions in CAB meetings (4) the number and the quality of suggestions and changes in research studies recommended by CAB members, and the extent they have been taken on board by SMRU researchers and collaborators (5) the extent of the CAB protecting the interests of the community it serves e.g. preventing exploitation (6) the representativeness of the CAB members (7) the sustainability of the CAB (8) the ability of the CAB to identify significant ethical issues and (9) the perception of the CAB by community members and researchers. These indicators are crucial for us to conduct a formal evaluation of the CAB and to design our community engagement strategy in years to come.

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MATERNAL SCREENING TO PREVENT CONGENITAL TOXOPLASMOSIS IN AUSTRIA: MODELING THE COST-MINIMIZING OPTION

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Toxoplasma gondii is a protozoal parasite that infects a significant proportion of humans globally, although prevalence varies widely among and within world regions. Mothers infected during pregnancy may exhibit no symptoms, but there is about a 50% risk of transmission to the fetus and possibility of mild to profound injury to the unborn child. Consequences of fetal infection include chorioretinitis, encephalitis, hydrocephaly, cerebral calcifications, hearing loss, and fetal death. In the USA, 91% of children with no prenatal or postnatal treatment had visual and/or cognitive impairment by age 12. In Europe, 3 countries have systematic prenatal screening programs for early intervention to prevent or reduce fetal injury and have seen large reductions in maternal infection, fetal infection, and fetal injury. The Austrian protocol for prenatal testing and medication was initiated in 1992. Using historical data from the Austrian Toxoplasmosis Register, which records serology history and birth outcomes for 1 387 680 pregnancies from 1992 to 2008, we compare lifetime societal costs of the Austrian protocol as implemented with lifetime societal costs in a hypothetical scenario without prenatal screening. In a decision-analytic model using a societal perspective, we include costs of treatment, lifetime care and accommodation of injuries, and lost productivity that would have occurred in a No Screening scenario with actual costs of screening, treatment, lifetime care and accommodation, and lost productivity in Austria from 1992 to 2008. Lifetime societal costs of the No Screening option would have been €198 per birth, for all Austrian births in the period, resulting in total costs of about €275 million for treatment and accommodation for children without early diagnosis and treatment. Maternal screening according to the Austrian protocol as implemented over the period, including costs of screening, maternal treatment, infant treatment, and lifetime costs for those children with CT in spite of the protocol cost €58 per birth, or roughly €80 million. The Screening option is cost-minimizing, saving €140 per birth, or about €200 million euros.

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TRANSLATING DATA FOR POLICY ACTION: ASSESSING HEALTH FACILITY CAPACITY FOR MALARIA CASE MANAGEMENT IN GHANA, KENYA, AND UGANDA

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To further accelerate gains made against malaria, more efforts that link research to local policy priorities are needed. This work is particularly important as more countries aim to improve their malaria case management guidelines. Most countries stipulate that parasitological confirmation for all suspected cases of malaria occurs before first-line antimalarials can be prescribed. Thus, assessing whether health facilities concurrently stock artemisinin-based combination therapies (ACTs) and malaria diagnostics (laboratory testing or rapid diagnostic tests [RDTs]) provides critical information about a country's overall capacity for malaria case management. Drawing from nationally representative facility-level data collected through the Access, Bottlenecks, Costs, and Equity (ABCE) project, we analyzed the availability of both ACTs and malaria diagnostics across levels of care in Ghana, Kenya, and Uganda. In 2012, nearly all hospitals had the capacity for both diagnosing and treating malaria. Among primary care facilities, however, results varied more by country. Ghana recorded the largest proportion of public primary care facilities which lacked diagnostics (47% of health centers and 68% of community-based planning and services [CHPs]). By contrast, public facilities in Uganda were more frequently limited by stock-outs of ACTs than deficiencies in testing (91% either had a microscope or RDTs). At the lowest levels of care in Kenya, dispensaries and clinics, availability of malaria testing was the primary constraint to proper case management (15% stocked ACTs but lacked diagnostics); however, over 90% of public and private health centers in Kenya had concurrent capacity for malaria testing and treatment. These findings show that the policy interventions for improving malaria case management are likely to vary across countries and levels of care. As more national guidelines require parasitological confirmation of malaria and call for universal access to ACTs, it is crucial to identify where specific gaps exist within a country's health system and then use these data to tailor evidence-informed strategies against malaria.

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USING STORIES TO SUPPORT MASS DRUG ADMINISTRATION FOR THE ELIMINATION OF LYMPHATIC FILARIASIS IN AGAM DISTRICT AND DEPOK CITY, INDONESIA

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In 2013, we developed a tool and process to support the implementation of mass drug administration (MDA) for lymphatic filariasis (LF) elimination in two districts in Indonesia where additional rounds of MDA were required. We aimed to understand MDA from the perspective of the recipients (community members) and the deliverers. In both locations (Depok City (urban) and Agam District (rural)), more than six rounds of MDA had been completed by 2012. In 2013, Agam District did not pass the Transmission Assessment Survey (TAS) and Depok City did not qualify for TAS. District stakeholders reported feeling discouraged when required to continue additional MDA rounds. In order to assist with this prolongation of MDA we developed a tool using a novel approach in social

data collection whereby people were asked to tell their direct experiences with MDA. Based on these narratives, respondents then provide insight into the influences and motivations for the reported behavior. In December 2013 and January 2014, 805 community members were interviewed following the last MDA round. Over 78% of the total sample had ever received the MDA, and 65% of those receiving the drugs, complied. Positive influences on compliance included being given the LF drugs outside of the house (AOR 2.74, p=0.004), exposure to informative media (AOR 2.10, p=0.002), perceived common goodness (AOR 1.5, p=0.019), being healthy (AOR 10.74, p=0.000) and knowing that others have complied (AOR 2.27, p=0.03). At the same time, 204 drug deliverers were surveyed. 58% of deliverers had participated in MDA more than 3 times and 93% reported previous MDA compliance. Results showed that MDA eligibility criteria were misunderstood; meaning less people were being offered LF drugs. This research demonstrated that specialized guidance could assist those districts that must prolong MDA past six rounds. The use of stories provided a more reliable prediction of compliance than the standard behavior survey data. The narrative capture tool developed in this study could be used to assess and advise districts struggling to reach sufficient coverage levels to achieve LF elimination.

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PREDICTING THE USE OF ORAL REHYDRATION SOLUTION (ORS) FOR CHILDREN WITH DIARRHEA: AN APPLICATION OF LOGISTIC REGRESSION AND CLASSIFICATION TREES

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Child diarrhea is a critical concern, for which oral rehydration solution (ORS) is a key management method. Logistic regression (LR) is one of the most common statistical methods used for prediction of key behaviours such as ORS use. With the large number of potential variables that could be included, variable selection is often a problem in LR. Classification trees (CT), a form of binary recursive partitioning, have been suggested as an alternative. CT can be used to identify complex interactions between predictors and present them in a tree diagram that is easy to interpret. Thus, CT may be used to build a predictive model as accurate and interpretable (or more) than LR. The aim of this study was to build and compare predictive models for ORS use using LR and CT, using data from the 2007 and 2013 Demographic Health Surveys of the Dominican Republic. Purposeful selection, which relies on univariate analyses to select variables, was chosen for LR. CT was used as a predictive model itself, and the variables in the tree output were used to build a LR. Purposeful selection and CT models ended up with almost the same list of predictors. Five of the six predictors in the CT were present in the purposeful LR (caregiver education, child age, feeding response, whether sought treatment/advice and whether used public health care for diarrhea), which may explain comparable prediction accuracy between the models. The CT had accuracy (Ac, 95% CI) of 67.9% (63.1-72.4), the tree LR had Ac of 71.9% (67.6-75.5), and the purposeful LR had Ac of 71.4% (66.8-75.5). The sensitivity and specificity were also similar between these models. Despite having similar performances, the CT approach was arguably more useful as the tree diagram better illustrated the order of hierarchy and interactions among the predictors, and how they collectively affected ORS use. While limited to two cross-sectional datasets, this study is one of the first to compare the utility of CT as a predictive model versus conventional method of LR for ORS use, and as a method to select variables for LR. This approach may be useful in identifying predictors in other health behavior studies.

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FACTORS ASSOCIATED WITH THE NON-USE OF INSECTICIDE TREATED NETS IN RWANDAN CHILDREN

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Insecticide treated bed nets (ITNs), when used properly, have shown to be highly effective in reducing malaria morbidity and mortality. However, factors related to individual, household and community may influence how ITNs are used. Our study examined factors associated with non-use of ITNs non-use among children under five years in Rwanda. We conducted a secondary data analysis of 2010 Rwanda Demographic Health Survey (DHS) data using multilevel logistic regression analysis. Responses from a total of 6,173 women aged 15-49 years living in 492 villages were included in the analysis. The ITN non-use among children under five years was 25%. Risk factors for ITN non-use included household with more than 5 members (OR=1.42; 95% CI=1.23-1.63), employed mother (OR=1.33; 95% CI=1.06-1.66), and lower village altitude (OR=1.36; 95% CI=1.14-1.61). Risk factors for ITN use included households with more than 3 nets (OR=0.39; 95% CI=0. 33-0.47), women with one to four visits to antenatal clinic (ANC) (OR=0.45;95% CI=0.29-0.69) and more than 4 ANC visits (OR=0.39; 95% I=0.21-0.70), mother married or living with partner (OR=0.43; 95% I=0.36-0.52), mother with any education (OR=0.77; 95% CI=0.65-0.91), and higher community wealth quintile (OR=0.71; 95% CI=0.59-0.84). Proper use of ITNs can decrease the burden of malaria in Rwanda. Factors related to ITN non-use at individual, household and community levels, including mother marital status, mother occupation, education, ANC attendance, number of mosquito bed nets, number of household members and, socio-economic status need to be addressed to ensure that ITNs continue to be effective in preventing malaria.

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BETEL OR ARECA NUT CONSUMPTION IN YAP, FEDERATED STATES OF MICRONESIA

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The areca or betel nut (AN), the fruit from the palm ***Areca catechu*** is the fourth most commonly used psychoactive drug after tobacco, alcohol, and caffeine globally. Worldwide, an estimated 600 million people of all age groups and social classes consume AN. Oral cancer mortality rates of up to 80% associated with AN consumption have been reported in the Western Pacific Region. Other adverse health effects associated with AN use include esophageal, pharynx, lung, pancreatic, and cervical cancers. Yap, one of the four islands that comprises the Federated States of Micronesia (FSM) has the most traditional and persistent AN practices. Oral cancer rates in Yap are the highest in FSM at 31.8 cases per 100,000 people. This study sought to characterize current AN consumption, preparation and health attitudes among the Yapese population. The surveys were conducted by trained Public Health staff, who randomly interviewed 524 Yapese using a standardized data collection instrument. The interviews were conducted from October 1-December 31, 2014. Demographic information, AN consumption practices, and smoking and drinking habits among AN consumers were collected. The survey participants comprised of a total of 524 individuals including 297 (56.7%) females. The mean age for all participants was 33.52 (range 13-90 years). 5.4% of participants

had completed professional education, 30.4% college education, and 48.7% high school. 87.9% of females and 90.7% of males responded yes to current AN consumption. The mean age at which participants started consuming AN regularly was 17.77 years (range: 2-53 years). Of all the participants, 22.1% indicated smoking tobacco and 48.5% indicated drinking alcohol. Of the current AN users, 81.4% agreed to stop consuming AN if they knew it was harmful to their health. According to the World Health Organization, AN alone is a cancer causing agent. Because a substantial number of participants indicated they would stop AN consumption if they were aware of harmful health effects, public health education on the adverse health effects of consuming AN should be an initial step implemented in Yap to reduce AN consumption in the population.

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USE OF INTERNET APPLICATIONS IN DATA MANAGEMENT OF THE DENGUE VACCINE INITIATIVE FIELD STUDIES

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The data management in epidemiological studies implemented in developing countries faces many challenges in communication with different language, imbalance of proficiency among the data team staff at study sites, differences in terms of cultural background and past experience, and so on. Adequate communication through training with the local site staff is a key component in proper data management and successful execution of the field project. Hence, relevant training should be taken in advance to avoid delay of the study launch as well as to minimize potential errors. However, some studies experienced substantial delayed with inevitable reasons, such as travel restriction to the site with natural disasters and political instability. From the experience of launch and management of the data system, we report the learning lessons obtained from data management training using internet applications and its utilization on relevant studies. Two types of internet application were implemented for the data management training. WebEx includes functions requisite for training through the video conferencing and sharing the computer screen. TeamViewer allows us control remotely the main computer of study site and able to implement the data management system and to resolve the unexpected problems. The pilot study after the on-line data management training has been done and the on-line training was also compared with offline training.In DVI with multiple field sites with different local characteristics with standardized study designs, the DM could gain experience and learning lessons from various data management systems implemented. As many information techniques have been adopted in such kinds of field studies, many methods for the data management from paperless data collection to web-based system became available. Accordingly, an intensive relevant training needs to be complemented. The best way to manage data staffs of the study sites is a face-to-face training with monitored practice. However, proper use of the on-line alternatives for communication and training could be taken to overcome long international travels and cost issues.

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YOUTH TRAUMA TRAINING - EXPLORING THE FEASIBILITY OF TRAINING ETHIOPIAN YOUTH IN BASIC TRAUMA PRE-HOSPITAL CARE

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Trauma continues to be a leading cause of death, globally accounting for approximately 11% of the global burden of disease. Low and middle income countries (LMIC) report an annual injury death rate due to trauma nearly double those of high income countries. Accordingly, trauma is a leading cause of morbidity and mortality in the LMIC Ethiopia. Many deaths occur in the pre-hospital setting due to airway compromise, respiratory failure, or uncontrolled hemorrhage and can be managed by basic first-aid techniques. Several studies have demonstrated the significant potential to decrease trauma morbidity and mortality through training laypersons in basic trauma resuscitation. In collaboration with Ethiopian academic partners, we piloted a basic trauma resuscitation course targeted towards Ethiopian youth as a proof of concept study. Classes of approximately 20 voluntary students aged 14-21 were given a multiple choice pre-test, taught a three hour course and then given an identical post test with a qualitative, free response survey attached. The course content was based off ATLS principles of ABCD (Airway, Breathing, Circulation and Disability) interventions modified for a low resource setting. We hypothesized that young adults are an effective layperson population that could easily learn and apply these techniques and can be targeted for future trauma education interventions in order to increase the number of first responders in Ethiopia. Following education intervention the students demonstrated improvement in test scores with a post test average score of 81.78% (SD=12.04, N=83) increased from a pre test average score of 49.17% (SD=13.73, N=83). A dependent t test demonstrated significant difference between pre and post intervention scores ($p < 0.01$). The trauma courses were well received by students and increased the number of capable responders in Ethiopia. This study encourages further trauma education interventions targeted towards youth in order to establish an organized trauma triage system in a resource challenged country.

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COMPARISON OF VISUAL INSPECTION WITH ACETIC ACID AND PAP TEST IN THE PREVENTION OF CERVICAL CANCER IN RURAL HAITI

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Cervical cancer has the highest incidence and mortality rate of all cancers in women in Haiti. Unlike many cancers, it is preventable to a large extent by effective screening programs. Papanicolau (Pap) tests are not readily available in resource-limited areas and require multiple steps prior to treatment. In a rural setting where the incidence of cervical cancer is high, visual inspection with acetic acid (VIA) provides a one step method to diagnose and treat cervical dysplasia in high risk patients in low resource settings and has been shown to decrease cervical cancer mortality by 31%. This technique is widely studied and shown to be an effective and safe way to prevent cervical cancer in rural settings and may also be used to provide early identification and treatment of cervical dysplasia in rural Haiti. Outpatient mobile clinics in Haiti were established in the rural village of Mussotte in March and April 2015. A total of 243 patients sought gynecologic care. VIA was performed by medical students supervised by physicians on patients ages 21-60 years of age. VIA screening and Pap tests were performed on 165 patients. A log was kept of all patients who received medical treatment, and the log was retrospectively analyzed. The purpose of this descriptive analysis is to compare the results of VIA and Pap test in the detection of cervical cancer. Of the 165 women screened with VIA and Pap test, 4 women had positive VIA results and were treated with cryotherapy. 1 woman had invasive cervical cancer. Pathologic analysis of Pap tests will be performed in the United States, and patients will be notified of their results. VIA positively identifies cervical dysplasia at least as often as Pap. The key advantages of VIA over Pap are a lower cost of testing and the ability to administer treatment contemporaneously. Given the relatively higher prevalence of cervical cancer in the rural setting, VIA is recommended as a cost-effective way to screen for cervical cancer in rural Haiti.

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EXPERIENCE USING FREE CLOUD SOFTWARE AS A MEDICAL TOOL ON A TROPICAL MEDICINE CLINIC

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Electronic records are currently changing the way epidemiology is gathering information. In tropical medicine the demand for such products is huge, as this group of diseases affects more than one billion people. Starting to use electronic records on a tropical medicine clinic in a small country in Central America such as panama does not need a lot of monetary resources. There are many different software that are offered for free or paid that can provide medical records or data base registry. On our clinic we are currently managing electronic tools such as: Electronic Medical Records with a data base, a photo library indexed for each patient, georeferencing system and various forms for reporting to the health authorities; they are all offered for free from google drive and fusion tables, they are easily accessed from the cloud and are exportable to other programs. Our clinicians have all the information about their patients available in any device and readably accessible. The patients are easily georeferenced to the fusion table map and easily filtered by diagnosis making this tool indispensable for monitoring the epidemiology of different infectious diseases. These kind of systems allow an easier data collection, patient's follow-up, data analysis, and also provide information to the medical community, health authorities and scientific societies. We have seen the many advantages of these tools in our clinical setting and we strongly believe that this model can be easily adopted by other clinics. In today's world it is necessary to take advantage of the technology we have at hand and every physicians should familiarize with the wide spectrum of tools at their disposal.

1346

LOGISTICS RELATED TO AN EBOLA TREATMENT STUDY IN WEST AFRICA

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With rapid response to the 2014 Ebola outbreak in West Africa, ClinicalRM initiated a treatment study for Ebola in West Africa, utilizing convalescent plasmapheresed plasma. Given the nature of this study, and the need for rapid response, logistical issues were overcome by providing rapid protocol development, mobility of personnel, equipment, including ***HopeMobiles*** (bloodmobiles), and the ability to leverage strong international partnerships to ensure effective and rapid study start-up. Preplanning involved consideration of personnel needed in the U.S. and Africa for effective execution of the study. ClinicalRM and partners provided full-time investigators, program and project managers, and a full research team. Challenges faced in Africa, from the lack of site Standard Operating Procedures (SOPs), training, as well as enhanced communication between the U.S. and Africa. These issues were overcome by utilizing ClinicalRM's extensive template library and by providing continuous SOP review by ClinicalRM project managers and quality specialists. Site communications and telephone line capacities were inadequate for large studies and international, daily conference calls. These issues were rectified with an appropriate carrier and a new platform for hosting meetings. Remote Data Capture could not be accomplished electronically due to internet bandwidth. We were required to use paper CRFs. One of the largest problems to overcome was that of transportation of the ***HopeMobiles*** from the manufacturing site in North Carolina to the destination in West Africa. With careful planning and one-of-a-kind international synergy, we were able to supply and ship our ***HopeMobiles*** to the study sites using a FedEx AN-124 transport aircraft and drive them directly to the sites. A U.S. engineer oversaw the setup of site equipment on board the ***HopeMobiles***, ensuring the technical efficiencies. Moreover, because ELISA could not be performed at the site in Africa, specialized labs at U.S. Army Medical Research Institute for Infectious Diseases (U.S.) committed to the study by providing RT-PCR and ELISA test methods. Along with our partners, we managed all aspects of operations and study startup.

1347

PRIVATE SECTOR PROVIDERS, KEY PARTNERS IN IMPROVING QUALITY OF FEVER CASE MANAGEMENT-INSIGHTS FROM MADAGASCAR

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In Madagascar, the private health sector distributes 80% of antimalarial drugs, primarily through drug stores, pharmacies and retailers. Diagnostic services, however, are practically non-existent in the private health sector, often leaving potentially life- threatening febrile illnesses mistreated. Population Services International is implementing a project to improve fever case management in the private sector. In addition to training private providers to correctly manage fever cases using Rapid Diagnostic Tests (RDTs), the project has built a dynamic system to supervise providers and improve their performance to ensure they meet international standards of Quality of Care (QoC). Private provider behavior is regularly assessed by a team of quality assurance officers (QAOs), using an assessment checklist approved by the Ministry of Health and WHO. The checklist provides QAOs with an overall QoC score, based on a series of critical steps that providers need to complete, from evaluating danger signs to making correct treatment decision according to diagnostic test results, as well as providing counseling and follow-up care through a referral system. It is used not only to track progress over time and plan next assessment visits, but also to identify specific gaps that can be addressed during each assessment visit, or through refresher training. Early results from the project show that monitoring for performance improvement can be achieved in the private health sector, and that tailored support to private health providers ensures high level of quality of care. Since the beginning of the project in Madagascar, 60 private providers have been trained on the use of RDTs, representing over 9,000 fever cases managed at 52 private facilities between April and December 2014. During that time, testing rate increased from 69.2% to 92.7%. The overall QoC score rose from 38% in late 2014 to 71.4% in early 2015. This project represents the first time such a system has been set up to monitor performance in the private sector.

1348

DELIVERING SEASONAL MALARIA CHEMOPREVENTION TO CHILDREN UNDER TEN AT SCALE IN CENTRAL SENEGAL: COSTS AND COST DETERMINANTS

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The World Health Organization (WHO) recommends seasonal malaria chemoprevention (SMC) for children under 5 in areas of highly seasonal transmission. Extending the age range could protect older children. We analysed the incremental financial and economic costs of delivering SMC to 180,000 children aged 3 months to 10 years from a health service perspective in 2010, the third year of implementation in rural and semi-urban central Senegal. In addition to analysing project accounts, we prospectively collected data from a representative, systematic sample of 405 community health workers (CHWs); all 46 health posts; and all

four district headquarters by introducing questionnaires in advance and administering them after each of three monthly rounds of SMC. Key informant interviews were used to understand CHW opportunity costs and data sources were triangulated to arrive at best estimates. Coverage estimates reflect a comprehensive analysis of administrative records and a cross-sectional household survey. Increasing the age range for SMC from under-5s to under-10s virtually doubled the target population, however, it only increased the target number of households by 13%; high coverage was achieved in both groups. The financial cost over one malaria season at average monthly coverage of 90% was $234,462, $0.50 per monthly course administered, or $0.32 per resident (all ages) in the catchment area. The economic costs were 19% higher at $278,407 or $0.59 per course administered. The average cost per course administered varied widely between health posts, with the smallest incurring substantially higher costs. Key determinants of variation between health posts in the total and average costs of SMC delivery were analysed with ordinary least squares regression, but neither previous experience with SMC nor geographic variables were consistent determinants. At a financial cost per capita of 1% of Senegal's general government expenditure on health per capita, SMC may be affordable. Costs were lower than estimated and projected in previous, smaller studies conducted only in under-5s, indicating economies of scale and/or scope in an extended age range.

1349

IMPROVING TRAUMA CARE SYSTEMS IN EAST AFRICA: AVOIDING THE BANDAID APPROACH

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Trauma is the leading cause of death in the first four decades of life worldwide, with an annual mortality rate higher than that of HIV, malaria, and TB combined. This burden falls disproportionately on Low Income Countries (LICs), where the rate of deaths secondary to trauma is the highest in the world. In LICs, there is severely limited access to adequate trauma care due to inconsistent quality of care, inappropriate distribution of resources, inadequate training, and poor infrastructure. Solutions to this problem must address multiple levels of a fragmented health system, rather than any one aspect alone. The Kenya Trauma and Injury Program (KTIP) is the Innovative Canadians for Change (ICChange) approach to addressing the burden of trauma in LICs. KTIP is working to develop and implement a national trauma framework and build the necessary administrative and technical capacity to train, implement, oversee, certify, and support selected existing and new infrastructure in all facets of trauma care in Kenya. The goal of KTIP is to collaborate with local experts to develop a thorough approach to trauma care, including preinjury policy work, prehospital, hospital, and rehabilitation services, which would be supported by a national trauma registry, trauma Geographic Information System (GIS) and trauma education systems such as Advanced Trauma Life Support (ATLS). Firstly, an assessment of the incident data of trauma regionally and nationally will improve the decision making of which sites on the GIS can act as a trauma "hub." Secondly, infrastructure and clinic readiness surveys will be done to gain a better understanding of the capacity and feasibility of bringing a facility to a standard that is adequate for essential trauma care. Incorporating a national trauma registry will increase understanding of the epidemiology of trauma, guide policy development and the monitoring and evaluation of interventions such as training of clinic staff in the handling of trauma on trauma morbidity and mortality outcomes. Using the model of KTIP, this presentation will explore the strengths, risks, and criticisms of innovative trauma care in East Africa.

1350

EVOLUTION AND FUNCTION OF THE DUAL-AFFINITY AMINO ACID TRANSPORTER, AASLIF IN *AEDES AEGYPTI*

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Aedes aegypti is the major vector of dengue, yellow fever and Chikungunya viruses, which put hundreds of millions of people at risk each year in endemic areas. Blood feeding is required for both virus transmission and mosquito reproduction. Amino acid transporters play critical roles in sensing blood-meal nutrients and transporting building blocks for egg development. We analyzed the arrangement of SLC7-family transporter genes in ***Ae. aegypti*** genome and found that these genes are tightly clustered in a specific genomic region. Homology modeling revealed that putative substrate-interacting residues are strictly conserved among insect Slif orthologs. Electrophysiological studies uncovered that AaSlif is a dual-affinity amino acid transporter that functions in a sodium-dependent and -independent manner. This property allows AaSlif to function in a wide dynamic range of substrate concentrations (from micromolar to high milimolar for L-argenine) and allows mosquitoes to quickly react to varied nutritional environments they encounter during their life history. Our study shows that AaSlif is a high-value-target for the development of novel mosquito control strategies.

1351

EFFECTS OF DIAPAUSE ON THE EXCRETORY PHYSIOLOGY AND EXPRESSION OF AQUAPORINS IN THE WEST NILE VECTOR *CULEX PIPIENS*

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The northern house mosquito ***Culex pipiens*** is the primary vector of West Nile Virus in North America. Only female adults survive winter in a dormant phase called diapause. Infected female mosquitoes can serve as reservoirs for the pathogens to overwinter, leading to potential disease outbreaks in the following spring. Desiccation is one of the biggest physiological challenges for insects that over-winter. A previous study found that diapausing ***C. pipiens*** conserve water by increasing the accumulation of cuticular lipids, thereby reducing evaporative water loss. However, it is not known if ***C. pipiens*** modulates its excretory system during diapause. My study aims to fill this gap of knowledge. Malpighian tubules (MTs) are the primary excretory tissues of insects. Water transport across MTs is mediated by a family of membrane-bound water channels called aquaporins (AQPs). I have measured the whole animal excretory capacity and mRNA levels of five AQP genes in diapausing (D) and non-diapausing (ND) mosquitoes. The D mosquitoes exhibit a significantly lower excretory capacity than ND mosquitoes. Moreover, the expression levels of some AQP mRNAs were significantly lower in D mosquitoes compared to ND mosquitoes. The results provide new insights into the molecular physiology of water balance in diapausing mosquitoes, and may also validate AQPs as targets for developing novel vector control methods that disrupt mosquito diapause and their transmission of pathogens.

1352

OXIDATIVE STRESS "MANAGEMENT" IS ESSENTIAL FOR *ANOPHELES* SURVIVAL POST *PLASMODIUM* INFECTED BLOOD MEAL INGESTION

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Anopheles mosquitoes like other dipterans lack the flavoenzyme glutathione reductase (GR) of the GSH pathway and therefore utilize

instead a Thioredoxin (Trx) system for "stress management". ***Anopheles gambiae*** and ***Anopheles*** stephensi mosquitoes have been shown to regulate proteins of the Trx system to protect midgut epithelial cells against reactive oxygen/nitrogen species (ROS/RNS) associated with ***Plasmodium berghei*** infection. However, this mosquito vector-parasite combination is not natural and may not necessarily reflect human malaria transmission biology in the field. Despite its importance, a complete understanding of the Trx pathway at the molecular level is missing. We used an ***ex vivo*** assay to examine the Trx response pathway following midgut exposure to ROS/RNS by measuring both protein and transcript expression levels of Thioredoxin-1 (AgTrx-1) by western blot analysis and qRT-PCR, respectively. We observed that protein levels of AgTrx-1 increase in midgut epithelial cells exposed to increased concentrations of a ROS inducer, tert-Butyl hydroperoxide, and transcript levels of AgTrx-1 and other genes of the Trx system were also up-regulated. Since it has been shown that ***P. berghei*** induces midgut cell damage in An. stephensi and An. gambiae, we anticipated the observed Trx pathway gene up-regulation at 24 hours post-infection with ***P. berghei***. We then compared this spectrum of responses to oxidative stress in a more natural vector-parasite combination of An. gambiae-***P. falciparum***. Transcriptomics and proteomics profiles were consistent with cells undergoing extensive redox regulation. Since it is known that ***P. falciparum*** does not induce marked midgut destruction in An. gambiae, these data suggest that ***An. gambiae*** must regulate ROS/RNS in response to an infected bloodmeal, that Trx system is crucial to this regulation, and that the cause of ROS/RNS is independent of midgut cell damage or apoptosis. We are now exploring approaches to perturb mosquito Trx regulation, which we predict will lead to unmanageable levels of ROS/RNS exposure to the parasite in the midgut, yet still allowing the mosquito to survive the dysregulation.

1353

A NOVEL SUPPLEMENT TO REDUCE *PLASMODIUM* DISEASE SEVERITY AND TRANSMISSION

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Nearly half of the world's population is at risk for malaria. Increased drug and insecticide resistance have demonstrated the need for novel methods of control, including treatments with dual therapeutic and transmission-blocking properties. We have established that a natural compound with roles metabolism and immunity in a wide variety of eukaryotes has significant therapeutic benefit in malaria. In our studies, oral supplementation of mice significantly reduced Plasmodium yoelii parasitemia and gametocytemia. Addition of the compound to Plasmodium falciparum-infected blood also reduced infection prevalence in exposed mosquitoes. Preliminary data indicate that this compound is perceived as a signal in the mosquito but not the parasite and thereby alters mosquito physiology to indirectly affect parasite development. Our data suggest that this natural compound affects the physiology of mammalian and mosquito hosts to reduce malaria parasite infection and transmission.

1354

MOSQUITOCIDAL PROPERTIES OF IGG AGAINST ESTABLISHED INSECTICIDE TARGETS IN THREE MOSQUITO DISEASE VECTORS

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Mosquito-borne diseases account for an estimated 1,434,000 deaths annually and 60,056,000 disability adjusted life years. The most successful strategy to control disease transmission is by targeting mosquito vectors through the use of chemical insecticides. As an alternative to chemical insecticides, we tested the effects of purified IgG against the extracellular domain of the glutamate-gated chloride channel (GluCl; the target of the insecticide ivermectin) on the survivorship of three mosquito disease vectors, ***Anopheles gambiae*** s.s., ***Aedes aegypti*** and ***Culex tarsalis***. Anti-AgGluCl IgG mixed in a single blood meal significantly reduced the survivorship of ***An. gambiae*** (LC_{50}=2.82mg/mL), as did serial blood feeds containing 1/10th of the LC_{50}. Anti-AgGluCl IgG blood meals did not affect the survivorship of ***A. aegypti*** or ***C. tarsalis***. However, injection of anti-AgGluCl IgG into the hemocoel equally reduced the survivorship of all three mosquitoes. Dot blot measurements of anti-AgGluCl IgG in the mosquito hemolymph following blood feeding revealed anti-AgGluCl IgG only in the hemolymph of ***An. gambiae***, suggesting that anti-AgGluCl IgG sensitivity requires antibody diffusion across the midgut. To analyze anti-AgGluCl IgG's mode of action, we fed ***An. gambiae*** blood meals containing both anti-AgGluCl IgG and ivermectin (IVM, GluCl agonist). Anti-AgGluCl IgG attenuated the mosquitocidal effects of IVM, suggesting that it acts as a GluCl antagonist. Our proof of concept showing the mosquitocidal properties of blood meals containing IgG against the target of IVM has prompted research into the mosquitocidal effects of IgGs against other insecticide targets. We have designed antibodies against the GABA-gated chloride channel (target of chlorinated cyclodienes) and the voltage-gated sodium channel (target of pyrethroids). Initial experiments suggest that blood meals containing IgGs against these insecticide targets also have mosquitocidal properties. Future studies will quantify the toxicity of these IgGs, both individually and in combination, as well as the mosquitocidal effects of blood meals taken directly from immunized cattle.

1355

IDENTIFYING SEMINAL PROTEINS THAT INDUCE REFRACTORY BEHAVIOR IN MATED *AEDES AEGYPTI* FEMALES

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Mosquito mating biology has important consequences for vector-borne disease transmission and control. The transfer of male seminal fluid to female mosquitoes during mating initiates a suite of physiological and behavioral changes in the female. By understanding the molecules responsible for these changes in the mated female we can identify potential targets for novel control strategies of mosquitoes, such as the dengue vector ***Aedes aegypti***. Injecting female ***Ae. aegypti*** with a homogenate of male seminal fluid proteins, extracted from the accessory gland tissue, causes the female to lay eggs sooner, lay more eggs, take more blood meals, become refractory to mating and live longer than virgin females. We narrowed the range of refractory-behavior-inducing candidates in ***Ae. aegypti*** to select proteins ranging from 6-30 kDa. We present this work, as well as our studies using RNAi to assess the functional significance of individual candidate proteins on female refractory behavior.

1356

THE ROLE OF THE MOSQUITO IMMUNE SYSTEM AND PFS47 IN THE ADAPTATION OF *PLASMODIUM FALCIPARUM* TO DIFFERENT ANOPHELINE VECTORS

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Plasmodium falciparum, the most fatal of the human malaria parasites, originated in Africa. It was dispersed relatively recently to other continents by humans, where it encountered diverse anopheline mosquito species. In order to obtain evidence of natural selection during the adaptation of ***P. falciparum*** to different vectors, we studied the compatibility between major malaria vectors from Africa (***Anopheles gambiae***), Southeast Asia (***A. dirus***) and the Americas (***A. albimanus***) with ***P. falciparum*** isolates collected in those regions. ***P. falciparum*** isolates from a given geographic region presented higher compatibility (i.e. higher infection intensity and

prevalence) with the anopheline of the same region, suggesting that *P. falciparum* underwent natural selection while adapting to different vectors. We tested the role that the mosquito immune system and the parasite gene Pfs47 may have had in the adaptation of *P. falciparum* to evolutionary distant anophelines. Disruption of the mosquito complement-like system by RNAi rescued infections in less compatible anopheline-parasite combinations. We have shown previously that *P. falciparum* can evade the complement-like system of some anophelines through its Pfs47 gene, which shows signatures of positive selection and a strong continent-level geographic population structure. Replacement of the Pfs47 haplotype in an African *P. falciparum* line with Pfs47 haplotypes that are frequent in other geographic regions was sufficient to change the compatibility of *P. falciparum* to evolutionarily diverse anopheline vectors by allowing the parasite to evade the mosquito complement-like system. The mosquito immune system appears to have been an important barrier for adaptation of *P. falciparum* to different anopheline species through selection of Pfs47, and this may have influenced the parasite's population structure and malaria epidemiology.

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DNA BARCODING OF ANOPHELINE MOSQUITOES FROM REMOTE MALARIA-ENDEMIC AREAS IN LORETO, NORTHERN PERUVIAN AMAZON

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Loreto is the most affected malaria endemic region in Peru; yet studies characterizing *Anopheles* species in high incidence areas are limited. Thus, we collected anophelines in the highest malaria risk areas in Loreto from February-May 2014. *Anopheles darlingi* was the most abundant species (1,649 adults; 95%) in nine communities surveyed in eastern Loreto; yet it was completely absent in seven communities in western Loreto where *An. benarrochi* was the most abundant species (224 adults; 96%). To further confirm our taxonomical identification results, we compared the morphological identification of a subset of specimens with their differentiation based on mtDNA cytochrome C oxidase I (*COI*) barcode. We analyzed 222 specimens from eastern Loreto comprising 5 species (*An. darlingi*, *An.* nr. *forattinii*, *An. mattogrossensis*, *An. nuneztovari*, *An. oswaldoi* s.l.) and unidentified *Anopheles*; and 58 specimens from western Loreto identified as *An. benarrochi*. *COI* sequences were compared with expertly identified specimens in the Mosquito Barcoding Initiative databank (Barcode of Life Database). Almost all *An. darlingi* specimens (99%) were molecularly verified; only one specimen was identified as *An. dunhami*. All *An.* nr. *forattinii* were confirmed as *An. forattinii; An. mattogrossensis* specimens were identified as *An. mattogrossensis* 1 (20%) and *An. mattogrossensis* 2 (80%). *Anopheles nuneztovari* and *An. oswaldoi* s.l. were all identified as *An. dunhami* except for one *An. oswaldoi* s.l. specimen identified as *An*. sp. nr. *konderi*. Unidentified *Anopheles* specimens (45) comprised *An. darlingi* (71%), *An. dunhami* (11%), *An. forattinii* (11%), *An. mattogrossensis* 2 (4%) and *An. mattogrossensis* 1 (2%). All *An. benarrochi* were identified as *An. benarrochi* B. We report two new country records for *Anopheles* in Peru: *An. mattogrossensis* 1 and *An. mattogrossensis* 2. Our results corroborate that *An. darlingi* is the dominant anopheline species and primary malaria vector in eastern Loreto, yet not found in western Loreto where *An. benarrochi* B is dominant. *COI*-based DNA barcoding proved useful in verifying and discovering anopheline species in the Peruvian Amazon.

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SILENCING OF XANTHINE DEHYDROGENASE (XDH1) SEVERALLY AFFECTS THE SURVIVAL OF BLOOD-FED *AEDES AEGYPTI* MOSQUITOES

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Xanthine dehydrogenase (XDH) is involved in uric acid synthesis in mosquitoes. This nitrogen compound serves as a depot for excess ammonia produced during the digestion of blood meal. Uric acid can be excreted directly or metabolized to allantoin, allantoic acid and urea through an amphibian-like uricolytic pathway. Surprisingly, we have recently observed that mosquitoes with alanine aminotransferase deficiency increase XDH1 transcript levels in mosquito tissues, and store provisionally high uric acid concentrations in the midgut as a strategy for survival. To better understand this metabolic regulation, we began to explore the effect of XDH disruption in *A. aegypti*. Two genes encoding XDH (XDH1 and XDH2) are present in the genome of *A. aegypti.* We analyzed XDH1 and XDH2 gene and protein expression by qRT-PCR and western blotting analysis in tissues from sugar- and blood-fed females. A differential XDH1 and XDH2 gene and protein expression was observed in mosquito tissues dissected during a time course. Next, we exposed females to blood meals supplemented with high doses of allopurinol. The effect of injecting dsRNA against XDH1 or XDH2 was also evaluated. The disruption of XDH by high allopurinol concentrations, as well as the silencing of XDH1 by RNAi caused a high mortality in *A. aegyti*. However, no mortality was registered in those blood-fed mosquitoes injected with dsRNA against XDH2 when compared with dsRNA-firefly luciferase-injected control. Surviving mosquitoes treated with allopurinol or injected with dsRNA-XDH1 showed a significant disruption in blood digestion, excretion and reproduction. Western blotting analysis indicated that surviving dsRNA-XDH1-injected mosquitoes showed a persistence of the major serine proteases in the midgut at 48 h after blood feeding and a significantly reduction in the uptake of vitellogenin by the ovaries of females with XDH1 deficiency. These data demonstrate that XDH1 plays a critical role during blood meal digestion and that nitrogen metabolism in blood-fed mosquitoes is much more complex than previously thought.

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AUTOPHAGY AS A FACTOR FOR DENGUE INFECTION IN THE VECTOR MOSQUITO, *AEDES AEGYPTI*

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The *Aedes aegypti* mosquito is the primary vector for transmitting dengue virus (DENV). DENV causes dengue fever, resulting in over 500,000 hospitalizations people worldwide annually. Programmed cell death (PCD) is often induced in virus-infected cells to limit infection in the host. In *Ae. aegypti*, PCD is activated by an initiator caspase, Dronc, which cleaves and activates other caspases including Casps7, responsible for activation of targets further downstream. However, our previous study found that upon knockdown of *Dronc*, DENV-refractory females showed decreased disseminated infection, and in those mosquitoes infected, lower titers of DENV-2. Further analysis revealed that Dronc also has a novel role in controlling a cellular recycling process, known as autophagy. During this process, the autophagy-related protein 8 (ATG8) is lipidated to double-membrane vesicles, known as autophagosomes. These vesicles surround cellular cargo and then fuse with lysosomes to degrade any contents. Using western blot analysis, we observed upregulation and lipidation of ATG8 following a blood meal, not only in fat body as previously shown, but also in midgut tissue. DENV must infect the midgut epithelium to become transmissible. Silencing *Dronc* and *Casps7*, resulted in decreased activation and lipidation of ATG8, suggesting that these caspases can control autophagy. Furthermore, to determine whether autophagy was

crucial to DENV-2 infection, we silenced several ATG genes involved in initiation and progression of autophagy. Preliminary silencing showed decreased levels of DENV genomic RNA detectable by qPCR, suggesting autophagy may influence DENV-2 infection. We also begin evaluation of the potential for this pathway to be targeted by chemical inhibition in order to inhibit DENV infection of the vector host.

1360

PROTEOMIC ANALYSIS OF MOSQUITO BLOOD CELLS REVEALS DISTINCT PHAGOCYTIC HEMOCYTE PROFILES AND IMMUNE ACTIVATION IN THE ABSENCE OF PARASITE CHALLENGE

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Insect blood cells (or hemocytes) are essential components of the innate immune response. Implicated in both cellular and humoral responses, hemocytes mediate pathogen clearance by phagocytosis or encapsulation and are responsible for the production of soluble immune factors. In addition, recent evidence suggests that hemocytes play critical roles in malaria parasite survival. However, despite their importance, critical aspects of their biology in mosquitoes remain uncharacterized. Using a novel method of enrichment, we isolated the phagocytic subset of hemocytes known as granulocytes (phagocytes) in the African malaria vector, ***Anopheles gambiae***, and quantified their proteomic profiles during homeostasis, blood-feeding, and following malaria parasite challenge. Integrating existing hemocyte transcriptional profiles, we demonstrate a high degree of similarity between our proteomic data and provide new information regarding hemocyte gene regulation. We observe that phagocytosis, blood-feeding, and ***Plasmodium falciparum*** infection promote dramatic shifts in phagocytic granulocyte protein expression indicative of changes in feeding status, cellular proliferation, and innate immune response priming. Of interest, we identified large numbers of hemocyte immune proteins induced in response to blood feeding alone, suggesting that these phagocytic blood cells may play an integral role in immune priming prior to pathogen challenge. Current experiments aim to address the role of candidate genes in blood cell proliferation/differentiation and ***Plasmodium*** development using dsRNA-mediated silencing. Together, these data provide a rich resource for hemocyte biology in ***An. gambiae*** and provide new insight into their biological roles that will serve as a foundation for their future study.

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DISSECTING THE ROLE OF MALE ACCESSORY GLAND TRANSCRIPTION FACTORS IN SHAPING THE REPRODUCTIVE SUCCESS OF *ANOPHELES GAMBIAE*

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In the fight against malaria, control strategies aimed at inducing sexual sterility in ***Anopheles*** populations are an attractive alternative to the use of insecticides due to evolution of resistance in mosquitoes. However, the development of these strategies is hampered by a profound lack of knowledge regarding many elements of ***Anopheles*** mating ecology. During mating in ***An. gambiae***, males synthesize a gelatinous, rod-shaped mating plug in their reproductive accessory glands (MAGs) that contains a complex cocktail of seminal fluid proteins, steroid hormones and lipids. Female receipt of this plug induces a suite of physiological and behavioral changes including increased oogenesis, stimulation of oviposition and refractoriness to further mating attempts. While our lab group has recently demonstrated that these changes are mediated in part by the steroid hormone 20-hydroxyecdysone, the role of additional ejaculate components in activating this female post mating response (PMR) remains largely unknown. Here we present evidence suggesting that highly expressed MAG transcription factors (TF) are a critical part of female PMR. Given their regulatory role in the complex protein expression network of MAGs, the targeting of TFs allows for a simplified identification of candidate genes critical for ***An. gambiae*** reproductive success. To determine the role these TFs play, we targeted them in males via RNAi mediated knockdown, and looked for post mating reproductive phenotypes in mated females. Compared to their counterparts mated to controls, females mated to TF knockdown males experienced a significant increase in egg infertility. Interestingly, this increase in infertility did not emerge until the second gonotrophic cycle, suggesting a possible time dependent impairment of sperm activation/storage mechanisms due to TF mediated alterations of male ejaculate components. Further characterization of this striking phenotype through transcriptional and morphological analyses is ongoing. This work represents a first step into identifying additional ejaculate components underlying the reproductive fitness of a major disease vector.

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HEMOLYSIN GENE EXPRESSION UNDER VARIABLE IRON REGIMES OF *ELIZABETHKINGIA ANOPHELIS*, A COMMENSAL BACTERIUM OF THE *ANOPHELES* MIDGUT

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Elizabethkingia anophelis predominates in the bacterial community in the ***Anopheles*** mosquito midgut and its presence may influence important mosquito physiological processes. Owing to hemolytic activity, ***E. anophelis*** may participate in erythrocytic lysis in in the mosquito midgut. The highly variable mosquito midgut conditions, particularly extreme fluxes in iron concentration and temperature before and after a blood meal, require that ***E. anophelis*** withstand stresses and efficiently regulate gene expression. However, iron uptake mechanisms such as sense and scavenge iron processes in this species and other related bacteria in the phylum Bacteroidetes are poorly known. Therefore, we investigated responses of ***E. anophelis*** responses to environmental iron status and explored the molecular mechanisms that these bacteria utilize to cope with iron alternation using high throughput, deep sequencing of mRNA transcripts (RNAseq). The differential regulated genes under low and high iron were analyzed and compared. A sensitive reporter analysis based on NanoLuc was utilized for studying representative gene regulation (such as hemolysins). Expression of hemolysin genes was significantly repressed when ***E. anophelis*** was grown under the iron-replete and high temperature (37 degrees C). ***E. anophelis*** cells increased more than three-fold in blood-fed mosquito than those in sucrose-fed ones. Furthermore, the hemolysin gene expression was down-regulated by the blood meal, indicating that it was actively involved in erythrocyte lysis ***in vivo***. Our results provide new insights into how ***E. anophelis*** responds and adapts to the midgut environment and reveals new functions impacting its mosquito host.

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GENES ASSOCIATED WITH REFRACTORINESS OR SUSCEPTIBILITY TO DENGUE-2 VIRUS INFECTION IN *AEDES AEGYPTI* FROM COLOMBIA, USING COMPARATIVE MICROARRAY-BASED ANALYSIS

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In recent years there has been considerable progress in our knowledge of dengue, particularly in vaccine development, the characterization of the immune responses and molecular properties of the virus. However, there are still many aspects that must be investigated in terms of its transmission by the principal vector, ***Aedes aegypti***. We have elucidates Dengue virus-vector relationships, specifically the innate immune response of ***A. aegypti*** to dengue virus infection. For this, we identified and selected two strains of ***A. aegypti***, from Cali, Colombia with different susceptibility to dengue infection: Susceptible (Cali-S, 96%) and refractory with midgut infection barrier (Cali-MIB, 50%).We used micro arrays to compare the global gene expression of the midguts of Cali-S and Cali-MIB after ingestion of sugar, a bloodmeal, or a bloodmeal containing Dengue-2 virus. A total expression of 3761 genes were observed. A differential expression between the two strains exposed to DENv-2 included genes in different functional groups: immunity, metabolism, proteolysis, redox, replication, transport, and unknown function. We have selected 12 genes to evaluate their differential expression at different time points (0, 8, 24, 36 and 48h) by qPCR. The qPCR analysis confirmed the predominance of down regulated genes in Cali-MIB in comparison with Cali-S strain, observed in the microarray at 30 hours post infection (h.p.i). However, early in the infection (at 0, 8 and 24 h.p.i), several of this genes were up regulated suggesting an early response to eliminate the virus in refractory strain. Finally, we used RNAi assays to evaluated the role of 4 genes from 12 selected before (Gram Negative Binding Protein-GNBP (AAEL009176), Niemann-Pick Type C-2 (AAEL015136), Galectin (AAEL009842) and Cathepsin-b (AAEL007585)) in DENv infection. We observed that the silencing of down regulated genes, GNBP, Cathepsin-b and Galectin, changed the phenotype of Cali-S strain from 96% to 8, 20 and 12% respectively. The up regulated gene, Niemann, changed the phenotype of Cali-BIM strain from 50% to 18%. This study validate the use of our field derived strains as a biological model.

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TARGETED DELIVERY OF MOLECULAR CARGO INTO THE MOSQUITO GERMLINE

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Genetic manipulation is a powerful technique for addressing research questions in arthropods of medical importance. Current approaches rely upon delivering DNA or endonucleases to preblastoderm embryos via embryonic microinjection. However, embryonic microinjection is technically challenging, is limited to a small number of arthropod taxa, and is inefficient even in optimized species. As such, there is a critical need to develop methods for arthropod genetic manipulation that are simple, accessible for many researchers and generally compatible for a large variety of arthropod species. During oogenesis, insects transfer molecules to developing oocytes by receptor-mediated endocytosis (RME). We are developing tools exploiting this phenomenon to specifically target DNA and protein cargo into the mosquito germline for heritable modification of the chromosomal genetic sequence. We will discuss progress in optimizing this technique and in transferring the technology to multiple mosquito species.

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STIMULATION OF A SERINE PROTEASE TARGETING THE LRIM1/APL1C COMPLEX REVEALS SPECIFICITY IN COMPLEMENT-LIKE PATHWAY ACTIVATION IN *ANOPHELES GAMBIAE*

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The complement-like pathway of the African malaria mosquito ***Anopheles gambiae*** provides protection against infection by diverse pathogens including bacteria, fungi, and rodent and human malaria parasites. Detailed analysis of complement activation in response to the Gram-negative bacterium ***Escherichia coli*** and ***Plasmodium*** showed that activation of the complement-like pathway is triggered by protein complex containing $TEP1_{cut}$, a processed form of the C3-like protein TEP1, and the leucine-rich repeat proteins LRIM1 and APL1C. Binding of $TEP1_{cut}$ results in the activation of a TEP1 convertase through the recruitment of a non-catalytic serine protease SPCLIP1. It is unknown how other pathogens are recognized by the mosquito's immune responses and if activation of the TEP1 convertase is a general requirement for eliminating all infections. In this study, biochemical and functional assays were performed to analyze the complement-like pathway responses to the Gram-positive bacterium ***Staphyloccocus aureus***. Western blot analysis revealed that like ***E. coli***, ***S. aureus*** also activates the TEP1 convertase. However, striking differences in the initial immune reactions promoted by the different Gram type bacteria were observed. In particular, it was found that ***E. coli*** specifically activates a protease that cleaves the C-terminus of APL1C. This study shows initial pathogen-specific reactions in the mosquito complement-like pathway. These unique responses converge on the same core mechanism involving the TEP1 convertase triggering pathogen neutralization.

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EVALUATION OF INNATE IMMUNE RESPONSE MECHANISMS ASSOCIATED WITH SUSCEPTIBILITY TO DENGUE VIRUS IN DIFFERENT FIELD POPULATIONS OF *AEDES AEGYPTI*

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Dengue is an infectious viral disease transmitted by mosquito vectors, for which there is no available vaccine or specific medical treatment. The main vector of dengue virus (vD) is ***Aedes aegypti***, a mosquito species extremely well adapted to the human urban environment. The ability of ***Ae. aegypti*** populations to become infected and transmit the virus (vector competence-VC) varies among populations and depends on genetic and environmental characteristics. Understanding the genetic basis of VC may help to identify potential targets and strategies to inhibit virus replication and transmission. Preliminary studies on differential expression in D-2 selected strains from field collected individuals in Cali, susceptible (Cali-S) and refractory (Cali-MIB), suggest that immune response genes associated with apoptosis play a role in the virus replication. This study evaluated if VC of the select strains is similar compared to the other serotypes. And assessed if the molecular mechanisms identified in select strains are a characteristic of field populations. The VC of the selected strains compared to the 4 virus serotypes was measured by artificial infection and immunofluorescence. The VC of ***Ae. aegypti*** collected from 6 locations in Cali was measured to D-2. Populations with different VC were selected to evaluate the expression by qPCR of Dronc, Caspase-16, Cathepsin-B and Niemann genes at 0, 24, 36, 48h post-infection. No statistically significant differences in the VC of the selected strains against the four serotypes

were observed. The mosquitoes collected in Cali showed differences on VC . Differential expression of the evaluated genes were observed among the evaluated times. The most refractory strain showed significant differences in gene expression between the treatments blood Vs blood +virus especially in Caspase -16.

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BASELINE SUSCEPTIBILITY OF *LUTZOMYIA VERRUCARUM* COLLECTED IN ANCASH, PERU TO THREE CLASSES OF INSECTICIDES BY CDC BOTTLE BIOASSAY

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Lutzomyia verrucarum is a vector of cutaneous leishmaniasis and bartonellosis in the Peruvian Andes. Since no effective vaccines exist for either disease, reduction of the burden of these diseases is accomplished through sand fly vector control using insecticides applied by thermal fog or Indoor Residual Spraying; however, there is no knowledge of susceptibility of sand flies, including ***Lu. verrucarum***, to commonly-used insecticides in Peru. The objective of this study was to determine the baseline susceptibility of ***Lu. verrucarum*** to three classes of insecticides using the CDC bottle bioassay method. Sand flies were manually collected with mouth aspirators in resting sites in Huichi and Choquechaca (Caraz, Peru) where bartonellosis and cutaneous leishmaniasis are endemic. Field-collected ***Lu. verrucarum*** adults were transferred live to the insectary; blood meals were provided to unengorged females using membrane feeding. Wild and artificially fed, blood-engorged females were individually placed into oviposition containers to obtain F1 progeny. Unfed, 3-5 day-old F1 and wild adult females were assayed with these three classes of technical-grade insecticides diluted in 100% ethanol: two pyrethroids, one organophosphate, and one organochlorine. On average, 23 females/bottle were tested and a minimum of three replicates/insecticide were performed. Diagnostic dosages (µg) standardized for ***Aedes aegypti*** by CDC (2010) were adapted for sand flies. Mortality was 99% for wild females from Huichi for deltamethrin (10 µg/40 min), permethrin (15 µg/30 min), fenitrothion (50 µg/40 min), and DDT (75 µg/30 min). For wild and F1 females from Choquechaca, mortality was 99% percent for deltamethrin (10 µg/30 min), permethrin (15 µg/30 min), fenitrothion (50 µg/40 min), and DDT (75 µg/30 min). Sand flies from both field sites in were susceptible to the three classes of insecticides tested, information which is critical to establish baseline insecticide susceptibility for management of ***Lu. verrucarum*** in this region. Our results show that the CDC bottle bioassay is a useful method to establish baseline insecticide susceptibility in sand flies in Peru

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EFFECT OF SPATIAL REPELLENTS ON DENGUE VECTOR ATTRACTANCY BEHAVIOR

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The ***Aedes aegypti*** mosquito is considered to be the principal vector of Dengue virus (DENV), which causes dengue fever, an arthropod-borne disease of global burden with incidence growing to estimates of approximately 50-100 million infections worldwide annually. Similarly, the expansion of ***Aedes albopictus*** has increased the risk of both DENV and Chikungunya transmission in temperate zones. Although efforts are underway for development of a DENV vaccine, current prevention relies on vector control through use of indoor and outdoor application of insecticides, specifically pyrethroids, through fogging and/or spray applications. However, pyrethroids that are characterized as spatial repellents – those that are highly volatile at ambient temperatures – have received interest as a novel delivery system for adult vector control. Understanding the behavioral effects these volatile pyrethroids have on target mosquito species will be critical to understanding the overall impact on vector populations and therefore expectations of efficacy against DENV transmission. This includes attraction to oviposition sites, which will influence next generation population densities. A significant decrease in ***Aedes spp.*** attractancy to potential oviposition sites after repellent exposure would indicate that there is considerable probability of impacting the overall vector population, human-mosquito contact and DENV transmission over time. This study quantified baseline levels of gravid ***Ae. aegypti and Ae. albopictus*** attractancy to oviposition containers in the laboratory using a 'sticky-screen' bioassay and established bacterial infusions, then repeated experiments following exposure of test cohorts to the volatile pyrethroid transfluthrin – a chemical widely used in mosquito coils and other household control products. Findings from this study will be used to begin defining implications of using spatial repellents for broader vector population effects.

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QUANTIFYING LLIN BIOEFFICACY: CAN COLORIMETRIC FIELD TEST (CFT) REPLACE THE WHO CONE BIOASSAY TEST?

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Under normal use, long-lasting insecticidal (mosquito) nets (LLIN) are expected to last up to three to five years. This is now recognized as an estimate since significant variation in durability has been observed. Programs implementing LLIN interventions are encouraged to conduct field monitoring of indicators such as LLIN survival, LLIN fabric integrity and LLIN insecticidal activity or bio-efficacy, to assess the actual rate of LLIN durability following distribution and use. World Health Organization (WHO) guidance recommends the use of a bioassay, the WHO cone test, to monitor bio-efficacy of the insecticide on LLINs. However, cone test accuracy depends on the capacity to routinely produce a high number of physiologically-uniform and insecticide-susceptible female mosquitoes, a challenging task. A non-mosquito alternative for measuring insecticide levels on the surface of a net is desirable. One possibility is the Colorimetric Field Test (CFT), a chemical method to estimate surface concentration of deltamethrin insecticide on LLINs. We used the CFT on 21 LLINs collected from the field in Madagascar, and compared results with standard cone test outcomes (five cone test positions / LLIN tested). By comparing classical bioassay and CFT results, we determined that CFT can distinguish between a LLIN with 'acceptable bio-efficacy' based on cone test criteria (mosquito mortality > 80%) and a LLIN scored as 'not acceptable' (mortality <80%). Using a CFT cut off value of 0.35 µg / sample (equivalent to 14% of deltamethrin content of a new net with a concentration of 55 mg/m2) we were able to correctly predict the WHO cone test results for 20/21 LLINs included in the comparison. Test accuracy was evaluated using Receiver Operating Characteristic (ROC) curve analysis. The area under the ROC curve was 0.954 and assay sensitivity and specificity were 0.92 and 1.00 respectively, indicating the CFT to be an excellent predictor of LLIN insecticidal content. We believe that the CFT is an easier and more economical option for programs seeking to monitor LLIN insecticidal activity loss as it allows for a larger number of nets to be tested using lower testing costs.

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INDOOR RESIDUAL SPRAYING (IRS) OF CLOTHIANIDIN IN COMBINATION WITH DELTAMETHRIN OR ALONE FOR THE CONTROL OF PYRETHROID-RESISTANT *ANOPHELES GAMBIAE* IN EXPERIMENTAL HUTS IN BENIN AND CÔTE D'IVOIRE

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One way to manage pyrethroid resistance in malaria vectors is to combine insecticides with unrelated modes of action so that insects resistant to one insecticide will succumb to the other. For IRS, identifying residual insecticides that can cover long transmission seasons from a single application is a research priority. The neonicotinoid, clothianidin represents a class of chemistry new to public health. IRS with Clothianidin alone or in a mixture with deltamethrin was evaluated in experimental huts in Benin and Côte d'Ivoire where ***Anopheles gambiae*** populations are resistant to pyrethroids. Monthly bioassays were carried out on substrates of mud, cement and plywood. On top of immediate mortality, clothianidin induced delayed mortality ***of An. gambiae;*** taking up to 120 hours to show full effect. Mortality among mosquitoes entering huts in Benin was 37% after 24h but reached 91% after 120h. Clothianidin applied alone or in mixture with deltamethrin gave high mortality (>80%) of resistant ***An. gambiae*** for ~9 months in cement and mud huts in Benin but produced marginal control (<40%) of pyrethroid-resistant ***An. gambiae*** in Côte d'Ivoire. In both areas, deltamethrin alone failed to achieve control of ***Anopheles*** mosquitoes entering the huts. Cone bioassays showed improved residual activity of the mixture over either insecticide applied alone in Côte d'Ivoire: the mixture killed >95% of both susceptible and resistant ***An. gambiae*** over 10 months whereas a drop below the WHO threshold (80% mortality) was observed just 2 months post spray with the single components. The differences between cone assay and hut observations will be subject for further investigation. Clothianidin shows promise as a new mode of action for malaria vector control when delayed mortality effects are taken into account. A mixture of clothianidin with deltamethrin showed very good activity in standard WHO Cone bioassays independent of any delayed mortality effects. The introduction of this new mode of action could be a timely addition to the limited portfolio of IRS insecticides.

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THE EFFICACY OF CHLORFENAPYR, DELIVERED AS A LONG-LASTING INDOOR RESIDUAL SPRAY, AGAINST PYRETHROID-RESISTANT *ANOPHELES ARABIENSIS* IN NORTHERN TANZANIA

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There is an urgent need for insecticides with novel properties and modes of action to combat pyrethroid-resistant mosquitoes. Additionally, there is a serious shortage of insecticides for indoor residual spraying that have a duration of effective action of 6 months or greater. Chlorfenapyr (CFP) is a pyrrole insecticide, which unlike neurotoxic pyrethroids, has a distinct mode of action targeting oxidative pathways in insect mitochondria and disrupting cellular respiration. Highly stable CFP formulations have been developed with an anticipated duration of efficacy of at least 6 months. In a succession of experimental hut trials and complementary laboratory bioassays the duration of the bioefficacy of CFP was assessed on both concrete and plywood-treated surfaces, and contrasted with the performance of a commercial formulation of Alphacypermethrin. Whilst the hut trials are on-going, at 3 months the mortality of unfed ***An. arabiensis*** caught from the CFP-sprayed hut is >80% 72h post-exposure, and in the laboratory the same level of mortality is observed after 6 months for several different CFP formulations. The results of the hut trials at 9 months will be presented and interpreted in the context of the distinctive house-entering, resting and exiting behaviour (and therefore insecticide exposure time) of ***An. arabiensis*** in the area.

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HIGH LEVELS OF *ANOPHELES GAMBIAE* S.L. RESISTANCE TO PYRETHROIDS, DDT AND MALATHION AND INDICATIONS OF REDUCED SUSCEPTIBILITY TO CARBAMATES IN OROMIA REGION - ETHIOPIA

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The insecticide susceptibility status of ***Anopheles gambiae*** s.l. (presumably ***An. arabiensis,*** the major malaria vector in Ethiopia) was assessed in 8 in Oromia region where indoor residual spraying (IRS) operations are supported by the President's Malaria Initiative. Mosquitoes were exposed to diagnostic dosages of DDT, deltamethrin, lambda-cyhalothrin, permethrin, alpha-cypermethrin, etofenprox, fenitrothion, pirimiphos-methyl, malathion, bendiocarb and propoxur using the standard WHO tube tests. Tests were conducted on 2- to 3-day-old, non-blood fed, adult female mosquitoes reared from larvae and pupae collected from different breeding habitats. Based on the mortality counts after a 24-hour holding period, the vector population was classified as susceptible, possibly resistant or resistant to an insecticide following the WHO criteria. Resistance to DDT (3-16% mortality), deltamethrin (12-51%), lambda-cyhalothrin (4.3-25.7%), permethrin (2.9-66%), alpha-cypermethrin (5-61%), etofenprox (8.7-55%), and malathion (26-93.7%) was reported in all eight sites. The vector was susceptible to bendiocarb in 7 of 8 sites and resistant in one site (mortality 87%). Propoxur resistance was also recorded in one site with a 75% mortality rate. The mortality rate to fenitrothion was 97% (possible resistance) in one site and ranged from 98 to 100% (susceptible) in the remaining sites. ***An. gambiae*** s.l. from all eight sites were susceptible to pirimiphos-methyl with a mortality rate of 100%. The findings of the study show that (1) the Ethiopian IRS program now has limited options of insecticides for public health use; (2) rational use of available insecticides and implementation of an appropriate insecticide resistance management strategy is critical; (3) a strong system for monitoring vector resistance to the insecticides in use should be part of the vector control program. The information generated from this study will contribute to the development of an insecticide management strategy and selection of alternative options for the Ethiopian IRS program. Overall, the study indicates that new insecticides are urgently needed for IRS.

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INCREASE LEVEL OF PYRETHROID AND DDT RESISTANCE IN THE MAJOR MALARIA VECTOR *ANOPHELES FUNESTUS* IN NORTHERN SENEGAL

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Insecticide resistance in ***Anopheles funestus***, one of the main malaria vectors, is threatening malaria control in Africa, notably in Senegal. Evaluation of the resistance profile and the mechanisms implicated are crucial to implement suitable resistance management strategies. Here, we explored the dynamic and evolution of insecticide resistance profile of ***Anopheles funestus*** populations in Northern Senegal and investigated the molecular basis of resistance observed. Insecticide susceptibility assays were carried out using 2-5 day old F1 adults generated from indoor-collected blood-fed female of ***An. funestus*** from Northern Senegal in 2014 and compared to 2011 samples. WHO bioassays revealed an increased level of pyrethroid and DDT resistance in ***Anopheles funestus*** population in 2014 with mortality to permethrin and DDT of 68.75% and 62% respectively compared to 91.19% and 83.36% in 2011. In addition, insecticide resistance was also observed to lambdacyhalothrin (89.82% mortality) and deltamethrin (88.23% mortality). However, this population remains fully susceptible to bendiocarb, dieldrin and malathion. Our results also show a reduced efficacy of the insecticide-treated bed net Permanet 2.0 (76%) on this ***An. funestus*** population whereas full efficacy was observed with Net Permanet 3.0, Olyset Net and Olyset Plus. The synergist assay with PBO suggests that while the cytochrome P450s genes have little involvement in DDT resistance they play the major role in the pyrethroid resistance, as a full recovery of susceptibility was observed after PBO pre-exposure. The increase level of pyrethroid and DDT resistance in Senegalese ***An. funestus*** populations represents a challenge to the control of this vector. However, the observed carbamate and organophosphate susceptibility offers alternative solutions for resistance management. Further molecular characterizations are on going to characterise the molecular mechanisms driving this increased resistance.

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EFFICACY OF A PYRIPROXYFEN-AUTODISSEMINATION STATION AGAINST *AEDES ALBOPICTUS* IN RESIDENTIAL AREAS

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The Asian tiger mosquito, ***Aedes albopictus,*** is an anthropophilic mosquito transmitting chikungunya and dengue. Suppression of ***Ae. albopictus*** populations is a challenge for mosquito control programs because immatures are found in numerous peridomestic artificial containers that are difficult to detect, access, and eliminate. ***Ae. albopictus*** uses cryptic habitats such as corrugated extension gutters, fence pots etc. as larval habitats, which are nearly invulnerable to penetrating by conventional pesticide spray. Our study was assessed the effectiveness of autodissemination stations to target the ***Ae. albopictus*** larval habitats in urban areas. This pull-and-push technology attracts gravid females to autodissemination stations, contaminates them with pyriproxyfen (juvenile hormone analogue), and disseminate the insect growth regulator to larval habitats using skip oviposition behavior. We assessed station efficacy in residential hot spots of ***Ae. albopictus***. Four treatment and four control plots (~ 3 acres each site) with >5 adult mosquitoes per BGS trap (hot spot) were selected for seasonal efficacy of the stations. Autodissemination stations were deployed in treatment plots for 8-12 weeks. Population density of eggs, larvae and adults of ***Ae. albopictus*** in controls and treatment areas were compared to assess the effect. Pupal mortality in the field samples assessed with 3rd instar ***Ae. albopictus*** bioassay were considered as another efficacy parameter. The findings may be helpful to develop a better management approach against container inhabiting mosquitoes.

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SMARTPATCHES: EXPLOITING MOSQUITO BEHAVIOR TO BUILD A BETTER BED NET

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When used correctly, bed nets can provide personal and community protection against ***Anopheles*** mosquitoes and consequently, against malaria. When nets fall short as a vector control tool, it can be attributed broadly to one of two issues: inequitable distribution due to cost of bed nets, and insecticide resistance in mosquitoes. To address these constraints, we have developed the SmartPatch. In its simplest form, the SmartPatch is a small piece of insecticide-treated fabric or netting that can be placed on top of an existing bed net. Because contact with host-seeking ***Anopheles gambiae*** is focused on this region, the SmartPatch should maximize protective effects while minimizing treated area. Because of the size and placement of the patch, it is less expensive and could be replaced more frequently than a full long-lasting insecticidal net (LLIN). It is also relatively protected from daily contact and as a result, the SmartPatch could potentially be treated with a wider range of actives than currently available for use on bed nets. As a proof-of-concept test and to better understand the relationship between treated area and efficacy, we have tested pyrethroid-treated patches ranging in size from 31 x 61 cm2 to the entire top of a bed net. We carried out these tests under laboratory and semi-field conditions using susceptible ***An. gambiae*** and measuring mosquito knock down and blood feeding relative to an untreated control and a full LLIN. We find intermediate levels of protection associated with all patch sizes. We also find that protection does not scale linearly with covered area and thus, the effects of the patch area greater than might be expected based on treated area relative to total area of a bed net. To follow up on these experiments, we are using mathematical models to explore the relationship between bed net efficacy and coverage. The goal of these simulations is to determine what conditions might favor the use of less expensive, less effective bed nets. Lastly, we are conducting trials with insecticide-resistance mosquitoes and non-pyrethroid patches, to evaluate the SmartPatch as a resistance-breaking tool.

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REDUCED EFFICACY OF LONG-LASTING INSECTICIDAL NETS IN AREA WITH PYRETHROID-RESISTANCE POPULATIONS OF *ANOPHELES GAMBIAE* S.L IN NE TANZANIA: AN EXPERIMENTAL HUTS STUDY

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The success of malaria vector control is threatened by widespread pyrethroid resistance. The extent to which insecticide resistance and underlining mechanisms impact the malaria vector insecticidal control is not clearly known. This study comparatively assessed the bio-efficacy of washed and unwashed PermaNet 2.0 long-lasting insecticidal nets (LLINs) against wild free flying pyrethroid-resistant ***Anopheles gambiae*** s.l. field populations in North Eastern Tanzania before and after development of insecticide resistance by the local vectors. Evaluation of the efficacy of PermaNet 2.0 LLINs was conducted in laboratory and in semi field (Experimental huts) following the standard WHOPES Protocol (WHO/CDS/NTD/WHOPES/GCDPP/2006.3). The evaluation criteria were

percentage mortality, blood feeding inhibition, exophily and deterrence effects. WHO method was used to detect resistance in wild ***Anopheles*** mosquitoes exposed to 0.05% deltamethrin. PCR based molecular diagnostics were used to identify mosquitoes to species and to detect kdr alleles. ***Anopheles*** mosquitoes were resistance to deltamethrin with mortality rate of 81.2% [95% CI: 76.8-84.9%]. Mortality rates induced by unwashed PermaNet 2.0 and PermaNet 2.0 washed 20x in this trial against An gambiae was 31%, 30% and 27% respectively. By contrast the mortality induced by unwashed and 20 washed PermaNet 2.0 nets against An gambiae in a trial conducted in this same area employing same huts when the vectors were fully susceptible to pyrethroids was 96% and 95% respectively. The reduced LLINs efficacy reported in this study indicates that resistance in Tanzania is capable of undermining the bio-efficacy of pyrethroid insecticide-based vector control interventions, especially LLINs. This emphasizes the need to identify alternative insecticides to replace or supplement pyrethroids also emphasizes the need for the development and implementation of effective and sustainable resistance management strategies.

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NEW MODE-OF-ACTION CHEMISTRIES FOR VECTOR CONTROL: SMALL MOLECULE INHIBITORS OF ARTHROPOD GPCRS

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New, safer mode-of-action chemistries are needed to control the arthropod vectors of infectious diseases. Continued control is threatened by the emergence of insecticide resistant populations of vectors and no new public health pesticides have been developed in recent decades. The Purdue Invertebrate Receptor Group (PIRG) is a collaborative effort to develop novel chemistries as interventions for the control and elimination of arthropod-borne diseases. G protein-coupled receptors (GPCRs) represent promising targets for insecticide discovery because of their critical role in the neurological processes of arthropods. We are pursuing rational drug design to discover small molecule inhibitors of arthropod GPCRs for vector control. To date, multiple arthropod vector genomes have been mined to identify suitable candidate GPCR targets. Using high-throughput chemical library screening and hit-to-lead optimization, we have identified potent antagonists of the D_1-like dopamine receptors (DARs) from the yellow fever mosquito, ***Aedes aegypti (AaDOP2),*** the malaria mosquito, ***Anopheles gambiae*** (***Ag***DOP2), the northern house mosquito, ***Culex quinquefasciatus*** (***Cq***DOP2) and the Lyme disease tick, ***Ixodes scapularis*** (***Is***DOP2). These chemistries exhibit greater than 100-fold selectivity for mosquito and tick DARs versus orthologous human and honeybee receptors ***in vitro***, and are highly toxic to mosquito larvae and adults ***in vivo***. Transcriptome analyses suggest that these antagonists disrupt GPCR-mediate signaling processes and operate via modes-of-action that are distinct from existing insecticides. Structure activity relationship studies are ongoing to explore multiple chemical scaffolds, develop a preliminary pharmacophore and identify leads for development. These studies demonstrate proof of concept for a target-based approach to deliver novel classes of vector-selective insecticides. Activities to expand the discovery pipeline via the development and incorporation of other GPCR targets from other species of arthropod vectors will be presented.

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MAPPING INSECTICIDE RESISTANCE IN MALARIA VECTORS: LATEST UPDATES FROM IR MAPPER®

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The emerging and rapid spread of resistance to major classes of public health insecticides threatens current malaria vector control efforts. Deployment of the most appropriate tools must be informed by up-to-date data on insecticide resistance in target malaria vector species. Launched in 2012, IR Mapper® (www.irmapper.com) is an online geospatial mapping platform built on a systematic review of peer reviewed, published literature to visualize insecticide resistance trends in ***Anopheles*** vectors. IR Mapper® utilizes an ArcGIS for JavaScript API platform and is updated monthly with newly published data from peer reviewed scientific publications on phenotypic (WHO susceptibility test and CDC bottle assay data) and resistance mechanisms (target site and overexpressed metabolic enzymes) data. Additional data meeting standard WHO or CDC test criteria from PMI, National Malaria Control Programmes, and other reputable sources are also included. The filter tools on IR Mapper® enable filtering by year, vector species, insecticide class, and resistance mechanisms. As of January 2015, IR Mapper® consisted of 9,433 unique field records from 57 countries and 48 ***Anopheles*** species or species complexes. 81.5% of countries have reported resistance to at least one of the four classes of insecticides; 76.0% have reported testing of resistance mechanisms. Filtering by time period highlighted that three times more reports of insecticide resistance in ***Anopheles*** were reported between 2000 and 2014 compared with the previous 45 years. IR Mapper® highlighted gaps in resistance monitoring, particularly in high burden countries like Democratic Republic of Congo and Nigeria. While insecticide resistance testing has increased in recent years, IR Mapper® is able to identify where data gaps on insecticide resistance exist in malaria vectors. IR Mapper® is a useful tool for visualizing temporal and spatial trends in ***Anopheles*** insecticide resistance and associated resistance mechanisms, and can be used to assist rational decision making on the deployment of vector control interventions and design of vector monitoring programs.

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SUSCEPTIBILITY OF *ANOPHELES GAMBIAE SENSU LATO* TO MALARIA CONTROL INSECTICIDES- RWANDA, 2011 AND 2013

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Insecticides are important malaria vector control tools, with pyrethroids being the most widely used class to treat nets and spray interior walls of homes.Widespread pyrethroid resistance emergence is a major threat to malaria control gains in Rwanda and throughout Africa. In 2011 and 2013, Rwanda conducted insecticide resistance monitoring of local malaria vectors at 12 sentinel sites in high malaria transmission districts with high LLIN and IRS coverage. Collected larvae were reared to adults and examined for knock-down and mortality using WHO tube bioassay. We analyzed a sample of female adults from field larval collections for the

kdr-east mutation (L1014S), a known resistance gene. We characterized 1,406 mosquitoes by polymerase chain reaction and identified 1,165 (82.9%) as ***Anopheles arabiensis*** and 241 (17%) as ***An.gambiae s.s***. We compared mortality rates observed in 2011 to 2013 and found a significant increase in resistance to lambda-cyhalothrin (98% vs. 63%%; p=0.0001), permethrin (86% vs. 66%, p=0.002), deltamethrin (100% vs. 87%, p=0.0003) and DDT (100% vs. 89%, p=0.001). Bendiocarb showed variable susceptibility with 58% of sites tested showing full susceptibility. No sentinel site had mosquitos resistant to fenitrothion. We genotyped 134 A***n. gambiae s.s*** mosquitoes for ***kdr***-east mutation and discovered 67 (50%exhibited homozygous sensitive (SS) alleles, while 35 (26%) and 32 (24%) mosquitoes exhibited heterozygous (RS) and homozygous resistance (RR) alleles, respectively. Of the 591 ***An.arabiensis*** genotyped, 425 (72%) exhibited homozygous sensitive (SS) alleles while 158 (27%) and 8 (1.4%) expressed heterozygous (RS) and homozygous resistance (RR) alleles, respectively. The results of this first insecticide resistance study in Rwanda reveal decreased pyrethroid and DDT susceptibility in 2013 compared to 2011 and susceptibility to carbamates and organosphosphates. These results demonstrate the need for Rwanda to continue monitoring insecticide resistance to inform effective insecticide management and sustain the gains made in malaria control as we approach pre-elimination.

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MODELING THE SPREAD OF *AEDES ALBOPICTUS* IN LOS ANGELES, CALIFORNIA

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The Asian tiger mosquito, ***Aedes albopictus***, is among the world's most invasive species. Its spread has been facilitated by rapid global transport of cargo, and it is now established on every continent except Antarctica. This species represents a "triple threat" to human health, being a day-biting pest, a competent vector of globally important dengue and chikungunya viruses, and a potential bridge vector of several zoonotic arboviruses. As a result of its importance, the biology of ***Ae. albopictus*** is also well-studied, but the fine-scale processes by which it becomes established in a given location are poorly understood. This is because even intensive surveillance systems yield limited information during the early phase of invasions when densities are low, and detection often occurs after populations are relatively widespread. Fine-scale spatial models for mosquito dynamics and movement offer a way forward, marrying our understanding of ***Ae. albopictus*** biology with surveillance paradigms and detailed data on the real landscapes where invasions occur. Here, we consider the ongoing invasion and establishment of ***Ae. albopictus*** in Los Angeles since 2011. We have used hierarchical modeling to account for heterogeneities in household-level suitability, then we modeled the stochastic dynamics of ***Ae. albopictus*** on this landscape using the suitability surface and a temperature-dependent, dynamical model for reproduction and spread. We then use the model to answer policy-relevant questions related to detection, control, and the feasibility of local eradication efforts.

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AEDES ALBOPICTUS MOSQUITO MICROBIOTA ASSEMBLIES: ROLE OF DEVELOPMENTAL STAGES AND LARVAL HABITATS

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Aedes albopictus is a major vector of dengue and other arbovirus worldwide and a sole vector for dengue in southern China. It's highly invasive and spreads rapidly. Microbiota-mosquito holobiont may play an important role in different aspects of mosquito life history, but the relationship between vector environments and vector microbiome assemblies is largely unknown. This study examined the hypothesis that microbiota of aquatic habitats is a key determinant of microbiota and larval and adult mosquitoes, and mosquito microbiota is modulated by sugar feeding, blood feeding, and exposure to environmental microbes. Using Miseq pyrosequencing of 16S rRNA gene V4 hyper-variable region, mosquito microbiota was examined from five natural larval habitats, and from adult mosquitoes of various ages and life histories. The results indicated that microbiota of water in different larval habitats and resulting mosquitoes differed significantly. Bacterial diversity generally decreased from aquatic stage to adults, while blood feeding reduced microbial community diversity. In mosquito larvae, bacterial species common to the five natural habitats were less than 30% of the total bacterial species OTU identified. Interestingly, we found that Wolbachia abundance was low in larvae, and increased with adult mosquito age. The relative densities of two main Wolbachia strains (wAlbA and wAlbB) in adult mosquitoes were examined by qPCR assays. The ratio of wAlbB to wAlbA gene copy number was around 106~108. Other bacterial taxa associated with human and pathogen vectorial capacity were also identified. The information obtained from this study would help to establish a metagenomic foundation for better understanding the impact of environmental microbial on vector development and disease transmission.

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TRANSCRIPTOME PROFILING BETWEEN PYRETHROID RESISTANT AND SUSCEPTIBLE POPULATIONS OF *AEDES ALBOPICTUS*

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The Asian tiger mosquito, ***Aedes albopictus***, is highly invasive mosquito species. It vectors a number of important human pathogens. The extensive usage of insecticides for public health vector control and agricultural pest control has posted strong selection pressure for increased resistance. However, very limited genomic information on ***Ae.albopictus*** insecticide resistance is available, hindering the development of sensitive molecular surveillance tools for insecticide resistance. In this study, we performed pyrethroid resistance bioassay in two field populations of ***Ae. albopictus*** from Southern China. We found one population from Shenzhen, southern China highly resistant to pyrethroid. With this population, the transcriptome profiling was conducted in 12 resistant individuals and 12 susceptible individuals using the Illumina HiSeq v4 PE100 technique. The transcriptome sequencing yielded more than 674 million clean paired end reads, with an average of 53 million reads per individual mosquito. A total of 19,733 (98.2% of the reference transcripts) transcripts were expressed in the tested mosquitoes. Among these, 742 transcripts were differentially expressed between deltamethrin resistant and susceptible mosquitoes collected from the same field site. Among these, top 50 differentially expressed genes included detoxification enzymes, cuticle protein and immune genes. A total of 439,306 non-synonymous variants were identified, among those, 5,245 SNPs differed significantly between resistant and susceptible populations in frequencies, and distributed in 1,925 genes. The most differential SNPs were found in cytochrome P450 detoxification genes. The transcriptome databases provide a valuable genomic resource for further genetic studies of this important dengue virus vector species. The differentially expressed genes associated with insecticide resistance identified in this study lay an important foundation for further functional analysis. The identified SNP markers will provide useful tools for future population genetic and comparative genomic analyses of ***Ae. albopictus*** vectors.

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GENOMIC ANALYSES OF DELTAMETHRIN RESISTANT AND SUSCEPTIBLE MOSQUITOES IN *ANOPHELES SINENSIS*

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Anopheles sinensis is a major malaria vector in Southeast Asia. Resistance to pyrethroid insecticides is becoming a common problem in this species. Previous study identified the ***An. . sinensis*** populations from Yunnan, China, showing high level resistance to deltamethrin, but total absence of knockdown resistance (kdr) mutation, suggesting resistance mechanisms other than kdr resistance in ***An. sinensis***. The aim of this study was to identify genomic variants, including single nucleotide polymorphisms (SNPs), multiple nucleotide variants (MNV), and insertion/deletion polymorphisms (INDELs) in ***An. sinensis***. Using massively parallel paired-end DNA sequencing, we generated 108 Gb of DNA sequence data from pooled deltamethrin resistant and susceptible mosquitoes, resulting in an average of 83.3X sequence coverage. Reads were mapped to approximately 94% of the ***An. sinensis*** reference genome. Using a stringent filtering method, we identified a total of 7.7 million variants, including 0.46 million variants located in codon regions. Over 140 thousand non-synonymous variants were identified. Approximately 6,000 differential SNPs were identified between deltamethrin resistant and susceptible population. Among those, 26 genes with the highest percentage of differential SNPs were identified, including detoxification genes, immune genes, cuticular protein, ordant protein, and ribosomal genes. We confirmed the finding by genotyping 40 mosquitoes at 20 highly differential SNPs. Furthermore, the two cytochromes P450 genes, CYP6Z3 and CYP4J10 previously identified with overexpression in resistant ***An. sinensis*** mosquitoes, were found having higher mutation frequencies at A513D of CYP4J10 and at G363S of CYP6Z3, respectively, in the resistant mosquitoes. The genomic variation described here will be a useful resource for future studies of genetic mechanisms of insecticide resistance.

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AN ANALYSIS OF CHROMOSOMAL INVERSIONS WITHIN THE *ANOPHELES* 1000-GENOMES PROJECT - MARKERS OF POPULATION STRUCTURE IN DISEASE VECTORS FROM THE PAST TO THE FUTURE

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The ***Anopheles*** 1000-genomes project has generated, in it's first phase, full-genome sequences for 765 individual mosquitoes. Samples collected cover two different species and sampling sites across the whole of Sub-Saharan Africa and genotypes have been called for more than 52m SNPs, enabling investigations of population structure and selection at an unprecedented scale. Yet despite the breadth of this dataset and the broad utility of SNP markers, it has been challenging to place this work in the context of the many decades of prior studies of the ***Anopheles*** species cluster. Work in the pre-genomic era frequently involved large structural variants; chromosomal inversions that can identify population structure, facilitate adaptation to differing ecological conditions, and can both prevent and facilitate genetic transfer between members of the species cluster. However, though inversions are readily identifiable via analysis of polytene chromosomes, they are difficult to call from the short-insert illumina sequence that makes up the Ag1000g data. Using multiple methods, including breakpoint identification, LD-clustering and admixture analysis, we have investigated chromosomal inversions in all sample groups of the ag1000g dataset, generating karyotypes for five of the seven major inversions that are thought to be represented in the Ag1000g dataset, and including additional rare inversions found on chromosomes 2 and 3. SNP markers have been identified for all of these inversions enabling future investigations of karyotype using cost effective genotyping methods. We will present a detailed analysis of the distribution of these inversions within the Ag1000g dataset, illuminating population structure at both intraspecific and interspecific levels. This will include sites showing reproductively isolated ***Anopheles gambiae*** / coluzzii, admixed ***A. gambiae*** / coluzzii,and evidence of allopatric structured populations within ***Anopheles gambiae*** s.s.

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CORRECTING GENOME MISASSEMBLIES IN MALARIA VECTORS BY PHYSICAL MAPPING

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The recently sequenced and assembled genomes of 16 anophelines allowed researchers to investigate the genomic basis of vectorial capacity. However, unmapped scaffolds may conceal gaps and misassemblies, which could cause incorrect or incomplete annotation of genomic sequences. Anchoring scaffolds onto chromosomes can correct these mistakes and can facilitate the development of high-quality reference genome assemblies that could be used for studying chromosome evolution and gene-order phylogeny. Using a combination of cytogenetic and bioinformatics approaches we mapped the genomic scaffolds to chromosomes of ***Anopheles*** arabiensis, An. stephensi, An. atroparvus, and An. albimanus. Each of the species was individually compared to An. gambiae. The comparative positions of genes within mapped scaffolds based on orthology relationships were plotted using the R program genoPlotR. Orientation of scaffolds on chromosomes was obtained from physical mapping data. This effort identified and corrected scaffold misassemblies in several species and developed chromosome-based genome assemblies. We estimated the number of chromosomal inversions between ***An. gambiae*** and each of the species and found that rearrangement rates are ~3 times higher on the X chromosome than on autosomes. The fast rate of X chromosome rearrangements is in sharp contrast with the paucity of polymorphic inversions on the X in anophelines. This finding could be indicative of a greater role of the X chromosome rearrangements in speciation of malaria mosquitoes.

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VECTORBASE: COMMUNITY RESOURCES FOR VARIATION, POPULATION AND COMPARATIVE GENETIC DATA ANALYSIS

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VectorBase is a NIAID-funded Bioinformatic Resource Center that provides resources to investigate vectors of human pathogens. Over the last year VectorBase has expanded to incorporate data from several species clusters (for a total of 22 ***Anopheles*** and 6 ***Glossina*** genomes) as well as the genomes of other insects supplying a total of 37 genomes representing 35 species. The availability of genomic, transcriptomic and proteomic data from multiple species permits a comparative approach to identify and characterize regulatory and biochemical pathways involved in diverse

phenotypes such as insecticide resistance, pathogen-host interactions and immunity, and arthropod phylogenetic relationships. Gene models and metadata are curated for all VectorBase species, and are used to recalculate gene trees for all updated organisms with each database release (bimonthly). Phylogenetic trees are constructed for all coding and ncRNA genes, orthologs and paralogs are identified, and gene and species trees are reconciled to identify duplication and speciation events. In addition to comparative omics information, variation data from a variety of wild populations and laboratory research strains are available for 14 species (12 ***Anopheles***, ***Aedes aegypti*** and ***Ixodes scapularis***). Variation data sets relating to insecticide resistance, host immune response to infection, and SNP chips used for large scale sample genotyping can be visualised in their genomic context using the VectorBase gene browser, and are linked to population and geodata for the originating samples via the Population Biology browser (PopBio) application. Since access to experimental sample metadata is a key requirement for efficient querying and visualization of large variation and population data sets VectorBase provides linkage to public repositories of reference sample metadata such as BioSamples to permit the provenance of samples to be determined, and provide convenient links to related sample data such as NCBI/ENA sequencing and expression data. All data provided by VectorBase are freely available at www.vectorbase.org.

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THE CREATION AND CHARACTERIZATION OF A TRANSGENIC *ANOPHELES GAMBIAE* LINE DYSFUNCTIONAL FOR SEXUALLY TRANSFERRED 20E

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Recent findings have demonstrated the importance of the insect hormone, 20-hydroxyecdysone (20E), in coordinating ***Anopheline*** reproductive biology. In the ***Cellia*** sub-genus of anophelines male-transfer of 20E during mating has been implicated in fertility, fecundity, mating refractoriness, and has been suggested to play an indirect role in ***Plasmodium*** development. Elucidating the downstream effects of male 20E transfer can therefore provide valuable insight into the biology of both reproduction and parasite development within the mosquito. Generation of 20E-depleted males is crucial to facilitate studies of this biological system. Unfortunately the tissue in which 20E is synthesized, the male accessory glands (MAGs), is generally difficult to target with RNAi based approaches; therefore targeting genes involved in 20E-synthesis is problematic. Here we present the characterization of an ***An. gambiae*** MAG specific promoter from the mating plug protein, Plugin, and demonstrate that this regulatory region is capable of driving MAG-specific expression of tdTomato. Further, we use this promoter to create a transgenic line in which expression of a 20E-deactivating enzyme is driven specifically in the MAGs. Males from this transgenic line, which we named Me2, are successfully depleted of 20E within their MAGs and transfer significantly less 20E to females during copulation. We are currently employing Me2 males to explore the role of male-transferred 20E in the female post-mating response, and its potential influence on ***Plasmodium falciparum*** development within ***An. gambiae.***

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THE EFFECT OF HYBRIDIZATION ON GENE EXPRESSION IN THE *ANOPHELES GAMBIAE* COMPLEX

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The accumulation of genetic incompatibilities that cause hybrid sterility and/or inviability between diverged populations is an important step in the formation and maintenance of species boundaries in the face of hybridization. While some chromosomal incompatibilities that cause hybrid sterility and inviability between ***An. gambiae*** and ***An. arabiensis*** have been identified, little is understood about the nature of these incompatibilities. Divergence in gene regulation, rather than genetic differences, is thought to account for a large proportion of phenotypic differences between species, and could also play a large role in hybrid sterility and inviability. Therefore, we analyzed gene expression in sterile F1 hybrid male pupae and compared it to parental males in crosses between ***An. gambiae, An. arabiensis,*** and ***An. quadriannulatus***. By analyzing genome-wide, allele-specific gene expression, we explored the roles of ***cis*** and ***trans*** regulatory divergence in the hybrid male sterility phenotype. The relationship between allele-specific expression, patterns of sequence evolution, and known hybrid sterility QTL was also explored. Our analysis provides insight into the divergence of the ***An. gambiae*** complex.

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SPECIATION, HYBRIDIZATION AND INTROGRESSION IN NATURAL *ANOPHELES GAMBIAE* AND *A. COLUZZII* POPULATIONS FROM THE AG1000G PHASE-1 DATA

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The ***Anopheles gambiae*** 1000 genomes (Ag1000g) project is opening unprecedented perspectives in the analysis of genome variation in natural populations of Afrotropical malaria vectors by providing high throughput sequence data on natural vector populations to be exploited to characterize patterns of diversity, linkage disequilibrium and recombination, population structure and gene flow, signals of recent selection, and demographic history. As a first step toward the full exploitation of the genetic data made available by Phase-1 of Ag1000g project, we focused on the SNPs that were found to show high allele frequencies difference (>0.9) between ***A. gambiae*** and ***A. coluzzii*** populations from Mali. Screening for variation in these SNPs in the first 765 wild-caught sequenced specimens from 8 sub-Saharan countries (i.e. Guinea-Bissau, Guinea-Conakry, Burkina Faso, Cameroon, Gabon, Angola, Uganda, Kenya) revealed evidence of: i) putative ***A. coluzzii/A. gambiae*** ancestral polymorphisms in an ***A. gambiae*** population out of its range of sympatry with ***A. coluzzii***; ii) ongoing gene flow between ***A. gambiae*** and ***A. coluzzii*** in the "far west" of their sympatric range, where a secondary contact region has been previously hypothesized; iii) introgression of mutations conferring insecticide resistance across species and between geographically distant populations. In order to facilitate large-scale and straightforward genotyping of additional ***A. gambiae*** and ***A. coluzzii*** populations, we developed a nucleic acid mass spectrometry assay based on a selection of the most informative SNPs and tested the novel approach on the initial 765 wild-caught sequenced specimens first, and on additional populations next.

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ANOPHELINE TRIGGERS OF FEMALE POST-MATING PHYSIOLOGY IN ABSENCE OF SEXUALLY TRANSFERRED ECDYSONE AND IMPLICATIONS FOR MALARIA TRANSMISSION

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The ability of anophelines to transmit malaria is influence by their sexual reproduction, with female fecundity, longevity and immunity shaped by post-mating physiological changes. In ***Anopheles gambiae,*** male sexual traits - transfer of a mating plug containing the steroid hormone

20-hydroxyecdysone (20E) - triggers major physiological events in females including loss of receptivity to mating and increased egg development. Correlated coevolution has driven the adaptation of these male traits in anophelines, with transfer of both a mating plug and 20E occurring exclusively in extant species of the subgenus ***Cellia***. We employed RNAseq to determine possible alternative triggers of female reproductive behaviors in other anophelines; focusing on the 20E-less and plugless ***Nyssorhynchus*** vector ***An. albimanus.*** We transcriptionally profiled the male accessory glands before and after mating as fluctuations in individual semen components my indicate factors crucial for mating success, and identified a number of unique proteins regulated by sex. Through reciprocal sequencing of the female uterus we identified potential interacting partners to these male peptides as well as pathways controlled by mating in the female. RNAi demonstrates a role for these male-female interactions in regulating ***An. albimanus*** female reproductive physiology and behavior. Furthermore, we show evidence of potential interplay between this divergent 20E-independent mating system and female physiology after blood feeding with implications for malaria transmission. Our data help elucidate the biological aspects relevant for vectorial capacity in different anophelines, and may facilitate control strategies aimed at reducing mosquito reproductive success.

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LANDSCAPE GENETICS OF *ANOPHELES ARABIENSIS* MOSQUITOES ACROSS KENYA

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Malaria transmission is affected by the movement of mosquitoes (over short distances) and people (over any distance). Active mosquito dispersal may depend on environmental and ecological factors, such as availability of blood meal source and larval habitats, suitability of ambient climate, and geographic distance and barriers. ***Anopheles*** arabiensis mosquito is presently one of the most widespread and important malaria vectors in Africa. Using a landscape genetics approach, we aimed to determine the relative contribution of geographic and ecological factors to genetic structure of ***An. arabiensis*** across Kenya. ***An. arabiensis*** larvae were collected from twelve sites in four ecological regions (Western Kenya highlands, Western Kenya lowlands, Great Rift Valley and Eastern Kenya coast). Ten microsatellite markers were used to genotype these mosquito populations. We are currently analyzing the population genotype data using the Bayesian Estimation of Differentiation in Alleles by Spatial Structure and Local Ecology (BEDASSLE) program to quantify the contributions of geographic and ecological factors to mosquito genetic structure. Understanding factors that promote and prevent gene flow of malaria vectors is critical to predicting the spread of insecticide resistance.

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ANOPHELES SPECIES DIVERSITY, BEHAVIOR AND SPOROZOITE RATES IN SIX STATES OF NIGERIA

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A survey of adult anopheline mosquito diversity was conducted from March through October 2014 in six States (Enugu, Plateau, Rivers, Jigawa, Nasarawa and Lagos) across different ecological zones of Nigeria. Monthly sampling was carried out over a 10-month period using human-baited CDC LT hourly cup-change method placed indoors and outdoors to measure mosquito biting-time and location from 18:00hrs to 06:00hrs. PSCs were used to sample indoor-resting mosquitoes. PCR identification was carried out on a subset of samples from each site. ***An. gambiae*** s.l. was tested for ***Plasmodium falciparum*** sporozoite infection using ELISA. A total of 23,740 ***Anopheles*** mosquitoes were collected between March and October 2014. 14,001 (58.98%) were caught using PSCs. Using CDC LT, 5,000 (51.3%) were caught indoors, while 4,739 (48.7%) were caught outdoors. Significantly higher numbers of anopheline mosquitoes were collected indoors than outdoors (χ^2=6.995; df =1; p= 0.0082). A total of 19,699 (83.2%) were ***An. gambiae*** s.l., 1,749 (7.4%) were ***An. funestus,*** 1767 (7.4%) were ***An. coustani,*** 162 (0.68%) were ***An. nili.*** and 6 (0.03%) were ***An. moucheti.*** The difference between the two major vectors was statistically significant (p<0.0001). ***An. gambiae*** s.l. was distributed across all the sites, while ***An. funestus*** was only found in Enugu, Plateau, Jigawa and Nasarawa States. ***An. nili*** was only present in Enugu, Jigawa, and Nasarawa States, with significantly higher densities in Enugu (p<0.0001). PCR analysis revealed that of the 2,984 samples analyzed from all the sites, 2,787 (93.4%) were ***Anopheles gambiae*** s.s., while 197 (6.6%) were ***An. arabiensis.*** The highest sporozoite infection rate of 5.5% was recorded in Rivers state, in ***Anopheles gambiae*** collected using both methods, followed by Enugu (3.7%). The predominance of ***An. gambiae*** s.l. in all the sentinel sites has been established. High diversity of ***Anopheles*** vectors was found in Plateau while ***An. gambiae*** s.l. was the only species found in the Rivers state. ***Anopheles moucheti***, was found only in Enugu. This shows the differences in the complexity of vectors and malaria transmission in the different ecological zones.

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MAJOR MALARIA VECTORS, *ANOPHELES FUNESTUS* AND *AN. ARABIENSIS*, BITE AT DIFFERENT TIMES OF THE NIGHT IN TWO VILLAGES IN NORTHERN MALAWI: IMPLICATIONS FOR MALARIA CONTROL

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The deployment of both long-lasting insecticidal nets and indoor residual spraying (IRS) has potential to alter vector behavior through selection or toxic effects. There are no recent data to indicate the behavioral patterns of major malaria vectors in Malawi. Therefore, human landing catches were carried out at one village each in Nkhata Bay and Karonga districts to investigate the biting behavior of ***Anopheles arabiensis*** and ***An. funestus*** from January to July 2014. At monthly visits, three houses were randomly selected, and teams of four people worked in pairs at each house, one person inside and the other outside. Using a flashlight and mouth aspirator, mosquitoes were collected as they landed on bare legs from 6:00 PM to 12:00 midnight. Another team of two continued from 12:00 midnight to 6:00 AM to complete the night's collection. Each hourly collection was placed in a separate paper cup, labeled by house, location and collection time. On a second night teams were rotated between houses and time period. An. ***funestus*** was predominant in Nkhata Bay (91.2%; n = 2567) compared to ***An. arabiensis*** (8.8%; n = 249). The latter species was predominant in Karonga (95.2%; n = 440) compared to An. ***funestus*** (4.8%; n = 22). ***An. arabiensis*** was active from 8 PM until 6 AM with peak biting between 1 - 2 AM, while An. ***funestus*** was active from 11 PM until 6 AM with peak biting at 5 AM. An. ***funestus*** predominantly was collected indoors while biting (65.8%; n = 1704) compared to outdoors (34.2%; n = 884), whereas ***An. arabiensis*** bit more outdoors (57.0%; n = 393) than indoors (42.0%; n = 296).

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ANTI-SALIVARY ANTIBODIES AS A MEASURE OF PROTECTIVE EFFICACY OF LONG-LASTING PERMETHRIN-IMPREGNATED CLOTHING AGAINST MOSQUITO BITES

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Mosquitoes are vectors of many infectious pathogens. Because mosquito bites may be unnoticed, it is difficult to monitor efficacy of mosquito avoidance measures in the field. However, mosquito saliva contains immunogenic proteins which elicit antibodies in humans and these are a direct measure of the exposure to mosquito bites. We have previously shown that long-lasting permethrin-impregnated clothing protects against tick and chigger bites in a double-blind randomized controlled trial in North Carolina outdoor workers. Here, we evaluated whether this clothing is protective against mosquito bites by measuring changes in antibody titers to mosquito salivary gland extracts. Paired serum samples were obtained from 64 participants. Antibody titers to ***Aedes atlanticus*** and ***Ae. albopictus*** salivary gland extracts were measured by ELISA. Antibody titers increased 10-fold between March 2011 and September 2012. The difference was 2-2.5 fold lower in subjects wearing treated uniforms than in control subjects. This suggests that long-lasting permethrin impregnated clothing provides protection not only against ticks but also against mosquito bites.

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TARGETING ECDYSONE SIGNALLING IN THE MOSQUITO: AN EFFECTIVE APPROACH FOR MALARIA CONTROL

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Chemical control of mosquito populations remains the most effective method to defeat malaria. New tools are however needed as malaria elimination is threatened by the spread of insecticide resistance. As ***Anopheles*** females typically mate only once in their lifetime, interfering with mosquito reproduction offers an exciting opportunity to interrupt malaria transmission. Besides the crucial role of 20-hydroxyecdysone (20E) in oogenesis, we have recently shown that in ***Anopheles gambiae***, male 20E transferred during copulation induces oviposition and refractoriness to further mating. Based on the multiple profound effects of 20E on the reproductive physiology of ***Anopheles*** females, we investigated the potential use of hormone agonists as mosquito chemosterilants against ***An. gambiae*** females. Here, we show that disrupting 20E signalling has a remarkable impact on a number of biological traits that are relevant to vectorial capacity; it significantly reduces longevity, completely abolishes egg development and drastically decreases insemination rates. By fitting our data into a mathematical model of the mosquito lifecycle, we demonstrate that manipulating hormonal balance significantly impacts mosquito population dynamics and malaria transmission; reducing mosquito population size and shifting its age structure. These results demonstrate that disruption of 20E signaling is an effective, new and complementary approach to the existing tools for mosquito control, and may provide the additional effort required to achieve malaria elimination. Importantly, as the role of 20E in reproduction is conserved among most anopheline vectors, applications based on this hormone could also translate to other important ***Plasmodium*** vectors.

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CHARACTERIZATION OF ANOPHELINE SPECIES COMPOSITION ALONG THE BHUTAN-INDIA BORDER REGION

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Bhutan is aggressively embarking on a path towards malaria elimination. Despite substantial progress, Bhutan remains vulnerable to imported malaria. The majority of cases are in Sarpang District, which shares a border with the state of Assam in India, and from where most cases likely originate. However, the anopheline species responsible for autochthonous malaria transmission have not been well characterized. Therefore, a comparison of the vector species in Sarpang was made with published vector records in neighboring Assam. An assessment of the anopheline species composition was undertaken from May to July 2014 in four Sarpang villages adjacent to the Assam border. Five sampling methods were employed: (1) human landing catches, (2) cattle-baited catches, (3) CDC light traps, (4) indoor resting catches and (5) resting boxes. Female anopheline mosquitoes were identified to species using a morphologic key. These results were compared to published literature on anopheline ecology and vectorial roles in Assam. The two suspected malaria vectors in Bhutan, ***Anopheles*** culicifacies (n=189) and An. pseudowillmori (n=205), were abundant in the Sarpong villages. However, in Assam, only An. culicifacies species B, a relatively incompetent vector, has been documented. In contrast, the primary malaria vectors of Assam, ***An. minimus*** and ***An. baimaii***, were absent in the Sarpang collections. If ***An. culicifacies*** is not a competent vector in Sarpang, the other recovered species--***An. pseudowillmori, An. maculatus*** and others may be the responsible vectors for malaria transmission in Sarpang. Nonetheless, molecular

methods are required to identify members of several of the sibling species in this region; however as yet, adequate equipment and trained personnel are not available to address this difficulty.

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UTILITY OF BARRIER SCREENS TO SAMPLE OUTDOOR MOSQUITOES IN AN AREA TARGETED FOR MALARIA ELIMINATION

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Malaria elimination strategies, employing reactive case detection and mass distribution of treated bednets, are being rolled out in southern Zambia. Entomological surveillance is essential to not only monitor the impact on vectors but also to identify where transmission may still occur. Two years of surveillance in Macha, southern Zambia indicate that vector densities indoors are very low, with high spatio-temporal heterogeneity, but recent data on outdoor transmission is lacking. To assess the utility of barrier screens for sampling outdoor vectors, eight households with above mean indoor densities of the vector ***Anopheles arabiensis*** were selected. Two barrier screens, set between houses and either mosquito breeding sites or animal enclosures, were rotated randomly though the households for a period of 40 nights during the rainy season of 2015. Barriers measuring 2 meters high and 5 meters long were constructed from polyester shade netting attached to poles, with a 'roof' to create 'eaves'. Mosquitoes were collected by mouth aspiration from each side of the barrier hourly from 18:00 to 06:00, recording the height of collection. More than 75% of anophelines caught were morphologically identified as ***An. gambiae s.l.,*** similar to the species composition indoors***.*** Molecular identities will determine whether these are indeed the vector species ***An. arabiensis***. Blood feeding rates were higher than that of indoor light trap catches. Human blood indices, infectivity and parity rates will be estimated. Whilst the majority of ***An. gambiae s.l.*** were collected between 22:00 and 05:00, when most people were recorded to be indoors and protected by bednets, almost 20% were caught between 19:00 and 22:00. A larger study surveying more houses over time would indicate the extent of this outdoor exposure. This study demonstrated that barriers screens can be used in an area of low vector density to collect anophelines outdoors and that densities are comparable to that of indoor host-seeking collections. This tool could be used to quantify residual transmission, estimations of which are essential in an area where elimination employs only indoor vector interventions.

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MALARIA TRANSMISSION IN TWO DIFFERENT RURAL ECOLOGICAL SETTINGS IN MALI

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Malaria transmission varies widely across divergent ecological settings. Here we compare entomological parameters for malaria transmission between two ecologically divergent zones in Mali: 1] one with distinct rainfall-based (seasonal) transmission (Dangassa) and 2] another with virtually year-round transmission (Koïla Bamana) because of irrigation to grow rice in the inner delta region of the Niger River. Standard control measures are used at both sites with the difference that their implementation and effectiveness have been monitored by the Millennium Villages Project (MVP) in Koïla Bamana, whereas in Dangassa, the only control measures are those provided by the government. Five entomological surveys were performed at each of the study sites simultaneously to collect mosquitoes using pyrethrum spray catches (PSCs) and human landing catches after obtaining ethical approvals. ***Anopheles gambiae*** s.l. was the major vector found in both localities and represented 99% (N=5466) of the mosquitoes collected. Overall, the mean density of mosquitoes per house from the PSCs (mean=15.0, 95% CI [14.4-15.6]) was higher in the rice growing area of Koïla Bamana than in the seasonal transmission setting of Dangassa (mean=5.9, 95% CI [5.5-6.3]). In contrast, the human blood index (HBI=percent of mosquito blood meals from human subjects) and the monthly entomological inoculation rate (EIR) were 6.2 (80.0% vs 13.6%) and 3.7 (1.43 vs 0.39) times higher, respectively, in Dangassa than Koïla. The higher ***An. gambiae*** s.l. densities in the rice growing area of Koila Bamana and the higher HBIs and EIRs in Dangassa may have resulted from a combination of: 1] intense implementation and monitoring of vector control measures by the MVP in Koila Bamana and 2] longer vector survival and less intense vector control in Dangassa.

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CHANGING VECTOR POPULATION DYNAMICS WITH AN EMERGING POPULATION OF *ANOPHELES FUNESTUS* IN A SETTING OF HIGH MALARIA CONTROL INTERVENTIONS IN THE GAMBIA

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The scale-up of key malaria control interventions like indoor residual spraying, use of insecticide treated bed nets and provision of artemisinin-based combination therapy has greatly reduced the burden of malaria in The Gambia. There is limited data on how these interventions have altered the ecology of major vectors in the country. Entomological sampling was conducted in the setting of an ongoing longitudinal cohort study in twelve villages across the country. In each village, fourteen houses were selected and indoor and outdoor mosquito collections were done using human landing catches and CDC light traps. Mosquito species were identified using morphological characteristics and by PCR. During the 2013 transmission seasons (June to December); a total of 12,815 adult ***Anopheles*** mosquitoes were collected. ***Anopheles gambiae*** s.l.complex was the predominant species (81.2%) in most villages. Significant densities of ***An.funestus*** species were identified for the first time in the country but only limited to the Central River region. There was significant heterogeneity in vector abundance between regions. Villages in central river region and the West coast had the highest mosquito densities despite having lower malaria parasite prevalence, whereas villages in the upper river region with higher malaria parasite prevalence had significantly lower vector densities. The peak vector density was in September and the peak biting time was at dawn (5:00 am). Significant outdoor feeding and resting behaviour was also observed. The overall sporozoite rate was 0.1 with the highest rates observed in the west coast (0.8) and upper river region (0.6). Mosquito survival (98% parity) and EIR (21 infective bites per person per year) were highest in the upper river region. In conclusion these observed changes in key entomological indices especially the emergence of ***An. fenestus*** species in this region have important implications for the epidemiology and control of malaria in The Gambia. Additional vector control strategies utilising both indoor and outdoor tools may be beneficial.

1400

A DIFFERENT ANGLE OF *AEDES AEGYPTI* PUPAL PRODUCTIVITY: SOCIAL AND BIOLOGICAL FACTORS IN TWO DENGUE HYPER-ENDEMIC CITIES

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Behaviors, beliefs and ecologic conditions in a community are of particular importance to dengue control given ***Aedes aegypti*** anthropophilic behavior. Since the reduction of household breeding sites is a key control measure available for dengue vector control, the objective of this study was to determine the social and ecologic factors related to pupal productivity in households of two hyperendemic cities in Colombia (Armenia and Arauca). 4000 households were randomly selected using cluster sampling with replacement after three attempts to contact. In each household a survey including knowledge, attitudes and practices about dengue; a characterization of all the potential breeding sites and subsequently counting of pupae was performed. Two statistical models were designed: a logistic model between absence/presence of pupae in a household and social and behavioural factors, and a negative binomial model for the counts of pupae per breeding site and the ecologic and behavioural factors. Risk factors in Armenia were the location of the household in areas of less altitude and more temperature (OR 6.2 p 0.02), having seen larvae in the household (OR 4.4 p 0.001), and not knowing about vector oviposition places (OR 1.8 p 0.001) was related with presence of pupae. Containers in which no frequency of emptying was reported or were filled with rain water were associated with a higher number pupae but those not having sediments, having the nearest tree further than 180 cm and a capacity of <20 liters, decrease significantly the number of pupae. In Arauca, hearing about dengue from parents and friends (OR 2.6 p0.03), report water storage for cooking or cleaning the house (OR 3.2 p0.001) and have washing machine (OR 1.5 p0.001) was associated with household positivity. Containers emptied 4 days or longer, not reporting this information and having less than half of the container filled with water was associated with a higher number of pupae. The related factors to pupal positivity and productivity within households are dependent on each environment and attention should be focused on the convergence of biological and social forces that drive productivity.

1401

SPACE-TIME CLUSTER ANALYSIS OF DENGUE FEVER OCURRENCE AND VECTOR DETECTION BY MOSQUITRAP IN SINCELEJO-COLOMBIA

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Dengue is the most common arthropod-borne viral disease in tropical and subtropical areas of the world and one of the most important in terms of morbidity, mortality and economic impact. In Colombia, the disease is considered a major public health problem. The main strategy to control the disease worldwide remain in the vector control and surveillance however, low correlation have been described between the infestation levels from different surveillance methods and the risk of dengue transmission. In the present study we describe a space-time cluster analysis of the dengue occurrence and the vector abundance in the city of Sincelejo-Colombia, in addition to infection rate estimations with dengue virus (DENV) in the mosquitoes. From May to August 2014, dengue vector ***Aedes aegypti*** was captured bi-weekly by MosquiTRAPs in seven districts of Sincelejo urban area. Captured mosquitoes were used for DENV detection and typing by RT-PCR. Space-time scans with SaTScan software, were used for detection of dengue cases and vector abundance clusters. Intersections were detected with Dengue cases and vector clusters, suggesting specific risk areas within district 5 of the city. District 5 captured mosquitoes represented 20.76% of the total amount of vectors, which is the highest percentage compare to the other districts. The general minimum infection rate was 24.4 per 1000 mosquitoes, but the highest value was estimated in district 5 with 32.1 per 1000 mosquitoes. All four serotypes were detected with DENV-3 (63%) as the most frequent. The simultaneous circulation of DENV serotypes and the high vector abundance within a specific zone suggest that this could potentially be a hotspot for dengue transmission in the city.

1402

SPATIO-TEMPORAL MECHANISMS OF COEXISTENCE IN URBAN MOSQUITOES

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Competitive asymmetry is the expected outcome of interspecific competition among mosquito species that utilize water-filled containers. But inconsistent with the expectations of competitive exclusion, mosquito species often co-occur with superior competitors within the same individual container in urban areas. Understanding the mechanisms behind species coexistence can provide insight into processes governing vector distributions and guide public health management efforts. Several important vector species, including ***Culex pipiens***, ***Cx. restuans***, and the competitively superior invasive ***Aedes albopictus***, coexist across neighborhoods that vary in socio-economic status (SES) in southwest Baltimore, Maryland. We tested predictions that temporal and spatial habitat segregation fosters coexistence of these species. Oviposition cups were deployed every three weeks in four neighborhoods of differing SES in Baltimore between May and November of 2014. ***Ae. albopictus***, ***Cx. restuans***, and ***Cx. pipiens*** constituted 80.4%, 10.9%, and 7.9% of the 61,534 total mosquito larvae collected, respectively. Coexistence of two or more species was seen in 19.9% (n=627) of ovicups. Abundances of both ***Ae. albopictus*** and ***Cx. pipiens*** peaked in late summer, whereas ***Cx. restuans*** abundances peaked in early summer. ***Aedes albopictus*** abundances in the highest SES neighborhood were approximately half that of the other three neighborhoods, while ***Cx. pipiens*** abundances varied among neighborhoods. ***Culex restuans*** abundances in the two highest SES neighborhoods were twice that of the two lower SES neighborhoods. These results suggest that both spatial and temporal habitat segregation may foster coexistence of ***Culex*** species with ***Ae. albopictus*** in southwest Baltimore, but the mechanism of coexistence (spatial vs. temporal) varies between species, even though they are ecologically similar. SES may affect abundances of all species through changes in larval habitat types. The coexistence of ***Ae. albopictus*** with at least one ***Culex*** species likely have real implications for disease risk and transmission due to differences in adult biting behaviors.

1403

EFFECT ON *AEDES AEGYPTI* FEEDING BEHAVIOR FOLLOWING PRE-EXPOSURE TO DEET

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DEET is the most widely used topical insect repellent in the world. Substantial evidence has documented DEET to provide effective protection against mosquito bites. Despite its substantial use, the exact mechanism

of action of DEET has yet to be fully elucidated. Moreover, reports from experimental studies indicate that mosquitoes can become less responsive to the chemical, thereby being less repelled. Either a genetic change or direct learning/habituation could be responsible for this altered sensitivity. As such, it is important to determine if previous exposure to DEET can alter mosquitoes' subsequent attempts at blood-feeding behavior. Here we pre-exposed cohorts of ***Aedes aegypti*** mosquitoes to various concentrations of DEET (0.10%, 0.12%, 0.14%, and 0.16%) or to ethanol as a control for a standard time period. The mosquitoes were then provided a blood meal source either immediately following exposure or after defined holding periods (one, three, six, and 24 hours post DEET exposure). The landing and probing rates were recorded every 30 seconds for the first five minutes and the engorgement level was assessed at the end of the 20 minutes blood feeding time. Results indicate that the mosquitoes that had been pre-exposed to DEET displayed no difference in landing and probing rates compared to mosquitoes that were pre-exposed to ethanol. However, 0.16% DEET caused test mosquitoes to imbibe significantly (van Elteren's test ***P***-value < 0.05) less blood as compared to the control population. This effect was observed in mosquito cohorts held for one, three, and six hours after exposure but disappeared when the test mosquitoes were allowed to blood feed at 24 hours post DEET exposure. Findings from this study indicate that DEET exposure can alter the ability of ***Ae. aegypti*** mosquitoes to imbibe blood to engorgement up to six hours post exposure. As the landing and probing rates were not affected, this result suggests that there is a possibility of short term increased vector capacity following DEET exposure that needs to be further evaluated.

1404

DOG HEARTWORM (*DIROFILARIA IMMITIS*) EXTRINSIC INCUBATION, MOSQUITO ECOLOGY, AND PUBLIC KNOWLEDGE: OPPORTUNITIES FOR IMPROVED MESSAGING AND RISK MANAGEMENT

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We present ***Dirofilaria immitis*** in a broad context of its extrinsic incubation period, vector ecology, and community impact. ***Aedes aegypti*** were infected with ***D. immitis*** to monitor extrinsic incubation period under temperature fluctuation near and above the ***D. immitis*** development threshold, 14°C. Infected mosquitoes were kept at 19 ± 9°C, 22 ± 4°C, 26 ± 4°C, and compared with constant temperature controls. ***D. immitis*** stages were identified under dissection and L3 development was compared between treatments. The ability of the standard Heartworm Development Unit (HDU) model to predict L3 development was tested against our novel hourly temperature model. L3 development differed between fluctuating and constant temperature treatments. HDU models underestimated L3 stage development at temperatures fluctuating around 14°C; hourly temperature models more accurately predicted L3 development. We then performed a community-level study to analyze vector species, infection rate, and residents' knowledge, attitudes, and practices in two counties of historical high and low ***D. immitis*** prevalence. Questionnaires regarding mosquito-borne disease and heartworm prevention were administered to consenting adults (n=100), and their yards were surveyed for mosquito breeding sites and larvae. Adult mosquitoes were collected in both communities for the duration of the study. Mosquito larvae and adults were identified, and ***D. immitis*** prevalence in adult mosquitoes was determined by PCR. Many residents (59.4%) were unaware that mosquitoes transmit ***D. immitis***; most owners (71.2%) administered heartworm preventive to their pets; and most non-compliant owners were unaware of the risk or decided that preventive was unnecessary. Mosquito species diversity was used to determine the role of key vector species in each community. We propose an updated model for spatiotemporal determination of dog heartworm risk, and describe a comprehensive approach to evaluate ***D. immitis*** vector and transmission dynamics in residential habitats. We use residents' responses to identify areas for improved messaging by public health officials and veterinarians.

1405

DISTRIBUTION AND SPECIES COMPOSITION OF THE *ANOPHELES CRUCIANS* COMPLEX IN SOUTH GEORGIA, USA

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Anopheles crucians Wiedmann (sensu lato) is an anopheline species complex that is distributed broadly across North America, Central America, and the Caribbean. It is a potential vector of ***Plasmodium falciparum*** and ***Plasmodium vivax***, as well as a potential vector of several arboviruses including EEE, VEE, SLE, WNV, and Tensaw virus. It has also been implicated as a vector of ***Dirofilaria immitis***, a filariod nematode that is a serious veterinary health problem among canines. Although the members of this species complex are morphologically indistinguishable as adults, it is possible to assign individuals at the species level within the complex using a previously described polymerase chain reaction (PCR) assay based on amplification of the internal transcribed spacer 2 region (ITS2). An understanding of species composition and distribution could be critical in elucidating the role that this complex may play as a disease vector as it is possible that each member of this complex may exhibit unique ecological and behavioral traits. Here we describe our initial efforts to determine the distribution and composition of the ***An. crucians*** complex within a community in the Southeastern United States. We employed PCR amplification of the rDNA ITS2 sequence to differentiate over 200 individuals from the ***An. crucians*** complex collected from 10 different sample locations in Lowndes county GA, USA. We observed individuals of this complex in sympatry in five of ten locations throughout the county with several of these locations exhibiting three of the species (A, C, D) in sympatry. Spatial and temporal distributions of members within this complex were explored. This work represents a first step in understanding ***An. crucians*** ecology in the state of Georgia. An understanding of species distribution and vector competence within the ***An. crucians*** complex could allow for the development of improved vector control strategies.

1406

DENGUE VACCINE INITIATIVE PROJECT: BURDEN OF DENGUE FEVER IN CHILDREN AND ADULTS OF PIEDECUESTA - SANTANDER, COLOMBIA

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Dengue infection is a major public health problem in Colombia, likely to be interested in early adoption of dengue vaccines. The largest dengue outbreaks in the past decade were in 2010 and 2013 with incidences of 587.7 and 473.2 cases per 100,000 inhabitants, respectively. Santander is a state in the northeastern Colombia, with significantly high incidence reported in 2013 as 979 cases per 100,000 inhabitants. In preparation for the upcoming dengue vaccine introduction, Dengue Vaccine Initiative (DVI) and the Universidad Industrial de Santander conduct the passive facility-based surveillance with a cost-of-illness (COI) survey to determine the true burden of dengue fever in Piedecuesta, Santander in Colombia. The passive fever surveillance was launched in August 2014 in the public hospital and the private clinic of Piedecuesta. From febrile patients between 1-55 years-of-age from the Piedecuesta area, acute and convalescent blood samples were collected 10-21 days apart. All subjects were tested using a rapid test (NS1/IGM/IgG) and IgM/IgG Capture ELISA. Subjects with dengue positive results on any of the tests were further

tested with RT-PCR. Since the launch, there were 407 subjects enrolled as of February 2015, from which 175 (43%) were found to be positive on the rapid test for dengue. About half (53%) of the subjects were female and 60% were under 24 years-of-age. From the sub-sample with complete lab-results (n=255) up to February, we have 130 (51%) subjects confirmed with dengue, including 66 (51%) primary and 64 (49%) secondary infections. Among the 130 cases with dengue confirmation, 93 (71.5%) were clinically diagnosed with dengue, 21 (16.2%) with severe dengue (DHF), and the rest with undifferentiated fever. Among 175 patients with NS-1 positive results, 99 patients agreed to participate in our COI survey. Once the needed sample size of 150 cases is reached, data analysis will begin and more data will be available for presentation by October, 2015. The data generated on dengue disease burden in Colombia would aid the policy makers for their decision making for upcoming dengue vaccine introduction in the country.

1407

STUDY OF ANTI-ENVELOPE ANTIBODIES IN POLYCLONAL SERA AFTER PRIMARY DENGUE VIRUS INFECTION

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The four serotypes of dengue virus (DENV) cause the most important and rapidly emerging arboviral diseases in humans. The envelope (E) protein is the major target of neutralizing antibodies (Abs). Previously, we and others reported that a significant proportion of anti-E Abs after primary DENV infection are cross-reactive to other flaviviruses, known as flavivirus group-reactive (GR), and recognize the highly conserved fusion loop residues of E protein. However, the characteristics of type-specific (TS) anti-E Abs, which is believed to account for the monotypic neutralization activity after primary infection, remain largely unknown. In this study, we investigated anti-E Abs in 10 individuals who experienced primary DENV1 infection during the 2001 outbreak in Hawaii. Depletion of cross-reactive Abs including complex-reactive (CR) and GR Abs did not affect the DENV1 neutralizing activities, whereas depletion of DENV1 TS Abs greatly reduced the DENV1 neutralization activities. Based on the endpoint titers to virion-ELISA after depletion, TS Abs were found to constitute 16% to 41 % of anti-E Abs and account for the TS neutralizing activity after primary DENV infection. In addition, the requirement of virions rather than solubilized E protein alone to deplete the neutralizing activities suggests the importance of neutralizing epitopes present on virions, which is in agreement with recent reports of potent TS neutralizing Abs recognizing quaternary epitopes on virions. These findings add to our understanding of antibody responses after primary DENV infection and have implications for dengue vaccine development.

1408

EVALUATION OF HUMORAL AND INNATE RESPONSES FOLLOWING A LIVE ATTENUATED DENGUE VACCINE IN FLAVIVIRUS-EXPERIENCED VOLUNTEERS

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The four mosquito-borne dengue viruses (DENV1-4) collectively cause nearly 100 million symptomatic dengue infections annually worldwide. Current dengue vaccine development is focused on tetravalent candidates that elicit a balanced immune response against all four serotypes since all cause disease but with unpredictable circulation patterns. In the United States, live attenuated tetravalent dengue vaccine (LATV) candidates with 3' UTR deletions developed at NIAID have been tested primarily in flavivirus-naïve (FV-) individuals. Since areas endemic for dengue and other flaviviruses are of importance for LATV use, we evaluated the safety and immunogenicity of a candidate, TV003, in healthy flavivirus-experienced (FV+) individuals. Neutralizing antibody responses to three of four DENV serotypes after a single dose of TV003 were significantly higher in FV+ subjects compared to FV- subjects. Interestingly, post-TV003 titers in individuals presenting as FV+ due to yellow fever (YF) vaccination were similar or greater to those in subjects who were FV+ due to prior vaccination with monovalent live attenuated dengue candidates or through prior apparent natural exposure. In both groups, a second dose of LATV within 6 months did not boost neutralizing antibody titers. These results are consistent with a cross-flavivirus anamnestic memory akin to what has been described for Japanese encephalitis virus and YF vaccinations. The basis for anamnestic flavivirus memory is not well understood. We hypothesize that the early plasmablast response to TV003 may differ in both kinetics and magnitude in FV+ compared to FV- subjects. Since early innate immunity plays a key role in establishing robust humoral responses to vaccination, we monitored blood cell differentials in FV+ and FV- subjects after a single dose of TV003. While neutrophils dropped after TV003 in FV- subjects, these cells remained stable after TV003 administration to FV+ subjects. Both phenotypes tracked with a select subset of serum cytokines. These results introduce new parameters that can be used to monitor LATV immunogenicity in addition to serum neutralizing antibodies.

1409

EBOLA VIRUS DISEASE PREPAREDNESS AND RESPONSE STRATEGIES IN ETHIOPIA AND LESSONS LEARNED FROM WEST AFRICAN COUNTRIES

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Shocking the world Ebola virus disease has claimed more than 10,000 lives during the current epidemic in West Africa making it the deadliest outbreak since Ebola was discovered in 1976. Evidently it has a global repercussion and response to contain spread of the epidemic .On 8 August the World Health Organization declared Ebola outbreak as a public health emergency of global Concern. The Ebola outbreak in West Africa is unprecedented in terms of its geographical scope. It began in Guinea in early 2014 and quickly spread to the neighboring countries. Inadequate health infrastructure and overall fragile health system has fuelled rapid spread of the outbreak in West Africa. The severity of the outbreak is exacerbated by lack of understanding about the disease by communities and lack of experience among health-care workers .Lack of adequate treatment facilities, rumor, fear and stigma has in turn led families to keep sick patients at home, risking further spread of the virus. Resistance to proposed response measures as well as traditional burial practices further aggravated high transmission of the outbreak with devastating economic and livelihood implications. Ethiopia by the virtue of its geographic position and being political capital of Africa and being a major transport hub in the east Africa region is very prone to the deadly virus due to importation from Ebola riddled countries. Hence it has swiftly enhanced its domestic preparedness to respond in a predictable manner in the event of Ebola outbreak .It has employed state of the art screening measures at main entry ports and has also been engaged in fighting the outbreak at its source by sending medical teams to Ebola-stricken nations. This paper reviews the current Ebola preparedness and response strategies in Ethiopia, challenges and crucial lessons learnt from West African countries and map out the strategies that are in place for improvement preventing future outbreaks.

1410

EXPRESSION OF NKG2D AND DNAM-1 LIGANDS DURING DENGUE INFECTION *IN VITRO*

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The first response against dengue virus (DENV) infection is regularly mediated by the innate immune response. Natural Killer (NK) cells are an important cell population that could be implicated in the response during the early stage of DENV infection, controlling DENV replication, resulting as a mild manifestation of the disease. NK cells usually recognize the surface of infected cells coated by antibodies using its CD16 receptor, and therefore capable of performing antibody dependant cytotoxicity (ADCC). However ***in vitro*** experiments suggest that they are also capable of direct recognition of DENV-infected cells in the absence of antibodies. Ligands of NK cells activating receptors such as NKG2D receptor could be induced during early establishment of DENV infection. A role of NKG2D and its ligands in DENV immune response is supported by genetic studies that showed the association of some alleles of MICB and MICA, two NKG2D ligands, and dengue disease severity. To verify if the ligands of some NK activating receptors, like NKG2D and DNAM-1, were altered by DENV infection, we performed ***in vitro*** experiments in which immature dendritic cells derived from peripheral blood monocytes from healthy volunteer donors were infected with a DENV-2 isolate from Panama. Expression of mRNA of NKG2D and DNAM-1 ligands was assessed by qPCR in non infected cells versus infected cells at 24 hours and 48 hours post infection. All currently described NKG2D (ULBP 1-6, MICA and MICB) and DNAM-1 (PVR and Nectin2) ligands were included in our analysis. Expression of surface protein ligands was evaluated by flow cytometry. Preliminary assays of Lamp1 expression as a marker of degranulation and intracellular IFNɣ production were also evaluated. Our preliminary results suggest that NKG2D and DINAM-1 ligands are induced during DENV infection ***in vitro***, the mechanisms of induction are yet to be identified and that this could allow for recognition of infected cells by NK cells.

1411

ASSESSMENT OF THE AGE-SPECIFIC BURDEN OF DENGUE IN MIRPUR, DHAKA: A CROSS SECTIONAL STUDY

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Dengue is the most significant mosquito born virus world-wide, with over two billion persons at risk for infection. In the summer of 2000, the first outbreak of 5000 cases dengue fever (serotype 3) occurred in Dhaka, Bangladesh. Since this outbreak, dengue fever has had a continued presence throughout Bangladesh. While previous studies have found that seroprevalence of dengue in Dhaka is currently approximately 80% in adults, the details of circulating serotypes and age at infection remain unknown. These data are necessary to assess the need and strategy for the introduction of dengue vaccines in Bangladesh, in particular into the extremely densely populated city of Dhaka (12 million persons). Specifically, this study determines the age-specific seroprevalence of dengue, the currently circulating serotypes, and quantitative neutralizing antibody titers in Mirpur, a densely-populated area of Dhaka. A cross sectional design is employed enrolling 700 individuals in four separate age categories: 6 month to 4 years, 4-10, 11-17, and 18-60 years. Individuals are randomly selected from household census and information on dengue risk exposure is obtained through a structured questionnaire. Dengue prevalence, serotype and antibody titer will be determined. Dengue seroprevalence is determined by IgG/IgM antibody testing; serotype and antibody level is determined by Plaque Reducing Neutralizing Antibody Titer (PRNT) assay. Differences in antibody titer among circulating serotypes and age strata will also be analyzed by analysis of variance methods according to the data distribution. Knowledge of which dengue serotypes are circulating in Dhaka, confirmation of previous exposure by age and intensity of infection will help determine burden of disease and guide safe introduction of a tetravalent live attenuated dengue vaccine in this population.

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NS1 CAPTURE ELISA WITH A SERUM HEAT DENATURATION STEP TO IMPROVE TEST SENSITIVITY IN PERSONS WITH SECONDARY DENGUE VIRUS INFECTION

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There is no licensed effective vaccine or anti-viral drug against dengue, and early diagnosis is important means to improve patient care as well as improve public health surveillance. The detection of dengue virus (DENV) non-structural protein 1 (NS1) in serum is widely used for the diagnosis of suspected dengue, worldwide. One of the limitations of the NS1 test is the reduced sensitivity in secondary DENV infections. Immune complex formation between DENV NS1 antigen and anti-DENV NS1 IgG antibodies is thought to be the cause for the decreased sensitivity of commercially available NS1 antigen capture enzyme-linked immunosorbent assays (ELISAs) observed in secondary DENV infections. Heat treatment of the serum sample could release complexed NS1 antigen by irreversible denaturation of antibodies and may improve the sensitivity of NS1 detection assays. However, this requires an assay that captures and detects heat-denatured NS1antigen. We have developed a NS1 capture ELISA using two serotype-cross-reactive, anti-NS1 monoclonal antibodies (MAbs) that bind specifically and efficiently to the heat-denatured DENV NS1. The performance of this assay was evaluated in a small panel of serum samples from patients with secondary DENV infection (n=15) and dengue negative cases (n=20), with and without heat treatment. Notably, heat treatment increased the optical density (OD) at least three-fold in dengue-positive serum samples and there was no increased OD in dengue-negative serum samples. Additionally, a considerable higher OD value was observed following heat treatment of artificially formed NS1-rabbit anti-DENV NS1 immune complexes compared to untreated ones. These findings suggest that our NS1 capture ELISA can be used to improve dengue diagnosis especially in patients with secondary DENV infections. Further evaluation of this assay in large panel of clinical samples is currently undergoing.

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DENGUE COHORT STUDY, ARARAQUARA, BRAZIL, 2015, BASELINE SEROPREVALENCE

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The number of reported cases does not fully represent the dengue burden. Access to health care, misdiagnosis and underreporting are factors that may influence dengue cases reporting. More accurate data on incidence are needed to support the evaluation of novel control strategies. A cohort study to assess the incidence of dengue among children and adolescents, from 2 to 16 years of age, in a low endemic setting has started in August, 2014. Data on the baseline seroprevalence will be addressed here. A random sample of children and adolescents was selected from the population of Araraquara, a city in Central-Western São Paulo State. Home visits were made to the families of selected children, to present the study and invite them to participate. The ones who agreed

to participate signed an Informed Consent Form. An interview on socio-demographic characteristics was carried out and blood samples from the selected children were drawn for dengue baseline serology. Dengue IgG antibodies were tested by enzyme-linked immunosorbant assay (ELISA). Families are being contacted weekly for fever surveillance. Fever cases are submitted to dengue diagnosis tests. 3,514 children and adolescents agreed to participate in the study. 50.5 % are girls. Their mean age is 7.9 years of age. The distribution according to age groups is 27.6% from 2 to 4 years of age; 36.7% from 5 to 9; 22.7% from 10 to 13; and 12.9% from 14 to 16. The baseline prevalence of IgG antibodies was 15.2%. There was no difference in prevalence according to gender. Regarding age groups, there was no difference in prevalence among the younger groups (from 2 to 13 years of age). Prevalence in the age group 14 - 16 was higher (18.6%), when compared to the younger group ($p = 0.047$). Despite the observed dengue transmission since 2000 in Araraquara, the seroprevalence among children and adolescents remains low. Surprisingly there was no increase in prevalence from 2 to 13 years of age, as observed in hyperendemic settings in Brazil. The state of São Paulo is facing its largest dengue epidemic ever in the 2015 transmission season. The follow up of the cohort will allow the assessment of incidence, as well as changes in prevalence.

1414

SPATIAL-TEMPORAL PATTERNS OF DENGUE AND CHIKUNGUNYA TRANSMISSION IN APARTADO, ANTIOQUIA, COLOMBIA. A PILOT CLUSTER STUDY

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Dengue and Chikungunya are two important arboviruses in tropical and subtropical urban and semi-urban areas. Of these arboviral threats, dengue is considered the most important, affecting an estimated 390 million people per year, with 500,000 episodes of severe dengue and >20,000 dengue related deaths. In Antioquia, department of Colombia, 26,027 cases of dengue were reported in 2010, 300 of which resulted in hemorrhagic fever. Chikungunya is now a threat in the Americas_as of January 2015, >1,135,000 suspected cases have been recorded in the Caribbean, Latin America and the United States. In Colombia, 249,013 cases have been confirmed since September 2014. Transmission of the dengue and chikungunya viruses is determined by factors related to their hosts, virus biology, their vectors (***Aedes aegypti*** and ***Ae. albopictus***) and the environment. With no effective vaccine and no treatment for dengue or chikungunya, control of these diseases is focused on suppresing their mosquito vector populations through environmental managment strategies and insecticide use. Examining patterns of viral transmission, and the factors that cause those patterns, will improve disease prevention and surveillance, ultimately reducing the burden of human infection. To gain insight into the patterns of dengue and chikungunya infection and the behavior of their vectors, we are conducting a pilot study of viral transmission in neighborhood clusters in Apartado, Antioquia, Colombia, a population endemic for dengue. This clustering method allows for the fine resolution of viral transmission dynamics within small geographic spaces during a short temporal scale. Data on human and mosquito infection, mosquito density and biometrics will be shown. These results will contribute to our knowledge of spatial-temporal patterns of dengue and chikungunya transmission, as well as the demographic and entomologic factors important for the spread of the virus. Our goal is to aid the local government in planning and applying strategies for vector and disease control.

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ESTIMATING THE SUPPLY AND DEMAND OF A BUTANTAN DENGUE VACCINE USING DIFFERENT INTRODUCTION STRATEGIES IN BRAZIL

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We modeled the vaccine demand, total implementation costs, and economic impact of Butantan's one-dose tetravalent dengue vaccine in Brazil for different introduction strategies in Brazil, using an integrated model approach. Input parameters on vaccine pricing, production capacity, introduction strategies, and related costs were derived from local Brazilian stakeholders. Country-specific epidemiological data were obtained from disease reporting systems. Thirty-year population projections were made with Brazil adopting by 2019. Brazil's highest dengue burden is among adults 19-46 years, and strategies targeting adults and children were modeled based on existing immunization age groups. Initial strategies targeting all ages or ≥18 year olds exceeded capacity and were considered not feasible. The demand for strategies targeting children ≤18 years was within capacity, but for adults 18-59 years, demand exceeded capacity by year 2024 except when stratifying by ages 19-31 (93.4M) and 31-46 (96.2M). At $5 per dose, the average annual cost of introduction ranged from $21.1-405.8M in the first 10 years to $52.6-810.4M in the last 10 years. The most affordable scenario was children aged 1-2 years, but this scenario had little disease burden impact (34% case reduction in last 10 years). Stakeholders prioritized vaccinating ages 7-60 years in the first 3 years followed by routine immunization in ages 2-6 years, but the expected capacity can only meet 76% of the demand over the first 3 years. Focusing on ages 2-46 years and staggering introduction over the first 5 years was feasible and had the greatest impact on reducing cases (90%) and deaths (79%) and on treatment cost savings (84%). Vaccinating adults followed by children yields the greatest vaccine impact. Vaccine price, introduction strategies, age, and production capacity are major drivers of the demand and require consideration when deciding vaccine introduction. Dengue vaccine has the potential to reduce cases and associated costs substantially based on various introduction scenarios.

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TRANSPLANTATION OF A COMPLEX QUATERNARY ANTIBODY EPITOPE FROM DENV3 INTO DENV4 REVEALS DETERMINANTS OF SEROTYPE-SPECIFIC NEUTRALIZATION AND DETAILS OF THE HUMORAL RESPONSE TO DENGUE INFECTION IN NON-HUMAN PRIMATES

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Despite an incidence of nearly 400 million infections per year, no approved therapeutics or vaccines exist to treat or prevent dengue virus (DENV) infection. Vaccination against DENV is complicated by the existence of 4 serotypes. Infection with one serotype does not confer immunity against heterologous serotypes. In fact, people exposed to secondary infections have an increased risk of severe disease thought to be a result of antibody-dependent enhancement of infection and aberrant cellular immune responses to the initial infecting serotype. As such, vaccination must induce a balanced protective response to all 4 serotypes simultaneously. To date this has been a challenge leading live-

attenuated tetravalent vaccine candidates have struggled with, indicating that new vaccine strategies may be needed. We recently reported on the discovery of a potent DENV3 human mAb (5J7) that recognizes a complex quaternary epitope present on the DENV3 virion. Using reverse genetics and structure-guided modeling, we have transplanted increasingly larger regions of the DENV3 mAb footprint into DENV4, to generate 3 recombinant DENV (from smallest transplant to largest: DENV4 M12, M14, and M16). These 3 rDENV4 were viable and demonstrated slight to moderate attenuation in cell culture systems. Importantly, the DENV3 mAb 5J7 bound and neutralized both DENV4 M14 and M16, indicating successful transplantation of the DENV3 epitope. Immune sera from people exposed to primary DENV4 and DENV3 neutralized DENV4 M14 and M16, demonstrating bivalent neutralization sensitivity of these viruses. Neutralization sensitivity was specific as DENV1 and DENV2 antisera did not neutralize rDENV4 M14 and M16. Inoculation of Rhesus macaques with DENV4 M14 or M16 resulted in detectible serum viremia in 3 of 4 animals receiving M14 and 1 of 4 animals receiving M16, and detectible DENV-specific IgM and IgG in a subset of animals. Taken together these results provide a more complete understanding of the development of complex antibody responses following DENV infection and have the potential to inform future vaccine design.

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GENERATING SUPPLY AND DEMAND ESTIMATES TO INFORM DENGUE VACCINE INTRODUCTION IN MEXICO, HONDURAS AND EL SALVADOR: A STRATEGIC DEMAND FORECAST

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Mexico, Honduras and El Salvador have recently reported growing incidence and mortality attributed to dengue hemorrhagic fever in the Americas. The growing burden of dengue in these countries has drawn attention to the need for an equitable dengue vaccine introduction. Currently, there are no studies that quantify the demand and supply of the dengue vaccine to inform the value of dengue vaccine introduction in Mexico, Honduras or El Salvador. Dengue strategic demand forecasts are a valuable decision-making tool that can evaluate the temporal demand of dengue vaccination, estimate the purchase costs of vaccination, and approximate the funding requirements for the vaccine according to different introduction scenarios. This study aims to generate new evidence on the potential demand and supply of two dengue vaccine candidates in Mexico, Honduras and El Salvador. We adopted an existing strategic demand forecast model to assess the potential demand of the vaccine candidates and estimate the purchase costs and disease impact of this demand in the three countries. Early results from extensive stakeholder consultations, including local government policymakers, program managers and vaccine researchers, indicate that, for a number of different scenarios, average national annual demand could be up to 105 million doses (entire endemic area) over 5 years. These levels of demand could result in substantial annual revenues over the same period. Higher vaccine demand requires increased capacity. Results rest on price, supply constraints, and timing of licensure. Governments in the three countries can increase their vaccine uptake by strengthening their national regulatory systems, expanding their healthcare infrastructure and programs to reach high-risk populations, and negotiating vaccine prices. Dengue strategic demand forecasts can aid overall strategic planning for vaccine introduction in the three countries.

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COST OF DENGUE VACCINE INTRODUCTION IN THE AMERICAS

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Planning for introduction of a novel vaccine relies heavily on financial considerations related to vaccine price, cost of production, and vaccine funding. Seven Latin American countries - Argentina, Brazil, Colombia, Ecuador, Mexico, Nicaragua and Venezuela - were selected to estimate the implementation cost of the Sanofi Pasteur three dose dengue vaccine and the potential financial impact at the national level. An economic supply and demand model was used to assess vaccine impact over a 30 year forecast. Country-specific data on age stratified case rates, treatment costs, vaccine coverage, and immunization costs was collected through national disease reporting systems and stakeholder interviews. Three survey versions were developed to align model inputs with stakeholder expertise. A variance range was applied to model parameters to account for differences across scenarios. Costs ranged from US$5-$15 per dose with the initial introduction strategy targeting children 2-14 year olds based on trends in disease burden. Results demonstrate that at highest potential price per dose, total cost of introduction to most countries was less than US$ 20M in the first three years. Highest cost was seen in Brazil and Mexico for the same time frame (US$1B; US$500M). At the lowest price per dose of US$5 over 3 years, highest introduction cost was for Brazil (US$419M) and less than US$ 2M for remaining countries. Vaccine purchasing costs had the highest financial impact in larger countries, estimated between US$460-$1370 for Brazil in the first five years and US$290-$870 in Mexico. Vaccine price represented the greatest portion of total production costs, with a difference of US$1.3 B between a US$5 per dose and US$15 per dose in the first three years in select countries. Understanding the financial requirements of a novel vaccine and the economic impact will service in accelerating uptake and increasing future health impact.

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CONSENSUS-BUILDING TO SUPPORT NOVEL VACCINE INTRODUCTION: METHODS AND LESSONS LEARNED FROM A STRATEGIC DEMAND FORECAST OF A DENGUE VACCINE IN BRAZIL

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We present methods used to model vaccine demand of a one-dose tetravalent dengue vaccine in Brazil. Understanding the potential demand and associated impact of this vaccine will inform introduction decisions and strategies. Public sector demand estimates were based on demographic, epidemiologic, and vaccine uptake assumptions derived from local stakeholder surveys, disease reporting systems and national and state-level census data. Using a combination of the above sources along with multi-level vetting and validation of model parameters, consensus was reached on a number of model parameters, including state-level age distribution of cases, vaccine coverage, and appropriate proxy vaccines. Consensus depended heavily on the strength of stakeholder survey questions and prioritization of stakeholder interests. Other factors that contributed to consensus agreement were early stakeholder involvement during data collection and participation of stakeholders with multidisciplinary backgrounds to reduce stakeholder bias. Disagreement remained on parameters related to target population and vaccine price.

Immunization program experts supported vaccine introduction through routine immunization in 1-2 year olds while vaccine manufacturers supported initial introduction in adults 15-49, the population considered high risk in Brazil. Disagreement remained regarding the public sector vaccine price and their willingness to pay. Where consensus was not reached, parameters were varied over multiple model iterations. Estimates of dengue vaccine demand are driven largely by supply constraints, vaccine price, timing of licensure, vaccine uptake after introduction, and population at highest risk for infection. Consensus was the result of a collaborative effort between investigators, internal staff, and participating stakeholders. Ongoing validation between parties was necessary to ensure plausibility of introduction strategies at every stage of analyses. Creating consensus around model parameters is critical for an impact study with a high level of unknown parameters and informs decision-making processes.

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CASE REPORT: ACUTE MYOCARDITIS CAUSE DEATH IN DENGUE PATIENT

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Dengue is a mosquito borne viral disease affecting 50-100 million people yearly. Despite global efforts neither an effective vaccine nor drug treatment are available. Infection with any of the four dengue virus serotypes 1-4 can cause outcomes that range from asymptomatic or mild disease to fatal dengue hemorrhagic fever/dengue shock syndrome. Typical signs and symptoms associated with most symptomatic infections include fever, headache, malaise, and body aches depending of the dengue serotype and the host immunological response. Severe manifestations are usually associated with subsequent infections and very rarely with first dengue infection. A 32 years old female was admitted to the clinic with fever, headache, body aches and malaise. No travel history, prior disease and no previous history of cardiac disease reported. The patient was previously treated at two outpatient health centers with medications to ameliorate fever and headache. She was admitted to the emergency room with hypotension, headache which increase by the hours, and immediately transferred to the Intensive Care Unit. Hypotension 80/60 mmHg of BP, cardiac frequency 120/min and breathing 32/min. Broad spectrum antibiotic treatment, antimalarial drugs and inotropics was administered inmediately. Results revealed Ig M positive reaction for dengue, hematocrit 41%, and platelets 35,000/ml. An abdominal ultrasound revealed evidence of cholecystitis and free liquid in the Douglas compartment, Leptospira test negative and smear for malaria negative. A cardiac ultrasound evidenced myocarditis and 25% of flow ejection. The patient clinical evolution was decreasing and in 36 hours after her admission to the clinic died, with multi organic dysfunction.The final diagnosis was myocarditis, pericardical effusion, and dengue shock syndrome, as the main cause of death. This is the first case of myocarditis due to acute dengue infection reported. It is important to educate individuals about the treatment and monitoring of vector borne virus disease and is convenient to perform the treatment in the same health facilit.

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PHYLOGENETIC ANALYSIS OF THE FOUR DENGUE VIRUS SEROTYPES THAT HAVE CIRCULATED IN PANAMÁ FROM 1993 TO 2014

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After 50 years of absence, Dengue virus (DENV) reemerged in Panama in 1993. Until 2014, a total of 55,970 Dengue cases with a case severity rate of Dengue Hemorrhagic fever (DHF) of 0.3% and of 48 deaths were reported. There is no information about the dynamic of the introduction, circulation and phylogenetic of the four serotypes of DENV in Panama. Since its reemergence, Dengue is endemic and all of the serotypes have circulated, with DENV-4 been reported only in 1999-2001. The reintroduction and replacement of serotypes has been associated with outbreaks. DENV-2 reemergence was responsible of the 1993 outbreak; during 1995 outbreak DENV-1 and DENV-2 co-circulated; in 2011 DENV-1 and 3 co-circulated and the reintroduction of DENV-2 was associated with an increase of the cases and the severity of the disease. Since then, DENV-2 is the main serotype in the country with the 90% of the PCR detected cases, and was also responsible of the 2013-2014 outbreak. Here we present the phylogenetic analysis of representative strains of each region of Panama from 1993 to 2014 of all four serotype of DENV and analyze the relationship with strains circulating in neighbor countries. We sequenced the coding region of the E envelope protein of at least two isolates by year of circulation for each serotype: 83 isolates for DENV-1; 38 for DENV-2; 15 for Denv-3 and 6 for DENV-4. Preliminary results show that the isolates of DENV-1 belonged to American/African genotype, DENV-2 to Southeast Asian/American genotype and DENV-3 to genotype III, DENV-4 isolates are still under analysis. Our data also show that the reintroduction of some of the serotypes included the circulation of different clades, related with clades circulating in neighbor countries. This suggests that because our geographic position, Panama could be a bridge between strains circulating in the North, South American and Caribbean regions. Future studies will evaluated the phylogeography dynamic of the Panamanian Dengue strains in the rest of the American continent to allow a better comprehension of the exchange and movements of DENV across the continent.

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FINDINGS FROM AN ACUTE FEBRILE ILLNESS ENHANCED SURVEILLANCE STUDY IN PUERTO RICO

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Dengue has been endemic in Puerto Rico for four decades, but little is known about other acute febrile illnesses (AFI) on the differential diagnosis of dengue. The Sentinel Enhanced Dengue Surveillance System (SEDSS) was implemented in two hospitals in southern Puerto Rico. Patients with fever or history of fever for <7 days were enrolled and followed through their illness. Blood, urine, nasopharyngeal, and oropharyngeal specimens were collected at enrollment and tested by RT-PCR and immunodiagnostic methods as appropriate for dengue viruses 1-4 (DENV); Influenza virus A and B; ten other respiratory viruses (ORV) including adenovirus, respiratory syncytial virus, metapneumovirus and parainfluenza viruses 1 & 3; enteroviruses; ***Leptospira*** spp., ***Burkholderia pseudomallei,*** and chikungunya virus (CHIKV). Of 31,525 AFI patients presenting from May 7, 2012 through September 30, 2014, 7,491 (24%) were enrolled. Of these, 26% were hospitalized, 50% were female, and the median age was 14 years (range: 0-103 years). An etiologic agent was identified in 46% (3,510), including DENV (13%), Influenza A or B (12%), ORV (10%), CHIKV (9%), enteroviruses (0.8%), Leptospira spp. (0.1%), and B. pseudomallei (<0.1%). In addition, 83 co-infections were confirmed by PCR, more than forty percent (42%) included DENV. Most (92%) of the 718 DENV PCR positive cases were DENV-1. Dengue patients were slightly older than other enrolled patients (median age: 15 versus 14 years, respectively), but younger than influenza patients (median age: 17 years). Dengue patients were more likely to be admitted than other

enrolled patients (OR = 2.89, 95% CI: 2.54-3.33) and influenza patients (OR = 3.57, 95% CI: 2.88-4.43). Almost half of all cases had a pathogen detected and most were caused by either a DENV, a respiratory virus (Influenza or ORV), or an enterovirus. Leptospirosis and melioidosis cases were sporadic and focal; study of these diseases may require study sites in high risk areas. Reasons for why dengue cases were more likely to be hospitalized will be studied further. We expect to use the SEDSS platform to conduct clinical research and evaluate current management practices.

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HIGH BURDEN OF DENGUE FEVER IN SOUTHERN COASTAL ECUADOR: EPIDEMIOLOGY FROM A PROSPECTIVE STUDY IN MACHALA IN 2014

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Comprehensive epidemiological studies that integrate passive and active surveillance of the dengue virus and vector are needed to inform the development of effective local control programs and novel interventions, including testing and licensure of new vaccines. Here we report the findings from the first year of a prospective study of dengue transmission conducted in Machala, Ecuador, a key surveillance site located near the Ecuador-Peru border. Individuals who presented with suspected dengue fever (index cases) at four sentinel clinics and the central hospital of the Ministry of Health were recruited into the study (n=204, mean age 21y, 48% female). Index cases with acute dengue infection (NS1 rapid strip positive) triggered an investigation of dengue cases in the index household and four neighboring households (n=329, 9-10 people per cluster, mean age 35y, 64% female). The primary endpoint was acute dengue infection, indicated by positive NS1 rapid strip, NS1 ELISA, or RT-PCR; secondary endpoints were recent secondary (IgG ELISA positive) and primary (IgM ELISA positive) dengue infections. Cases were deemed febrile if ear temperatures were >37°C. Dengue transmission peaked in mid-May following the onset of the hot, rainy season and an increase in adult female ***Ae. aegypti*** densities; the weekly incidence of acute cluster and index cases was significantly correlated ($p<0.05$). Children 10 to 14 years of age had the highest burden of dengue. In index cases, the prevalence of afebrile and febrile acute dengue infections was 11.1% and 20.2%; recent secondary and primary infections were detected in 46.1% and 57.2% of cases. In cluster cases, the prevalence of afebrile and febrile acute dengue infections was 11.8% and 4.4%, with recent secondary and primary infections detected in 37.9% and 25.2% of cases. DENV-2 was the dominant serotype, although all four serotypes (DENV1-4) were detected. Findings indicate that there is a high burden of symptomatic and inapparent dengue infections in this region, highlighting the need for improved surveillance and disease control interventions.

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PERSISTENT SYMPTOMS OF DENGUE: SYSTEMATIC REVIEW AND ESTIMATES OF THE INCREMENTAL ECONOMIC BURDEN IN MEXICO

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Dengue imposes a substantial disease burden to health systems in most tropical and subtropical countries worldwide. Symptomatic dengue infections are considered as mainly acute illnesses with three phases: a 1-7 day febrile phase, a critical phase affecting a small share of patients with possible hemorrhagic manifestations, and a 2-3 day recovery phase leading to a quick improvement of symptoms. But some dengue patients present persistent symptoms including profound fatigue, depression, and weight loss, a possibility acknowledged by WHO since 1997. If persistent symptoms affect a non-negligible share of the population, previous studies probably underestimate the burden of dengue. We performed a systematic review of literature of primary studies in English, Spanish, and Portuguese, in PubMed, Web of Science, LILACS, and SciELO since 1980. We identified a total of 31 articles, 11 of which (from Brazil, Cuba, Peru, and Singapore) satisfied our criteria (full-text available, empirical data, scientifically valid approach, and external validity). Persistent symptoms were usually associated with female and older age, and additional medical expenses. We found a significant relation between the share of patients (s) reporting persistent symptoms that may result in work loss (fatigue, asthenia, or difficulty working) and time (in months, t): $s=\exp(3.12-.38*\ln(t))$, $p=.03$, $r2=.24$. Using these predicted values, we updated recent estimates of the economic burden of dengue in Mexico, addressing uncertainty in the loss of productivity and additional expenses using Monte Carlo simulations. Our estimates suggest that persistent symptoms represent about US$38.2 (US$19.3-US$57.0) million annually in additional costs (US$0.35 per capita), or a 23% increase over previous estimates of economic burden in Mexico (US$170 million, US$1.56 per capita). While there is uncertainty in our estimates due to limited data, our results show a substantial neglected economic burden of dengue due to persistent symptoms. Given the breadth of the literature by continent and demographics, similar patterns likely extend to other dengue endemic countries.

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MIR-34A IS AN ANTI-FLAVIVIRAL MICRORNA THAT ENHANCES THE INNATE IMMUNE RESPONSE THROUGH REPRESSION OF THE WNT PATHWAY

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MicroRNAs (miRNAs) are small, noncoding RNAs that modulate gene expression post-transcriptionally and have been demonstrated to regulate a broad range of cellular processes. Our research is focused on identifying pro- and anti-flaviviral miRNAs as a means of elucidating cellular pathways that support or limit viral replication. To this end, we have screened a library of known human miRNA mimics for their effect on replication of three flaviviruses--dengue (DENV), West Nile virus (WNV), and Japanese encephalitis virus (JEV)--using a high throughput immunofluorescence screen. Of more than 1200 miRNA mimics screened, 61, 117, and 217 miRNA mimics were found to significantly inhibit infection of DENV, WNV, and JEV, respectively, with 23 blocking replication of 2 or more viruses. Members of the miR-34 family--miRNAs that have been extensively characterized for their inhibitory effects on the Wnt/β-catenin signaling pathway--demonstrated strong anti-flaviviral effects. Previous research has suggested that crosstalk occurs between the Wnt signaling and type I interferon (IFN) pathways. Therefore, we investigated the role of type I

IFN induction in the inhibitory effect of miR-34a and confirmed that miR-34a enhances the activation of IRF3 phosphorylation and translocation to the nucleus, induction of IFN-responsive genes, and release of type I IFNs from transfected cells. Importantly, enhancement of innate immune signaling by miR-34a does not occur in the absence of a stimulus, such as viral infection. This characteristic, along with the extensive ***in vivo*** characterization that has already been performed on miR-34a mimics as anti-cancer agents, makes this miRNA an attractive candidate for investigation as an ***in vivo*** antiviral treatment. Current research is focused on investigating the effect of miR-34a against WNV infection a mouse model. These findings highlight the opportunities for using miRNAs to discover novel cellular pathways and factors involved in supporting or limiting virus replication.

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RECRUITMENT AND CHARACTERIZATION OF A LOCAL DENGUE IMMUNE COHORT IN PORTLAND, OR

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Dengue virus (DENV) is the most important arthropod-borne virus affecting humans worldwide. The specific correlates and determinants of natural long-term DENV immunity are not known, without which designing and evaluating candidate DENV vaccines must proceed in the absence of reliable targets and endpoints. Large cohorts of naturally dengue immune individuals are vital resources for defining the critical correlates of dengue immunity. While investigators have recruited international DENV immune cohorts to study natural DENV immunity, local DENV immune cohorts offer several advantages over internationally based cohorts, including: 1) recruited individuals are unlikely to be confounded by repeat infection, with only long-term immunity present, 2) adult recruits can provide large volume serum and cell samples serially can be taken and processed locally and contemporaneously, 3) given time, a local cohort is expected to include donors with diverse DENV exposures that vary over time, geography, and DENV serotype. Here we present the design, execution and initial characterization of a DENV immune cohort based at Oregon Health & Sciences University in Portland, OR. We use previously validated recruitment approaches including traditional media, social media, email and direct physician contact to identify and recruit DENV immune individuals from local college student populations, cultural centers, and medical clinics expected to serve populations enriched for DENV exposure. Additionally, we focused recruitment efforts in the Portland VA Medical Center, which has a patient population expected to be enriched for very remote DENV exposure. From this cohort we expect to subsequently develop a baseline understanding of the correlates of long-term DENV immunity, specifically rates of neutralizing antibody decay and persistence of DENV immune cells. This cohort will provide new high-value immune sera and cells for both dissecting components of protective DENV immunity and validating targets and correlates of long-term DENV immunity.

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TOWARDS REFINING THE GLOBAL DENGUE MAP: SEROPREVALENCE VS. CASE DATA

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Good understanding of the transmission dynamics of infectious diseases is important to design effective control strategies. In particular, good estimates of the force of infection (the per capita hazard of infection), and of the basic reproductive number (R0) are critical because they give insight into the level of control that is required to reduce incidence and eventually block transmission. Given that population based serological studies (the gold standard) are very scarce, recent efforts to generate a global dengue map have been based on crude case counts and occurrence (presence/absence) data. However, inference from case counts is often misleading due to the poor correlation that exists between infection and symptomatic disease, and to differences that exist between and within surveillance systems. Here, we build on existing frameworks to estimate yearly forces of infection and R0 from age-specific incidence data and census data. We then use publicly available data to produce detailed updated maps of the transmission hazard and R0 of dengue in Thailand, Brazil, Mexico and Colombia. Preliminary analyses show very good agreement between our estimates and those obtained from age-stratified serological surveys from a range of settings. These include historically hyperendemic settings (Rayong, Thailand) and settings where dengue more recently re-emerged (Recife, Brazil; and Piedecuesta, Colombia). Furthermore, when applied to data from re-emergent settings, model estimates correctly the date of re-emergence. Additional analyses will include comparisons to additional serological data and to previously published estimates. Good characterization of dengue epidemiology will be fundamental to design optimal vaccination strategies and control interventions. This framework provides an opportunity to reconstruct recent dengue trends at a local scale and to refine the global dengue map using data that is readily available from multiple surveillance systems.

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DENGUE INFANT COHORT STUDY IN BRAZIL

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Dengue disease is the most prevalent arthropod-borne viral disease in humans and is a global and national public health concern in Brazil. Prospective studies are important to estimate incidence and to guide control measures. The aim of this study was to estimate the prevalence of dengue in pregnant women and the incidence in infants from a community in Rio de Janeiro (RJ). Prospective study of 0-2 years-old children who live in Manguinhos, an urban slum community in RJ, going on since May 2012. Pregnant women at third semester followed by the local Family Health Program were recruited. Epidemiological, clinical data and blood samples were obtained for dengue serological survey. The infants had programmed pediatric visits for evaluation of fever history and blood collection for serological tests (IgM). Mothers were instructed to contact the study staff, in case of infant´s fever. Active febrile surveillance was made by telephone calls, facebook and home visits. Pediatric visit is scheduled within 7 days after the onset of fever. The study was approved by the local Ethical Board No. 308/2011 CAAE: 0048.0.009.031-11. In two years, 502 pregnant women were included in the study with median age of 25 years-old (15 to 44). 90.5% tested positive for dengue IgG. There were 8 stillbirths, resulting in a total of 494 newborns eligible for follow-up. Until March 2015, 25.5% were lost for follow-up, most of them due to address change. From 374 infants on followed-up, 33.3% were infected by DENV in the 1st year of life. The high prevalence of positive IgG in young women is expected in hiperendemic areas as RJ where DENV circulates since 1986. The high incidence of dengue observed in infants in the 1st year of life raises the question about the protection to dengue by the maternal transmission of neutralizing antibodies. However, it can also be a result of the recent introduction of DENV-4 in Rio. The maintenance of this cohort is important to confirm our preliminary results and to determine the ideal age of life for vaccine trials. It also enables the study of environmental and individual factors associated to dengue infection.

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DYNAMIC EXPANSION FACTORS ENABLE REAL-TIME USE OF SURVEILLANCE DATA

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Surveillance systems generate data on disease incidence at a delay but public health decisions require current data. Recent work has focused on reducing reporting delays by converting to electronic records and centralized databases. For large systems these changes are expensive and time consuming, and there will always be some delays as records are reviewed for quality and updated based on laboratory tests. We propose a method to adjust case counts for under-reporting resulting from delays in the surveillance process. Our approach estimates dynamic expansion factors from statistical models of the surveillance system based on either individual case data or aggregated counts. We demonstrate the large-scale application of these methods using data from the Thai passive notifiable disease surveillance system (R506) for the years 2013-2015. To generate dynamic expansion factors we construct a country-wide contaminated Weibull model of reporting times with time-specific and province-specific parameters. We will present results from the 2015 dengue season and characterize the ability of dynamic expansion factors to accurately predict finalized case counts. Key lessons from this exercise are that 1) dynamic expansion factors increase the utility of reported surveillance data; 2) in a system with multiple reporting units creating accurate dynamic expansion factors requires characterizing surveillance process variation among units and over time; and 3) a model-based characterization of the reporting system can be an important resource for the management of the surveillance system. Future work will focus on integrating reporting and population dynamics into joint forecasts.

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ANTIGENIC IMPACT OF GENOTYPIC VARIATION WITHIN THE DENGUE 1 ENVELOPE PROTEIN

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Dengue virus is a mosquito-borne human pathogen with four serotypes, each consisting of multiple genotypes. These genotypes tend to be broadly associated with different geographical regions, but do co-circulate in some cases. Despite the immense effort being made toward the development of a vaccine capable of protecting against all four serotypes, relatively little has been done to investigate the importance of genotypic variation. To address this issue, a panel of dengue 1 viruses was generated consisting of a West Pac 74 backbone with envelope sequences derived from representative strains within the other dengue 1 genotypes swapped for that of West Pac 74. Replication kinetics were assessed in Vero 76, C6/36 and U937+DC-SIGN cells, and virulence was assessed in a type I/II interferon receptor knockout mouse model. A panel of human monoclonal antibodies and dengue immune sera was used to assess the impact of genotypic variation in a U937+DC-SIGN neutralization assay. Combined, these results indicate that the genotypic variation in the envelope protein had minimal impacts on viral replication. There, was, however, a noticeable impact on antigenicity, indicating that geographical differences in the prevailing genotype of dengue 1 may need to be taken into account when designing a vaccine or interpreting the results of clinical trials.

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MODELING POPULATION SUSCEPTIBILITY TO DENGUE INFECTION USING A "MEMORY" TERM

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Models for interacting multi-pathogen systems are challenging to analyze. Dengue, a viral pathogen estimated to cause 400 million infections each year worldwide, has four identifiable serotypes. Infection with one serotype is thought to confer permanent immunity to that serotype and temporary cross-protection against infection with other serotypes. A central challenge in modeling dengue fever is accurately estimating the (largely unobservable) population of individuals who are susceptible to infection. We propose a new approach to accounting for the susceptible population over time based on observed dengue case data. We use standardized weighted sums of prior case counts (a.k.a. ``memory terms", since they retain a memory of recent level of relevant infections) for all serotypes as an approximation to deviations from mean susceptibility. This method provides a simple way to include complex dynamics in otherwise standard statistical time-series models. We apply and evaluate this method in two contexts. First, we use memory terms to predict dengue hemorrhagic fever outbreaks in Thailand. Including the memory term in prediction models results in modest improvements in the average cross-validated prediction errors. Second, we use memory terms to draw inference about the existence and duration of cross-protection between dengue serotypes. Using simulated data, we demonstrate that our method can detect the presence of cross-protection in a wide array of four-strain disease settings with sensitivity ranging between 65% and 95%.

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ASSOCIATED FACTORS TO DENGUE INFECTION FROM A COHORT OF NAÏVE SUBJECTS IN AN ENDEMIC AREA

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Dengue is a public health problem in Mexico. The study of factors that contribute in dengue infection is important to optimize the promotion and prevention programs. However underreporting of cases and the asymptomatic infections makes it difficult. Objective: To determine associated factors to DENV infection in naïve subjects from two Mexican dengue endemic localities. Between August and November of 2014, we visited naïve subjects from a Mexican cohort enrolled between June and November 2011. We interviewed and took blood sample from subjects who accepted to participate in this follow-up. ELISA IgG indirect (Panbio) was performed to evaluate DENV infection during this period. Binomial regression was performed to evaluate associated factors to the seroconversion. An adjustment of standard errors was made considering the 125 houses as cluster units. 196 (79.4%) of 247 naïve subjects from original cohort agreed to participate. 90 were IgG positive (45.9%) and 1 was IgG indeterminate. Only 16 subjects remembered to have a dengue medical diagnosis (17.8%), and only 3 subjects were detected by the surveillance system. The associated factors were age (Prevalence Ratio 0.97; IC95% 0.95-0.99; p=0.016) and the occupation; compared with the students, the independent workers (PR 1.93 IC95% 1.46-2.56, <0.001) and the subjects who stay at home the majority of time (housewife, unemployed, retired, and disabled) (PR 1.62; IC95% 1.01-2.59, p=0.047) had more risk to get DENV infected; the employed workers were marginally associated (PR 1.41 IC95% 0.85-2.34, p=0.183). Also, the exposition to a cohabitant with a medical dengue diagnostic was marginally associated (PR 1.37 IC95% 0.98-1.93, p=0.07). Other variables like gender, education, locality, work/study out side the locality were not

associated. There was a high rate of DENV infection in these localities during 2.5 years of follow-up. The occupation factor could be related with the time that subjects spend in places where the infection is occurring (home or workplace).

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A HIGH-THROUGHPUT MICROASSAY FOR DETERMINING ANTIBODY RESPONSES TO 15 CLINICALLY IMPORTANT SPECIES OF FLAVIVIRUSES

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Flaviviruses, such as dengue virus (DENV) and West Nile virus (WNV), are arthropod-borne RNA viruses that are infectious disease risks to half of the human global population. Viruses within the Flaviviridae family share a similar genomic organization and replication strategy, yet are able to cause a wide range of clinical disease in humans, from undifferentiated febrile illness to the more severe forms, hemorrhagic fever and encephalitis. Serological assays for diagnosis and surveillance are generally directed towards one specific virus and disregard the potential for antibody cross reactivity between nearest neighbors. Here, we developed a comprehensive assay for flaviviruses that includes whole virus preparations and recombinant antigens from several clinically significant species. The antigens include capsid, pre-membrane/membrane, envelope, NS1, and NS3 proteins from 15 species that may co-circulate within the same geographic region. Using the microarrays, we examined serum IgG and IgM responses from humans infected with DENV serotypes 1-4, WNV, and Japanese encephalitis virus in order to examine specificity and cross reactivity of antibody responses among the viral antigens. We further employed sera from dengue vaccine studies to demonstrate the utility of using multiple viral antigens for obtaining a detailed analysis of antibody responses to vaccines. The small test volume requirement (1-10 microliters), multiplexing capacity, and high-throughput nature are all valuable features of this microassay. We propose that the data obtained from these flavivirus-focused protein microarray assays will provide a wealth of information into the human antibody response during natural infections and in response to vaccination.

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DENGUE VACCINE INITIATIVE: OVERVIEW OF THE FIELD OPERATION

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IVI within the Dengue Vaccine Initiative (DVI) consortium handles the field operation of the program. From 2011 to 2013, DVI conducted field studies in Colombia, Thailand, and Vietnam, likely early adopter countries of dengue vaccine. A complete package of the study including the passive fever surveillance, serological survey, cost-of-illness survey (COI), willingness to pay survey (WTP), and healthcare utilization survey (HUS) is conducted in Thailand and Colombia. In Vietnam, the COI and WTP surveys were conducted in 2011-2012. Since 2014, DVI has shifted the focus from the middle-income countries to the lower-income countries in Africa and Asia. The recent publication of the WHO Global Strategy for Dengue Prevention and Control in 2012-2020 emphasized the need for true burden of dengue by 2015. While there are documented disease transmission and vector presence, not much data from population-based studies are available, especially from Africa. Dengue may be considered for the GAVI vaccine investment strategy. Three countries, Burkina Faso, Gabon, and Kenya in Africa, as well as Vietnam and Cambodia, were selected for field studies. Most of these countries are GAVI-eligible with some documented disease transmission or outbreak history with existing infrastructure and local commitment for dengue research. In Vietnam, the study of the passive fever surveillance and the serosurvey are launched in Nha Trang in Aug. 2014 and data are forthcoming. In Burkina Faso, the set of DVI study including the passive fever surveillance with the COI survey embedded, HUS to complement the surveillance, and the serosurvey, was launched in Ouagadougou in Dec. 2014. In Gabon, the same set of study is launched in April of 2015 in Lambaréné. In Cambodia, launch of similar work package with the serosurvey in a community-based cohort design is planned in May, 2015. The similar work package is also in plan in Kenya. More information on the progress made in these sites of DVI field operation will be presented at the conference. By conducting DVI study in these sites, we hope to generate further evidence on burden of dengue for low-income countries.

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DETECTION OF DENGUE VIRUS IN EGG-HATCHED MOSQUITOES COLLECTED IN A CITY OF THE CENTRAL PORTION OF SÃO PAULO STATE, BRAZIL

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Dengue is a viral disease transmitted by ***Aedes*** mosquitos, which affects nearly 390 million people worldwide. It is endemic in more than 100 countries and the infections result in 500,000 hospitalizations and 20,000 deaths each year. Dengue is an arbovirus that belongs to ***Flaviviridae*** family, genus ***Flavivirus***, and presents four antigenically distinct serotypes (DENV 1-4). Virus surveillance in mosquitoes is important to track the spread of a virus after its introduction. ***Ae. aegypti*** and ***Ae. albopictus*** mosquitoes were obtained from ovitrap collected eggs. Traps were installed monthly in Araraquara, São Paulo/Brazil, from February 2014 to January 2015. Mosquitoes were grouped according to collection site, species and gender, and tested for the presence of four dengue serotypes using Hemi-Nested-Multiplex-RT-PCR. Field-collected eggs produced 8,667 ***Aedes*** mosquitoes, which were grouped in 1,114 pools. The presence of DENV was detected in 26 pools among the 187 that were analyzed. There were 25 amplifications that indicate the presence of DENV-1, DENV-2, DENV-3 and DENV-4 in pools of ***Ae. aegypti***. Among them were 15 male and 10 female pools. There was one pool of ***Ae. albopictus*** that was positive for DENV-1. Apparently, the four dengue serotypes are circulating in Araraquara. However, for complete confirmation of these results, our next step is viral isolation and nucleotide sequencing of the positive samples. But our results seem to be in accordance with the recent outbreaks in Brazil, caused by serotypes 1 and 4. The verification of vertical transmission in Araraquara seems to confirm a global trend and can be related with the maintenance of virus transmission in less favorable periods for the vectors and disease transmission.

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SPATIAL AND TEMPORAL HETEROGENEITY IN DENGUE SEROTYPE CIRCULATION AND DYNAMICS OF RISK ACROSS PERU

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Dengue is one of the most aggressively expanding mosquito-transmitted viruses approaching 400 million infections annually. Complex transmission dynamics pose challenges for predicting location, timing, and magnitude of risk or spatial scale of risk when an outbreak occurs; thus models are needed to guide prevention strategies and policy development locally and globally. Empirical data needed to guide model development and a better understanding of risk dynamics suffer from critical gaps. Weather regulates transmission-potential via its effects on vector dynamics, yet an empirical perspective clarifying how weather impacts transmission in diverse ecological settings is lacking. Four antigenically related but distinct virus serotypes co-circulate in hyperendemic regions causing (1) short-term heterologous immunity and long-term homologous immunity against re-infection; (2) wide range in clinical disease from asymptomatic to self-limiting dengue fever to life-threatening dengue hemorrhagic fever- a spectrum that varies across serotypes/strains and with number, sequence, and relative timing of sequential infections from different serotypes; and (3) a general lack of serotype-specific information in surveillance data. This lack of data obstructs an understanding of spatial scales and networks of virus spread beyond community cohort studies. We developed a high-resolution empirical profile of (1) the local weather-disease connection, (2) serotype-specific dengue disease, human susceptibility, and force of infection, and (3) serotype-specific transmission networks across Peru, a country with considerable ecological diversity. For 1990-2012, we tracked the introduction and spread of the 4 dengue serotypes across Peru, examining serotype-specific spatial dynamics amidst the role of weather in regulating transmission, heterogeneity in spatial networks, and factors that regulate cycles in serotype replacement spatially. Results provide new insights regarding the spatial scales and dynamics of risk that will enable more effective strategies for separate or integrated delivery of vector control and vaccines.

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POTENTIAL IMPACT OF DENGUE VACCINATION IN DENGUE ENDEMIC AREAS: INSIGHTS FROM TWO LARGE-SCALE EFFICACY TRIALS

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With about 4 billion people living in endemic areas and 100 million apparent infections occurring each year, dengue is a major international public health concern. To date, no specific treatment is available for this disease but the Sanofi Pasteur vaccine candidate has in 2014 successfully demonstrated its protective efficacy against symptomatic dengue during the 25 month active phase of two Phase 3 efficacy trials performed in five Asian countries (Indonesia, Malaysia, Philippines, Thailand, Vietnam) and 5 Latin American countries (Brazil, Colombia, Honduras, Mexico, Puerto Rico), based on the observations obtained from a total of 31,144 subjects. In order to best use this vaccine, it is important to evaluate its ability to reduce dengue burden in endemic areas and mathematical models of transmission can provide valuable insights on the matter. Results observed during the active phase 3 efficacy trials were used to fit an age-structured, host-vector and serotype-specific compartmental model previously developed. The estimation was performed using Approximate Bayesian Computation (ABC) method based on Sequential Monte Carlo. Several scenarios of cross-interactions between serotypes and vaccine efficacy were tested. We notably considered vaccine efficacy based on: 1) difference in efficacy between serotypes; 2) increase in efficacy after subsequent doses; 3) protection conferred to naïve and primed populations. The results obtained provide insight on the optimal age for vaccination for the 10 countries included in the two trials. We also considered the impact over time of different vaccination programs including routine and catch-up vaccination. In conclusion, the analysis performed on the basis of these two large vaccine efficacy trials suggests that the dengue vaccine candidate has the potential to significantly impact dengue burden in endemic countries.

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SEROLOGICAL AND VIROLOGICAL INVESTIGATION OF YELLOW FEVER OUTBREAK IN SOUTH OMO ZONE, ETHIOPIA

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South Ari district of South Omo zone reported unknown illness with clinical presentations of fever, headache, nausea and bloody vomiting since December 2012. On the basis of clinical symptoms, a viral hemorrhagic fever was suspected and outbreak investigations were carried out. This abstract presents findings of the serological part of the outbreak investigation. A total of 30 serum samples were collected either from in-patients admitted at the Zonal Hospital as a result of having at least two of the clinical manifestations typical of the outbreak or active searches of suspected cases and contacts in the affected areas. As part of further investigation of the outbreak and elucidating if possible expansion of the yellow fever outbreak exists in the neighbouring/nearby districts/zones to the affected area, 42 serum samples collected from patients of acute febrile illness (AFI) seen at a health facility and Hospitals in these areas were also also investigated. The 30 serum samples were screened for yellow fever IgM by ELISA and 7 cases (23.3 %) were found to be positive. Differential diagnoses for other flavi viruses (Dengue, West Nile and Zika viruses) and also for RVF, CCHF and Chikungunya viruses showed no positivity. None of the samples were positive by PCR . Two samples (6.6%) had a positive IgG test for flavi viruses. Of the 42 serum samples from AFI patients in the neighbouring/nearby district/zones to the affected area, 3 (7.14%) were positive for west Nile IgG and 2 (4.76%) were positive for yellow fever IgG. The serological and virological investigation revealed that the cause of the outbreak to be a yellow fever virus. IgG positivity for falviviruses in adjacent/nearby districts and zones to the affected area necessitates identifying which specific virus of the flavi virus group is circulating and may require determining yellow fever epidemic risks as well as strengthening of surveillance in the broader vicinity of the affected area.

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CDC WEB-BASED YELLOW FEVER VACCINE COURSE: USE BY STATE AND TERRITORIAL HEALTH DEPARTMENTS IN THE UNITED STATES

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In 2010, the Centers for Disease Control and Prevention (CDC) Travelers' Health Branch (THB) launched the free web-based course, ***Yellow Fever: Information for Health Care Providers Advising Travelers,*** to provide state and territorial health departments (STHDs) with an effective training tool to educate health care providers about yellow fever (YF) and best practices for YF vaccination. To purchase and administer YF vaccine (YFV) in the United States, a nonfederal provider must possess a STHD-issued YF stamp or practice in the same clinic as a YF stamp owner. However, practices for authorizing use of YF stamps vary among STHD YF stamp designation programs. We examined characteristics of these programs and their use of CDC's web-based course. THB invited 53 STHDs to participate in a survey during December 2011. Topics covered included eligibility for YF stamp ownership; training requirements for first-time stamp applicants, renewing stamp owners, and non-stamp-owning YFV providers. Survey response data were analyzed using SAS Enterprise Guide 5.1. Overall, 31 (58%) of 53 STHDs participated in the survey. Among the 31, 28 (90%) are aware of CDC's YFV course. All 31 responding STHDs issue stamps to physicians, 11 (35%) to nurse practitioners, 9 (29%) to pharmacists, and 7 (23%) to physician assistants. Thirteen (43%) of 30 STHDs that responded to the question require current stamp owners to renew their stamp yearly or less frequently. Ten (32%) of 31 responding STHDs require training in YF or travel medicine before issuing a YF stamp; 9 specifically require completion of CDC's YF course. Six (20%) of 30 STHDs require non-stamp-owning YFV providers to complete the course. Most responding STHDs are aware of CDC's YFV course but do not require proof of YF training from first-time stamp applicants or from non-stamp-owning YFV providers. However, among STHDs requiring proof of YF vaccination training, CDC's YF course is the most often utilized resource. With a revised course in development, THB plans to re-administer the survey and assess changes in YF programs and in STHD use of the course as a training requirement for health care providers who administer YFV.

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GLOBAL DISTRIBUTION AND ENVIRONMENTAL SUITABILITY FOR CHIKUNGUNYA VIRUS

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Chikungunya is an acute febrile illness caused by the Chikungunya virus (CHIKV), which is transmitted to humans by Aedes mosquitoes. Although Chikungunya is rarely fatal, patients can experience debilitating symptoms that last from months to years. Despite expanding worldwide geographical distribution, knowledge of the global burden and extent of CHIKV remains alarmingly sparse. Here we exhaustively assess and map the global occurrence of Chikungunya using an established modeling framework previously used for dengue. We also predict the global environmental suitability and estimate population at risk of CHIKV transmission. We identified 89 countries with good evidence for CHIKV occurrence and several countries with environmental suitability and previous reports of CHIKV but with a general lack of reliable data and poor surveillance leading to indeterminate conclusions on presence or absence. We estimate 1.3 billion people living in areas at-risk of CHIKV transmission. These observations provide a basis for assessing clinical surveillance and burden estimation of Chikungunya globally, and an initial evaluation on the global distribution that can be refined as CHIKV continues to expand.

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CHARACTERIZATION OF A GENETIC DETERMINANT FOR THE MOUSE NEUROVIRULENCE OF YELLOW FEVER VIRUS IN *AEDES AEGYPTI*

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The loss of neurovirulence of wildtype flaviviruses in vertebrate hosts has been considered as a critical phenotype for the development of live attenuated strains of flaviviruses as vaccine candidates. Using yellow fever virus (YFV) 17D vaccines as a model, one of the genetic determinants for mouse neurovirulence of YFV has been mapped to the wildtype specific epitope Thr^{173} in the envelope (E) protein. Whilst the functional importance of the E protein with respect to antibody neutralization and attenuation of the YFV 17D-204 strain in mice has been demonstrated ***in vitro*** and ***in vivo***, it is still unclear whether or not the E T173I genetic mutation contributes to the loss of the biologically transmissibility by mosquitoes that is a characteristic of the YFV 17D vaccine strain. We evaluated the phenotypic changes caused by the T173I mutation with the cDNA infectious clones of the Asibi strain and the 17D+Asibi M-E chimera in ***Aedes aegypti*** through ***per os*** infection. The Asibi E T173I mutant and the 17D+Asibi M-E E T173I chimeric mutant were orally administered with the control mosquito groups receiving either the Asibi strain alone, the 17D+Asibi M-E chimera, or the 17D strain. Mosquitoes were collected for analysis at 7, 10, and 14 days post-infection. Comparison of the percentage of infection and dissemination as well as the replication kinetics was performed between wildtype and mutant viruses by detection of infectious viruses using tissue culture infectious dose 50. The phenotypic change caused by the mutation on infection and dissemination in mosquitoes will be discussed.

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ARIPO VIRUS IS AN INSECT-SPECIFIC FLAVIVIRUS THAT CAN ENTER AND REPLICATE IN VERTEBRATE CELLS AND PRECLUDES SUPERINFECTION AND DISSEMINATION OF WEST NILE VIRUS *IN VIVO*

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To date, at least 25 distinct insect-specific flaviviruses have been isolated from and/or detected in mosquitoes collected worldwide. Although insect-specific flaviviruses are abundant in nature, little is known about their ecological niche, their place in the evolutionary history of flaviviruses, genetic determinants that render them unable to replicate in vertebrate cells, or to what extent they affect transmission of vertebrate-pathogenic flaviviruses in nature. Herein we describe the isolation and characterization of a novel insect-specific flavivirus (tentatively named Aripo virus; ARPV) that was isolated from a pool of Psorophora albipes mosquitoes collected in Trinidad. The complete ARPV genome was determined and phylogenetic analyses showed that ARPV clusters with other insect-specific flaviviruses that fall within the pathogenic mosquito-borne flavivirus clade. ARPV antigen was significantly cross-reactive with Japanese encephalitis virus (JEV) serogroup antisera by both immunofluorescence assays and hemagglutination inhibition assays. Infection with ARPV significantly

reduces superinfection rates and prevents dissemination of West Nile virus ***in vivo*** in ***Aedes aegypti*** mosquitoes. Experimentally, we also determined that ARPV infection is efficiently transmitted vertically in ***Ae. aegypti*** mosquitoes at least to the F3 generation. A 2 nm single particle cryo-EM reconstruction revealed that ARPV is structurally similar to other flaviviruses, having a very smooth surface with envelope protein trimer-dimers oriented in a head to tail manner, across its surface. We also show using transmission electron microscopy, confocal microscopy, and PCR based molecular techniques, that ARPV can attach and enter vertebrate cells via clathrin mediated endocytosis, as well as replicate its genome in these cells. Preliminary data suggests this virus is restricted from replication in vertebrate cells because it is incorrectly assembled in these cells.

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INFERRING THE YELLOW FEVER FORCE OF INFECTION FROM THE OBSERVED AGE DISTRIBUTION OF CONFIRMED CASES

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Yellow fever is an arboviral disease, transmitted between humans and non-human primates and mosquitoes of various Aedes spp. in Africa. Disease severity shows a broad spectrum with high rates of fatality amongst the more severe cases, but symptoms tend to be non-specific, particularly in milder cases leading to huge underreporting and highly uncertain disease burden. As yellow fever is a sterilising infection, the age distribution of observed cases can give clues to the yellow fever transmission intensity. Using data on confirmed human cases of yellow fever reported between 2005 and 2013 to the Yellow Fever Surveillance Database covering 20 countries in western and central Africa in conjunction with data on population demographics and age-dependent vaccination coverage we estimated the force of infection that could give rise to the observed age distribution of cases under a number of different assumptions regarding the age-dependence of the exposure. Point estimates of the force of infection were low, but comparison with estimates derived from other data suggest upper confidence bounds obtained might put useful bounds on the extent of transmission. Results were sensitive to the assumed age distribution of the population and vaccination coverage and strongly depended on the age-dependence of exposure assumed, highlighting the need for robust sensitivity analyses. Models assuming age-independent force of infection fitted better in western Africa, but a higher exposure in adults than children fitted better in central Africa. In western Africa, the annual risk of infection to a susceptible person was estimated to be below 2.3%, while the annual infection risk in central Africa was estimated to be below 0.5% for children and 1.2% for adults. The fact that in different areas different assumptions about the age-dependence of the infection risk gave the best fit to the data indicates an indiscriminate exposure in villages in western Africa, where the transmission intensity is highest, but more limited occupational exposure through activities such as wood clearing in central Africa with a lower overall transmission intensity.

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DISTINCT SPLENIC CELLULARITY IN WILD-TYPE YELLOW FEVER VIRUS AND 17D VACCINE STRAIN INFECTION AND ITS IMPLICATION ON IMMUNOGENICITY/PATHOGENESIS

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Yellow fever virus (YFV) is a mosquito-borne flavivirus that causes hemorrhagic fever in humans and poses a serious health threat in developing countries. Control of YFV relies upon the live-attenuated 17D vaccine strain, derived in the 1930s. The mechanism for 17D attenuation and immunogenicity remains unclear. In our pathophysiologically relevant mouse model, WT-YFV infection causes severe viscerotropic disease in type I IFN receptor deficient mice whereas 17D vaccination induces long-term, protective immunity against WT-YFV challenge. CD169 (Siglec-1) is a glycoprotein expressed on tissue-resident macrophages such as splenic marginal zone macrophages (MZMs) and hepatic Kupffer cells. Immunologically active CD169+ macrophages play an important role in the capture of antigens, induction of innate immune responses and peptide presentation to T and B lymphocytes. Interestingly, the expression of CD169 was dramatically increased in 17D-infected mice, consistent with a role in the induction of effector and memory immune response. In contrast, after a transient upregulation, CD169+ cells disappeared following infection with WT-YFV. Parallel to this finding, GL-7, an activation marker for B and T cells, expression in splenic follicles is also different between the two. Together with the evidence for enhanced CD169 transcript levels in the PBMCs of human 17D-vaccinees, the stark differences in CD169+ cell populations and lymphocyte activation may infer a mechanism for pathogenesis for WT virus or strong immunogenicity of 17D which we are currently exploring.

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THE ROLE OF PIWI-INTERACTING RNAS IN *CULEX QUINQUEFASCIATUS* CELLS AND MOSQUITOES DURING WEST NILE VIRUS INFECTION

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Arboviruses are maintained in an enzootic cycle between a vertebrate host and an arthropod vector and have evolved to cope with both the complex vertebrate immune system and arthropod antiviral defenses. In order to develop efficient preventive measures it is important to understand both virus-host and virus-vector interactions. Our understanding of arthropod antiviral defenses has increased immensely over the last 15 years, and RNA interference (RNAi) is considered the most important antiviral mechanism. There are three individual components of the RNAi system, namely the small interfering RNA (siRNA), the micro RNA (miRNA) and the PIWI-interacting RNA (piRNA) pathways. The siRNA pathway is predominantly considered an antiviral mechanism, while miRNAs have important roles in regulation of gene expression and piRNAs are important for the protection of germ line DNA from retrotransposons. While the siRNA pathway has been considered the most important antiviral response in mosquitoes, the antiviral role of piRNAs is becoming more and more evident. A number of studies have implicated virus-derived piRNAs in the control of alpha-, bunya- and flaviviruses in Aedes mosquitoes. Culex mosquitoes are important vectors of arboviruses, such as West Nile virus, but they are considerably less studied than ***Aedes*** mosquitoes, vectors of for example dengue virus. In the present study we investigated the role of the PIWI pathway in antiviral defenses of ***Culex*** mosquitoes. We characterized expression of putative Piwi pathway components in ***Culex quinquefasciatus*** and ***C. pipiens*** mosquito tissues, as well as ***C. quinquefasciatus*** Hsu cells. Interestingly, while all six PIWI genes were highly expressed in ovaries, only selected PIWI genes were expressed at high levels in mosquito midguts and Hsu cells, possibly indicating the importance of these PIWI proteins in antiviral defenses rather than just germ line protection. The putative antiviral role of individual PIWI proteins was then determined in Hsu cells by silencing of Piwi gene expression and subsequent virus infection. This is the first study to implicate the piRNA pathway in ***Culex*** antiviral defenses.

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EXPERIMENTAL WEST NILE VIRUS EVOLUTION USING A NATURAL TRANSMISSION MODEL: DETERMINING THE ROLE OF VECTOR-HOST INTERACTIONS IN SHAPING VIRAL POPULATIONS

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West Nile virus (WNV) is a dynamic, evolving entity that can adapt to a wide range of environments and infect many different host species. It is primarily maintained in an enzootic cycle by Culex species vectors and avian hosts. Microhabitat-specific aspects of WNV transmission, such as how often the vectors and hosts interact, may drive evolution on a local scale. We have developed an ***in vivo*** model for WNV transmission using ***Culex*** mosquitoes and young chickens to 1) characterize intrahost WNV evolution during transmission and 2) to determine the degree to which the frequency of transmission influences WNV population structure and infection phenotype. We will use next generation sequencing to track changes to the viral populations in mosquito saliva and chick serum during several rounds of transmission. Our data suggests that shorter durations of mosquito infection (7 vs 21 days post infection) leads to higher viremia in chicks upon mosquito bloodfeeding. Multiple cycles of more frequent mosquito-to-bird transmission may lead to the selection of WNV variants that can be transmitted sooner (i.e. decreased extrinsic incubation periods) and increase vectorial capacity. Ultimately, this could help determine how periods of rapid transmission can change the genetic foundation of a WNV population and give rise to new genotypes.

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THE IMPACTS OF CYCLING TEMPERATURE ON WEST NILE VIRUS TRANSMISSION IN CALIFORNIA'S CENTRAL VALLEY

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West Nile virus (WNV), a flavivirus from the family Flaviviridae, is of public health concern due to its ability to cause disease in both humans and animals. It is transmitted between birds by mosquitoes, typically those from the genus ***Culex***. The time from a mosquito's consumption of an infected blood meal until it becomes capable of transmitting the virus by bite is termed the extrinsic incubation period (EIP) and is temperature-dependent. The EIP can be expressed in terms of degree-days, a measure of the cumulative temperature above a zero-transmission threshold. The current model for WNV transmission in California was based on the assumption that the number of degree-days to transmission is the same under all conditions and that a model calculated using only constant temperatures accurately captures the effects of daily temperature cycling found in nature. However, research on other vector-borne pathogens, such as ***Plasmodium*** and dengue virus, has shown that extrinsic incubation rates and vector competence depend on the range of daily temperature cycles in addition to the daily mean temperature. We examined the impacts of daily temperature cycling on WNV transmission using temperatures typical of the viral amplification season from March-July in a hyperendemic area of California's Central Valley. In addition to the daily air temperature profiles, we considered the impact of realistic mosquito exposure temperatures for night-biting ***C. tarsalis***, which are sheltered from afternoon peak temperatures. We found that the combination of extrinsic incubation time and daily mean temperature explained temporal changes in vector competence, but when transmission was expressed as a function of degree-days, vector competence was consistently higher for the July temperature treatment. Taken together, these results suggests that extrinsic incubation rates depend on both cumulative temperature (degree-days) and other features of the diurnal temperature cycle.

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AVIAN VIRULENCE AND VECTOR COMPETENCE PROFILES OF EMERGENT LINEAGE 2 WEST NILE VIRUS STRAINS

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The continuing global dissemination of West Nile virus (WNV) has been highlighted by the recent geographic expansion of lineage 2 (L2) West Nile strains from sub-Saharan Africa into eastern and southern Europe. An NS3-H249P substitution, previously unassociated with L2 WNVs, was initially identified in L2 WNV isolates (NS10) made during a Greek 2010 human WNV disease outbreak. In L1 WNV strains, an NS3-249P substitution has previously been associated with increased viremia and mortality in corvids as a result of positive selection. In order to assess similar avian pathogenic associations with the NS3-249 loci in L2 WNV backbones, alternative polymorphisms (Pro/ His) were generated at the NS3-249 site in NS10 and South African 1989 (SA89) L2 WNVs. Resulting mutant viruses were assessed directly in a highly susceptible American crow (AMCR) model. The parental NS10 and SA89 strains displayed peak viremias of 9.5 and 7.5 log10 PFU/mL sera with 100% and 33% mortality, respectively. The NS10 mutant, NS10 NS3-249H, exhibited reduced peak viremia (8.7 log10 PFU/mL sera) and mortality (80%) relative to the parental NS10. Reciprocally, the SA89 NS3-249P virus exhibited a bolstered peak viremia (9.6 log10 PFU/mL sera) and an increase in mortality (100%) relative to the parental SA89 WNV. In order to assess if altered vector competence is associated with compensation for lower avian viremias elicited by polymorphisms at the NS3-249 loci and/or whether this loci could modulate vector competence, three parental L2 WNV isolates containing a His at the NS3-249 loci (SA89, Hungary 2004 and Uganda 2009) were compared to NS10 in colonized ***Culex pipiens*** and ***C. quinquefasciatus*** mosquitoes. Initial studies have indicated that all four L2 WNVs display similar vector competence profiles following oral exposure to varying doses of the viruses and extrinsic incubation periods in mosquito species were similar. These results suggest that the emergence of the L2 NS3-249P substitution has likely resulted from avian selection and its subsequent effect on modulating the force of transmission has been independent of inherent direct vector competence effects.

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RAPID DETECTION METHODS OF KOREAN SACBROOD VIRUS

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Sacbrood virus (SBV) is one of the most serious honeybee viruses. The virus causes failure to pupate and death in both larvae and adult bees. Recently, Korean Sacbrood virus (KSBV) caused a great loss in Korean honeybee (Apis cerana) colonies. Although KSBV shows high homology with SBV strains, it has unique motifs and causes different symptoms. Therefore, a simple, sensitive and specific method for detecting KSBV is needed urgently. In this study a reverse transcription loop-mediated isothermal amplification (RT-LAMP) and a novel micro PCR-based detection method, termed ultra-rapid real-time PCR (URRT-PCR) were applied for rapid detection for korean sacbrood virus (KSBV) from honeybees (Apis cerana) infected with SBV in Korea. The LAMP could be detect the virus in RT-LAMP reactions containing 10^2copies of pBX-KSBV within 30 min, which was 10 times more sensitive than a RT-PCR assay. The URRT-PCR showed high sensitivities which were able to detect 10 copies in the standard

assays. In the application of URRT-PCR detection to an KSBV-infected honeybee, the shortest detection time was 10 min 12 sec, including reverse transcription. In addition, these methods could be distinguished between KSBV and other closely-related SBV strains, These rapid methods were rapid molecular-based diagnostic tools and useful tool for the rapid and sensitive diagnosis of KSBV infection of honeybees.

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THE PREVALENCE AND GENETIC DIVERSITY OF SAPOVIRUS IN A NESTED CASE-CONTROL STUDY OF A PERUVIAN PERI-URBAN COMMUNITY

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Human sapovirus (SaV) belongs to the caliciviridae family and a causative agent of acute gastroenteritis. Recently, occurrence of SaV has been increasing worldwide with emerging SaV GI.2, but its attribution and diversity among infant diarrhea has not been reported. A birth cohort study to look at infant diarrhea was conducted between June 2007 and May 2010 in a peri-urban shanty town in Lima, Peru. Stool samples from 300 diarrheal episodes and 300 age-season matched non-diarrheal samples were selected from archived specimen bank generated from this cohort study. The detection rate of SaV were 12.3% in diarrheal and 5.7% in non-diarrheal stool samples using real-time PCR. The attributable fraction of SaV was higher in the second year (13.1%) than that in the first year of life in children (2.6%). Ten known genotypes (8 GI.1, 2 GI.2, 2 GI.6, 7 GI.7, 2 GII.1, 4 GII.2, 2 GII.4, 1 GII.5, 2 GIV, and 5 GV.1) and one novel genotype in GII (n=5) were found. Symptomatic reinfection causing by different genogroups (n=5) and genotypes (n=3) were observed in eight children and it suggest SaV may develop genotype specific immunity. This study indicates SaV contributes to community acquired diarrhea, especially in the second year of life of infants in developing area. Multiple genotypes circulating in one community and possible genotype specific immune response could complicate the SaV vaccine development.

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DEVELOPMENT OF MOLECULAR ASSAYS FOR THE DETECTION OF SOUTH AMERICAN HEMORRHAGIC FEVER VIRUSES

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Of the several viral hemorrhagic fevers (VHF) in South America, there are five that are caused by arenaviruses. Due to the necessity of higher biocontainment, a diagnosis is usually confirmed through reference laboratories. However, diagnosis can be complicated by other VHF such as those caused by dengue and yellow fever viruses, requiring the development of new molecular tools. Individual qRT-PCR virus-specific assays with synthetic RNA or DNA positive control standards were developed for ***Junin***, ***Chapare***, ***Guanarito***, ***Machupo***, and ***Sabiá*** viruses. In certain assays, DNA standards were utilized due to incomplete ***in vitro*** transcription of the target amplicon (RNA). Parameters tested during assay development included sensitivity, specificity as a function of melt curve, linearity, and reproducibility. Each assay was found to be sensitive enough to detect between 10 and 100 genome equivalencies per reaction and formed a single melt peak, indicating primer target specificity. To compare the assay performance of RNA versus DNA standards, both types of synthetic positive controls for the ***Junin*** assay were evaluated. Both standards showed comparable performance with respect to sensitivity of detection, specificity, PCR efficiency, linearity, and reproducibility. In separate studies, we demonstrated the utility of these assays for the detection of viral RNA content using a preparation made from irradiated inactivated virus as a surrogate to live virus. To test the effectiveness of viral detection from a clinically relevant matrix, inactivated ***Junin*** virus was serially diluted then spiked into normal human serum. Total viral RNA was extracted and analyzed using the established ***Junin*** qRT-PCR assay. The recovery of viral RNA from serum was within 0.5 logs of calculated genomic equivalencies to that of viral RNA recovered from positive controls. This demonstrates the applicability of the ***Junin*** qRT-PCR assay with clinically relevant matrices. Our findings show that these assays are a sensitive method for the detection of arenavirus causing VHF and could potentially be developed for diagnostic testing.

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DETECTION OF CORONAVIRUSES IN BATS IN CAMBODIA AND LAO PDR

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South-East Asia has been identified as hot spot for emerging zoonotic diseases. Over the past decade, bats have been recognized as natural reservoir for an increasing number of zoonotic viruses such as SARS-coronavirus, or Nipah virus. Hence, there is the necessity to identify known and novel infectious diseases in bats which may constitute a risk to the human population. In our study, we assessed and characterized the presence of coronaviruses (CoVs) in bats at interfaces with human populations in Lao PDR and Cambodia. Bat samples (organs, swabs, urine, feces) were collected in several provinces of Cambodia and Laos from 2010 to 2014 and tested for the presence of coronavirus by RT-PCR targeting the RdRp gene. Positive samples were sequenced and phylogenetic analyses of RdRp fragments (430 bp and 1500bp) were conducted to determinate their relationship with other known coronaviruses. A total of 83 coronavirus positive samples were detected in microchiroptera and fruit bats, including 35 αCoV and 48 βCoV. All βCoV clustered with the group of bat βCoV-HKU9 strains (78 to 99% of nucleotide identity). Detected αCoV clustered with different αCoV bat strains and 2 samples were closely related to PEDV strains (93% and 96% of nucleotide identity respectively) responsible of severe diarrhea in pigs. Such results showed the presence and diversity of coronaviruses circulating in bat population in Cambodia and Lao PDR. This is the first report of coronavirus detection in bat population in these two countries. Further molecular epidemiological studies and complete genome sequencing, especially the spike protein gene, will shed more light on diversity of those coronaviruses and help to estimate the potential zoonotic risk.

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DISTRIBUTION OF THE CIRCULATING ROTAVIRUS GENOTYPES BEFORE AND AFTER ROTARIX® VACCINATION AT SENTINEL SITES IN SUDAN

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Globally, rotavirus infection is responsible for 37% to 53% of hospitalizations due to diarrhea in children below 5 years of age where rotavirus vaccination is not routinely administered. In Sudan, rotavirus has accounted for 33% of diarrheal cases from 2008 to 2010. These data supported the decision of the Sudanese Ministry of Health to introduce rotavirus vaccine (Rotarix ®, monovalent G1P[8]) into the national immunization program (NIP) in July 2011. This study evaluated the impact of rotavirus vaccine on the distribution of rotavirus genotypes. Group A rotavirus EIA positive stool samples obtained prior (n=358; 2011) and post (n=120; 2013) vaccine introduction, were genotyped using two-step RT-PCR and DNA sequencing. Stool samples were collected from three sites in Sudan: SDKK, SDFO and SDGG. No significant change was observed in the prevalence of diarrhea after vaccination. However, Rotavirus G9 frequency increased from 0.3% to 67.5% post vaccination. Almost half of rotavirus G9 strains identified in mixed infections with rotavirus G2 strains. Post vaccination, the frequency of genotype G1 decreased from 9.5% to 5.8%. Additionally, rotavirus P[8] rate increased from 12.3% to 32.5% post vaccination. We noted change in the wild type sequence within 87.5% of the P[8] strains post vaccination. G1 completely disappeared from samples from SDKK and SDFO while G9 was predominant in both sites. At SDGG site, a dramatic decline in G2 was noted (82.9% to 6.1%), combined with an increase of G9 from 0.8% to 32.7%. For VP4,a reduction in P[4] (62% to 4.1%) in conjunction with increased P[8] (16.3% to 53.1%) was observed at SDGG. Rotavirus P[4] remained the dominating genotype at the other two sites. Out data suggests that the Rotarix ® vaccine did not reduce the overall prevalence of the virus in patients in Sudan. However, the selective pressure of vaccine administration resulted in the changes within circulating genotypes. Whether rotavirus G9 and mutated P[8] strains emerged as a result of vaccination and replaced endemic rotavirus G1 and P[8] strains in Sudan remains to be seen.

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CO-INFECTIONS WITH MONKEYPOX AND VARICELLA ZOSTER VIRUSES, THE DEMOCRATIC REPUBLIC OF THE CONGO, 2010-2013

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Monkeypox (MPX) is an acute febrile rash illness that is endemic in the Democratic Republic of the Congo (DRC). Human MPX is caused by a zoonotic orthopoxvirus (OPXV), monkeypox virus (MPXV). One febrile rash illness that can be mistaken for MPX is chickenpox, caused by varicella zoster virus (VZV). In 2010, Tshuapa District, DRC, implemented an enhanced surveillance system for human MPX, which incorporates the collection and laboratory testing of at least two lesion specimens per patient. Between December of 2010 and April of 2013, 611 suspect MPX patients were investigated. Of those, 216 (35.4%) patients were positive for MPXV and 405 (66.3%) were positive for VZV. 71 (11.6%) patients had specimens that were positive for MPXV and VZV, indicating a co-infection. Patients with co-infections ranged in age from 1-42 years (mean 13.8 years); 38 were male and 33 were female. Co-infections were observed in ten of the twelve Tshuapa health zones. The majority (n=47, 66.2%) of co-infected patients had a reported occupation of "child" or "student". In addition to rash and fever, the most common symptoms reported by co-infected patients were fatigue, headache, and chills. The rash burden for co-infected patients averaged to 97 lesions per patient with the thorax, arms, and legs being the sites with the highest lesion counts. The rash burden for these patients was higher than those seen with VZV alone (average=86) and lower than those with MPX alone (average = 129). While MPXV and VZV co-infections have been reported in the past, this is the first time these patients have been examined and documented to this extent. The surveillance protocol of collecting at least two specimens from distinct lesions may assist in the identification of co-infections. Laboratory results from these specimens show biological evidence for MPXV and VZV co-infection, where one distinct lesion yields one virus. Understanding and documenting differences in symptoms, disease severity, and diagnostic results for febrile rash illnesses will help guide case investigation procedures and clinical case management in areas where both viruses circulate.

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EVALUATION OF THE GENEXPERT FOR HUMAN MONKEYPOX DIAGNOSIS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Monkeypox virus (MPXV), a zoonotic ***Orthopoxvirus***, is endemic and causes human infections in the Democratic Republic of Congo (DRC). Molecular diagnostics for human monkeypox are currently limited to real-time quantitative PCR assays (qPCR) in sophisticated laboratory settings, and a simpler system designed to perform well in field conditions is needed to promptly diagnose new infections. Here, we evaluated the accuracy and utility of a multiplex MPXV assay using the GeneXpert platform, a portable rapid diagnostic device that may serve as a point-of-care test to diagnose infections in the endemic areas. The Multiplex MPXV assay includes a MPXV specific qPCR test, Orthopoxvirus (OPXV) generic qPCR test and an internal control qPCR test. In total, 106 diagnostic specimens (32 crusts, 74 vesicular swabs) were collected from suspect monkeypox cases in Tshuapa District, DRC under national surveillance guidelines. The specimens were tested with the GeneXpert MPXV assay and an OPXV qPCR assay at the Institut National de Recherche Biomedicale (INRB) in Kinshasa. Aliquots of each specimen were tested with a second GeneXpert system and a q-specific MPXV qPCR assay at the Centers for Disease Control and Prevention (CDC). The results of the MPXV qPCR were used as the gold standard for all analyses. The OPXV assay at INRB showed a sensitivity of 88% and a specificity of 51%. The GeneXpert MPXV assay at INRB had a sensitivity of 100% and specificity of 96%, and the GeneXpert MPXV assay at CDC similarly showed a sensitivity of 100% and specificity of 100%. The GeneXpert assay was equally sensitive with both crust and vesicle samples. The GeneXpert monkeypox test incorporates

a simple methodology and the results were sensitive and specific for monkeypox, suggesting its viability as a diagnostic platform that can be easily employed in established labs and/or field settings.

1456

RUBELLA VACCINATION IN INDIA: IDENTIFYING BROAD CONSEQUENCES OF VACCINATION INTRODUCTION, KEY KNOWLEDGE GAPS AND RECOMMENDATIONS FOR ADDRESSING THEM

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Rubella is a mild childhood disease, but infection during early pregnancy may cause fetal death, spontaneous abortion, or the birth of an infant with congenital rubella syndrome (CRS). In 2014, India announced their plan to introduce rubella-containing vaccine (RCV) into India's national measles immunization program. The success of India's rubella vaccination program will depend on maintaining a critical threshold of vaccination coverage. Empirical analyses have shown that `insufficient' levels of RCV coverage can result in an increase in CRS incidence in the short term, by increasing the age of infection without sufficiently reducing incidence of rubella cases. Using a deterministic age-structured model that accounts for state-specific demography and vaccination coverage levels, we simulated rubella dynamics across India in the presence and absence of the introduction of RCV into the public sector. We show that the effect of introducing the rubella vaccine at coverage levels equal to current measles coverage on the incidence of CRS is highly sensitive to the basic reproductive number for rubella in India. As the assumed basic reproductive number of rubella increases, the risk of an increase in CRS incidence post vaccine also increases. The basic reproductive number for rubella in India is an unknown parameter due to poor surveillance of rubella incident cases and a lack of rubella serological surveys among the general population in India. The second part of the analyses therefore uses simulation tools to explore optimal serological survey designs and analysis techniques to strengthen inference of rubella epidemiologic parameters in India. All results are conservative to model assumptions and limitations, and tend to underestimate of the benefits of public sector vaccine introduction.

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THE SPEED AND DIRECTION OF EBOLA SPREAD IN GUINEA, LIBERIA AND SIERRA LEONE

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In speed outpacing control efforts, the Ebola virus disease (EVD) epidemic in West Africa has spread across Guinea, Liberia, and Sierra Leone infecting an estimated 25,515 of individuals and claiming over 10,500 lives as of 5 April 2015. The outbreak likely began in early December 2013 in the southeastern district of Guéckédou, Guinea, and variety of risk factors have contributed to the human-to-human spread of EVD including caring for the infected, involvement in funeral preparations of infected corpses, and healthcare infrastructure. At the population level, mobile populations, porous borders, and commercial air travel patterns have influenced the frequency and breadth of EBV transmission. However, little is known about the speed and pattern of EVD spread in the current epidemic. The goal of our analysis was to calculate the velocity of spread of EVD in Guinea, Liberia, and Sierra Leone. Publically available data from the World Health Organization (WHO) were used for this analysis, which we restricted to confirmed cases of EVD. Using a surface trend analysis, the speed and direction of EVD diffusion was calculated for each district. The average speed of EVD spread across Guinea, Liberia, and Sierra Leone was 13.8 km/week, and varied from 1.9 km/week to 69.6 km/week. There was a radial pattern of diffusion from the initial EVD-affected districts that bordered Guinea and Liberia. Other spatial patterns of spread were present, which could likely be explained by the translocation of infected individuals. Understanding the movement of EVD is useful for identifying the timing and placement of treatment and containment efforts. These methods can be applied prospectively and also to other infectious diseases, to understand the broad pattern of spatial and temporal spread.

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EXTRACELLULAR MICROVESICLES MEDIATE RECEPTOR-INDEPENDENT TRANSMISSION OF NOVEL TICK-BORNE BUNYAVIRUS

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Severe Fever with Thrombocytopenia Syndrome virus (SFTSV) is a newly recognized member of the family ***Bunyaviridae*** genus Phlebovirus. The virus was isolated from patients presenting with hemorrhagic manifestations and an initial case fatality rate of 12-30% was reported. Knowledge concerning exploitation of cellular processes by SFTSV is currently limited. Recently, we reported that the nonstructural NSs protein of SFTSV is a potent inhibitor of interferon (IFN) responses. We also found that SFTSV NSs relocalizes key components of the IFN pathway into SFTSV NSs-positive cytoplasmic structures and that these structures co-localize with the early endosomal marker Rab5 but not with Golgi or endoplasmic reticulum markers. In this study, we have investigated the role these structures play during SFTSV infection. We conducted live cell imaging studies in cells stably expressing the SFTSV NSs to gain further insight into the role and trafficking of these cytoplasmic structures during virus infection. Astonishingly, we found that some of the SFTSV NSs-positive cytoplasmic structures were secreted to the extracellular space and endocytosed by neighboring cells. Electron microscopy and biochemical analyses of secreted structures isolated from SFTSV NSs-expressing cells and SFTSV infected cells, revealed that these structures were positive for SFTSV NSs, and the host protein CD63, a protein associated with extracellular microvesicles. Our study also revealed that the isolated CD63-immunoprecipitated extracellular microvesicles produced during SFTSV infection contained high numbers of viral RNA copies and that the viral RNA was efficiently delivered to uninfected cells and were able to sustain SFTSV replication in the absence of infectious virus. Overall, our results suggest SFTSV exploits extracellular microvesicles to mediate viral RNA receptor-independent transmission to host cells. To our knowledge, this is the first report describing exploitation of microvesicles to mediate virus infection among members of the ***Bunyaviridae*** family and other arboviruses, and open the avenue for novel therapeutic strategies against SFTSV.

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MEASLES OUTBREAK IN NAUGAON BLOCK, UTTARKASHI DISTRICT, UTTARAKHAND, INDIA, APRIL-AUGUST, 2014

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Although India is working toward measles elimination, measles still prevails as an outbreak prone disease reported with 310 outbreaks and 10059 cases till August, in the year 2014. There is a need to understand

the epidemiology of measles outbreaks to help achieve elimination. In June, 2014, Uttarakhand state reported 18 children with fever and rash, including one death in Naugaon block, Uttarkashi district. Suspecting measles, an outbreak investigation was conducted from July 30 - August 6, 2014. A suspected measles case was defined as onset of fever and rash with ≥1 of cough, coryza, or conjunctivitis during April 1-August 5, 2014 in a resident from either of two affected villages in Naugaon block. We did house to house survey for active case finding and assessed measles vaccination coverage among 9 -59 months old children. Measles vaccination status was determined by vaccination card or mother's history with record verification of at least one dose of measles vaccine. We assessed risk factors by 1:1 unmatched case-control study. Controls were persons aged <15 years from case-patient's house or neighbourhood without fever, rash, cough, coryza, or conjunctivitis. Case management with vitamin A was also assessed. We performed serologic test using ELISA for measles-specific IgM antibodies. There were 65 cases with one death; median age was 4 years (range: 8 months-21 years). Age specific attack rates were highest in 1-4 (23/49, 41%) and 5-9 years groups (21/70, 30%). Measles vaccination coverage was 51% (37/73). 75% (44/58) of cases and 57% (33/58) of controls not being vaccinated for measles (OR: 2.36, 95% CI: 1.07-5.34) and sharing the room with > 2 children (OR: 5.1, 95%CI: 1.1-35.9) were significant risk factors. Among cases, 14 (21.5%) were managed with vitamin A. Among 17 serum samples tested, 14 were positive. Low vaccination coverage and close contact with the cases led to propagation of this outbreak. Outbreak response immunization, case isolation, case management with vitamin A and > 95% coverage with two doses of measles vaccine are needed.

1460

ARTESUNATE-AMODIAQUINE IS ASSOCIATED WITH REDUCED EBOLA MORTALITY

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Malaria treatment is recommended for all suspected cases of Ebola virus disease (EVD), either presumptively or based on malaria diagnosis. In Foya Ebola Treatment Center (ETC), Lofa County, Liberia, the first-line anti-malarial, artemether-lumefantrine (AL), ran out for a two-week period in August 2014; during this time patients received artesunate-amodiaquine (ASAQ), which includes amodiaquine, a compound with anti-Ebola virus activity ***in vitro***. With standardized line-list data we estimated the relative risk of mortality in confirmed EVD patients prescribed ASAQ compared to either AL or no anti-malarial therapy, using unadjusted and adjusted regression models. Between 5 June_21 October 2014, 382 patients with confirmed EVD were admitted to the Foya ETC, with 194 prescribed AL and 71 prescribed ASAQ at admission. Patients prescribed ASAQ were similar to those prescribed AL or no anti-malarial therapy. Sixty-four percent (125/194) of patients prescribed AL died, compared to 51% (36/71) for ASAQ. In adjusted analyses, ASAQ prescription reduced mortality risk by 31% (risk ratio 0.69, 95% confidence interval, 0.54_0.89) compared to AL, with a stronger effect in individuals without malaria. Age, cycle threshold value at admission, total number of ETC inpatients on the day of patient admission, and IV rehydration were associated with risk of dying in the adjusted model. Artesunate-amodiaquine may provide substantial protection against EVD mortality compared to AL. While more pre-clinical and clinical research is needed to understand these biologically plausible findings, health policy makers should consider recommending ASAQ for all EVD patients regardless of malaria status.

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A TENTATIVE NEW PHLEBOVIRUS ASSOCIATED WITH A DENGUE-LIKE FEVER ILLNESS IN PANAMA

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The genus Phlebovirus (Phlebovirus, Bunyaviridae) is composed of approximately 70 viruses that are classified into two broad groups: the Sandfly fever group and the Uukuniemi group. Recently, a third distinct group within the genus was described and is composed of two mosquito-specific viruses. In Panama only two members of the Sandfly fever group, Chagres and Punta Toro Viruses, has been related with human disease. In order to detect the circulation of both members we develop a human retrospective cross-sectional survey from samples collected through the dengue surveillance program in 2009 to 2014. Viral RNA was extracted from acute sera samples, with a previous dengue negative results, and tested using phlebovirus genus-specific RT-PCR as described previously. Amplicons generated were purified directly and sequenced in both directions with the RT-PCR primers. The segment analyzed is based in a higly conserved gene thougth the pheblovirus genus. After its identification using Basic Local Alignment Search Tool Software consensus sequences from the partial L gene of the phleboviruses, sequences of interest were aligned with representative homologous sequences from the GenBank library using the MUSCLE program as amino acids. An optimal maximum likelihood tree was then generated with GTR+G model. One thousend bootstrap replicates were calculated. Phylogenetic analyses were conducted using MEGA*, version 6. Four samples from 2009 and two from 2014 were RT-PCR phlebovirus positive. Phylogentic analysis from the partial L segment detected in human samples indicated that the virus belongs to the Naples serocomplex that had not been previously detected in Panama. Because 30% of received acute samples from the dengue surveillance program are negative for dengue virus, it seems likely that human infections with other arboviruses, specially with Phlebovirus Sandfly fever members are more frequent than is now being recognized. More studies to sequence the completed genome of the virus and increased the resolution of evolutionary analysis are being conducted.

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SAFETY AND IMMUNOGENICITY OF THE HETEROLOGOUS PRIME-BOOST EBOLAVIRUS VACCINE REGIMEN CHAD3-EBO Z AND MVA-BN® FILO IN HEALTHY UK ADULTS

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Viral vectored vaccines using chimpanzee adenoviruses and Modified Vaccinia Ankara (MVA) vectors have demonstrated efficacy against Ebolavirus challenge in non-human primates. This Phase 1 trial was designed to assess the safety and immunogenicity of 2 novel Ebolavirus vaccines in healthy UK adults: the monovalent chimpanzee adenovirus 3 vector encoding Zaire Ebolavirus glycoprotein (ChAd3-EBO Z) and the multivalent MVA encoding multiple filoviral proteins (MVA-BN® Filo). 60 healthy adult volunteers were recruited in Oxford, UK and vaccinated with a single dose of the ChAd3-EBO Z vaccine at three dose levels (groups 1-3): $1x10^{10}$ viral particles (vp), $2.5x10^{10}$ vp and $5x10^{10}$ vp (n = 20 per group). Of these subjects, 30 received a heterologous boost 3 to 10

weeks later with MVA-BN® Filo at 2 dose levels: 2.2x10^8 TCID50s (n=18) and 4.4 x 10^8 TCID50s (n=12). Safety was assessed over the subsequent 4 weeks. Antibodies were measured by ELISA, T cell responses by ELISpot and flow cytometry assays were performed. The trial was then extended to include 4 additional groups (groups 4-7) to assess the effect of a reduced prime boost interval (either 1 or 2 weeks), and the effect of a further reduced dose of MVA-BN® Filo (4.4 x 10^7 TCID50s). In groups 1-3, both ChAd3-EBO Z and MVA-BN® Filo were well tolerated in all subjects, with short-lived, predominantly mild adverse events, and a low rate of febrile reactions. Both ChAd3-EBO Z alone, and the heterologous prime boost regimen were immunogenic at all dose levels with induction of both antibodies and T-cells on ELISpot and flow cytometry. Vaccination in the extension groups 4-7 is ongoing. Safety and immunogenicity data from these groups will be presented. Initial assessment of ChAd3-EBO Z and MVA-BN® Filo show these vaccines to be safe and immunogenic. These results suggest that this regimen is suitable for further evaluation as a tool to protect healthcare workers, and aid control of Ebola outbreaks.

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COMPARISON OF IMMUNOLOGICAL MEDIATORS IN DENGUE PATIENTS OF TWO ANCESTRAL GROUPS, COLOMBIA

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Colombia is a country with endemic transmission of dengue; besides its population has racial mixture diversity. On the other hand, studies shown that host genetic susceptibility can contribute in clinical outcomes. We developed a cross sectional study and we compared serum levels of immunological mediators between two Colombian populations: Afro-Colombians and Mestizos each group with 48 individuals. In the acute serum sample were measured the following cytokines: IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, TNF-α, IFN-γ; chemokine: MCP-1, MIP-1α, MIP-1β, RANTES, IP-10, Eotaxin and growth factors: basic FGF, G-CSF, GM-CSF, PDGF-BB, VEGF. We found that dengue hemorrhagic fever and thrombocytopenia were more frequent in mestizo that in Afro-Colombian patients. Levels of IL-2, IL-5, IL-7, IL-9, IL-12 IFN-γ and IL-17 were statistically significant higher in Mestizo with dengue virus infection. The current study shown differences in levels of cytokines with pro-inflammatory function or related to cellular and humoral immune responses. These are important findings because can contribute to knowledge about the immunological response to dengue infection according to the diversity racial.

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COMPARISON OF SEROPREVALENCE OF TETANUS COMPARED TO REPORTED VACCINATION STATUS AMONG CHILDREN AGED 6-59 MONTHS IN THE DEMOCRATIC REPUBLIC OF CONGO, 2013

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Previous demographic surveys have determined immunization status through vaccination cards and maternal recall, however this may not be the most reliable method for obtaining vaccination status in children. We proposed a novel method for adding a seroprevalence study in conjunction with the 2013 Demographics and Health Survey (DHS) in DRC, to assess population immunity. We screened 8,117 children aged 6 - 59 months for IgG antibodies against tetanus using dried blood spots (DBS) collected during the study. These results were compared to reported data on vaccination status. The data was broken down by sex and age to explore differences between reported rates and seroprevalence. Overall for tetanus immunity, we found a seroprevalence of 36.0%, children with reporting complete tetanus vaccination (3 doses) by cards or maternal recall was 60.5%. Children with vaccination cards seen had the highest rates of positive antibody response compared to those with a card not seen and maternal recall (55.6% vs. 37.8% vs. 27.4%, respectively). Additionally, as number of doses received increases (1 to 3), there was a slight increase in seroprevalence results for results reported by card and maternal recall (56.3% to 58.6%, respectively, and 41.9% to 44.6%, respectively). Children reporting never vaccinated had a 15.6% seropositivity, if the received 1 or 2 doses (considered drop-outs), they conferred slightly higher immunity (19.5% and 24.4%). Similar results were seen when looked at by sex. Children 12-23 months had slightly higher immunity, but had a greater range between if reported by card or maternal recall. The immunity for tetanus was higher for those with vaccination cards compared to those that had maternal recall. However, overall maternal recall and vaccination status, both overestimated the seroprevalence of tetanus. This data should be used to show the improved information on coverage rates and where immunity gaps exist that could be obtained using seroprevalence studies in place of or in coordination with reported coverage surveys.

1465

MOLECULAR CHARACTERIZATION OF THREE VIRUS BELONING TO GUAMÁ SEROGROUP (ORTHOBUNYAVIRUS, *BUNYAVIRIDAE*)

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The Guamá virus serogroup (***Bunyaviridae***: ***Orthobunyavirus***) is among the first groups of viruses identified in the Brazilian Amazon, in Belém, Brazil, in the 1950s. The prototype, the Guamá virus (GMAV, strain BE AN 277) was isolated from the blood of a sentinel monkey ***Cebus apella*** in the Oriboca Forest (Pará State, Brazil) in 1955. The Bimiti virus (BIMV), originally isolated in Port of Spain, Trinidad in 1955, after some years, it was isolated in Brazil (Utinga Forest, Belém municipality, Pará State) in 1966 from ***Proechimys guyannensis*** (prototype BE AN 100519). The Moju virus (MOJUV, strain BE AR 12590) was initially isolated in 1959 in a forest area of the "Agronômico do Norte" Institute (nowadays EMBRAPA - Brazilian Agricultural Research Corporation), in Belém, Pará, Brazil from a pool of ***Culex*** (***Melanoconion***) sp. mosquitoes. GMAV has relevance for public health because it causes acute febrile disease in humans characterized by fever of sudden onset, headache, arthralgia, myalgia, photophobia and asthenia. This study aimed to perform the genetic characterization of the three prototype virus strains of the Guamá virus serogroup isolated in the Brazilian Amazon: GMAV, BIMV, MOJUV. These viruses were sequenced and we got complete sequences of the S-RNA, M-RNA and L-RNA segments of them. The genomic organization of these viruses is compatible with the organization of members of ***Orthobunyavirus***, except for the absence of the NSs protein. It was identified for the Guamá group conserved motifs for the N gene of other ***Orthobunyaviruses***, some even involved with viral replication, as well as, conserved motifs for L-RNA segment called I, II, III and IV regions and the motifs of the Region III (Pré-A, A, B, C, D, and E). Based on nucleotide- and aminoacid- sequences of the three RNA segments, the higher degree of genetic similarity exhibited occurred between the GMAV and BIMV. The most divergent for all genomic segments evaluated was the MOJUV. This relationship is according to phylogenetic analysis for S-RNA, M-RNA, and L-RNA segments. Moreover, the findings of this study agree with the serological analysis previously described for the Guamá virus serogroup.

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EXAMINING DISCREPANCIES IN MEASLES VACCINATION AND IGG SEROPOSITIVITY AMONGST CHILDREN AGED 12-23 MONTHS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Steady monitoring and evaluation of national immunization programs is vital to uncovering discrepancies in coverage that leave populations susceptible to vaccine-preventable diseases. In countries with weak surveillance programs, examination of biomarkers for antigen exposure may provide insights into the reach and effectiveness of vaccination campaigns, as well as the baseline immunity conferred upon unvaccinated groups via passive or active immunization. Using dried blood spots collected during the 2013 Demographics and Health Survey (DHS), we compared the seroprevalence of anti-measles IgG with survey responses regarding vaccination history for 1,766 children aged 12-23 months in the Democratic Republic of Congo (DRC). Of those children with reported measles vaccination from either vaccination card or maternal recall, 62.1% tested positive for measles antibodies (n= 1,155). Within this group, 69.2% of children with vaccination cards (n= 362) and 58.8% of children with maternal declaration of vaccination (n= 793) tested positive for measles antibodies. IgG seropositivity was 36.6% amongst those children reporting no measles vaccination (n= 543). These data suggest high background exposure of young children in DRC to measles, as well as a large discrepancy between measles vaccination and the presence of anti-measles IgG. It is difficult to discern whether the presence of antibodies in vaccinated children in the study is due to effective vaccine response or other unverified exposure to measles, considering the high seroprevalence observed amongst unvaccinated children. Given the high efficacy of the measles vaccine in controlled settings, our results implicate the potential for haphazardly filled vaccination cards, poor maternal recall, inadequate vaccine storage conditions, and interference of preexisting conditions (malaria, malnutrition, immunocompromised state) on the effectiveness of the vaccine to elicit an immune response. Intervention strategies should focus on improvements in cold-chain maintenance alongside general activities to improve vaccine coverage in DRC.

1467

PLASMODIUM FALCIPARUM AND BLOOD BRAIN BARRIER: LOCAL ADHESION-INDEPENDENT EFFECTS

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This presentation intends to analyze the effects of ***Plasmodium falciparum*** on the Blood Brain Barrier leading to cerebral malaria through adhesion independent events. ***Plasmodium falciparum*** prevails to be one of the leading causes of mortality and morbidity in sub-Saharan Africa. In its severe form, this infection can lead to a severe case of morbidity called cerebral malaria. This exposition addresses the following question: What is the working mechanism of the blood brain barrier? What are local adhesion-independent activities in relationship with ***Plasmodium falciparum***? What are the effects of these activities on the blood brain barrier? To answer these questions, this study critically examines research works and literatures concerning this problem. Moreover, it will offer recommendations for possible elimination of malaria in sub-Saharan Africa.

1468

INVESTIGATING THE MECHANISMS MEDIATING IMPROVED OUTCOMES FOR S-NITROSOGLUTATHIONE REDUCTASE (GSNOR) NULL MICE IN EXPERIMENTAL CEREBRAL MALARIA

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Severe and fatal malaria are associated with decreased nitric oxide (NO) bioavailability. NO bioavailability is mediated through both soluble guanylate cyclase and post-translational S-nitrosylation. Endogenous levels of S-nitrosothiols (SNOs) are negatively regulated, in part, by S-nitrosoglutathione Reductase (GSNOR). Therefore, we hypothesized that the increased levels of SNOs in the GSNOR knockout (KO) mice would lead to improved outcomes during experimental cerebral malaria (ECM). In support of this hypothesis, we have previously shown that GSNOR KO mice had improved survival in ECM. The GSNOR KO mice had improved Rapid Murine Coma and Behavioural Scores (RMCBS), and improved maintenance of the blood brain barrier. To further investigate the mechanisms mediating the improved outcomes, we first measured plasma levels of endothelial activation markers (VWF, Ang-2, sICAM-1, and sVCAM-1) throughout the course of infection. There were no significant differences between the GSNOR KO and wild type (WT) mice at any of the time points measured. However, there were significantly higher levels of IFN-Gamma on D4, and D5 post infection in the GSNOR KO mice compared to the WT mice ($p<0.001$). This suggested that the GSNOR KO mice had altered immune responses to ***Plasmodium berghei*** ANKA (PbA). In order to evaluate the role the hematopoietic compartment, specifically T cells, in mediating improved outcomes for the GSNOR KO mice, bone marrow transplants were performed. WT mice were irradiated and received either GSNOR KO or WT bone marrow. Eight weeks following the transplant, the mice were infected with PbA and followed for survival. There was no significant difference in survival between the mice receiving GSNOR KO or WT bone marrow. This suggests that a compartment outside of the hematopoietic system is mediating protection in the GSNOR KO mice. We are currently investigating this hypothesis using reciprocal bone marrow transplants. Although the mechanism mediating protection is still being investigated, these findings suggest a potential target for novel therapeutics for the treatment of cerebral malaria.

1469

PLASMODIUM FALCIPARUM FATTY ACID METABOLISM IN LIMITED GLUCOSE GROWTH CONDITIONS

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Despite the importance of fatty acid (FA) metabolism, relatively little is known about these pathways in ***Plasmodium falciparum***. Acyl CoA Synthetase (ACS) enzymes activate exogenous FAs, and these enzymes have expanded to a thirteen-member, polymorphic gene family in ***P. falciparum***, suggesting an important role for scavenged FAs. Previously, we have used [3H]-hypoxanthine incorporation assays to demonstrate restricted parasite growth in limited media conditions, including restricted glucose and limited fatty acids. Knockdown of individual ACS family members exacerbates this growth defect, suggesting a link between FA and carbon metabolism in the parasite. We determined that glucose-

deprived parasites exhibit FA oxidation (FAO), as measured by release of 3H20 from parasites grown in 3H labeled palmitic and oleic acid. Restricted glucose growth conditions induced a significant increase in FAO as compared to uninfected red blood cells, while parasites grown in the standard RPMI1640 did not exhibit FAO. We will update these initial findings with the results of metabolic labelling studies and metabolite profiling. In addition, we are using Seahorse XFe Bioanalyzer to better understand parasite carbon and FA metabolism under these restricted growth conditions. Fluorescent sensors detect changes in the pH and oxygen consumption of cells in real time, allowing us to measure parasite metabolic flux in response to specific substrates. Our observations of FA metabolism point to an important role for exogenous FAs and offer a potential explanation for the previously reported expansion and positive selection of the ACS gene family in ***P. falciparum***. Characterization of parasite growth under limited nutrient conditions offers insight into the parasite's metabolic flexibility and ability to survive in different host microenvironments.

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PLASMODIUM FALCIPARUM EXPRESSES FEVER-INDUCED REGULATORS TO MAINTAIN PARASITE LOADS AT APPROPRIATE LEVELS FOR HOST AND PARASITE SURVIVAL

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Plasmodium falciparum infection in a non-immune human host portends a significant risk of death. The human spleen mechanically clears a proportion of circulating ring-infected erythrocytes (rings). This innate process potentially favors host survival by reducing the pace at which initial steps of infection proceed. Because preventing death of infected hosts increases the chances of parasite transmission this should also favor parasite survival. Here, we show that ***P. falciparum*** expresses PIKA-1, a protein that associates with Ring Erythrocyte Surface Antigen to form a 390 kDa complex and changes the properties of late rings. PIKA 1-expressing rings are recognized and eliminated in the spleen more efficiently after exposure to febrile temperatures. As compared to wild-type PIKA-1-expressing parasites, αPIKA-1 mutants grew faster ***in vitro***. In addition, RBC infected with αPIKA-1 mutant rings were retained less effectively in biomimetic spleen-like filters and in human spleens perfused ***ex vivo***. These phenotypic traits were expressed only after exposure of rings at 41°C. Wild-type PIKA-1-expressing parasites were retained more effectively by the spleen when the parasite had been exposed to 41°C while there was no such difference after exposure of wild-type and mutants at 37°C. Phagocytosis of infected RBC was greater with wild type parasites than with αPIKA-1 mutants. We propose a model where parasite expansion is initially rapid until parasite loads are high enough for transmission then slowered down by PIKA-1 (probably in collaboration with other parasite components) when parasite loads reach fever-inducing levels associated with severe complications. PIKA-1 can thus be viewed as a fever-induced sensor of high parasite loads that reduces parasite expansion when death of the host becomes a short-term risk. PIKA-1 and RESA would thus confer ***P. falciparum*** a key selective advantage and may be original operators of parasite-host co-adaptation toward mutual survival.

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MALARIA SYSTEMS BIOLOGY AT THE HOST-PATHOGEN INTERFACE

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The Malaria Host-Pathogen Interaction Center (MaHPIC) is a large systems biology consortium that uses multi-'omic data integration approaches to study malaria infections and specifically the interactions of diverse ***Plasmodium*** species with their non-human primate (NHP) and human hosts. We are designing and implementing longitudinal investigations in NHPs, to build upon existing knowledge of host-parasite interactions for several primate species of malaria parasites with diverse biological characteristics. We are investigating which features (e.g. genes, transcripts, proteins, lipids, metabolites, immune profile) of the host and parasite during the course of experimental infections are associated with differences observed in malarial disease progression and severity. We are comparing different parasite species in the same host using ***P. cynomolgi*** and ***P. coatneyi*** infections of ***Macaca mulatta*** (rhesus monkey) to model ***P. vivax*** and ***P. falciparum***, respectively. We are also studying the same parasite (***P. knowlesi***) in two different hosts, using ***M. mulatta*** (susceptible) and ***M. fascicularis*** (refractory), to identify features that confer protection against disease. Finally, we are directly studying ***P. vivax*** and ***P. falciparum*** experimental infections using New World monkeys and conducting high-resolution metabolomics analyses involving natural human infections and clinical investigations being led from South America, Southeast Asia and Sub-Saharan Africa. Altogether, we aim to identify novel host and parasite factors involved in malaria disease progression in NHPs, and translate these findings to what is observed in humans. Furthermore, we aim to address biological questions using NHPs that for ethical reasons cannot be approached in clinical studies. The MaHPIC includes intensive informatics to generate and release datasets for use by the broad scientific community, and mathematical modeling and computational analysis to guide ongoing research and to integrate and make sense of the large amount of information being generated and compared. We will present the status of the project with data featured from a series of infections.

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MALAWIAN CHILDREN WITH CEREBRAL MALARIA DO NOT HAVE MARKERS OF KIDNEY INJURY

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Renal failure is a common manifestation of severe malaria in adults but is rarely reported in children. A recent study of children in 9 African countries found elevated blood urea nitrogen to be an independent predictor of mortality, and other studies have found that elevated creatinine is a marker for poor prognosis in children. None of these children were found to have frank renal failure. Because assessments of renal function are limited in resource-challenged settings, it is possible that clinically relevant kidney injury is more widespread in children with severe malaria than has been recognized. This study seeks to determine whether Malawian children with cerebral malaria (CM) had evidence of kidney injury. Creatinine concentration was measured in stored plasma samples from 79 children aged 6 months to 12 years who were enrolled in an autopsy study at the University of Malawi College of Medicine. Creatinine concentrations were compared between differently defined groups. Groups 1 and 2 were patients with clinically defined CM during life who either had malarial retinopathy (n=25) or did not (n=17). Groups 3 and 4 were patients with clinically defined CM during life and either with (n=31) or without (n=13)

sequestered parasites on autopsy. Only 2 patients, both with autopsy confirmed non-malarial encephalopathy, had creatinine concentrations meeting the WHO criteria for kidney injury. There were no statistically significant differences in the creatinine concentrations between Groups 1 and 2, or between Groups 3 and 4. These findings suggest that kidney injury is not common in Malawian children with cerebral malaria. However, because functional impairment is often significant before elevations in plasma creatinine manifest, we will be expanding this study to include novel early kidney injury biomarkers KIM-1 and NGAL.

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ATTEMPTS TO REVERSE ADAPT AOTUS DERIVED *PLASMODIUM VIVAX* TO *IN VITRO* CULTURE BY *EX VIVO* CO-CULTIVATION WITH HUMAN ERYTHROCYTES

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Plasmodium vivax malaria is widely distributed around the world. The lack of ***P. vivax*** continuous ***in vitro*** culture has limited the advance of research in this area. ***P. vivax*** has proven difficult to maintain in continuous culture, due in part to special culture conditions and its preference for invading reticulocytes. In the past, adaptation of malaria parasites to Aotus monkeys, have been carried out by replacing human by Aotus erythrocytes in a stepwise fashion. Moreover, it has long been known that Aotus serum does not support ***in vitro*** culture of ***P. falciparum***, and that leukocytes removal by filtration is important to avoid phagocytosis. However, during filtration, an important number of parasites are retained. In order to compare the use of filtered vs retained back-flush recovered parasites, on the effect of parasitemia of ex-vivo cultures, and test the hypothesis that co-cultivation of Aotus adapted ***P. vivax*** parasites with malaria naïve human RBCs, reverse adapts Aotus parasites to ***in vitro*** culture: First, we tested the effect of adding malaria naïve human or Aotus RBCs to ex-vivo cultures over parasitemia; then, tested the effect of media supplementation with or without 0.5 % glucose or 5 % lipids, and, lastly, the effect of adding reticulocytes at different time intervals over parasitemia. For this purpose, we used Aotus derived ***P. vivax*** SAL-1 parasites, filtered or retained by a Plasmodipur® device, re-suspended in McCoy's media at 5% HCT, plate it in 4 well plates and incubated under a 5% O2, 5% CO2, 90% N2 atmosphere at 37 Celsius. Thin smears were prepared and stained with Giemsa to determine % parasitemia and staging. Preliminary results indicated that Aotus ***P. vivax*** iRBCs co-cultivated with human RBCs or filter retained had higher and longer parasitemias when compare to Aotus RBCs alone or Plasmodipur® filtered respectively. In conclusion: Parasitemia in ***P. vivax*** Aotus derived ex-vivo cultures is enhanced by co-cultivation with human erythrocytes. In general Plasmodipur® filter retained parasites had higher and longer ex-vivo parasitemias with or without media supplementation.

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INTRINSIC VARIATIONS IN CULTIVABILITY OF *PLASMODIUM FALCIPARUM* MALARIA PARASITES

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An understanding of variations in drug resistance, pathogenesis, and immune evasion in malaria often relies on culture-adapted ***Plasmodium falciparum*** parasites. Before making population-based inferences, parasite collections require standard methods and precise definition of successful ***in vitro*** adapted parasites to assure good representation. Patient blood was collected from 31 ***P. falciparum***-infected individuals at the Goa Medical College and Hospital (Bambolim, Goa, India) during the 2012-2013 malaria season. After dividing samples into whole blood or washed blood, they were further distributed in different media or at different hematocrit levels , in duplicate, to give 8 test flasks per original patient sample. ***In vitro*** adaptation was considered successful when two sequential 4-fold increases in parasitemia were observed within 96 hours, after culture for as long as 30 days. Later, separately, cryopreserved parasites from the day of patient contact were tested again for adaptability but this time in parallel using identical host RBC and culture media. Overall, 70% of the tested patient samples were successfully culture-adapted. Cultures setup at 1% hematocrit and 0.5% Albumax adapted most easily, even with whole blood inoculations. Success was not limited by low patient parasitemia, patient age, recent treatment with antimalarials, or even significant delays in sample processing. The speed to adaptability appeared to be an intrinsic property of the parasites. Microsatellite analysis confirmed that adaptation favored some clones over others. Our findings can help assure more complete descriptions of culture-adapted parasite collections. Such precision is expected to improve population-based extrapolations of findings from cultured parasites to questions of public health importance.

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RETINOPATHY-POSITIVE CEREBRAL MALARIA IS ASSOCIATED WITH GREATER INFLAMMATION, BLOOD-BRAIN BARRIER BREAKDOWN AND NEURONAL DAMAGE THAN RETINOPATHY-NEGATIVE CEREBRAL MALARIA

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Cerebral malaria (CM) is the most severe neurological complication of malaria, and is associated with a case fatality rate of up to 15%, while 24% of survivors develop long-term neurocognitive sequelae. Examination of the ocular fundus in children with CM has revealed a characteristic retinopathy that has both diagnostic and prognostic significance. Children with CM can be characterized as retinopathy positive (RP) or retinopathy negative (RN), but little is known about the pathophysiologic differences between these two groups. Understanding the differences between RP and RN CM may help shed light on the pathophysiology of malarial retinopathy. We compared markers of endothelial activation, platelet activation, angiogenesis, oxidative stress, ischemia, inflammation, and neuronal damage in Ugandan children with CM admitted to Mulago Hospital, Kampala, Uganda who were RN (n=91) or RP (n=170). RP children had higher CRP (p=0.008) and ferritin (p<0.001) levels than RN children. RP children had an elevated CSF/plasma albumin ratio

(p<0.001) and elevated CSF tau protein levels (p=0.024) as compared to RN children. Additionally, RP children had significantly elevated total (p<0.001) and sequestered (p<0.001) parasite biomass levels compared to RN children, but there was no significant difference in circulating parasite biomass levels. RP CM is associated with greater parasite sequestration, inflammation, blood-brain barrier breakdown, and neuronal damage than RN CM.

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MATERNAL TO FETAL MICROCHIMERISM IS ASSOCIATED WITH PLACENTAL MALARIA AND RISK OF MALARIA DURING INFANCY

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Placental malaria (PM) is associated with increased risk of malaria during infancy, which may be the result of acquired ***in utero*** malaria-specific tolerance. Microchimerism (Mc) refers to the harboring of cells or DNA derived from a genetically disparate individual, a phenomenon most easily observed during pregnancy. We hypothesize that PM, with its associated damage to the trophoblast barrier, increases the prevalence and level of maternal to fetal Mc. In addition, we hypothesize that maternal Mc may increase malaria susceptibility in infancy via direct immune activity of maternally-derived microchimeric cells or a Mc-dependent increase in fetal tolerance to malaria antigens. We conducted a nested case-control study of women with (n=18) and without (n=21) PM and their paired infants, drawn from a larger birth cohort from Muheza, Tanzania. To investigate Mc, we first genotyped the women and infants for MHC class II alleles to identify an informative marker of maternal cells in the infant background, looking for a non-shared, non-inherited maternal allele (NIMA). We are currently assaying for presence and level of Mc in cord blood using a previously validated quantitative PCR-based method, targeting the NIMA for each maternal-infant pair. Among 8 controls and 10 cases thus far surveyed, PM is associated with presence of Mc (OR=10.5, p=0.07) as well as level of Mc (2/100,000 cells vs 2,647/100,000 cells, p<0.001). In addition, both presence and level of Mc predict risk of parasitemia during infancy independent of PM status (AOR=2.04, p=0.04; per 1% increase in Mc cells: AOR=1.08, p<0.001). This preliminary data suggests a possible association between PM and increased maternal to fetal Mc, as well as an association between maternal Mc and susceptibility to malaria during infancy.

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CHILDREN WITH SEVERE MALARIAL ANEMIA AND SICKLE CELL DISEASE HAVE DECREASED PARASITE BIOMASS AND INFLAMMATION BUT INCREASED ANGIOPOIETIN-2 LEVELS COMPARED TO CHILDREN WITHOUT SICKLE CELL DISEASE

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Malaria is an important cause of morbidity and mortality among children with sickle cell disease (SCD, genotype HbSS) living in malaria endemic regions. While sickle cell trait (HbAS) has been shown to confer protection against infection by the ***Plasmodium falciparum*** parasite, and children with sickle cell trait or SCD have a lower risk of malaria infection than children without SCD (HbAA), children with SCD have higher rates of severe anemia and death when hospitalized for malaria. The reasons for increased morbidity and mortality from severe malaria in children with SCD are not known. In the present study, we assessed biomarkers associated with severe malaria pathogenesis in children with severe malarial anemia (SMA) admitted to Mulago Hospital in Kampala, Uganda and compared these biomarkers in children with SMA who had SCD (n=19) or did not have SCD (n=172). Children with SCD were older and had lower hemoglobin levels and higher leukocyte counts than children without SCD. Children with SCD also had significantly lower sequestered and circulating parasite biomass, and evidence of a significantly reduced inflammatory response, including decreased C-reactive protein and alpha-1-glycoprotein levels, and decreased levels of multiple pro-inflammatory cytokines (TNF-α, IL-1Rα, IL-10, IL-12, G-CSF) and chemokines (MCP-1, MIP-1α, RANTES). In contrast, as compared to children without SCD, children with SCD had significantly higher levels of angiopoietin-2, a marker of endothelial cell disruption and increased vascular permeability that has been associated with increased mortality in severe malaria. Children with SMA and SCD have decreased parasite biomass and a reduced inflammatory response when compared to children without SCD, but have evidence of increased endothelial cell disruption. Further study is required to assess whether the reduced inflammatory response and increased endothelial cell disruption correlate with increased morbidity or mortality in these children.

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ESTABLISHING AN *IN VITRO PLASMODIUM VIVAX* CULTURE FROM COLOMBIAN CLINICAL ISOLATES

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One problem, which has delayed better understanding of the host-parasite interaction for ***Plasmodium vivax***, has been the inability to maintain an ***in vitro*** culture for facilitating tests, such as drug susceptibility, red blood cell-parasite interaction and the evaluation of new vaccine candidates. It was proposed that an ***in vitro*** culture of this parasitic species isolated from patients living in an endemic area of the Córdoba department should be established due to high ***P. vivax*** incidence. Three variants of each RPMI 1640 and McCoy's 5A media were used; reticulocytes were obtained from cord blood and hemochromatosis patients for maintaining and growing cultures. Both cord blood reticulocytes and hemochromatosis patients' reticulocytes were enriched by 70% Percoll density gradient. Infected samples having more than 50% rings were processed and enriched by 46% Percoll density gradient and samples having more than 70% mature trophozoites by 70%. Parasitised erythrocytes were maintained in a 37°C anaerobic microenvironment using the candle jar system. Average starting parasitaemia observed per crop was 1.58%. Parasites were viable for up to 120h when RPMI-1640 culture medium was tested, whereas viability increased by up to 140h when culturing the same base medium modified in our laboratory. Twenty samples which were viable for up to 168h were processed once it has been established that our RPMI 1640 medium variant was as efficient as McCoy's 5A medium and more efficient than that proposed by Russell ***et al***., in 2011. A final 257.38% average reticulocyte efficiency was obtained once the samples from umbilical cord blood and hemochromatosis patients had been enriched by 70% Percoll gradient. Reticulocytes were viable until week 4 when they were kept at 4^0C (1.8% average daily loss). Two invasion assays were performed by adding reticulocytes to culture maintained at 6% haematocrit when schizonts were observed in culture samples; two possible erythrocyte invasion routes were observed in one of these two trials. Colombian ***P. vivax*** clinical isolates were thus susceptible to growth in the culture media tested and it was feasible to adapt them to laboratory conditions.

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DRUG-INDUCED LIVER INJURY AFTER TREATMENT WITH ARTESUNATE-ATOVAQUONE-PROGAUNIL FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* INFECTION

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Drug-induced liver injury (DILI) is a common phenomenon and often underreported in remote settings without laboratory monitoring, such as those found in malaria-endemic areas. Atovaquone-progaunil has been known to cause subclinical and transient elevation in liver enzymes in clinical trials of malaria prophylaxis, but reported liver injury is rare. Only one case report in the literature details an episode of hepatitis after atovaquone-proguanil prophylaxis. Given AP's otherwise excellent safety profile, the significance of an associated subclinical transaminitis is unknown but purportedly, there have been reports where liver function test abnormalities have limited treatment with AP. The mechanism by which atovaquone-progaunil may cause hepatic injury is unknown, but is of concern now that atovaquone-progaunil is being increasingly deployed at treatment doses in multidrug resistant settings of ***Plasmodium falciparum***. Here, we report an episode of drug-induced liver injury in a Cambodian farmer after taking a course of artesunate-atovaquone-progaunil and a single dose of primaquine for ***P. falciparum*** mono-infection. This incident serves as an important safety signal to remain pharmacovigilant, particularly if this efficacious combination regimen is more frequently deployed in settings of multidrug resistant ***P. falciparum*** such as Cambodia.

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THERAPEUTIC EFFICACY OF ARTEMISININ-BASED COMBINATION THERAPY IN THE TREATMENT OF UNCOMPLICATED MALARIA IN TWO ECOLOGICAL ZONES IN GHANA AFTER EIGHT YEARS OF DEPLOYMENT

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Malaria remains a major public health problem in Ghana, accounting for 44% of all outpatient clinic visits, 37.6% of all admissions, and 22.3% of all under-five deaths in 2013. Case management based on prompt diagnosis and adequate treatment remains the main focus of malaria control in Ghana. Artesunate-amodiaquine (AA) combination was deployed in 2005 as first line drug for the treatment of uncomplicated malaria. This was followed in 2008 with artemether-lumefantrine combination (AL) and dihydroartemisinin-piperaquine (DHAP) as alternative first line drugs for patients unable to tolerate AA. Since 2005 ten sentinel sites, representing the ecological zones of Ghana, have been monitoring the therapeutic efficacy of artemisinin-based combination therapies (ACTs) in the country using the World Health Organization's (WHO) protocols for antimalarial drug efficacy assessment. After 8 years of ACT deployment in the country, per protocol analyses for two ecological zones studied (savannah and forest) showed significantly higher day-28 pcr-uncorrected cure rate for AA compared with AL. In the savannah zone, pcr-uncorrected cure rates were 97.3% (95% CI: 90.7, 99.7) for AA and 56.3% (95% CI: 37.7, 73.6) for AL (p<0.001). In the forest zone pcr-uncorrected cure rates were 98% (95% CI: 89.4, 99.9) for AA and 85.2% (95% CI: 77.7, 91.0) for AL (p=0.031). Kaplan-Meier survival analysis for the savanna zone showed day-28 pcr-uncorrected cumulative cure rates of 97.4% (95% CI: 89.9, 99.3) for AA and 60.1% (95% CI: 41.8, 74.3) for AL (p<0.001) whilst the forest zone showed cumulative cure rates of 98.1% (95% CI: 87.1, 99.7) for AA and 85.2% (95% CI: 77.6, 90.4) for AL (p=0.030). These preliminary findings suggest greater day-28 efficacy of AA over AL in terms of preventing treatment failures in the savannah and forest zones of Ghana after 8 years of ACT deployment.

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FITNESS LOSS AND COMPENSATION IN ANTIFOLATE AND ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Malaria drug resistance is a major public health problem on a global scale. At present, the effectiveness of antimalarials is dwindling due to the spread of multidrug-resistant strains. However, the evolutionary process to change from drug-sensitive wild-type to drug-resistant mutants is under evolutionary constraints. One of the examples is the antifolate drug family. Antifolates inhibit the folate pathway enzymes that produce metabolites required for nucleotide synthesis. Antimalarial pyrimethamine inhibits dihydrofolate reductase (DHFR), a key folate enzyme. Four canonical mutations at the dhfr gene abrogate the effectiveness of pyrimethamine. Interestingly, the gains of these four dhfr mutations follow step-wise order, and, most important of all, there is trade-off between antifolate resistance and fitness in the evolutionary trajectory from wild-type to the quadruple mutant. Even though these mutations are highly beneficial under pyrimethamine, the mutants suffer from poor fitness when drug pressure is removed. To overcome this problem, malaria parasites accumulate multiple copies of the gene encoding rate-limiting folate enzyme GTP cyclohydrolase I (GCH1). Extra GCH1 from gene amplification was originally selected for slight improvement in antifolate resistance. The parasite co-opted the gain of extra GCH1 and used this mechanism to compensate for loss in fitness from dhfr mutations. In addition, extra GCH1 increases the overall robustness, which allows additional dhfr mutations and reduces the drug sensitivity level of other antifolates. A similar issue was also encountered in artemisinin resistance, an emerging problem in Southeast Asia. Parasite isolates with decreased sensitivity to artemisinin suffer from fitness loss. The parasites produce fewer progenies, corresponding to the loss of one round of mitosis. These parasites are metabolically impaired, resulting in growth arrest under stress. The loss in fitness might explain why the parasites cannot achieve higher level of artemisinin resistance like in the cases of chloroquine and antifolate.

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METABOLOMIC ANALYSIS OF *PLASMODIUM VIVAX* CHLOROQUINE RESISTANCE

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Chloroquine is the main drug used in the treatment of uncomplicated vivax malaria. Reports of chloroquine resistant (CQ-R) ***Plasmodium vivax*** malaria from the Western Pacific region followed by reports in the Americas and Asia have questioned its widespread use. However, the mechanisms associated with CQ-R malaria are not yet fully understood. We used plasma samples from individuals ≥18 years of age with ***P. vivax*** monoinfection treated at the Fundação de Medicina Tropical Heitor Vieira Dourado in Manaus, Brazil during 2011-2012. Chloroquine resistance was defined as recurrence of parasitemia with detectable plasma concentrations of CQ ≥100ng/dl. 33 individuals with CQ-sensitive

and 16 with CQ-R ***P. vivax*** malaria were included. Samples underwent randomization followed by Liquid Chromatography/Mass Spectrometry. We used xMSanalyzer for data extraction and obtained 21,360 ***m/z*** features. We then performed linear regression controlling for platelet and total bilirubin levels, as they were significantly associated with the outcome and for age and gender as they may have an impact in the metabolome. We adjusted for multiple hypothesis testing by the Benjamini-Hochberg false discovery rate (FDR) method. The linear regression model resulted in 18 ***m/z*** significantly different between both study groups (t-test, FDR<0.20). We then created a partial least squares discriminant analysis (PLS-DA) model without controlling for covariates in an effort to further explore our results. This model detected 354 ***m/z*** with a Variable Importance in Projection ≥2. Features detected by linear regression and PLS-DA had an overlap in 17 ***m/z***. We tested the 354 ***m/z*** from PLS-DA for pathway enrichment using ***mummichog***, an algorithm for pathway-level annotation of metabolomics data, and found alterations in steroid biosynthesis, glycerophospholipid and tryptophan metabolism (all p<0.05). Our analyses likely measure the net metabolic host-pathogen interaction rather than separating host or pathogen pathways. Although not evidence of causality, our effort yields potential biochemical pathways associated with chloroquine resistance in ***P. vivax*** from the Brazilian Amazon.

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INADEQUATE EFFICACY OF CLINDAMYCIN PLUS QUININE COMPARED TO ARTEMETHER-LUMEFANTRINE IN THE TREATMENT OF CHILDREN WITH UNCOMPLICATED FALCIPARUM MALARIA IN WESTERN KENYA

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Background: Clindamycin plus quinine is a non-artemisinin-based antimalarial drug combination. The evidence on the efficacy of clindamycin plus quinine compared with other antimalarial drugs is scanty, inconclusive and outdated. An open-label randomized controlled trial to evaluate the efficacy and safety of oral clindamycin plus quinine vs. artemether-lumefantrine in the treatment of children below 5 years of age with uncomplicated falciparum malaria was conducted in western Kenya. Methods: 384 children aged 6 to 59 months with uncomplicated malaria were enrolled at Homa Bay District and Ahero Sub-District Hospitals in western Kenya and randomized (1:1) to receive oral clindamycin plus quinine or dispersible artemether-lumefantrine tablets and followed for 28 days. The primary endpoint was parasitological cure rate after 28 days of follow up, unadjusted and adjusted by genotyping to distinguish recrudescence from new infections. Secondary endpoints included parasitological failure on days 3, 14, 21 and 28, parasite clearance rates, gametocyte carriage, recovery of haemoglobin concentration from baseline at day 28, and incidence of adverse events. Results: The unadjusted cure rate was 32.6% in the clindamycin plus quinine group and 78.9% in the artemether-lumefantrine group. The risk of treatment failure after adjusting by genotyping was significantly higher in the clindamycin plus quinine group (55.6%) compared to the artemether-lumefantrine (2.9%) group (risk difference 52.7, 95% confidence interval 43.0-62.4, p<0.0001). Parasitological clearance was significantly lower and the proportion of early treatment failures significantly higher in the clindamycin plus quinine group. Adverse events did not differ between the two groups. Three serious adverse events occurred in the clindamycin plus quinine group. Conclusion: The cure rate with clindamycin plus quinine in our study was significantly lower than artemether-lumefantrine. These findings question the recommendation of quinine-based combinations as second-line drugs for the treatment of malaria.

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IMPACT OF K13 MUTATIONS ON PARASITE FITNESS AND SUSCEPTIBILITY TO OZONIDES

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Artemisinin (ART)-based combination therapies (ACTs) have contributed substantially to reducing the burden of malaria. Thus, alarms were sounded when efficacy studies in Western Cambodia documented the emergence of ART resistance in ***Plasmodium falciparum***. Recent reports have demonstrated that mutations in the K13 propeller domain associate strongly with slow parasite clearance rates in patients and confer elevated survival of drug-exposed ring-stage parasites ***in vitro***, as reported previously. Several K13 alleles have emerged in Southeast Asia and are now spreading across the Greater Mekong Subregion. In addition to the different levels of resistance that single K13 mutations may confer, we hypothesize that the impact of each mutation on parasite fitness is decisive in establishing the viability of a given haplotype in malaria-endemic settings. In this work we have determined the fitness cost imparted by several mutations in the K13 gene on parasite growth. We used genetically modified, otherwise isogenic, parasites with a selection of K13 propeller mutations and conducted direct growth competition experiments. We found that mutations in K13 exert a growth deficit on mutant parasites compared to their wild-type parental counterparts. We also assessed susceptibility of K13 mutant parasites to the next-generation long half-live ozonides OZ227 and OZ439, which are currently being evaluated as alternatives to ART. We identified cross-resistance between OZ277 and DHA (dihydroartemisinin), the active metabolite of all ARTs, whereas OZ439 demonstrated excellent potency against all mutant and wild-type K13 parasite lines tested. These data suggest that the major K13 mutations present in Cambodia do not protect parasites from OZ439 action, highlighting the potential benefit of replacing ARTs with ozonides.

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A COMPARISON OF NEW AND EXISTING *IN VITRO* METHODS FOR ASSESSMENT OF ARTEMISININ RESISTANCE BY *PLASMODIUM FALCIPARUM* RING SURVIVAL

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As artemisinin (ART) resistance in ***Plasmodium falciparum*** spreads through Southeast Asia and threatens malaria control programs worldwide, assessment of phenotypes ***in vitro*** is necessary to survey parasite populations and study resistance mechanisms. A Ring Survival Assay (RSA) developed by Witkowski and colleagues in 2013 became instrumental to study ART response ***in vitro***. The method includes treatment of cultures with heparin, selection of late stage schizonts by Percoll gradient, and treatment of 0-3h ring stage parasites with sorbitol prior to a six hour drug pulse. These three chemical treatments, however, do not guarantee the exclusive drug exposure of 3h rings. We offer two alternative methods, one requiring less resources and one offering improved specificity for 3h rings. The first alternative acquires young rings through only sorbitol synchronization, lessening the list of needed chemicals from three to one. Additionally, this method uses only twenty microliters or less of parasite culture per assay. For instances where samples are scarce, this method provides an opportunity to assess phenotype ***in vitro*** that would otherwise not be possible. Our second alternative adapts the procedure of Boyle and colleagues, reported in 2010, involving filtration of late schizonts with a 1.2 µm filter to provide invasive merozoites to uninfected erythrocytes. In contrast to the original method, filtration provides a sharper synchronization of ring stage parasites without chemicals and allows drug incubation of exactly 3h-old rings. This enhanced specificity offers increased precision for determinations of

ring survival percentages. All three methods (the original, filtration, and sorbitol-only) tested on the same lines yielded comparable results of ring survival percentages: of a resistant line, percentages were 6.87%, 8.88%, and 6.92%, respectively; of a sensitive line, 0.55%, 0.68%, and 0.76%. A comparison of needed resources and procedural logistics for each method will be presented. The availability of three RSA methods provides flexibility in evaluating ART response in a variety of scenarios.

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PREDICTIONS OF PIPERAQUINE SYSTEMIC EXPOSURE IN YOUNG CHILDREN UNDER DIFFERENT DOSING REGIMENS FOR CHEMOPREVENTION: A SIMULATION STUDY TO INFORM DOSING

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Dihydroartemisinin-piperaquine, the first-line artemisinin combination therapy regimen, is under evaluation for intermittent preventive treatment (IPT). Dosing approaches in IPT have been inferred from treatment trials, and fail to adequately consider differences in PK/PD relationships, adherence, and safety concerns that exist with repeated dosing in chemoprevention. Further, we have recently demonstrated that young children are systematically underdosed in treatment settings. To better inform dosage selection of DP for IPT, we simulated PK profiles of candidate DP chemoprevention regimens based on a previously developed population model of piperaquine (PQ) in the setting of acute treatment of Ugandan children aged 6 months to 2 years. Regimens were simulated for children weighing over 10 kg (reference group), and included standard and 1.5x standard 3-day monthly regimens, bimonthly and weekly dosing, and use of a loading dose. The current approach (240 mg x 3 doses monthly) led to a predicted geometric mean steady-state trough capillary plasma level of 29 ng/mL, with 94%, and 47% of children maintaining levels ≥ 10 ng/mL and 30 ng/mL, respectively. Increasing doses by 50% lead to a predicted trough of 43 ng/mL, with 98% and 71% maintaining ≥ 10 ng/mL and 30 ng/mL. Notable improvement in exposure was seen with weekly 240 mg dosing, leading to a steady-state of 67 ng/mL, with 99.7% and 85% maintaining troughs ≥ 10 ng/mL and 30 ng/mL. Importantly, predicted maximum concentrations were 42% lower with weekly dosing compared to standard dosing. Previous studies have demonstrated that individuals have a higher risk of breakthrough malaria in the first few months of chemoprevention. The use of a loading dose with weekly dosing was associated with trough levels at the end of one month that are 94% of steady-state trough levels, compared to 70% without a loading dose. With corroborating clinical data, these alternate dosing approaches may prove favorable in terms of exposure, adherence and safety. This approach provides a systematic information-driven method to evaluate candidate antimalarial chemopreventive dosing regimens.

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ANALYSIS OF POLYMORPHISMS IN ARTEMISININ RESISTANCE ASSOCIATED GENES IN *PLASMODIUM FALCIPARUM* SAMPLES FROM SOUTH AMERICAN AND AFRICAN COUNTRIES

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The emergence of ***Plasmodium falciparum*** resistance to artemisinin (ART) in Southeast Asia threats the disease control. ART resistance has been associated with mutations in some candidate genes and monitoring these events as well as its impact in treatment outcome may contribute to malaria control. This study aimed at analyzing ART-resistance associated genes ***pfATPase6***, ***pfAP2μ*** and ***pfK13*** in ***P. falciparum*** samples from South American and African countries collected in health facilities in Brazil, after informed consent. After sequencing with sense and antisense primers, sequences of 24 samples were aligned and compared to the reference ***P. falciparum*** 3D7 available in PlasmoDB. An empirical Bayesian method was applied using the M8 model to assess codons under selection. Datasets used for selection pressure analysis were built with sequences from this study and worldwide reference sequences retrieved from PlasmoDB and GenBank. The following mutations in ***pfATPase6*** gene were observed: R37K, L402V, E431K, N569K, A630S, G632E, G639D, H747Y and I898I. In ***pfAP2μ***, W250R and D3Y in one sample each, V127V in nine and S478S in one. Amino acid insertion was seen in three samples and deletion in other three. For ***pfK13*** gene, K189T was present in three samples, K189N in one. One sample harbored G112E and A578S. Overall, all genes are under negative selection, showing ω=0.4 for ***pfATPase6***, ω=0.12 for ***pfAP2μ*** and ω=0.5 for ***pfK13***. Several negatively selected codons were observed in all three genes, but few positive selected codons were seen within ***pfATPase6***, at positions 141, 402, 431, 569, 574 and 639 with significance level of 0.001. Interestingly, in the ***pfK13*** gene all codons under strong purifying selection were located within the Kelch motif. Mutation K189T was found in isolates from South America and Haiti in agreement with reports of this SNP in African countries. Despite the absence of C580Y mutation, it was found the A578S, whose structural analysis shows that could affect K13 gene function. These results generate a baseline data on three candidate genes associated with ART resistance that could be useful in view of the risk of ART resistance spread worldwide.

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GENETIC MODIFICATION VIA CRISPR/CAS9 IN *PLASMODIUM FALCIPARUM* MULTIDRUG RESISTANCE PROTEIN1 (PFMDR1) CONFER RESISTANCE TO A PIPERAZINE-CONTAINING ANTIMALARIAL AGENT IN BOTH ASEXUAL AND SEXUAL PARASITE STAGES

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ACT451840 is a piperazine-containing antimalarial compound developed by Actelion Pharmaceuticals Ltd. with support from the Medicines for Malaria Venture. This compound is an exquisitely potent inhibitor of ***Plasmodium falciparum*** asexual blood stage parasites, with an IC_{50} value of <1 nM. Single-step drug-selection studies yielded resistant asexual blood stage parasites with 10 to 200-fold shifts in IC_{50} values compared to the sensitive parental line. Whole-genome tiling arrays and Illumina-based sequencing on resistant clones uncovered a host of novel point mutations in the ***P. falciparum*** multidrug resistance protein 1 (PfMDR1), resident on the parasite's digestive vacuole membrane. Using CRISPR/Cas9-based ***pfmdr1*** gene editing, we confirmed that the ***pfmdr1*** point mutations mediated resistance. Furthermore, when compared to wild-type isogenic parasites, ACT451840-resistant parasites became more susceptible to mefloquine, lumefantrine, halofantrine, and quinine, but not chloroquine or its active metabolite, and displayed a slight fitness cost ***in vitro***. We also observed potent activity of ACT451840 on mature ***P. falciparum*** gametocytes. Unexpectedly, stage V gametocytes harboring Cas9-introduced ***pfmdr1*** mutations acquired resistance to this compound, suggesting that PfMDR1 can also impart resistance to mature gametocytes. The potent asexual and gametocytocidal properties of this compound merit further investigation.

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DEVELOPMENT OF ANTIBODY-BASED IMMUNO-ASSAYS FOR QUALITY CONTROL OF ARTEMISININ-BASED COMBINATION THERAPIES

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Prompt treatment of uncomplicated ***Plasmodium falciparum*** malaria with artemisinin-based combination therapies (ACTs) is paramount to avoid clinical complications and deaths as well as to our ability to control this disease. Approximately 392 millions of ACT treatment courses have been procured to the public and private sectors in 2013, largely contributing to the globally decreasing malaria mortality rate. Yet, a significant fraction of antimalarial drugs are sold over-the-counter in poorly regulated settings, favoring the uncontrolled distribution of falsified or substandard antimalarial drugs in large quantities. Up to a third of antimalarial available in endemic countries can be of unacceptable quality, turning life-saving medicines into life-threatening substances. ACTs quality control (QC) is needed at all levels of the supply chain, and affordable analytical tools which can be easily deployed in settings with limited resources to perform routine quantitative analyses are currently missing. To address this issue, we have developed a set of monoclonal antibodies directed against key active pharmaceutical ingredients (APIs) of ACTs, such as artesunate, amodiaquine, and piperaquine, while work is ongoing to generate additional ones directed against dihydroartemisinin, artemether, lumefantrine and primaquine. We further developed and validated indirect competitive Enzyme-Linked Immunosorbent Assays (iELISAs) based on monoclonal antibodies against artesunate and amodiaquine for the rapid quantification of the corresponding molecules in pharmaceutical formulations. iELISAs are easy-to-handle as well as highly specific and sensitive, displaying a lower limit of detection between 1.0 and 3.9 ng/ml. Finally, proof-of-concept studies evaluating the performance of iELISAs as compared to high-performance liquid chromatography-mass spectrometry for the quantification of APIs in ACTs of varying quality are underway. iELSA could be used to instate or reinforce ACT QC at local levels and could be relatively easily deployed in simple laboratories to contribute ensuring the safety of key antimalarial drugs.

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ARTEMISININ RESISTANCE AT THE CHINA-MYANMAR BORDER AND ASSOCIATION WITH MUTATIONS IN THE K13-PROPELLER GENE

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The recent emergence and spread of artemisinin (ART) resistance in the Greater Mekong Subregion (GMS) of Southeast Asia greatly threatens malaria control and eradication. The K13-propeller gene (K13) has been lately identified as a promising maker to confer ART resistance. Investigation of K13 gene in parasites from the China-Myanmar border area, where ART use has the longest history, has revealed high prevalence of polymorphisms. Elucidation of their importance in ART resistance is highly needed. A total of >11,000 suspected malaria cases from three studies during 2008-2013 in this area were screened and 248 were recruited for clinical assessment. The day 3 positive rates in two studies with dihydrartemisinin-piperaquine treatment were below 10%, whereas in one study with artesunate monotherapy reached 23.1%. To validate the predictability of K13 mutations for potential ART resistance, we selected 24 day 3-positive cases and 33 day 3-negative cases from these three efficacy studies. The NN insertion at codon 137 and the predominant F446I mutation as well as the total mutations in the propeller domain were significantly associated with the day 3 parasite positivity ($P < 0.05$). To determine whether the suspected ART resistance in the study region could be further corroborated by ring-stage survival assay (RSA), we culture-adapted 19 isolates from day 3-positive cases and 25 from day 3 negative cases. The median percentage of viable parasites in isolates from the day 3 positive group was ~10 times greater than that of isolates from the day 3-negative group (0.5%) ($P < 0.0001$). Parasites with mutations in the propeller domain had a ~9 fold higher ring-stage survival rate ($P<0.05$). The NN insertion and F446I mutation also significantly associated with day 3 parasite positivity. From association studies between clinical data of day-3 positivity, ***in vitro*** ring-stage susceptibility, and K13 polymorphisms, we provide strong evidence indicating emergence of artemisinin resistance at the China-Myanmar border and K13 as a promising marker for artemisinin resistance.

1491

PRIMAQUINE: METABOLITE ACTIVITY AND SCHIZONTICIDE DRUG COMBINATION ACTIVITY ON ASEXUAL AND SEXUAL STAGES OF *PLASMODIUM FALCIPARUM*

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Primaquine (PQ) used with Artemisinin Combination Therapies (ACTs) field studies in Southeast Asia and Africa has shown effective ***Plasmodium falciparum*** gametocyte clearance which is critically important for reducing the risk of malaria transmission and potentially mitigating the threat of drug resistant infections. Currently, the World Health Organization recommends adding a single 0.25 mg/kg dose of PQ on the first day of ACT treatment in areas under threat of ART resistance. PQ antimalarial activity ***in vivo*** has been historically attributed to the potential activity of its metabolites as well as perturbation of mitochondrial function. The former was investigated via 1) a PQ metabolite mixture generated ***in vitro***, 2) and synthesized PQ analogs from Walter Reed Army Institute of Research. The presence of potential PQ metabolites in the mixture was analyzed using LC-MS and gametocytocidal activity tested on transgenic male and female ***P. falciparum*** lines expressing green fluorescent protein in gametocytes using a flow-cytometry based drug sensitivity assay. The PQ analogs' activities on sexual and asexual stages were tested with the same gametocytocidal sensitivity assay and SYBR Green I drug assay, respectively. In addition, since PQ dosage is now a recommended addition to ACTs, we looked at the effect of PQ in combination with various antimalarials (chloroquine, mefloquine, piperaquine, lumefantrine, naphthoquine) on ***in vitro*** growth of asexual ***P. falciparum*** strains and gametocytocidal activity on ***P. falciparum*** gametocytes.

1492

ATESUNATE/SULPHADOXINE/PYRIMETHAMINE COMBINATION SHOWS EQUAL ACTIVITY TO OTHER ACTS AGAINST *PLASMODIUM BERGHEI* IN MICE AFTER CO-TRMOXAZOLE PRETREATMENT

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Co-trimoxazole(COT) therapy has been found to reduce the high early mortality rate in HIV-infected adults on ART in low- and middle-income countries hence the WHO recommended that the use of COT be scaled up in HIV-infected patients starting or receiving antiretroviral therapy. However, resistance due to drug pressure and subsequent cross- resistance between COT and sulphonamides such as sulphadoxine/pyrimethamine (S/P) which could otherwise be useful as part of ACTs is a concern. Knowledge of the effect of atesunate combination with S/P on malaria parasites previously exposed to COT would be instructive. COT pre-treatment was administered to 30 adult albino mice divided into 6 groups and weighing between 15-25g for 14 days after which the animals were inoculated with chloroquine sensitive ***Plasmodium berghei*** . Subsequently, the anti-malaria activities of curative doses of Artemether/Lumifantrine (AL), Atesunate/Sulphadoxine/Pyrimethamine (AS/SP), Atesunate/Amodiaquine (AS/AMQ) and Atesunate/Mefloquine (AS/MQ) combinations against ***Plasmodium berghei*** were investigated in each of 4 groups respectively. One of the two remaining groups serving as controls was administered chloroquine(CQ) and the other distilled water, respectively.The average parasite densities on day 0 were 28045.5 ± 26718.2, 36462.0 ± 7070.0, 65651.7 ± 27686.5, 78809.1 ± 6160.8 respectively for the groups administered AL, AS/SP, AS/AMQ and AS/MQ; On day 1, these values decreased to 11980.0 ± 11980.0**, 7838.4 ± 4038.7**, 7767.7 ± 4202.3**, 12677.0 ± 2893.1**respectively ($P < 0.01$, Vs Distilled water); there was complete parasite eradication by day 3. Total parasite eradication occurred in the CQ group by day 2 from an initial value of 76746.3 ± 28457.1, while there was an increase from 46459.4 ± 13949.2 to 130499.4 ± 936.2 between days 0 and 13 in the distilled water group with the mice in this group dying off by day 17. Atesunate combination with S/P was as active as other ACTs.Validation of this theory in humans may provide a wider range of ACTs useful even among patients on prior COT therapy.

1493

RESISTANCE ANALYSIS OF THE ANTIMALARIAL MK-4815: LOW FREQUENCY OF RESISTANCE AND CROSS-RESISTANCE PROFILE CONSISTENT WITH ROBUST CLINICAL CANDIDATE

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Due to the emergence and increased prevalence of multi-drug resistant malaria, new drugs are urgently needed for combination therapy to effectively treat and eliminate malaria. Currently there are several new fast-acting antimalarial candidates in development, yet there is a dearth of long-acting compounds with good oral bioavailability, potency against multi-drug resistant strains, and low potential for resistance generation. One such candidate for combination therapy is MK-4815 (2-aminomethyl-3,5-di-***tert***-butylphenol), a Mannich base previously shown to be potent ***in vitro*** and ***in vivo*** and equally effective against wild-type and multi-drug resistant strains of ***Plasmodium falciparum***. We assessed the potential for MK-4815 to generate resistance and MK-4815's ***in vitro*** efficacy against multidrug resistant strains. First we examined the frequency of resistance after continuous drug pressure and were unable to select for resistant parasites, suggesting that the resistance frequency for MK4815 is less than 10^{-8} as found for atovaquone. Next the potential for drug resistance generation was assessed by step-wise pulse exposure to MK-4815 in the W2 clone of ***P. falciparum***. The base-line IC_{50} for MK-4815 in W2 is 7 ng/mL. We started with 5x IC_{50} concentration and applied four-day pulses of drug followed by wash out and a recovery period to allow parasites to grow. Once recovery was established, exposure continued with higher concentrations of MK-4815. Ultimately parasites recovered following 96 hr at 1,000x IC_{50}. Decreased susceptibility to MK-4815 was minimal and not stable. A three-week period without drug pressure was enough for the parasite response to return within 3-fold of baseline. In addition, we evaluated the IC_{50}'s of MK-4815 against multidrug resistant strains and found that MK-4815 has no discernable cross-resistance patterns with other anti-malarial drugs. The results of our studies suggest that MK-4815 has a low potential for selecting resistance in ***P. falciparum***; these data as well as its favorable pharmacokinetics, safety, and efficacy profiles, make MK-4815 an excellent drug combination candidate.

1494

TEMPORAL TRENDS IN SULPHADOXINE-PYRIMETHAMINE RESISTANCE MARKERS IN *PLASMODIUM FALCIPARUM* IN NIGERIA 2002 - 2014

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Intermittent preventive treatment of malaria during pregnancy (IPTp) and in infants (IPTi) with sulphadoxine-pyrimethamine (SP) is a major strategy for malaria control in African countries where malaria is endemic, including Nigeria. However, the implementation of this strategy is faced with challenges such as timing of SP administration and rising levels of parasite resistance to SP in the general population. SP resistance is associated with mutations in the genes of dihydropteroate synthetase (dhps) and dihydrofolate reductase (dhfr). Three Pfdhfr mutations N51I, C59R and S108N, known as the triple mutation, and the Pfdhps mutations A437G and G540E, known as the double mutation, collectively form the quintuple mutations. The quintuple mutation and an additional mutation on the dhps (A581G) are known to confer high level of SP resistance. The World Health Organization (WHO) has recommended that prior to implementation of IPT-SP in any region with moderate to high malaria transmission, the prevalence of these markers of resistance with special emphasis on K540E should be determined and IPT-SP commenced only in regions with a prevalence rate less than 50%. Recent data show growing concerns of SP resistance in Nigeria with emerging novel dhps haplotypes and the triple mutant (IRN) dhfr haplotypes but their effect on efficacy of IPT-SP is unknown. There is paucity of data on SP resistance markers in Nigeria. Therefore, there is need for continuous monitoring of these resistance markers over the years to provide comprehensive data that will guide implementation of IPT-SP in Nigeria. We identified molecular markers of SP resistance by direct PCR sequencing in 1200 malaria positive blood spots collected from pregnant women and children attending hospitals across Southwest, Southeast, South south and Northeast Nigeria. Prevalence of markers in each site, and temporal patterns in these markers from 2002 to 2014 will be presented.

1495

ASYMMETRICAL BIS-QUINOLINES AS CHEMOTHERAPEUTIC AGENTS FOR MALARIA

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Millions of people are afflicted by malaria each year, and hundreds of thousands die. Historically, the most successful synthetic antimalarial drug was chloroquine, as it was safe, inexpensive, and highly efficacious. However, plasmodial resistance to chloroquine now greatly limits its utility. Other 4-aminoquinoline drugs, such as the bis-quinoline antimalarial piperaquine, often exhibit activity against chloroquine-resistant malaria. A series of "asymmetrical bis-quinolines" are presented here that are highly active against chloroquine-resistant and chloroquine-sensitive ***P. falciparum*** malaria, with IC_{50} values that are in several cases remarkably potent. We also present an initial structure-activity relationship series for this new series of potentially useful chemotherapeutic agents for malaria.

1496

EFFICACY OF ARTEMETHER-LUMEFANTRINE IN DIORO, MALI: AN AREA WITH INTENSE TRANSMISSION

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Few studies have examined the efficacy of ACTs in Mali after the introduction of Artemether-Lumefantrine (AL) and the ban on monotherapy for treatment of uncomplicated malaria. In Dioro, Mali, we performed a prospective study to examine the efficacy of the AL combination for the treatment of uncomplicated ***P. falciparum*** malaria. Subjects enrolled in this study were between 2 and 15 years of age and had asexual parasitemias between 2,000 and 199,000 per µl. Giemsa-stained thick smears were used to examine asexual and sexual parasites before treatment and to estimate the asexual parasitemias on follow-up days 1, 2, 3, 7, 14, 21, 28, 35 and 42. Filter paper blots were used to collect parasite DNA for PCR to distinguish between recrudescence and re-infection. The Dapi assay was used to estimate the IC50s of ***P. falciparum*** isolates collected at the time of diagnosis. To estimate parasite clearance time, we considered only volunteers who were observed continuously during the 3 days of treatment. Two endpoints were considered for ACT efficacy: 1] parasite clearance on or before Day 7 and 2] lack of recrudescence between days 8 and 42. All 77 volunteers cleared their asexual parasitemias on or before Day 7 and 4 (5%) had late parasitologic failures between Days 28 and 42. In addition, 5 (9%) of the 53 patients examined for the PCT had persistent asexual parasites ≥ 48 hours after beginning treatment. There was a positive correlation between the initial parasitemia and the PCT with delayed clearance (≥ 48 hours after beginning treatment) more frequent in subjects with higher parasitemias (p=0.006). Molecular studies and IC50s are currently being performed to determine whether the 4 late parasitologic failures represent recrudescence of the original parasite(s) or new infections and to test for antimalarial resistance based on elevated IC50s.

1497

DYNAMICS OF DRUG RESISTANCE PATTERNS TO COARTEM USED IN SENEGAL

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Artemether-lumefantrine (AL) was adopted as the recommended treatment for uncomplicated ***Plasmodium falciparum*** malaria for Senegal in 2006. Because the monitoring of drug efficacy is essential to ensure efficacy and recognize resistance when it appears, the efficacy of AL for the treatment of uncomplicated malaria in children aged 2 to 20 years has been evaluated in Thiès (Senegal). From September 2011 to November 2014, we have studied the treatment of uncomplicated malaria using AL in children with fever or a history of fever who have uncomplicated ***Plasmodium falciparum*** malaria. In this study, parasite samples have been examined using ***ex vivo*** testing to estimate IC50s for different antimalarials and followed for 6 weeks in accordance with World Health Organization (WHO) guidelines. Follow-up visits are performed on days 1, 2, 3, 7, 14, 21, 28, 35 and 42 to evaluate clinical and parasitological results. A total of 246 subjects have now been enrolled in Thiès and Dakar. There have been no early treatment failures and all patients have cleared asexual parasites from the blood on or before day 3. Polymerase chain reaction (PCR)-uncorrected adequate clinical and parasitological response rate (UACPR) was 98.8% at day 28 as well as at day 42. PCR-corrected ACPR at day 42 was 98.8%. We found no mutation at dhpsK540E in Senegal, high level of mutation in pfmdr1 - Y184F and decreased parasite sensitivity to artesunate and Lumefantrine. Coartem still show efficacy in Senegal. Anti-malarial being used in ACT except Lumefantrine, including artemisinin derivates, amodiaquine, mefloquine are ***ex vivo*** sensitive to falciparum.

1498

CONSEQUENCES OF RESTRICTING ANTIMALARIAL DRUGS TO RAPID DIAGNOSTIC TEST POSITIVE FEVER CASES AND CAUSES OF NON-MALARIAL FEBRILE ILLNESS

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Current malaria treatment guidelines dictate that RDT-negative children do not receive ACT but should be evaluated for other causes of fever. This practice could lead to increased frequency of malaria and anemia in children who are denied ACT. 511 febrile children aged 3 to 59 months presenting with fever or history of fever within 48hours were enrolled in southwest Nigeria and followed up for 28 days. Malaria parasite was evaluated using SD-Bioline RDT (HRP-2 based) and microscopy of Giemsa stained blood film. Enrollees were investigated and treated for other causes of fever. RDT result was used to determine ACT treatment. RDT+ve children received artesunate-amodiaquine (ASAQ™) at standard dosage supervised. The mean age of enrollees was 26.41 ± 15.67 months. Malaria parasite was detected by RDT in 308 (60.3%) and by microscopy 227 (44.2%) enrollees. Parasite density ranged from 20 - 611,600/µL (Geomean = 7762/µL). 40/193 (20.7%) children with patent parasitemia on D0 had parasite recurrence on D28 while 18/232 (7.8%) of those who were free of patent parasitemia on D0 had parasitemia on D28 (ρ=<0.0001). The sensitivity and specificity of SD-Bioline RDT were 95.2% and 55.7% respectively while positive and negative predictive values were 70.19% and 94.6%. 12/493 (2.4%) blood cultures yielded bacterial growth while 84/392 (21.4%) mid-stream urine cultures yielded bacterial growth. 5/218 (2.3%) children had malaria-bacteremia co-infection while

22.5% (41/182) had malaria plus urinary tract infection. Other causes of fever among the children include measles, acute respiratory infection, diarrhea diseases and mumps. ASAQ cleared the malaria parasitemia promptly and was well tolerated. 78.8% of 189 parasitemic children who completed D28 follow up had adequate clinical and parasitological response. Two children who tested positive and receive ASAQ were referred to hospital because of severe anemia. The results of this study did not reveal any severe consequence sequel to the use of RDT result as basis for treatment.

1499

TERTIAN DURATION OF ARTEMISININS SELECTS FOR DELAYED PARASITE CLEARANCE WITH SINGLE 48 HOUR LIFECYCLE ARTEMISININ EXPOSURE

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In the 1990s many artemisinin combination dosing trials ranged from 2-7 days in duration. The three day or 48 hour dosing duration was just as effective as five or more days. The correct rationale at that time was that greater compliance would be achieved with tertian dosing rather than five or more days. This three day regimen was effective until the prolonged parasite phenotype was noted in Palin Cambodia. The three day or 'tertian' dosing equates to a single 48 hour ***Plasmodium falciparum*** lifecycle. The consequence of dosing most of the artemisinin component of an ACT within a single ***P. falciparum*** 48 hour lifecycle is selection for parasites with Kelch-13 gene mutations. Ring-stages of parasites in the peripheral blood at drug initiation only receive a single effective dose coincident with the sensitive trophozoite-stages, because two artemisinin doses (at initiation and the last dose) target the naturally more resistant ring-stages. Sequestered trophozoites in the tissues at drug initiation receive two effective trophozoite-stage drug doses at 0 and 40-48 hours resulting in greater reduction of these trophozoites in contrast to the rings. The parasite has been selected by the short pharmacodynamics of artemisinin behaviour within a single lifecycle to favour a mutant Kelch-13 gene to permit survival beyond a single cycle so that if the quinoline partner drug in an ACT is failing as well, then elimination of remaining parasites is compromised. Clinical drug failure measured at day 28 or 42 is failure for both ACT drugs, and should therefore be termed treatment failure. Importantly the tertian ACT 48 hour dosing regimens including the 60 hour every 12 hour artemether/lumefantrine dosing is presently selecting for additional ***foci*** of mutations outside Southeast Asia. Effective artemisinin combination therapy with present drugs now requires doubling the duration to six days. This allows initial ring-stage populations to encounter artemisinin drug exposure at least three times at the more sensititive trophozoite-stage rather than once. Incremental increase of an extra day of therapy is not sufficient.

1500

NEXT-GENERATION SEQUENCING PLATFORM BASED ON MALARIA RAPID DIAGNOSTIC TESTS FOR SURVEILLANCE OF ANTIMALARIAL DRUG RESISTANCE

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Antimalarial drug resistance is a constantly present threat towards one of the major tools necessary for rolling back malaria, namely efficient antimalarial drugs. It has occurred on several occasions and rendered the health systems close to helpless towards the malaria parasites, in particular towards ***Plasmodium falciparum*** malaria. For reasons that are not entirely understood by modern science, ***P. falciparum*** never ceases to be one step in front of the research community, repeatedly disarming the forces that try to combat the disease. The current global malaria situation situation involves ***P. falciparum*** parasites that are resistant towards artemisinin-based combination therapies in Asia, as well as global resistance towards sulphadoxine-pyrimethamine and chloroquine. With very limited antimalarial drugs remaining to effectively prevent malaria in children and pregnant women, as well as treat malaria-infected individuals, the local situation regarding antimalarial drug resistance needs to be monitored. With the help of molecular markers of resistance in terms of single nucleotide polymorphisms representative of tolerance or resistance of ***P. falciparum*** parasites towards specific antimalarial drugs, monitoring the antimalarial drug resistance situation becomes feasible. Furthermore, by applying used malaria rapid diagnostic tests, the source of ***P. falciparum*** DNA is acquired at very low costs and in plentiful numbers. Also, by applying a next-generation sequencing technology, the cost of sequence analysis is brought down 20-50 fold in comparison to commercial Sanger sequencing. Lastly, by applying indexing of the samples analysed, sequences can be traced back to a specific sample and a specific place of origin, allowing action to be taken in terms of fine-tuning policy guidance at the local level. This platform allows for real-time monitoring of antimalarial drug resistance in a cost-efficient and extremely high throughput manner.

1501

EVALUATION OF THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ADMINISTERED BY MASS CAMPAIGN IN SOUTHERN SENEGAL

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Seasonal Malaria Chemoprevention (SMC) is a new strategy for the control of malaria in children involving monthly administration of sulfadoxine-pyrimethamine plus amodiaquine (SPAQ) to prevent malaria. In Senegal, the southern regions where malaria is highly seasonal, and transmission is intense are the most suited areas for SMC with 620,000 children under 10 years of age eligible for SMC. It is however important that scaling up of SMC by national Malaria Control Programmes is evaluated to document its coverage, the safety profile, the impact on malaria morbidity and drug resistance molecular markers. In 2014, to monitor malaria morbidity surveillance, 230 health structures were listed by district, with their catchment populations, (a total pop about 2 million) based on the 2012 census. A survey sample of 32 health post, 16 districts hospitals were selected with probability proportional per size, by systematic random sampling, checked with respect to geographical coverage, malaria cases and malaria incidence rates to ensure the sample was representative. One month after the 3 SMC rounds with an administrative coverage of more than 95%, 2000 children under 10 and 1750 adults into 45 villages selected by PPS were surveyed to document SMC coverage and drug resistance markers. A case - control study has been conducted in 2 districts to measure the efficacy of SMC treatments. 225 malaria cases and controls who do not have malaria have been recruited concurrently, and the dates of the doses of SMC they received noted by trained fieldworkers. The strengthened passive pharmacovigilance system detected only 80 mild adverse events and 2 serious adverse events (1 Lyell and 1 Steven - Johnson syndromes) after 1.843 million SMC treatments. The analysis is on going and efficacy results, impact on malaria morbidity and on drug resistance markers will be presented.

1502

THERAPEUTIC EFFICACY OF ATOVAQUONE-PROGUANIL AND ARTESUNATE ATOVAQUONE-PROGUANIL FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN AREAS OF MULTIDRUG RESISTANCE IN CAMBODIA

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We recently reported a clinical failure rate of greater than 54% for dihydroartemisinin-piperaquine, the last currently available artemisinin combination therapy (ACT) for the treatment of ***Plasmodium falciparum*** malaria in Cambodia. Few alternatives remain unfortunately. Atovaquone-proguanil (AP) is a safe, well-tolerated drug for the treatment of drug resistant ***P. falciparum***, and has recently been used in limited areas of Cambodia as part of public health interventions. There have been recent though unconfirmed reports of developing clinical AP resistance in Pailin province, as well as reports of mutations to the cytochrome bc-1 complex associated with atovaquone resistance. We are currently comparing two fixed-dose 3 day regimens of AP, with or without a 3 day course of co-administered artesunate (ASAP) for the treatment of uncomplicated malaria. This randomized, open label clinical trial will be conducted in up to 200 patients with ***P. falciparum*** or mixed ***P. falciparum/P. vivax*** infection at two sites in Cambodia. Enrollment began in Anlong Veng on the Thai-Cambodian border in December 2014, and will start in Kratie, on the Vietnamese border in May 2015. A single 15mg dose of primaquine is given on day 1, and subjects are followed for recurrence for 42 days. To date, 40 volunteers have been screened, 23 randomized to AP and 17 randomized to ASAP. Preliminary results indicate only 1 of 31 evaluable subjects (3.2%) completing 42 day follow up to date have had P.f. recurrence, while 8 (25.8%) have had a ***P. vivax*** recurrence. One subject in the ASAP group suffered a serious adverse event with suspected drug-induced liver injury. An interim report, including both efficacy, and safety results pertaining to the use of single low dose primaquine in subjects with G6PD deficiency, will be presented.

1503

MEASURING DIFFERENCES IN THE *IN VITRO* SUSCEPTIBILITY OF AFRICAN *PLASMODIUM FALCIPARUM* ISOLATES TO DIHYDROARTEMISININ USING A FLUORESCENT-READOUT PULSING ASSAY

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Artemisinin-based combination therapy (ACT) is currently the most effective treatment strategy against ***Plasmodium falciparum*** malaria. There have been reports from Southeast Asia of parasite resistance to artemisinin and its derivatives, and there are fears that resistance to this class of drugs will spread to Africa. Artemisinin resistance is recognised by a relatively slow parasite clearance rate in patients receiving an ACT or artemisinin***. In-vitro*** or ***ex-vivo*** methods are conventionally used to determin the IC_{50} value - the drug concentration that inhibits parasite growth by 50%- for parasite lines in the laboratory. To date, there have been no consistent correlations between half lives of ***P. falciparum*** treated with artemisinin ***in-vivo*** and ***in-vitro*** IC_{50} estimates from standard 48-hour artemisinin susceptibility assays. This study investigates alternative methods of assaying artemisinin resistance by ***in vitro*** exposure to dihydroartemisin (DHA) - the major metabolite of all ACT. Artemisinins have short half lives (1-2h) ***in-vivo*** and so short drug pulses were applied to parasites in an effort to better mimic ***in-vivo*** conditions. A 6hr drug pulse assay and a standard 48hr assay were used to observe any differences that may exist in IC_{50} values between field isolates from Kenya (HL1204), Ghana (HL1210) and Nigeria (HL1212). In all three isolates tested, the mean DHA IC_{50} values for the 6hr pulse assays were higher than that of the standard 48hr assays. This trend was seen as a shift to the right in the dose response curve. This approach revealed differences in IC_{50} values for DHA among the isolates. Field isolates from Ghana (6 fold higher than 3D7 lab strain) and Nigeria (3.4 fold higher) showed elevated mean DHA IC_{50} value compared to that from Kenya (1.7 fold higher than 3D7). Results also showed that DHA IC_{50} values varied among experimental replicates, reflecting the polyclonal nature of the isolates used in this study. There is an urgent need to develop ***in-vitro*** assays that correlate with parasite artemisinin response ***in vivo,*** as this will be useful in elucidating the molecular basis of artemisinin resistance.

1504

CONTRIBUTION OF *PLASMODIUM FALCIPARUM*, *P. VIVAX* AND *P. MALARIAE* TO CLINICAL MALARIA CASES IN NORTH SUMATERA PROVINCE, INDONESIA

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Indonesia, a country situated at the southeastern tip of Asia, has a total population of 245 million, of which 61% live in malaria endemic areas. Most of the endemic regions are in stable transmission zones although with low transmission risk. In 2012, WHO reported 2,051,425 malaria cases from Indonesia, comprising 22% of the malaria burden in Southeast Asia. All species of ***Plasmodium*** have been reported in the country with ***Plasmodium falciparum*** and ***P. vivax*** the predominant species. In order to carry out studies of ***in vivo*** efficacy of antimalarial treatment in North Sumatera, we conducted passive and active case screening by RDT and microscopy examination in Batubara regency, Langkat regency and South Nias regency. These semi-forested areas comprise dispersed small- to medium sized communities practising a mixture of permanent and shifting agriculture, as well as foraging in the forests. Two malaria parasite species, ***P. falciparum*** and ***P. vivax***, have previously been reported in these sites. Among febrile patients, ***P. vivax*** was the most common cause of malaria, followed by ***P. falciparum***. Demographic data and the features of clinical malaria in these communities will be presented, as well as the prevalence of each parasite species in febrile adults and children. We also describe the first PCR-confirmed clinical cases of ***P. malariae*** infection from this part of Indonesia.

1505

THE PREVALENCE OF MALARIA PARASITAEMIA IN SEVERELY MALNOURISHED AND WELL NOURISHED CHILDREN PRESENTED AT MULAGO HOSPITAL UGANDA

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Malnutrition and malaria remain the major causes of morbidity and mortality in young children mostly in sub-Saharan Africa. We hypothesized that the prevalence of ***Plasmodium falciparum*** will be higher in malnourished children compared to well nourished children. This study was case and control study whereby 100 samples of blood from severely

malnourished (cases) and 100 from well nourished (controls) children who presented to Mulago Hospital were examined for ***P. falciparum*** using light microscopy. The prevalence of Plamodium faciparum in malnourished children was 6% with mean geometric parasite density of 88/µl of blood, while that of well nourished children was 17% with average geometric parasite density of 1755/µl of blood caused by a mono-infection of ***P. falciparum***. Stratifying both groups with malaria and associating them with age, children between 2- 36 months had high prevalence of malaria in both the cases (66.8%) and control (88.2%). The findings showed that malnourished children are less likely to suffer from malaria as compared to well nourished ones (P- value = 0.0148). The low prevalence of ***P. falciparum*** in malnourished child may be due to lack of essential nutrients needed by malaria parasites to thrive in the red blood cells. In addition, the presence of parasites in both cases and controls may due to the fact that partial immunity for malarial infection is just developing.

1506

MALARIA IN DONATED BLOOD IN CHIPATA DISTRICT HOSPITAL, EASTERN ZAMBIA

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Although malaria is preventable and treatable, it still claims 584,000 lives every year globally with children aged under five years having the highest burden. ***Plasmodium falciparum*** the parasite that cause malaria is one the most commonly encountered blood parasite. This parasite is transmitted by a female Anopheline mosquito, but can also be transmitted through blood transfusion and results in transfusion transmitted malaria (TTM). TTM poses a considerable risk of illness to the major recipients of blood. Unfortunately the blood collected is not routinely tested for malaria; hence the aim of this study was to determine the prevalence of malaria parasites in donated blood. A cross-sectional study where 600 donated blood samples from Chipata District Blood bank in Eastern Zambia were tested using rapid diagnostic tests (RDTs) for ***P.falciparum***. Out the 600 sample 74 were positive; hence the study showed 12.3% prevalence of malaria in donated blood. The study revealed prevalence 12.3%, therefore, it is important that there are preventive measures for all potential routes of transmission if the goal to eliminate it is to be achieved. Although the RDTs used have a 14 day antigen lag, hence is need to conduct a large study were microscopy and molecular methods are employed to detect parasites.

1507

TRENDS IN PROVIDER BEHAVIOR CAPTURED THROUGH ROUTINE MONITORING OF CASE MANAGEMENT OF FEVER AMONG ACCREDITED PROVIDERS IN UGANDA

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Coverage of fever case management interventions remains low across sub-Saharan Africa, including Uganda. While many caregivers seek treatment for symptoms of fever in the private sector, private sector outlets may not have adequate diagnostics, training, waste management, and first line quality assured treatments to ensure appropriate case management. Malaria Consortium together with FIND, PSI and WHO are implementing a project creating a private sector market for quality assured RDTs in malaria endemic countries from April 2013 to date targeting private sector. Accredited outlet types including drug shops, pharmacies and clinics have been established, and participating members received training in integrated case management for febrile illnesses, supportive supervision, quality assured malaria rapid diagnostic test kits, and waste management services. Malaria Consortium UNITAID Private Sector RDT project implements a program monitoring system that collects routine data on a monthly basis. Data is captured by accredited private providers in a client register on phones and or patient slips, and which includes information on symptoms (e.g. fever), assessment (e.g. RDT results), and case management (i.e. referral, treatment). The register facilitates tracking numbers of patients, number of treatments administered, outcome of treatment and the extent to which providers are providing correct case management according to the treatment algorithm. Patient register data were entered into a database for calculation of indicators across the project. Trends in provider behavior over time will be presented. Implications for improving quality of care in the private sector, and approaches to effectively monitoring private providers will be discussed.

1508

POTENTIAL IMPACT OF RAPID DIAGNOSTIC TEST DIAGNOSTIC ERROR ON INCREASED MALARIA MORTALITY

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It is crucial to investigate whether increased adoption of rapid diagnostic tests (RDTs) could have adverse effects on malaria mortality due to the high absolute number of initially untreated cases. Malaria rapid diagnostic tests (RDTs) have been recommended by the World Health Organization to obtain a specific malaria diagnosis before treatment of malaria. RDTs are now being used widely in malaria-endemic regions, including use by community health workers. Published estimates of RDT sensitivity for ***Plasmodium falciparum*** malaria typically range from 85-95% ((check)). In addition, real world sensitivity is often lower and treatment decisions may not be made correctly for both positive and negative RDT results due to limited education of many users, poor training for proper use and limited quality assurance. Globally, a 5% false negative rate in an RDT represents 25 million ((confirm)) untreated malaria cases. It is currently unknown what happens to these untreated cases. The mortality for delayed treatment in travelers is estimated to be ((confirm XX)), but is unknown in the developing world setting. The rationale for the use of RDTs will be presented along with methods to reduce the risk for mortality. Such methods include serial testing for a negative result, instructing of the patient about the possibility of a false negative result and for the patient to return promptly for persistent or worsening symptoms. In fact, presumptive treatment should be reconsidered in some situations. The myth of increasing artemisinin-resistance will be addressed and well as the potential real risk for resistance-induction with long half-life antimalarials. Lastly, methods to conduct quality assurance for RDTs, as well as to detect clinical relevant problems with RDTs will be described.

1509

COMPARISON OF BLOOD SMEAR WITH SERUM MOLECULAR TESTING FOR THE DIAGNOSIS OF MALARIA AMONG FEBRILE KENYAN CHILDREN

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In endemic countries, accurate malaria diagnostics are needed to both correctly identify cases and exclude this as the etiology of nonspecific acute febrile illness in children. Molecular testing is the most sensitive diagnostic test for malaria, but this is typically performed using whole blood. Serum may provide a useful alternative; however, few data have been published on the performance of malaria molecular diagnostics using this specimen type. To compare the performance of blood smear with malaria molecular testing on serum, we randomly selected samples from 194 children who were enrolled in an ongoing, acute febrile illness surveillance study in coastal and western Kenya. Patients presented

within the first 3 days of fever. Blood smear was performed at the time of presentation. Serum samples were tested using a multiplex molecular test (referred to as the UFI assay), which detects all five ***Plasmodium*** species with the specific detection of ***Plasmodium falciparum***. Malaria was detected in 37 (19.1%) patients by blood smear and 59 (30.4%) patients using the UFI assay (p=0.01). Five patients with positive blood smears were negative for malaria in the UFI assay, whereas 27 patients only had malaria detected by molecular testing. Compared to the UFI assay, the sensitivity of blood smear was 54.2% and specificity was 96.3%. Among the 59 samples positive for malaria in the UFI assay, 48 were confirmed as ***P. falciparum*** (81.4%), 6 (10.2%) demonstrated evidence of a mixed infection with ***P. falciparum*** and another species, and 5 (8.5%) were positive only for non-falciparum malaria. Patients who tested positive for malaria by either method were significantly older than patients who tested negative (mean 5.7 yo (range 1-15.75) versus 4.4 yo (range 1-17.5), respectively; p<0.01). Using archived serum, the UFI assay detected malaria in significantly more febrile Kenyan children than peripheral blood smear performed at presentation. This study demonstrates the utility of serum for malaria molecular testing and suggests that malaria may be underdiagnosed if blood smear is relied upon.

1510

DEVELOPMENT OF A POINT OF CARE MOLECULAR TEST FOR *PLASMODIUM VIVAX*

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Malaria is a major public health problem in tropical and subtropical countries with almost 200 million cases reported annually. In the American continent ***Plasmodium vivax*** extends from Mexico to northern Argentina and is responsible for the majority (>70%) of the ≈670,000 reported annual cases. In addition to its known high rate of post-treatment recurrence, recent reports indicated that ***P. vivax*** could be responsible for severe malaria cases. The elimination/eradication efforts include the development and implementation of specific and sensitive diagnostic tests. Several antigen-based Rapid Diagnostic Tests are currently being used in endemic areas with good results when parasite loads are high (≥2,000 parasites/μL), yet the sensitivity is poor when parasitemia is <200 parasites/μL. This is particularly evident with ***P. vivax***. Consequently, our objective was to develop a sensitive field-applicable molecular test for ***P. vivax*** capable of detecting low parasite burdens. We designed primers and probes targeting 18S rRNA of ***P. vivax*** for isothermal DNA amplification using Recombinase Polymerase Amplification coupled with a lateral flow immunochromatographic strip (RPA-LF). Blood DNA from infected patients from an endemic are in the Peruvian Amazon was extracted using the Qiagen® kit. Preliminary results in a small number of clinical samples (n=10) (30 min, 40 °C) showed that these primers specifically amplified ***P. vivax***. There was no cross reactivity with ***P. falciparum*** (n=6). We are currently evaluating the sensitivity of RPA-LF with the goal of detecting samples with low parasite burden. The utilization of lateral flow (LF) strips allowed detecting ***P. vivax*** infections with the naked eye, which makes this diagnostic test potentially applicable at the point of care.

1511

HIDDEN PREVALENCE OF SUB-MICROSCOPIC INFECTIONS: THE ROLE FOR MOLECULAR DIAGNOSTICS IN MADAGASCAR

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Community prevalence of infection is a widely used, standardized metric for evaluating malaria endemicity status at the population level. Conventional methods for measuring prevalence include light microscopy (LM) and rapid diagnostic tests (RDT), but their detection thresholds leave them inadequate for assessing low density parasitaemias. The significance of sub-microscopic malaria infections is poorly understood in Madagascar, a country targeting pre-elimination status by 2017. The country's distinct epidemiological zones experience different patterns of endemic/epidemic transmission, and their underlying parasite reservoirs remain poorly quantified. A population prevalence survey of 2141 individuals was conducted across 25 villages in the western highland fringe region of Ampasimpotsy, Tsiroanomandidy district, in March 2014. LM and the SD Bioline Combo ***Pf/Pan*** RDT were used to screen for infection. PCR-based molecular evaluation by a ligase detection reaction-fluorescent microsphere assay (LDR-FMA) was subsequently conducted from filter-paper blood spots. Prevalence of ***Plasmodium*** infection diagnosed by LM, RDT, and PCR was 2%, 5% and 20%, respectively. This diagnostic discordance was greatest for ***Plasmodium vivax*** infection, which ranged from 0.2% prevalence by LM, to 11% by PCR. Diagnostic sensitivities and prevalence of sub-microscopic infections are examined in relation to patient age, fever, and history of fever. These observations of high prevalence of sub-microscopic infections in western Madagascar strongly call for wider assessment of the parasite reservoir in other regions of the country. The deadly epidemic during early 2015 highlights the threat that these reservoirs can present, particularly in apparently low endemic settings such as southern Madagascar. Pre-elimination will require a shift in focus towards attacking the parasite reservoir, including these sub-microscopic infections. The diagnostic disparities observed from this study corroborate calls globally for a transition towards higher-sensitivity diagnostics for epidemiological monitoring and assessing intervention impact.

1512

CLINICAL IMPACT OF MALARIA RAPID DIAGNOSTIC TESTING IN A NON-ENDEMIC SETTING

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Children who develop malaria after returning to non-endemic settings are at high risk for critical delays in diagnosis and initiation of appropriate antimalarial therapy. Laboratory diagnosis traditionally depends on thick and thin Giemsa smears, requiring highly trained lab personnel. Rapid diagnostic testing (RDT) shows excellent sensitivity and negative predictive value, superior to blood smears, and does not require specific expertise. Yet RDT has not been adopted by most US clinical laboratories. An FDA-approved immunochromatographic assay for the rapid detection of ***Plasmodium*** antigens was introduced at the Children's Hospital of Philadelphia on August 1, 2007. RDT is performed on all samples tested for blood parasites, followed by routine blood smears. Use of RDT dramatically decreased time to report a positive result with any ***Plasmodium*** species (from 9.8 to 1.7 hours) and with ***P. falciparum*** (from 10.2 to 1.6 hours). The objective of this retrospective cohort study is to measure the impact of RDT on the management of children with malaria at an urban tertiary care children's hospital serving a large immigrant

population. Among 71 children admitted with laboratory-confirmed malaria from 2000 to 2014, we will compare clinical management and outcomes of malaria cases before and after RDT introduction while adjusting for disease severity and patient age. Primary outcome variables derived from electronic medical record abstraction include time to positive malaria result report, time to initiation of antimalarial therapy, time to clearance of parasitemia, need for exchange transfusion, ICU admission, and length of hospital stay. We hypothesize that implementation of RDT is associated with a significant reduction in time to initiation of antimalarial therapy, time to resolution of parasitemia, and length of hospital stay. The clinical impact of RDT has not previously been reported in a non-endemic setting. This research will emphasize the utility of RDT as a critical tool for the optimal management of patients with malaria in non-endemic settings. We anticipate completion of this ongoing study by August 2015.

1513

ASSESSING THE MARKET LANDSCAPE FOR AN INFECTION DETECTION TEST AIMED AT MALARIA ELIMINATION

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PATH's Diagnostics for Malaria Elimination toward Eradication (DIAMETER) team is focused on bridging the gap between the rapidly evolving set of infection detection requirements that support elimination and the limitations of existing diagnostic tests. As the managing partner for Infection Detection Test (IDT) Development Initiative, the DIAMETER team is working to create a high quality, low cost, easy to use, and highly sensitive ***Plasmodium falciparum*** (Pf)-specific HRP2 IDT capable of identifying low density Pf infections that current rapid diagnostic tests (RDTs) cannot detect. In Phase 1, we worked with experts to draft a preliminary target product profile, landscaped potential pipeline technologies, and conducted field work to understand use scenarios for improved diagnostics. In Phase 2, we managed technical feasibility activities for next generation tests aimed at identifying individuals with low-density malaria infections. As a subset of this work, we sought to understand how the market will respond when such a test is available for use. Ultimately, the goal of our market research activities was to support the definition, commercialization, and implementation of the most promising, cost-effective, and impactful diagnostic technologies for malaria elimination while promoting investment and development of a healthy IDT market. First, we had to define the factors that could influence supply and demand of the proposed IDT. We assessed early market demand for the IDT by developing various demand scenarios. For instance, the IDT's intended use and whether the IDT would replace existing RDTs would impact product uptake by countries, donors, and implementing agencies as well as manufacturers' volumes. We modeled key factors, capturing project assumptions and develop these scenarios by engaging with subject matter experts and Ministry of Health malaria staff. Additionally, we conducted a comprehensive review in several focal countries to understand elimination strategy impact on the supply and demand issues associated with RDTs. We will present our methodology, findings, and implications related to IDT development decision.

1514

INCREASING USE OF AND ADHERENCE TO MALARIA DIAGNOSTIC TESTS THROUGH ENHANCED NATIONAL CASE MANAGEMENT TRAINING IN CAMEROON

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The national malaria strategy of Cameroon calls for at least 80% of suspected malaria patients (fever or history of fever) to receive a malaria test, and less than 20% of negative tests to receive anti-malarials. To improve testing and adherence rates towards these targets, the malaria program organized a two-day malaria case management training for providers and laboratory technicians in public health facilities between February and June 2014. As confirmatory diagnosis and adherence to negative tests were not routinely collected we placed consultation registers between October 2013 and September 2014 in 157 public health facilities across five of the ten regions in Cameroon. Patients of any age seeking care for any cause were recorded in the registers. Information was collected on demographics (age, gender), symptoms, malaria testing (microscopy or rapid diagnosis test), and any treatment received. A multivariable analysis was performed to assess whether patient symptoms and healthcare worker participation in the training were associated with test uptake and adherence to test results, adjusting for patient demographics, month of consultation, and health facility. During the study period, 126,462 patients were recorded across the 72 facilities that filled the register, of which 28% had suspected malaria. Overall, 69% of suspected malaria patients consulting before the training received a malaria test versus 76% after (p<0.001). However after adjusting for potential confounders, patient consulting after the training had reduced odds of being tested (OR=0.88, 95% CI=0.82-0.95) compared to those consulting before. Among patients who tested negative and reporting receiving a treatment, 22% received an anti-malarial before training compared to 17% after (p<0.01), consistent with increased adjusted odds of adhering to test results after training (OR=1.44, 95% CI=1.12-1.84). Over the course of the study, test uptake and adherence increased, however uptake remained below the national target. Further studies should explore whether additional efforts, such as mentorship, would be sufficient to improve test uptake to national targets.

1515

EVALUATION OF MALARIA RAPID DIAGNOSTIC TESTS AND GIEMSA LIGHT MICROSCOPY IN HIGHLY MALARIOUS DISTRICTS, AMHARA REGION, ETHIOPIA, 2014

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Malaria remains a leading health challenge in Ethiopia. A confirmatory diagnosis with a rapid diagnostic test (RDT) or microscopy is recommended for all suspected malaria cases, and understanding factors affecting the comparability of these diagnostics is important for good case management. We assessed factors related to RDT (SD BIOLINE) and microscopic diagnosis of malaria. A cross-sectional study was conducted in eight high malaria districts of Amhara Region from May 15 to June 15, 2014. All suspected cases presenting to health facilities were eligible for inclusion. With an estimated 9% malaria prevalence, sample size was calculated at 892 using Buderer's formula and 1,000 suspected cases were sought from ten health centers; 987 and 990 were tested for malaria by RDT and microscopy, respectively. The median age was 21 years old (range 1-88 years); 53% were male; 669 (67%) were over 15 years old, 172 (17%) were under 5 years old. The malaria positivity rate by RDT and microscopy was 17.1 % (169) and 16.5% (163), respectively. Compared to the microscopy standard, RDTs showed a sensitivity of 83.9%, specificity of 96.0%, positive predictive value (PV) of 80.4%, and negative PV of 96.8%. The level of agreement (Kappa value) between first and second microscopy reader was 0.74 (p-value < 0.001). In a multivariate logistic regression model, factors associated with higher infection included: male sex (OR=1.49, 95% CI=1.06-2.12), recent reported fever (OR=1.57, 95% CI=1.06-2.33), and having received malaria health education (OR=1.61, 95% CI=1.12-2.32). Children under 5 years old had a lower risk of malaria infection (OR=0.32, 95% CI=0.17-0.60). In this malarious area of Amhara Region, the specificity and negative-PV of the RDTs were excellent; however, the sensitivity and positive-PV were lower (83.9% and 80.4%, respectively). As malaria control improves and prevalence declines, the PV of a negative-RDT will be excellent, but the PV of a positive

test will become very low. Continued quality assurance and training of laboratory technicians on RDTs and microscopy will be critical to maintain comparability of these two important diagnostic methods.

1516

USE OF MALARIA RAPID DIAGNOSTIC TESTING AND ACCEPTABILITY OF RESULTS: AN ASSESSMENT AMONG MEDICAL LABORATORY SCIENTISTS IN NIGERIA

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Confirming suspected cases of malaria through Rapid Diagnostic Tests (mRDT) is expected in secondary and tertiary health facilities when malaria microscopy is not feasible. Unavailability of trained microscopists, non-availability of functional microscope and quality reagents, and in some cases, high client load are challenges to quality malaria microscopy. Medical laboratory personnel sometimes question the validity of mRDT results leading to their low use and acceptance. This study assessed the perception of medical laboratory scientists on the validity and acceptance of mRDT results in secondary and tertiary health facilities in Nigeria. This cross sectional study purposively sampled 66 medical laboratory scientists attending a professional meeting. A pre-validated self-administered questionnaire was used to assess attitude towards validity and acceptability of results obtained through the use of mRDTs. The mean age of respondents was 43±8 years. The majority (98%) had heard of mRDT but only 67% use mRDT in their health facility. Sixty four percent (64%) agreed that mRDT results are valid and should be accepted by all health care providers, however only 49% agreed that mRDT has specificity and sensitivity levels comparable to microscopy. Although 83% of respondents agreed that mRDT results are useful for clinical decisions, 60% disagreed that mRDTs should be used in secondary and tertiary health facilities where microscopy is not feasible. Fifty percent claimed to have had challenges using mRDTs and 26% were related to the discordance with microscopy results. A large majority, 94% agreed that mRDT use skills should be incorporated in the school curriculum for medical laboratory scientists. Distrust of mRDT results persist among medical laboratory scientist and may exist among other health care professionals. Integration of mRDT training into medical professional curriculum should be advocated for, to increase use and acceptance of mRDT results. The current in-service training on mRDT for primary health care workers should be strengthened in secondary and tertiary health facilities in Nigeria.

1517

ASSESSMENT OF A STANDARD MALARIA MICROSCOPY TRAINING PROGRAM ON THE PERFORMANCE OF MEDICAL LABORATORY SCIENTIST IN NIGERIA

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Rapid and precise diagnosis of malaria is an essential element in the effective case management and control of malaria. Malaria microscopy is used as the gold standard for malaria diagnosis, however results remain poor as positivity rate is consistently over 90%. The President's Malaria Initiative (PMI) through the Malaria Action Program for States in Nigeria supports 9 states to build capacity for malaria microscopy. This study demonstrates the effectiveness of in-service training on malaria microscopy amongst medical laboratory scientists. The training was based on WHO basic microscopy training manual. The training utilized a series of didactic lectures and examination of quality teaching slides using a CX 21 Olympus binocular microscope. Purposive sampling was used to enroll 109 medical laboratory scientists across 5 states in Nigeria. Evaluation of the training using a pre and post-test method was based on: written test questions; reading photographic slide images of malaria parasites; and prepared slides. There was a significant improvement in the mean post-test scores for the written test from 38±9% to 71±12%. Mean post-test score for computer based picture speciation test (63±16%) and picture detection test (89.2±10.0) were significantly higher than the mean post-test score for slide reading speciation test (38±20%) and slide reading detection test (70.7±15.3). The mean counting post-test score improved significantly from 4.2±8.0% to 27.9±14.0%. Parasite detection and speciation using enhanced visual imaging was significantly improved compared with using direct microscopy. Regular in-service training and provision of functional and high resolution microscopes are needed to ensure quality malaria microscopy.

1518

REAL-TIME QUANTITATIVE PCR AS THE GOLD STANDARD FOR MALARIA DIAGNOSIS: EVIDENCE FROM THE PERUVIAN AMAZON, A MALARIA HYPOENDEMIC REGION

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The diagnosis of malaria requires the identification of malaria parasites or ***Plasmodium*** antigens/products in patient samples. While conventional microscopy remains the gold standard, quantitative real time PCR (qPCR) despite technological and cost challenges remains an important tool for quantification and confirmation of low density parasitemia and mixed infections. With the goal of finding potential human reservoirs of malaria transmission asymptomatic, clinically immune, ***Plasmodium***-parasitemic individuals_we compared qPCR (***Plasmodium*** 18S rDNA target) to light microscopy on blood samples obtained from a longitudinal cohort of six communities in the Peruvian Amazon. Of 7,494 samples 868 (11.6%) were positive by qPCR; of these, 73% were ***P. vivax***, 27% ***P. falciparum***. By microscopy, only 320 (4.3%) were positive; 95% were ***P. vivax***, 5%. ***P. falciparum***. Sensitivity and specificity of microscopy were 24% and 17%, respectively, compared to qPCR. No mixed infections were detected by either method. The 526 asymptomatic infections had very low parasite density (16/µl; SD ± 18.47) detected only by qPCR. Most asymptomatic subjects (40%) were 30-50 years old. Compared to the light microscopy, qPCR or any other molecular tool, with appropriate quality controls, should be implemented at the public health level for monitoring programs and control of malaria. Further work to determine if treatment of qPCR-positive/light microscopy-negative individuals has an impact on malaria is important to carry out.

1519

DIAGNOSTIC TESTING AND ADHERENCE TO ANTIMALARIAL MEDICATION: EVIDENCE FROM A RANDOMIZED CONTROLLED TRIAL IN UGANDA

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Artemisinin-combination therapies (ACTs), currently the only effective treatment for malaria, have contributed to large declines in the morbidity and mortality burden of the disease over the past decade. Though it is a short 3-day treatment for malaria, 34 percent of patients do not complete the full course of ACTs. This is not only harmful for the individual, but also increases the risk of widespread pathogen resistance to the drugs. Adherence to ACTs is complicated by the fact that, historically, both health workers and patients have relied primarily on the patients' symptoms to diagnose malaria. Since the symptoms of malaria overlap with several other common diseases, patients are likely to be uncertain about whether

they are actually suffering from malaria, which reduces the expected benefit of completing the treatment. We conducted a randomized controlled trial in Central Uganda to examine whether patients who received a confirmed diagnosis of malaria, using a rapid diagnostic test (RDT), are more likely to finish their medications. Among a sample of 1,525 patients purchasing ACTs at private drug shops, we randomly offered 573 (37%) a free malaria rapid diagnostic test and then visited all patients at their households three days later to assess whether they had completed the treatment. Of those who were offered and consented to testing, 68% tested positive for malaria while, overall, 66% of patients finished the full course of drugs. Compared to those who were not offered the test, a positive malaria test did not increase the odds of completing the treatment, not even among young children for whom the risk of malaria mortality is highest. However, patients who received a positive malaria test had 25% fewer tablets remaining at the follow-up survey than those not offered the test, indicating that a confirmed diagnosis encouraged some patients to complete a few additional doses of the drug. We also show that patients who still felt unwell on the second day of treatment had approximately twice the odds of completing the treatment which suggests that many people may stop taking the medicines because they feel better and believe that they are cured of malaria.

1520

STRUCTURAL CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* HRP2: ANALYTICAL CHALLENGES IN *PLASMODIUM FALCIPARUM* HRP2-BASED IDT DEVELOPMENT

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With improved performance over rapid diagnostic tests (RDTs), infection detection tests (IDTs) currently under development will provide improved diagnostic support for global ***Plasmodium falciparum*** (Pf) elimination campaigns. By targeting Pf-specific histidine rich protein 2 (PfHRP2), which persists several weeks in peripheral blood, prototype IDTs are designed to detect low-level and episodic Pf infections. Commercially available PfHRP2-based RDTs have a high degree of variability in their clinical and analytical sensitivity. The naturally-occurring sequence variations of PfHRP2, especially the frequency and types of tandem amino acid repeats found in various isolates, may affect the performance of these tests, especially at low levels of parasitemia. As we begin to characterize the performance of IDT prototypes in the product development pipeline, we recognize that evaluation of these tests using available PfHRP2 reference standards that are not equivalent to endogenous PfHRP2 may provide a relative, but not truly definitive, quantitative measure of PfHRP2 test performance. The aim of the present study is to investigate the effects of the sequence and repeat number variation of PfHRP2 proteins on the binding of PfHRP2-specific antibodies, and to examine potential shortcomings of current reference standards used for IDT test development. Recombinant PfHRP2 proteins that possess or lack an affinity purification tag were evaluated for binding to PfHRP2-specific antibodies in an in-house developed sandwich enzyme linked immunosorbent assay. Our preliminary data show that the detection limit of PfHRP2 proteins lacking an affinity purification tag is much lower than for those carrying an affinity purification tag, indicating that the extra unnatural amino sequence might hinder the access of antibodies to their epitopes. Evaluating the effect of PfHRP2 sequence variations on the binding of PfHRP2-specific antibodies used for IDTs is currently ongoing. The present study will provide new evidence to enable rationale selection of reference standards in support of IDT development for detecting low level Pf infections.

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A SEMI-QUANTITATIVE PAPER-BASED TEST FOR PREDICTING PROGRESSION TO SEVERE MALARIA

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Severe malaria affects thousands of children in sub-Saharan Africa annually, with the majority of these children under the age of five. Parasites sequester in microcapillaries and cause hypoxia that may lead to dysfunction of vital organs. Unless treatment is given quickly, severe malaria can lead to mortality or permanent complications for the child. Retinopathy and other suitable methods to predict progression to severe malaria are not feasible for low-resource settings due to cost and infrastructure requirements. Additionally, the gold standard for malaria diagnosis, blood smear microscopy, is not indicative of disease progression. Recent studies have shown that plasma concentration of ***Plasmodium falciparum*** histidine-rich protein 2 (pHPR2), a protein secreted by parasite-infected erythrocytes, can identify children with uncomplicated malaria that might progress to severe malaria. We have developed a low-cost 2D paper network which semi-quantitatively reports the concentration of pHRP2. The device works with one drop of whole blood, costs about 1 USD, and has a limit of detection of 10 ng/mL, which is lower than current RDTs on the market. It involves minimal user input and runs within 30 minutes. Pilot tests have assessed the ability of the device to semi-quantitatively differentiate levels of pHRP2 using spiked human blood from various donors. Once validated, our device could potentially allow for simultaneous diagnosis of malaria and prediction of progression to severe malaria. Therefore, we believe the test could provide clinicians with a low-cost tool to make informed decisions for parasitemic children.

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IMPACT OF MALARIA RAPID DIAGNOSTIC TESTS ON PATIENT CARE: RESULTS FROM THE ACT CONSORTIUM

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Malaria rapid diagnostic tests (RDTs) are intended to have a beneficial impact on management of suspected malaria, including that patients with confirmed malaria receive artemisinin-based combination therapy (ACT) and patients without malaria receive non-antimalarial treatment. The ACT Consortium includes several studies designed to test operational strategies for ACT and RDT implementation in various settings, providing an opportunity to draw on data from multiple projects that have introduced RDTs across a range of clinical, social, and epidemiological contexts, and in public, private retail, and community health service sectors. To assess the impact of RDTs on patient treatment and satisfaction at the consultation, data were examined from nine studies comparing scenarios where RDTs were made available to control scenarios where RDTs were not made available. Where RDTs were introduced, the proportion of patients tested ranged from 39% to 99%; figures were similar for children under 5 years and for older patients. Prescription of ACT was lower in nearly all RDT scenarios (range 8-64% versus 15-99% in scenarios without RDTs), driven mostly by reduced prescription for RDT-negative patients (range 3-45% versus 18-98% for RDT-positive patients). An exception to this pattern was seen in public facilities in a high-transmission setting where RDTs were irregularly available in the control arm. The impact of RDTs on prescription of systemic antibiotics varied, ranging from 15-73% in scenarios without RDTs and 21-75% in scenarios with RDTs available, and was slightly higher for RDT-negative patients (range 29-78% versus 13-65% for RDT-positive patients). There was no clear pattern observed for prescription of other treatments, polypharmacy, or patient satisfaction. This ongoing analysis

aims to understand factors associated with variation in case management outcomes in order to offer more tailored guidance for RDT introduction in other areas.

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MODELLING THE COST-EFFECTIVENESS OF INTRODUCING MALARIA RAPID DIAGNOSTIC TESTS IN THE PRIVATE FOR-PROFIT SECTOR

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During the last five to ten years, major changes have occurred in the diagnosis of malaria in primary level public health facilities in endemic countries, as malaria rapid diagnostic tests (mRDTs) have been widely deployed. There are now increasing calls for mRDTs to be made available outside formal public health facilities - and in particular through the private for-profit sector, where a high proportion of people with suspected malaria seek care. Private sector antimalarial providers include private hospitals and clinics, though in most settings the majority of antimalarials are distributed through drug retailers, primarily drug stores and small pharmacies, which very rarely provide blood tests. It is common to find that a high proportion of patients sold antimalarials at such outlets are not parasite positive, while many other clients who are parasite positive do not receive appropriate antimalarial treatment. Private sector mRDT introduction on a large scale has already begun in several African countries, though robust evidence on the impact and value for money of these strategies is not yet available. Fundamental questions remain as to where and how mRDTs should be introduced, and what the impact will be on costs, individual health outcomes and public health. We have developed a cost-effectiveness decision tree model of management of non-severe febrile illness, adapting and expanding previous models to cover the private sector, and updating them by drawing on parameters from recent published and unpublished data. The model documents care-seeking pathways, test accuracy, and the effect of test results on treatment choice and adherence to treatment. Results will be presented on the impact on treatment outcomes, costs, and cost effectiveness of different mRDT roll-out packages, for those with malaria and those with other febrile illnesses. Cost-effectiveness will be reported in terms of cost per disability adjusted life year (DALY) averted and cost per death averted. The overall cost implications for implementing agencies, across a range of malaria transmission and health system settings, will also be discussed.

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MALARIA RAPID DIAGNOSTIC TEST MARKET TRENDS IN SUB-SAHARAN AFRICA, 2009-2014

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In 2012, the WHO launched the Test, Treat, Track initiative recommending confirmatory testing prior to antimalarial treatment. National malaria control programs (NMCP) across sub-Saharan Africa (SSA) subsequently aligned national guidelines with this recommendation. Strategies to scale up testing using malaria rapid diagnostic tests (mRDT) were introduced by NMCPs. We use multi-country outlet survey data collected between 2009-2014 by ACTwatch to describe mRDT markets in Benin, the DRC, Kenya, Madagascar, Nigeria, Tanzania, Uganda and Zambia. A census of outlets with potential to distribute antimalarials was conducted among a representative sample of administrative units. In addition to an antimalarial audit, information was captured for malaria microscopy and all mRDTs in stock including retail price and amount distributed in the past week. The private sector distributes the majority of antimalarials in all project countries except Zambia. Although modest improvements in mRDT availability have occurred, testing availability among antimalarial-stocking private sector outlets remains low, ranging from 4% in Madagascar to 25% in Uganda. Subsequently, while majority of antimalarials are distributed by the private sector, the public sector provides the majority of testing. The price of an mRDT among private sector outlets is generally higher than the price of a pediatric pre-packaged quality-assured artemisinin combination therapy (QAACT). However, the private sector price of an mRDT is generally lower than the price for one QAACT adult equivalent treatment dose. Confirmatory testing prior to antimalarial treatment is currently limited across high burden countries in SSA due to limited availability at the private sector outlets where most antimalarials are distributed. Where mRDTs are available, financial incentive to test before treating with QAACT is apparent with respect to adult testing and treatment. There is generally no such incentive to test young children before QAACT treatment. Implications for policies and strategies to implement WHO and national guidelines on confirmatory malaria testing will be discussed.

1525

SUSCEPTIBILITY OF DIFFERENT STRAINS OF MICE TO LIVER-STAGE INFECTION WITH *PLASMODIUM BERGHEI* SPOROZOITES BY USING *IN VIVO* IMAGING SYSTEM

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The liver stages of ***Plasmodium*** parasites are important targets for the discovery and development of prophylactic drugs. A sensitive and useful mouse model with an ***in vivo*** imaging system (IVIS) to monitor the liver stage was established. The transgenic ***P. berghei*** parasite expressing the bioluminescent reporter protein luciferase was utilized to visualize and quantify parasite development in BALB/c, C57BL/6, C57BL/6 albino, C3H, and ICR mice. These inbred strains of mice were tested to study the differences in susceptibility to ***P. berghei*** hepatic infections previously monitored with the liver resection methods. As an additional endpoint, blood stage parasitemia was monitored by flow cytometry. A real-time IVIS instrument determined the exposure level of luminescence measured from luciferase expressing of sporozoites through development in the hepatocyte and assessed through liver, peritoneum, skin, and hair coat. The luminescence values (photon counts/min) measured from the anatomical liver location in untreated mice infected with 10,000 ***P. berghei*** sporozoites at 24 hours post inoculation were 8.15×10^5 for C57BL/6 albino, 2.12×10^5 for C3H, 0.91×10^5 for C57BL/6 WT, 0.58×10^5 for BALB/c, and 0.08×10^5 for ICR. The high grow-up rate of sporozoites in hepatocytes was also found in C57BL/6 albino, C57BL/6 WT, and C3H mice, and low rate was shown in BALB/c and ICR mice. In addition, similar to previous studies, the susceptibility of female mice to liver-stage infection is higher than that of male mice. In summary, this data supported the use of highly susceptible mouse strains (C57BL/6 albino, C57BL/6 WT, and C3H) at the WRAIR to study ***Plasmodium*** hepatic infection by IVIS monitoring. The study indicates that by using IVIS, the C57BL/6 albino, C57BL/6 WT, and C3H mice infected with ***P. berghei*** sporozoites are preferable for investigating the discovery and development of prophylactic antimalarial drugs.

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NICOTINAMIDE ENHANCES ANTIMALARIAL EFFECTS OF CHLOROQUINE, PYRIMETHAMINE AND ARTEMISININ *IN VITRO*

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Malaria is a dangerous infectious disease of humans and other animals caused by protozoan microorganisms belonging to the genus ***Plasmodium***. The malarial parasite causes different symptoms including fever, fatigue, vomiting, headaches, yellow skin, seizures and even death. ***Plasmodium falciparum*** represents the most virulent form of human

malaria, causing about a half million deaths per year. Despite the attempts to develop various antimalarial vaccines, chemotherapy is still a major approach of malaria prevention and treatment. Systematic use of common antimalarial drugs together with the high genetic variability of the malaria parasite leads to the appearance of resistant ***Plasmodium*** strains. Therefore, identification and development of new antimalarial compounds and treatments remains an important problem of malarial parasitology. Nicotinamide (vitamin B3) is a water soluble amide derivative of nicotinic acid, which has been used at high doses for a variety of therapeutic applications. It has been demonstrated earlier that nicotinamide inhibits ***P. falciparum*** growth. However, its antimalarial effect, in combination with other antimarial drugs, has not been described to our knowledge. In this work, we analysed the antimalarial effects of nicotinamide in combinations with chloroquine, pyrimethamine and artemisinin on the blood stages of ***P. falciparum***. Our results demonstrate that combinations of nicotinamide with chloroquine, pyrimethamine or artemisinin lead to synergetic antimalarial effects ***in vitro*** and significantly enhance the anti-parasite potential of these drugs. Moreover, treatment of uninfected red blood cells with high dose of nicotinamide (20 mM) does not provoke LDH release, demonstrating its non-toxicity for erythrocytes. These results suggest that nicotinamide might be useful not only as a vitamin supplement but also as a strong enhancer of the anti-parasitic effect of common antimalarial drugs, including chloroquine, pyrimethamine and artemisinin. *Equal contributions

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DEVELOPMENT OF ANTIMALARIALS WITH SERCAP PROFILE FROM DOS DERIVED COMPOUNDS

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Despite increased efforts in the last decade, the antimalarial drug discovery and development pipeline still lacks compounds with non-erythrocyte stage activity and target diversity. We previously performed a high throughput screen (HTS) on approximately 100,000 diverse small molecules from the Broad Institute's Diversity Oriented Synthesis compound collection which contains unique core structures more likely to have novel antimalarial mechanisms of action. The present study describes the discovery and optimization of a novel series from our HTS study displaying potent activity against a panel of clinical strains harboring resistance to known antimalarial drugs as well as agents in clinical development. The compound series inhibits phenylalanine t-RNA synthetase activity, a novel molecular target, and shows robust ***in vivo*** efficacy in erythrocytic, hepatic and sexual stages with a single dose. We also present extensive pharmacokinetic and preclinical safety data that supports the progression of this novel antimalarial agent towards clinical development to treat malaria.

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DIFFERENTIAL KINETIC PROFILES AND METABOLISM OF PRIMAQUINE ENANTIOMERS BY HUMAN HEPATOCYTES

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The clinical utility of primaquine (PQ), which is used as a racemic mixture of two enantiomers, is limited due to metabolism-linked hemolytic toxicity in G6PD deficient individuals. The current study aimed to investigate differential metabolism of the enantiomers in light of the suggestions that toxicity and efficacy might be largely enantioselective. Using cryopreserved human hepatocytes, ^{13}C-labeled (+)-, (-)- and (±)-PQ were separately incubated. Substrate depletion and metabolite production were monitored via UHPLC-MS/MS. The initial half-life was 217 and 65 min for (+)- and (-)-PQ; with an elimination rate constants (λ) of 0.19 and 0.64 h^{-1} respectively. The ***in vitro*** intrinsic clearance was 2.55 and 8.49 (µL/min)/million cells which when upscaled ***in vivo*** yielded 6.49 and 21.6 (mL/min)/kg body mass respectively for (+)- and (-)-PQ. Extrapolation to ***in vivo*** human hepatic clearance was performed using the well-stirred liver model. The rate of hepatic clearance of (+)-PQ was only 45% that of (-)-PQ. Two major primary routes of metabolism were observed as earlier reported: oxidative deamination of the side chain terminal amine; and hydroxylations on the quinoline moiety. The major deaminated metabolite, carboxyprimaquine (cPQ), a putative PQ terminal alcohol (***m/z*** 261), a cyclized side chain derivative from the aldehyde (***m/z*** 241), cyclized carboxylic acid derivative (***m/z*** 257), a quinone-imine product of hydroxylated cPQ (***m/z*** 289), cPQ glucuronide (***m/z*** 451), and the glucuronide of PQ alcohol (***m/z*** 437) were preferentially generated from the (-)-PQ. The major quinoline oxidation product (***m/z*** 274) was preferentially generated from (+)-PQ. A prominent conjugate (***m/z*** 422) (seemingly a glycosylated PQ, but still under investigation) was preferentially generated by (+)-PQ. An accumulating metabolite (***m/z*** 480) thought to be a carbamoyl glucuronide of PQ was exclusively generated from (+)-PQ. In the light of the desire to establish clinical differences in PQ enantiomers, these current findings may provide important information that may lead to clearer understanding of PQ-induced hemolysis and possible enantioselective safety.

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NITRIC OXIDE DONOR DIHYDROARTEMISININ DERIVATIVES AS MULTITARGET AGENTS FOR THE TREATMENT OF CEREBRAL MALARIA

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A series of hybrid products in which the dihydroartemisinin scaffold is combined with NO-donor furoxan and NONOate moieties were designed, synthesized and studied as potential tools for the treatment of experimental cerebral malaria (ECM) in C57BL/6 mice infected with ***Plasmodium berghei*** ANKA. Five synthesized products were able to dilate rat aorta strips precontracted with phenylephrine with a NO-dependent mechanism. All hybrid compounds showed preserved antiplasmodial activity ***in vitro*** and ***in vivo*** against ***Plasmodium berghei*** ANKA,

comparable to artesunate. Compound 8, selected for additional studies, was capable of increasing survival of mice with late-stage ECM from 33.3% to 63.6% compared with artemether. Artemisinin-NO-donor hybrid compounds show promise as potential new drugs for treating cerebral malaria.

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DIFFERENTIAL DECAY OF ANTIMALARIAL ACTIVITY OF ARTEMISININ AND ITS DERIVATIVES WHEN INCUBATED IN PHYSIOLOGICALLY RELEVANT CONDITIONS

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Artemisinins are peroxidic antimalarial drugs which are known to be very potent, yet chemically highly unstable: they degrade quickly in the presence of ferrous iron, Fe(II)-heme or biological reductans. Less known is how their chemical decay relates to antimalarial activity. We incubated dihydroartemisinin (DHA) and artemisinin (ART) in a range of conditions relevant to both treated patients and in-vitro assays (PBS, plasma or erythrocytes lysate for different durations and pHs) and measured their residual activity on ***P. falciparum in vitro*** using the pLDH method. Chloroquine was also used to verify if drug instability was related to the presence of the endoperoxide. A significant reduction of the antimalarial activity of DHA was observed after incubation in plasma or serum, and to a lesser extent in erythrocytes lysate or PBS. ART showed a different behavior: its antimalarial activity was significantly reduced by incubation with erythrocytes lysate but was minimally affected by PBS, plasma or serum. Moreover, the serum-enriched medium (10% human serum or 10% albumax) customarily used for ***in vitro*** cultures also affected DHA, and to a lesser extent, ART efficacy. Decreasing pH from 7.6 to 7.2 led to a decrease in artemisinins degradation and this increased activity. The presence of reductans such as ascorbic acid or N-acetylcysteine in the erythrocytes lysate strongly reduced artemisinins activity and CO binding to Fe(II)-heme conferred partial protection. Adding ascorbic acid to plasma had no effect on artemisinins activity. Chloroquine was unaffected in any of the tested ***in vitro*** conditions. Biological results correlated well with chemical degradation quantified as degradation rate constant. These results suggest that instability is likely due to the endoperoxide, but C10 substitutions can further modulate the stability of the molecules. Particular care should to be taken in conducting and interpreting ***in vitro*** studies, and in storing these compounds. Moreover, conditions such as hemolysis or acidosis associated with malaria severity may contribute to artemisinins instability and reduce its effectiveness.

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LEAD CANDIDATE SELECTION OF BROAD-SPECTRUM ANTIMALARIAL ACRIDONES

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We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced ***Plasmodium*** infection in addition to efficacy against blood stage parasites. We have been successful in producing extremely potent new lead candidates with pico molar IC50 values against MDR resistant parasites, as well as full protection of liver stage infection at comparable dosage with primaquine. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

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PROVEBLUE (METHYLENE BLUE): A PROMISING ANTIMALARIAL DRUG

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In 2011, the World Health Organization recommended artesunate as the first-line treatment for severe malaria. In recent years, several studies have reported clinical failures or at least extended parasite clearance times in Asia. There is an urgent need to discover partners for combination with artemisinin derivatives. Proveblue (PVB) (international patent no. PCT/FR/2007/001193), which is a methylene blue preparation that complies with the European Pharmacopoeia and contains limited organic impurities and heavy metals of recognized toxicity, was demonstrated to possess ***in vitro*** antimalarial activity (at a geometric mean 50% inhibitory concentration [IC50] of 3.62 nM) against 23 ***Plasmodium falciparum*** strains that were resistant to various other antimalarials. The IC50 for PVB ranged from 0.88 nM to 40.2 nM with a mean of 5.3 nM in Senegalese isolates collected from November 2013 to January 2014 at the Hôpital Principal de Dakar. PVB exhibited noticeable ***in vitro*** synergistic effects in combination with mefloquine and quinine and high synergistic effects associated with dihydroartemisinin, the active metabolite of artemisinin derivatives. Treatment with 1 to 10 mg/kg of weight of PVB for five days significantly reduced or prevented cerebral malaria in mice. PVB demonstrated high efficacy in cerebral malaria in comparison with dihydroartemisinin or quinine. These results confirm the therapeutic potential of Proveblue, which could be integrated into the pipeline of new antimalarial combination therapies.

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RESISTANCE SELECTION APPROACH TO IDENTIFY AND VALIDATE TARGETS FOR ANTIMALARIAL DRUG DISCOVERY

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The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and prevent the development of drug-resistance. As part of the Malaria Drug Target Identification Project efforts, we have adopted a chemogenomic approach to identify the targets of the most prominent compounds from chemically diverse libraries. Study compounds were selected based on availability, purity, potency in a multi-drug resistant isolate, and lack of known mechanism of action towards the mitochondrion or folate biosynthesis. To further eliminate overlap with known targets, we performed cross-resistance testing against a panel of drug resistant parasite lines with well-characterized mutations in diverse targets. Here we present studies of two molecules: a drug-like compound from the Malaria Box, MMV007564, and a probe-like molecule from a Diversity Oriented Synthesis library, BRD3842. ***In vitro*** resistant lines were generated by single-step selection and whole genome sequencing employed to identify genetic variants contributing to the resistance phenotype. Novel variants were identified in the Pf cyclic-amine resistance locus (PfCARL) (MMV007564), and the phosphatidylinositol-4-OH kinase (PI(4)K) (BRD3842), both of which have been previously implicated in the mechanism of action for compounds

chemically distinct from those in this study. These results demonstrate that multiple chemical classes are able to inhibit common parasite targets and suggest that there are a limited number of pathways in the parasite susceptible to inhibition and/or that contribute to drug-resistance. Drug-development strategies that counteract these common escape mechanisms may therefore become invaluable in extending the usable lifetime of future therapies.

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TARGETING FREP1 TO BLOCK MALARIA TRANSMISSION THROUGH SMALL MOLECULES

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Inhibiting ***Plasmodium*** development in mosquitoes will block malaria transmission. Fibrinogen-related protein 1 (FREP1) has been demonstrated to be critical for parasite infection in mosquitoes. Here, we further determined that FREP1 protein binds ***P. falciparum***-infected red blood cells (iRBCs) and ookinetes. We propose that small molecules disrupting the interaction between FREP1 and ***Plasmodium*** will prevent parasites from infecting mosquitoes. To test this hypothesis, we developed an ELISA-based method to screen a fungal extract library, and obtained one candidate fungal extract (Chapel SA-3) that inhibited about 98% of the FREP1-***Plasmodium*** interaction. The inhibition was further confirmed by indirect immunofluorescence assays (IFA). We also verified the inhibition specificity between FREP1 and ***Plasmodium***. Notably, feeding Chapel SA-3 to mosquitoes significantly inhibits ***P. falciparum*** infection in midgut. The candidate fungal extract does not affect the development of ***P. falciparum*** gametocytes or ookinetes. Therefore, we conclude that disruption of the interaction between FRPE1 and ***Plasmodium*** effectively reduces malaria infection in mosquitoes. Targeting FREP1 with fungal small molecules is thus an effective novel approach to block malaria transmission.

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HUMAN PATHWAYS FOR METABOLISM OF PRIMAQUINE: IMPLICATIONS IN TOXICITY AND EFFICACY OF 8-AMINOQUINOLINE ANTIMALARIALS

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Primaquine (PQ), an 8-aminoquinoline, is the drug of choice for the radical cure of ***Plasmodium vivax*** malaria and utility for prevention of malaria transmission. Efficacy and hemolytic toxicity of PQ have been attributed to the metabolites. Reactive nature and low quantities pose major challenge for identification and quantification of these metabolites in clinical and experimental studies. We have recently addressed this challenge with application of PQ labeled with ^{13}C-stable isotope and analysis of the metabolites with UPLC integrated with QTOF-MS. Chemical synthesis of key metabolites have provided necessary standards for confirmation of structures and quantification. Comprehensive experimental and clinical studies with primary human hepatocytes, the recombinant human enzymes (CYPs and amine oxidases) and healthy human volunteers along with use of specific enzyme inhibitors have been useful in phenotyping of key PQ metabolites and their relative quantification. The results have been utilized for prediction of pathways for metabolism of PQ. Human metabolism of PQ follows two distinct pathways. The major pathway is initiated by oxidative deamination of PQ by monoamine oxidase B to carboxy PQ. Carboxy PQ is further metabolized through CYP mediated pathways and phase II metabolism generating reactive quinoneimine metabolites and glucuronide conjugates. This pathway determines characteristic pharmacokinetic and pharmacodynamic properties of PQ. Another pathway mediated through CYPs (predominantly CYP2D6) generates multiple mono hydroxylated metabolites. CYP2D6 mediated oxidation of PQ occurs at different positions on the quinoline ring. An orthoquinone product of a hydroxyl product 5-OH-PQ was identified as the major CYP2D6 metabolite, a likely marker for the 5-hydroxylation pathway. Pharmacological studies suggest 5-OHPQ as highly reactive and may be responsible for the efficacy/toxicity of PQ. Direct metabolism of PQ through phase II glucuronide conjugation and excretion in urine was also observed. Critical analyses of these pathways have helped in understanding the mechanism of efficacy and toxicity of PQ

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A PROTOCOL TO EVALUATE THE EFFECTIVENESS AND FEASIBILITY OF REACTIVE TARGETED PARASITE ELIMINATION (TPE) TO REACTIVE CASE DETECTION (RACD) AS A COMMUNITY LEVEL INTERVENTION IN SWAZILAND

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Reactive case detection (RACD), which is testing and treatment of individuals residing near passively detected index cases, is recommended for malaria elimination yet some available diagnostics have limited sensitivity and there are logistical challenges including cost. Treatment without testing or targeted parasite elimination (TPE) may be a more effective and feasible approach for reducing and interrupting transmission. As an operational research study embedded into malaria program activities, we plan a cluster-randomised control study design trial to evaluate reactive-TPE in the low transmission setting of Swaziland. The primary aim is to compare the impact of reactive TPE versus RACD on malaria incidence, with a hypothesis that TPE will result in lower cumulative malaria incidence. A total of 58 high risk localities or clusters will be included in the trial (population 124,737) and randomized to either TPE or RACD. Over 2 years, individuals residing within a radius of 200 m from an index case will be targeted for either RACD or TPE within 1 week of index case presentation. Incidence will be measured using passive surveillance with subsequent household follow up to identify which cluster the index case resides in. The study is powered to detect a difference in cumulative incidence of 1.1/1000 in the TPE arm versus 2.2/1000 in the RACD arm observed in 3 previous malaria seasons in RACD study area. Secondary outcome measures of effectiveness will include: seroprevalence and prevalence (in a cross sectional survey at the end of the study), proportion of imported incident cases, and transmission potential (utilizing genotypes). Secondary outcome measures of feasibility will include: coverage, adherence, serious adverse events, acceptability, and cost-effectiveness. Findings will inform malaria elimination strategies for Swaziland and other countries pursuing malaria elimination.

1537

SEASONAL VARIATION IN ACCESSING BIOMEDICAL HEALTHCARE FACILITIES IN AN AREA OF LOW MALARIA TRANSMISSION IN RURAL ZAMBIA: POTENTIAL IMPACT ON MALARIA ELIMINATION PROGRAMS THAT USE REACTIVE CASE FINDING

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Many malaria elimination programs use reactive case finding or diagnose and treat malaria in symptomatic patients presenting at a biomedical healthcare facility and then track asymptomatic cases living in the vicinity of an index case. The premise behind these programs is they are less expensive than communitywide mass diagnosis and treatment campaigns. However, if symptomatic patients are unwilling or unable to travel to a biomedical facility for the initial diagnosis, the program cannot effectively cut transmission due to missed pockets of asymptomatic carriers. Anecdotal evidence indicates that in the low-transmission area of Macha, Choma District, Southern Province, Zambia, people are less able to reach a government health center or regional hospital during the heavy rainy season, which coincides with the period of highest malaria transmission. We tested this hypothesis through transforming interview data on all pathways and routes used in the dry and rainy seasons to reach local healthcare providers (biomedical and traditional) in four villages into GPS coordinates and maps. Additional information on the highest level of use (footpath, bicycle, motorcycle, oxcart or car/truck), and average time needed to reach providers was used to calculate signficant differences in access to biomedical resources providing malaria diagnosis and treatment. Results can help identify communities where environmental factors serve as barriers to accessing a government-run malaria elimination program in an area of very low transmission.

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NEW INFECTION DETECTION TESTS TO ENABLE IDENTIFICATION OF LOW DENSITY INFECTIONS: THE ACCESS FRAMEWORK

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As malaria prevalence declines due to successful malaria control programs, more sensitive diagnostic tests are need to identify an increasing number of low-density infections that contribute to the infectious reservoir. PATH's Diagnostics for Malaria Elimination towards Eradication (DIAMETER) team is committed to enabling access to the most appropriate diagnostic tools to support malaria elimination, bridging the gap between rapidly evolving elimination tactics and diagnostic capabilities. As the managing partner for the Bill & Melinda Gates Foundation-funded Infection Detection Test (IDT) Development Initiative, the DIAMETER team is working to create a high quality, low cost, easy to use, and highly sensitive ***Plasmodium falciparum*** (Pf)-specific HRP2 IDT capable of identifying low density Pf infections that current rapid diagnostic test cannot detect. In this poster, we will review progress on the IDT's development and outline next stages in the product development value chain, focusing on the transition from technical feasibility phase to operational feasibility and product development. We will identify remaining questions and propose a plan to resolve these while advancing the IDT toward commercialization and impact. Finally, we will review remaining challenges and outline key elements of the access framework including manufacturing, forecasting, procurement, distribution, delivery, and governance. Included in this review are: results from prototype IDT evaluation; an update on regulatory pathways; an assessment of HRP2 issues that impact standard selection; a revised market assessment; and an updated target product profile.

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PRIVATE SECTOR SURVEILLANCE AND RESPONSE: LESSONS FROM WESTERN CAMBODIA

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Tracking data on malaria cases treated by Cambodian public health facilities (HFs) and village malaria workers (VMWs) is part of routine surveillance. However, patients who first present to private providers remain largely untracked and unreported, limiting effective planning and monitoring of national malaria control efforts. Data from a national health survey suggests 75% of recipients prefer seeking treatment from the private sector due to perceptions of prompter service and constant availability. The National Strategy for Public and Private Mix (PPM) directs private providers in endemic areas where no drug resistance has been documented (zone 2) to provide early diagnosis and treatment. However, private providers in areas where drug resistance has been documented (zone 1) can test, but must refer cases to a public HF or VMW. Private providers must report all suspected malaria cases (diagnosed, treated or referred) to the national malaria information system. Private providers taking part in PPM gain access to supplies available at local HFs. The Control and Prevention of Malaria (CAP-Malaria) Project strengthens linkages between public and private providers and improves the quality of care provided by the private sector. The project works with 167 registered private providers in 3 operational districts (ODs) in Western Cambodia, engaging them in public sector response. In 2014, 114 suspected malaria patients in zone 1 were referred from 56 private providers to HFs and VMWs. 102, (89%) reached the designated service points; of those, 93 patients (91%) were diagnosed with malaria. In Sampov Meas (zone 2), 2,733 suspected patients were tested and 1,574 were found malaria positive and treated by private providers while 2,852 were tested and 1,112 found positive at public HFs. In comparison, 39%, 27% and 34% of malaria cases in Sampov Meas were managed by private providers, public HFs and VMWs, respectively. Recognizing the strong role of the private sector, CAP-Malaria, with the provincial health department and ODs, engaged private sector partners in a campaign for intensive health education, net distribution, and active case detection.

1540

INCREASING ACCESSIBILITY OF MALARIA SERVICES AMONG CROSS-BORDER POPULATIONS IN THE GREATER MEKONG SUB-REGION

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While malaria morbidity and mortality have declined significantly in the Greater Mekong Sub-region countries, the emergence and spread of artemisinin-resistant malaria (ARM) in border areas has raised alarm. A potential source of transmission and a cause of the spread of resistant parasites in the region is population mobility. Cross-border workers often have limited knowledge of malaria risk, making them highly vulnerable to malaria. Because many are unfamiliar with the local health services, they may not seek services and thus remain untreated and unknown by health authorities, inadvertently carrying the parasite from one country to another. To improve accessibility of malaria services by cross-border populations in the GMS countries, the Control and Prevention of Malaria Project (CAP-Malaria) is engaging local authorities along the borders between Cambodia, Thailand, and Myanmar through a Twin-Cities approach that aims to increase coordination of activities and contain the spread of ARM. With the project's support, Twin-Cities working groups were established in four pairs of cities located across a border, engaging representatives from the district level, from health facilities, and from

among community-based malaria workers. The working groups meet on a quarterly basis to share data on malaria, discuss and develop joint work plans, and monitor implementation of activities. The groups also conduct exchange visits and maintain a map of health service delivery points along the border areas to facilitate access to services. Health providers in the area take advantage of bilingual malaria control materials developed by the CAP-Malaria Project. Preliminary observations suggest that the Twin-cities approach improves awareness of malaria among mobile and migrant populations, facilitates their access to malaria services, and promotes a supportive environment for quality services. It also holds the potential for improved coordination at the local level, coordination that is critical should the spread of ARM be contained.

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ENGAGING THE PRIVATE SECTOR IN MALARIA SURVEILLANCE: A REVIEW OF STRATEGIES AND RECOMMENDATIONS FOR ELIMINATION SETTINGS

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In malaria elimination settings, all malaria cases must be identified, documented, and investigated. To facilitate complete and timely reporting of all malaria cases and effective case management, engagement with private providers is essential, particularly in settings where the private sector is a major source of healthcare. Research on the role and performance of the private sector in malaria case management and reporting is limited. Moreover, effective strategies for private sector engagement in malaria diagnosis, treatment, and reporting in elimination settings remain unclear. A purposive sample of 21 key informant experts in malaria elimination, surveillance, and private sector engagement were interviewed. An extensive review of grey and published literature on malaria surveillance and private sector engagement in malaria testing, treatment, and reporting was also conducted. Additional literature research was conducted for six country case studies on eliminating (Swaziland and Vietnam) and neighboring, non-eliminating settings (Cambodia, Mozambique, Myanmar, and Zambia). The private sector is a diverse and often unaffiliated group of providers that can be categorized based on their profit or business model (for-profit vs. nonprofit) and their regulation status within a country (formal vs. informal). Because the private sector varies from country to country, conducting a baseline assessment of the private sector is critical to understanding its composition, size, geographical distribution and quality of services provided. Facilitating reporting, referral, and training linkages between the public and private sectors and making malaria a notifiable disease are effective strategies to improve private sector involvement in malaria surveillance. The private sector can also be organized and better engaged through social franchising, effective regulation, professional organizations, and government outreach. This review highlights the importance of engaging private sector stakeholders early and often in the development of malaria elimination strategies.

1542

PREDICTIVE MALARIA RISK MODELING USING AGGREGATE CASE DATA FOR IMPROVED INTERVENTION TARGETING IN HONDURAS

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Countries in Central America recently committed to eliminate malaria by 2020. Honduras, a low endemic country in the region, plans to reach elimination by strengthening surveillance, improving case management, and targeting interventions. To help target elimination efforts in Honduras, we used national surveillance data reported in aggregate to predict spatial patterns of malaria transmission. The numbers of confirmed malaria cases, reported by 294 of 298 municipalities between 2003 and 2013, were combined with social, demographic, and ecological variables in a Bayesian hierarchical model to predict incidence by municipality and year. Incidence was predicted because of the potential for underreporting. Predicted incidence in 2013 was compared with reported incidence, used to estimate the population at risk, and used to evaluate the spatial distribution of malaria interventions. Highest risk of malaria was concentrated in the Northeast along the border with Nicaragua. In 2013, predicted incidence was highest in two of the 294 municipalities, Wampusirpi (32 cases/1000 persons) and Puerto Lempira (18 cases/1000 persons), in which predicted incidence was higher and lower than reported incidence, respectively. These two municipalities were expected to account for a third of all malaria cases and 0.5% of the total population in Honduras. Higher incidence was associated with lower elevation, greater distance to roads and rivers, and distribution of long-lasting insecticide treated nets (LLINs). In 2013, LLINs were targeted to high-risk municipalities in the Northeast, while indoor residual spraying was targeted to low and medium risk municipalities in other regions of the country. Focusing interventions, improving case management, and implementing active surveillance in municipalities at highest risk, may help reduce the burden of malaria in Honduras. Predictions based on aggregate case data can be used to direct malaria resources. However, these predictions are still coarse in resolution. Georeferenced case reports from a strong passive surveillance system could be used to target malaria elimination activities even further.

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PRELIMINARY RESULTS FROM THE BIOMARKERS FOR MALARIA ELIMINATION (BIOME) STUDY

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The Bill & Melinda Gates Foundation Accelerate to Zero strategy aims to eradicate malaria swiftly to minimize the negative effects and setbacks caused by malaria resurgence and the spread of drug resistance. Acceleration will require identifying individuals who harbor and transmit low density infections. The proportion of low density infections varies inversely with transmission intensity; when a high transmission region has a rapid decrease in prevalence, it can become more difficult to detect and treat infected individuals. Active infection detection (ID) tactics such as mass test and treat, focal test and treat, and reactive ID following an index case aim to find and treat low density infections. However, existing tools used for detecting clinical malaria lack the sensitivity to detect low density infections. Other tools lack the ease of use, affordability, rapid time to results, and portability required for large scale field use. To improve the efficiency of active ID tactics, new, low cost, portable tools with improved limits of detection (LOD) are being developed and are called malaria infection detection tests (IDTs). Very limited data exist regarding the concentration of target biomarkers and their kinetics over the course of a ***Plasmodium falciparum*** (Pf) infection. Thus, informed IDT product development requires a greater understanding of the fraction of PCR positives we can detect with a new IDT at 10x improved LOD, and of the relevance of the undetected population to transmission at this LOD. The BIOME study is generating clinical data to better characterize the extent and relevance of the human Pf reservoir stratified by individual biomarker concentrations. This study builds evidence for validation of key target product profile performance criteria for an HRP2-based IDT. The focus of the study is to generate data to select a LOD for the IDT sufficient to interrupt transmission. The study is a comparative evaluation of the concentration of DNA, HRP2, total NA, and asexual stage parasites in the peripheral blood of individuals with low density infections. Here, we present the preliminary results from our Thailand and Uganda cohorts.

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ASSESSING READINESS TO ACCELERATE TOWARDS MALARIA ELIMINATION: A COMPARATIVE ANALYSIS OF THE DECISION-MAKING ENVIRONMENT IN KENYA, ETHIOPIA, SENEGAL AND ZAMBIA

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Optimizing national malaria control activities and accelerating towards malaria elimination will require a unified critical pathway in which robust evidence generation and national policy guidance are complemented by appropriate financing, planning and operations, and governance. The opinions of national decision makers and implementers in four sub-Saharan African countries with varying malaria epidemiology, health and regulatory systems, and national malaria targets were collected and analyzed in order to assess opportunities and barriers to accelerating progress along this critical pathway. Attitudes towards the feasibility and desirability of national malaria elimination targets were also assessed. In each country, Ethiopia, Kenya, Senegal and Zambia, interviewers conducted on average 35 semi-structured interviews with stakeholders including policymakers, regulators, donors, procurement officials, academic researchers, NGO representatives, health care workers, and community advocates. Stakeholder responses were qualitatively coded and ranked using quantitative indicators including formal power, informal influence, knowledge of national malaria targets and support for malaria elimination as a national target. Thematic content analysis revealed new information about what stakeholders in each country perceive to be the strengths and challenges of their national malaria control programs and what adjustments are needed along the critical pathway to accelerate towards malaria elimination. The need to address population mobility, increase private sector engagement and strengthen and expand human resources for case management emerged as major themes. Attitudes towards the feasibility and desirability of national malaria elimination targets, and levels of knowledge and approval of new tools and approaches for elimination, varied by country and by organizational and individual perspective. In addition, interview responses yielded numerous proposals for improving malaria implementation efforts that may merit additional consideration by policymakers.

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SO YOU SAY YOU WANT ELIMINATION? USING COMMUNICATION AND ADVOCACY TO ADVANCE MALARIA ELIMINATION IN THE AMERICAS

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A qualitative assessment of the communication component of National Malaria Control Programs (NMCPs) in 11 countries in Latin America and the Caribbean was conducted from 2013-2014. The assessment surveyed the overall programmatic objectives, existing resources for communication, malaria stakeholders, target audiences, ongoing communication activities, and gaps. Among the main findings of the assessment showed challenges in providing information to decision-makers to support and sustain efforts, expanding and strengthening partnerships beyond the health sector, and reaching special populations that have a higher burden of disease including indigenous communities and migrant workers. Overall, communication activities were found to be focused at the community level with less attention paid to decentralized health system professionals, and very few if any activities directed at the policy-level. Two examples of the role of effective communication at the policy-level include Suriname, which made significant strides against malaria from 1995-2015 and is now on the path to elimination with help from a National Malaria Board that brings together technical advisors, civil society actors, and other government ministries to help guide the policy making process. In Brazil, new legislation was passed in 2014 to require companies completing infrastructure projects in the Amazon region to engage in a series of activities, including social mobilization, to assess the impact and mitigate the risk of greater malaria transmission in the surrounding area. As more countries in the Americas transition towards elimination, malaria communication will have a sizable role to create a shared understanding among key decision-makers and malaria partners of what it means for countries to be working towards elimination and beyond, in terms of funding levels, technical interventions, multi-sectoral engagement, and sustained surveillance and reporting requirements. Channels and mechanisms that have worked in some countries should be explored for use in others, especially at the policy-level to ensure long-term commitment.

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MASS TESTING AND TREATMENT FOR MALARIA IN MODERATE TRANSMISSION AREAS IN AMHARA REGION, ETHIOPIA

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In moderate malaria transmission areas, strategies to clear parasites from populations are a means to reduce infection and transmission. A malaria mass testing and treatment (MTAT) intervention was implemented in six intervention villages in Amhara Region, Ethiopia, at the beginning of the 2014 malaria transmission season. Intervention villages were purposively selected and matched with control villages based on the incidence of passively detected ***Plasmodium falciparum*** (***Pf***) and mixed malaria during the 2013 malaria transmission season. All households in the intervention villages were targeted. Participants received a rapid diagnostic test (RDT) and RDT-positive individuals received artemether-lumefantrine (***Pf***, mixed) or chloroquine (***Plasmodium vivax*** [***Pv***]). Of 9,130 households in the intervention villages, 7,974 households (87.3%) were visited by 93 field teams over a period of three weeks. The average household had 4.4 individuals and the average number of households visited per day per team ranged from 3.7 to 8.5. Of the 35,389 individuals living in the households, 30,712 (86.8%) received an RDT. Of these, 47% were <20 years of age, 67% slept under a mosquito net last night, 52% resided in a household that received indoor residual spraying (IRS) in the last 12 months, and 1% spent ≥1 night away from home in the last month. Among tested individuals, 421 (1.4%) were RDT-positive. The prevalence of ***Pf*** or mixed RDT-positivity was 1.0% (0.7% ***Pf***, 0.3% mixed), ranging from 0.1% to 4.6% by village; 58% of ***Pf*** or mixed infections had no fever or history of fever in the preceding 24 hours. Of individuals with a ***Pf*** or mixed RDT result, 61% were <20 years of age, 64% slept under a mosquito net last night, 44% resided in a household that had received IRS in the last 12 months, and 8% spent ≥1 night away from home in the last month. Spatial clustering of RDT-positives varied by village. The incidence of passively detected, RDT-confirmed malaria cases at the health post in each village will be compared before and after the intervention and between intervention and control villages to evaluate the impact of the MTAT intervention and implementation costs will be estimated.

1547

EVALUATING THE ANNUAL COSTS OF IMPLEMENTING CASE INVESTIGATION TO SUPPORT MALARIA ELIMINATION IN SOUTHERN PROVINCE, ZAMBIA: A MICRO-COSTING ANALYSIS

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The Zambian Ministry of Health and partners are implementing a package of interventions and surveillance systems in Southern Province to support the national malaria control and elimination agenda. Case investigation (CI) is part of this package. With CI, if a malaria case is detected at a health facility or in the community, a community health worker (CHW) then visits the household of the malaria case and other nearby households, tests all individuals using a rapid diagnostic test (RDT), and treats all RDT-positives with artemether-lumefantrine. We estimated the cost of implementing CI in 173 health facility catchment areas (HFCAs) in ten districts in Southern Province during the 2014 calendar year. Primary data on unit costs and quantities of various resources used during implementation (e.g. drugs, RDTs, other supplies, labor) and key programmatic outputs (number tested, number testing positive, number treated) were obtained from programmatic records and the health management information system. At the HFCA level, average annual costs were estimated at US$2,142. Main cost categories include: labor at the HFCA (33%), mobile phone talk time for CHWs (15%), equipment (15%), RDTs (13%), artemether-lumefantrine (5%), and other supplies (19%). On average, 6.7 CHWs conducted CI activities in each HFCA (average population 7,432). On average, 127 households were visited per HFCA in 2014, 560 individuals were tested, and 86 (15%) were RDT-positive and treated. The average cost per household visited was $46, per individual tested was $11, and per individual testing positive and treated was $232. The estimates reported here are preliminary and do not yet include additional costs associated with training CHWs on CI implementation and supervision. Costs of surveillance and interventions implemented to support malaria control and elimination are useful for budgeting, identifying opportunities to improve program efficiency, and informing additional analyses on the cost-effectiveness and budget impact of different approaches to malaria elimination.

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DESIGNING A SUFFICIENT SURVEILLANCE SYSTEM AGAINST RE-ESTABLISHMENT OF MALARIA IN A SPATIALLY CONNECTED MODEL

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Malaria transmission is spatially heterogeneous, and movement of individuals connects locations over local, regional, and global scales. Elimination planning must therefore account for this interconnectivity and its ability to reintroduce parasites to previously-cleared areas or to stall progress at low prevalence. One important component of elimination efforts is surveillance systems in areas of low-prevalence and sub-regions which have at least temporarily achieved elimination. Robust and timely surveillance may allow sufficiently vigorous and rapid response to the reintroduction of parasites to prevent re-establishment of malaria in cleared areas. Options for this surveillance may include clinic-based surveillance, active and passive case detection, mass screening, parasite genetics, and immune serology. We implement a spatial simulation model for malaria transmission in the EMOD framework across varying spatial scales and in varied transmission settings, with interconnectivity through human movement and spatially heterogeneous baseline transmission. This spatial simulation is then used to demonstrate the operational impact of surveillance systems once a sub-region has at least temporarily achieved elimination. Areas with different baseline transmission rates, potential for transmission following re-introduction, population densities, local mosquito populations and feeding behaviors, and primary health care coverage require different levels of investment in surveillance systems to ensure robust maintenance of elimination.

1549

THE IMPACT OF CLIMATE VARIABILITY ON MALARIA INCIDENCE RATES IN LORETO, PERU

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Despite a decade of reduction (2001-2010), data shows that during 2011-2014, malaria has increased about 5 folds in Loreto. Past studies indicate that climate variables can play a crucial role in malaria transmission. The purpose of this work is to understand why certain populated centers report a higher parasite incidence rate compared to other ones, and which specific climate variable is the most associated with a higher malaria incidence. Annual parasite incidence (API) and weekly NASA climate data of the 2000-2014 period from the 315 populated centers that serve as surveillance reporting units within Peru were analyzed using a generalized linear model with a Poisson distribution and a link log. The analysis focused on comparing the top 10 percentile in terms of API from the whole period as compared to the rest. During the years 2000-2014 Loreto reported 539,315 malaria cases, which were mostly due to ***Plasmodium vivax*** (81%). The average API at the district-level was 51 cases per 1,000 inhabitants (95% Confidence Interval [95% CI], 41-61). The reporting units were located on average at -4.5 degrees of latitude from the Equator (range, -8.4– -0.14). The average microclimate was significantly different across reporting units ($p<0.01$) regarding surface temperature (range, 294.9– 300.8 K), 2 meter above ground temperature (294.9- 300.8 K), specific humidity (range, 0.012– 0.019 kg vapor * kg^-1 air), and surface pressure (range, 91965-100065 Pa). The API rates are the log odds of ranking among the top 10th percentile malaria endemic populated centers in Loreto during the years 2000-2014. The API increased with latitude (OR, 1.11; 95% CI 1.10-1.12) and decreased with surface pressure (OR, 0.99; 95% CI 0.99-0.99), 2 meter above ground humidity (OR, 2.59*10^-10; 95% CI, 2.51*10^-13-2.66*10^-7), surface temperature (OR, 0.98; 95% CI, 0.97-0.99), and soil moisture (OR, 0.77; 95% interval, 0.59-0.99). The observed and regression-estimated API were poorly correlated (Pseudo R^2: 5%, $p<0.001$). Further studies will be conducted on weekly malaria incidence rates at each populated center to understand the potential impact of microclimate variability.

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ACTIVE SURVEILLANCE THROUGH PEER REFERRAL TO IDENTIFY POPULATIONS AT HIGHER RISK FOR MALARIA IN ZAMBEZI REGION, NAMIBIA

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As malaria transmission declines, infection risk is increasingly clustered in individuals or groups of individuals with specific occupations or behaviors. Often these individuals will remain uncaptured through passive surveillance due to poor health care access or asymptomatic infections. With the goal to eliminate malaria by 2020, Namibia faces the challenge of identifying these higher risk populations in order to better target interventions. Motivated by the novel patient-initiated peer referral approach to identify undiagnosed HIV cases, we designed a strategy to screen and interview individuals connected through their social networks to malaria cases

from February to May of 2015. RDT positive index cases, which served as "seeds" to begin the peer referral recruitment chains, were identified at 6 randomly selected health facilities within Zambezi Region in northern Namibia; recruitment was embedded within a concurrent case-control study at these health facilities to identify and refine risk factors. All eligible cases (15 years and older) were randomly assigned to distribute referral coupons within either their social network or their household. Each referral in the social network group had to fit the risk criteria of regularly sleeping or working outdoors during evening and morning mosquito biting hours. Individuals receiving coupons from either group were asked to visit the health facility to participate in an interview- assisted survey and provide a blood sample for malaria testing via RDT and DBS. Participation was tracked to assess the linkage to his or her recruiter. Recruitment and return were incentivized with LLINs, travel reimbursement, or mobile phone airtime. In this presentation, we discuss the feasibility of this study design in a rural setting characterized by transportation barriers as well as the challenges of peer centered recruitment during a season with low case numbers. We also evaluate the ability of active surveillance through peer referral to identify individuals with specific high risk behaviors and current infections diagnosed by PCR, through comparison to a concurrent household survey.

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MODELING THE OPTIMAL INTERVENTION MIX FOR MALARIA ELIMINATION IN DIFFERENT SPATIALLY CONNECTED TOPOLOGIES

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Elimination of malaria is not complicated, in principle, if we have unlimited resources at our disposal. In practice, logistical, political, and financial constraints couple with the complex etiology of the disease to cloud the path forward. While there are many means at our disposal to address the issue, it is not clear how to combine them for optimal efficacy, especially when we account for the fact that local variation in the characteristics of endemic settings may significantly impact the best choice of intervention mix. To address this question, we used the EMOD malaria model as the basis for a prioritization tool that recommends intervention mixes as a function of a region's current attributes. The results are based on simulations of the impact of combining interventions, such as vaccines, insecticide treated nets, drug campaigns, and case management, analyzed through a context of feasibility: grounded by data and experience, what are achievable levels of coverage, what does administration cost, and are there implementation synergies that could favor certain combinations? Spatial heterogeneity is another important factor in planning for elimination, particularly in environments that are susceptible to reimportation, and was accounted for by running the simulations across spatially connected regions, each of which may be distinct in terms of transmission intensity and access to care. While the primary results were calculated using simulations of existing intervention standards, e.g., RTS,S in the case of vaccines, hypothetical scenarios provide insight into what might be achievable if new products become available and suggest how elimination strategies might be adapted in response.

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SCALE-UP OF CASE INVESTIGATION AND REACTIVE CASE DETECTION EVALUATIONS IN THAILAND: RESULTS FROM FIVE PROVINCES

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Case investigation and reactive case detection (RACD) activities are implemented in many low transmission settings and recommended by the World Health Organization as a key strategy for malaria elimination. Determining the origin of infection, known as case investigation, and screening household members and neighbors of passively detected malaria cases for infection, known as RACD, requires substantial programmatic and human resources. By evaluating the program performance of case investigation and RACD activities, gaps in reporting completeness and timeliness can be identified to determine if improvements are required. Thailand is implementing case investigation and RACD and aims to eliminate malaria by 2024. Based on the findings from a pilot evaluation conducted, the Thailand Bureau of Vector-Borne Diseases (BVBD) scaled-up the use of a standardized monitoring and evaluation (M&E) tool to identify best practices and gaps in case investigation and RACD to inform active screening efforts in Thailand. Data will be presented on the five evaluation provinces covering a range of transmission endemicities. Routine surveillance data on case investigation and RACD reporting completeness, timeliness and coverage from 2013 and 2014 are analyzed from the national malaria information system as well as from district-level malaria clinics, malaria posts and border malaria posts. Questionnaires were administered to surveillance program personnel regarding RACD operations and procedures to understand their knowledge, practices and the challenges to conduct RACD. Costing data for personnel, commodities, and other services related to case investigation and RACD expenditures were collected and analyzed to determine the primary cost drivers and operating costs for RACD. Preliminary findings from the evaluation provinces have informed the BVBD on where program efficiencies can be achieved, including the need for additional RACD-specific trainings. M&E of case investigation and RACD will assist the BVBD in improving RACD program effectiveness and help Thailand achieve its goal of malaria elimination.

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DEVELOPMENT OF A PREGNANCY-SPECIFIC SEROLOGY TEST: A PATH TOWARDS A NEW TOOL TO MEASURE MALARIA TRANSMISSION IN THE CONTEXT OF ELIMINATION

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New metrics for malaria transmission are needed for malaria elimination. ***P. falciparum*** infection during pregnancy is associated with a strong antibody response against VAR2CSA (pregnancy-specific antigen expressed by the parasite on the erythrocyte membrane that binds to placental Chondroitin Sulphate A), suggesting that detection of these antibodies in pregnant women at antenatal clinics could be used for surveillance of malaria. To select antigens with the highest potential for a VAR2CSA-based serological test, we measured IgGs in Mozambican pregnant women, as

well as in non-exposed individuals, using a quantitative suspension array technology that included 46 peptides from both conserved and semi-conserved regions of VAR2CSA, 3 recombinant proteins (DBL3x, DBL5ε, DBL6ε) and non-pregnancy specific antigens. Dynamics of antibody responses during pregnancy were also determined to assess acquisition and longevity of antibody responses. We first excluded those antigens that were a) poorly recognized by plasmas from pregnant women with high antibody levels against a VAR2CSA-expressing parasite line (CS2) (n=106); b) recognized by Mozambican men (n=102) and Spanish individuals (n=100) and c) not associated with antibody acquisition in women infected with ***P. falciparum*** during pregnancy (n=252, longitudinal cohort with 3 time-points per women). Among the 25 antigens selected, antibodies against 17 peptides, DBL3X and DBL5ε mirrored falls and rises in malaria prevalence in Manhiça during 2003-2012 (n=654). Finally, 9 out of the 17 peptides, DBL3X and DBL5ε were selected based on high boosting of antibody by malaria infection, low time to double the levels when infection occur (rapid generation of antibodies) and short half-life (detectable during one pregnancy). We are currently exploring the value of VAR2CSA serology to detect recent changes in ***P. falciparum*** exposure associated with the use of intermittent preventive treatment with different antimalarials. This pregnancy-specific serological test could be placed into action to provide information for malaria surveillance in elimination campaigns.

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OVERVIEW OF MALARIA SURVEILLANCE SYSTEMS IN ELIMINATION SETTINGS: LESSONS FROM THE PAST, AND ASSESSING CRITICAL PROGRAM NEEDS

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Achieving malaria elimination requires a well-designed surveillance system which can guide targeted efforts and selection of impactful strategies. However, challenges remain to design systems to fit countries' needs and constraints. A systematic literature review of surveillance systems from countries that have eliminated malaria or have a national elimination policy in place was conducted to build a stronger evidence-base on what technical elements are essential to efficiently support elimination. The review evaluated the system structure and surveillance activities in each country while focusing on what data were collected, what output was generated to direct programmatic response, and what technical components are required. We reviewed 10 countries worldwide that eliminated malaria between 1965 and 2014, and 9 countries that are in the process of eliminating. According to the review, every country that successfully eliminated malaria described a system that had strong passive case detection as its backbone. The passive systems included individual case reporting and detailed investigations, parasitological confirmation of cases, ***foci*** identification, staff at district or village level specifically assigned to assist in surveillance activities, and entomological data collection. 7 out of the 10 countries that have eliminated also described conducting reactive case detection with responses varying depending on country context, and 8 out of 10 had a response mechanism incorporated into their surveillance systems that triggered programs to target interventions based on current data (i.e. implementation of indoor residual spraying dependent on ***foci*** investigation). In comparison, not all of the 9 eliminating countries had individualized reporting or thorough case investigations directing their interventions. However, those countries have begun to adopt technologies such as web-based or mobile tools that might enable a quicker response. As countries work to strengthen surveillance for elimination, lessons learned from historical experience should be used to highlight and prioritize components critical for success.

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THE PROBABILITY OF ACUTE ILLNESS FOLLOWING *PLASMODIUM VIVAX* PRIMARY INFECTION AND RELAPSE IN A COHORT OF CHILDREN FROM PAPUA NEW GUINEA

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The probabilities of clinical illness following ***Plasmodium vivax*** primary infection and relapse are unclear in people living in endemic areas. A major difficulty lies in the inability to distinguish primary infections and relapses. We previously analysed genotyping data to show that the seasonal pattern of the incidence of primary infections and relapses differed in a cohort of children in Ilaita, Papua New Guinea. The differential seasonality can be used to gain leverage to estimate the probability of clinical illness following primary infection and relapse in the same cohort. The children, aged one to three years at enrolment, were followed up over 16 months. Illness was detected during active case detection every two weeks and carers were encouraged to visit the study clinic if the child was ill at other times. ***P. vivax*** illness was defined as fever or reported fever in the last 48 hours with a parasite density of 500/µl or greater. We relate the number of observed ***P. vivax*** cases in each age-group and each two month time period to the expected numbers of primary infections and relapses, and use the expected cumulative number of lifetime genotypes seen as a proxy for clinical immunity. The expected numbers of primary infections and relapses are derived from a simulation model, parameterized by previous analyses of the same cohort and including the seasonal pattern of primary infections, differential biting by age by weighting by body surface area, the durations of blood-stage infections, the number and timing of relapses, and treatment. We assume relapses occurring when a blood-stage infection by the same genotype is present do not cause illness and are not counted. The estimated probability of acute illness declined with the cumulative number of genotypes seen. For the ages in this cohort, the probability following primary infection ranged from 0.3 to 0.05, following the first relapse, 0.07 to 0.0006, and for the second or later relapse, the probability was low. These results can inform estimates of the burden of ***P. vivax*** disease and provide building blocks for mathematical models for predicting the impact of interventions against ***P. vivax***.

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ABSENCE OF EFFECT OF HETEROZYGOUS HEMOGLOBIN S ON THE PREVALENCE OF PLACENTAL MALARIA AND LOW BIRTH WEIGHT

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Heterozygous hemoglobin S (HbAS), or sickle trait, protects children from life-threatening falciparum malaria. The mechanism of this protection is unclear, and evidence suggests that HbAS reduces expression of, ***Plasmodium falciparum*** erythrocyte membrane protein 1 (PfEMP1) and thereby attenuates adherence of infected erythrocytes (IEs) to extracellular ligands. Such binding of IEs to ligands is central to the pathogenesis of placental malaria, wherein the expression of the PfEMP1 variant VAR2CSA mediates IE binding to chondroitin sulfate in the placenta; thus, placental malaria serves as an ***in vivo*** model of PfEMP1-mediated sequestration of IEs. We hypothesized that HbAS would be associated with reduced risks of placental malaria and its sequelae, including low birth weight (LBW). We tested this hypothesis in a cross-sectional study of 850 delivering

women in Southern Malawi. Demographic, clinical, and antenatal records were collected from enrolled women along with infant birth weight. Placental histology was scored for markers of malaria, and parasites were detected in peripheral and placental blood by real-time PCR. The overall prevalence of HbAS was 3.7%. The prevalence of ***P. falciparum*** was 8.5% in peripheral blood and 12.7% in placenta by PCR and 24.4% by any histological evidence. The prevalence of LBW (<2500g) was 11.2%. There were no significant associations between HbAS and placental ***P. falciparum*** (OR: 1.29, 95% CI: 0.48, 3.41) or any histological evidence of placental malaria (OR: 0.98, 95% CI: 0.40, 2.34). ***P. falciparum*** densities, as estimated by PCR, were also similar between groups. Furthermore, mean (standard deviation) birth weights of infants born to HbAS (2947g [563g]) and HbAA (2991g [465g]) mothers were similar, as was prevalence of LBW (OR: 0.82, 95% CI: 0.24, 2.73). Across a range of parasitologic, clinical, and histologic outcomes, HbAS did not confer protection from placental malaria or its adverse effects. Although HbAS reduces cytoadherence of IEs to endothelium, the absence of protective effects in this study suggest that HbAS does not attenuate the ability of IEs to sequester in the placenta.

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DISTRIBUTION AND PREVALENCE OF MALARIA IN CHILDREN UNDER THE AGE OF FIVE IN THE DEMOCRATIC REPUBLIC OF THE CONGO, 2013-2014

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The Democratic Republic of the Congo has one of the highest malaria burdens in the world. Over 8,000 children under the age of 5 in 540 population-representative clusters were tested for malaria in the 2013-4 Demographic and Health Survey. Infection was ascertained by microscopy, rapid diagnostic tests (RDT), and PCR for the ***Plasmodium falciparum*** lactate dehydrogenase gene. Weighting for populations, 34.1% of children were PCR-positive for ***P. falciparum*** malaria. In contrast, 22.7% and 30.9% were positive by microscopy and RDT, respectively. The prevalence of microscopic gametocytemia was 1.4%. Malaria was common in all 26 national health divisions. PCR prevalence, like microscopy, increased with age and was higher in those living in rural areas compared to urban areas. PCR prevalences ranged from 10 to 68%. We estimate that prevalence of mono-infections with P. malaria or P. ovale was approximately 1%. Of the RDT-positive samples, 18% were both PCR- and microscopy-negative, probably due to persistence of HRP-2 in the circulation. This high burden of malaria was found despite the fact that insecticide-treated net (ITN) ownership increased from 7% of households in 2007 to 70% in 2013.

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ESTIMATING THE MALARIA ATTACK RATE IN TANZANIAN MILITARY CAMP: RISK FACTORS ASSOCIATED WITH MALARIA EPIDEMIOLOGY IN MILITARY CAMPS IN TANZANIA

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In Tanzania, malaria ranks number one cause of morbidity and mortality, accounts for over 32% of the National disease burden. There is high heterogeneity of malaria transmission. Imported cases by travellers to and from different areas in Tanzania poses a great challenge in malaria epidemiology. The aim of this study was to identify risk factors associated with malaria attack rate among individuals when entering a training camp in a highly endemic area. Tanzania People's Defence Forces (TPDF) Recruits eligible to study in Mgambo National Service camp -Tanga, were randomly selected by multistage sampling; consented and followed for six months. Fortnightly malaria smear was collected. Blood samples for serological tests were collected. Microscopy was a gold standard method for malaria diagnosis. Data was subjected to univariate and multivariate analysis, logistic regression model was used to identify the risk factors. Among 549 recruits who were involved in this study, 31.7% (174) were malaria positive, of which 80.5% (442) were male recruits. Female recruits had 54% significantly reduced chances of being malaria positive [OR: 0.46; 95% CI: 0.28 - 0.77; P=0.003]. Among positive cases, those who didn't sleep under treated net previous night were found to have significantly increased odds of being malaria positive [OR: 7.71; 95% CI: 1.01-58.61; P=0.048]. Travelling outside the camps in the past one month had increased odds of being malaria positive [OR: 1.25; 95% CI: 0.87-1.79; P=0.232]. There was significant difference between malaria positivity and place of travel [X^2=40.1; P=0.015]. This study revealed failure to use bed nets and travel are major drivers of malaria. Identification of gaps in net use, knowledge, relevant types of human movement and development of strategies addressing travel is highly recommended. Mgambo NS being a high malarial endemic area is ideal site for malaria drug prophylaxis or vaccine studies, where TPDF recruits from various transmission intensity areas train under similar exposure environment.

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A COMPARISON OF HOUSEHOLD SURVEY SCREENING FOR MALARIA AMONG OLDER CHILDREN (5-14YRS) AND ADULTS VERSUS YOUNG CHILDREN TO DETERMINE FINE-SCALE HETEROGENEITY IN AN AREA OF HIGH TRANSMISSION

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There are increasing calls for more targeted malaria control interventions that take into account transmission heterogeneity. Capturing such heterogeneity and identifying hotspots accurately at the sub-district and district level however remains a challenge. Current parasitaemia indicators

for household surveys are based on children aged 6 to 59 months. While there is an increasing interest to expand parasitaemia screening in household surveys to older children or adults, its added value remains unclear. We investigated whether screening older age groups in addition to young children within households could improve precision in mapping heterogeneity and malaria hotspots. We conducted a monthly continuous ('rolling') population based household-level malaria indicator survey (rMIS) in an area of ~400km2 in Chikwawa district, southern Malawi. Malaria parasitaemia screening was conducted in three age groups: 6-59 months, 5-14 years, and ≥15 years, and based on a pLDH/HRP2 rapid diagnostic test (First Response® Malaria Ag. pLDH/HRP2 Combo Card Test, Premier Medical Corporation Ltd., India). Further collected household information included socioeconomic status, long-lasting insecticide-treated net and indoor residual spraying coverage. A total of 1,197 children aged 6-59 months, 1,506 aged 5-14 years, and 1,1881 aged ≥15 years were sampled. Parasite prevalence was 16.7% (95%CI 14.5, 19.2) in the youngest age group, 11.8% (95%CI 10.1, 13.7) in the 5 to 14 years age group and 5.4% (95%CI 4.5, 6.3) in the ≥15 year group. This data was used to create geospatial maps of parasite prevalence for both individual age groups and combined groups, using generalized linear geostatistical models. Comparative analyses of these maps suggest that the older age groups do not increase the accuracy or precision in mapping malaria hotspots. These findings thus suggest that in settings of high but varying malaria transmission the screening of older children and adults in addition to children under the age of 5 in household surveys will not contribute to a more accurate picture of heterogeneity and identification of malaria hotspots.

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CLINICAL SPECTRUM OF DISEASE IN ADULTS AND CHILDREN WITH *PLASMODIUM KNOWLESI* MALARIA IN A DISTRICT SETTING IN SABAH, MALAYSIA

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Plasmodium knowlesi is now the most common cause of malaria in Malaysia, but prospective studies describing the clinical spectrum have only detailed adult disease. In our prospective study (2012-ongoing) at 3 district hospitals in Sabah, Malaysia, previously untreated, non-pregnant patients of any age hospitalised with PCR-confirmed malaria includes: 368 ***P. knowlesi*** (32 [8.7%] children ≤ 12 yrs), 152 ***P. vivax*** (63 [41.5%] children), 56 ***P. falciparum*** (11 [19.6%] children), 14 ***P. malariae***, and 1 mixed ***Pk/Pf***. Preliminary data include a lower baseline parasite count for ***P. knowlesi*** malaria patients (median 2274/μL, IQR 537-9252) than ***P. falciparum*** (9762/μL, IQR 2030-24010, ***P***<0.001), and ***P. vivax*** (4319/μL, IQR 1588-9608, ***P***=0.011). Parasite counts were higher in adults with ***P. knowlesi*** compared to children (2427 vs. 890/μL, ***P***=0.013). In ***P. knowlesi*** (WHO criteria) anaemia was present in 37% of adults vs. 86% in children (***P***<0.001), however was less prevalent than in ***P. vivax*** overall (60%, ***P***<0.001). Acute kidney injury (AKIN criteria and/or creatinine >132mmol/L) was found in 42/285 (14.7%) with ***P. knowlesi*** including 42/263 (16%) adults and 14/27 (52%) of those with severe disease, compared to ***P. vivax*** overall (12/140, 8.6%, ***P***=0.073). In those with ***P. knowlesi*** 28/366 (7.7%) had severe malaria (modified WHO 2010 criteria): 28/334 (8.4%) in adults but none in children. In comparison, severe disease was seen in 5/152 (3.2%, ***P***=0.062) with ***P. vivax*** and 4/56 (7.1%, ***P***=0.839) with ***P. falciparum***. 207/359 ***P. knowlesi*** patients were treated with artemisinin combination therapy, including 39 with prior intravenous artesunate, and the remainder with oral chloroquine. There were no treatment failures in knowlesi malaria patients seen at 28 days. There was one ***P. knowlesi*** malaria death, with delayed parenteral artesunate due to misreported hyperparasitaemia. Overall ***P. knowlesi*** malaria predominantly affected adults, with AKI (16%) and severe malaria (8.4%) commonly developing in this age-group. Although anaemia was more common in children than adults with ***P. knowlesi***, parasitemia was lower and severe anaemia, AKI or other severe disease was not seen.

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PROGRESS TOWARDS ACHIEVING MILLENNIUM DEVELOPMENT GOAL AND ROLL BACK MALARIA TARGETS IN GHANA

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Ghana has been implementing the Roll Back Malaria Strategy since 2003, and is currently operating with the 2008- 2015 strategic plan. The overall goal of the 2008 - 2015 malaria strategic plan is to reduce both malaria deaths and malaria burden by 75% by 2015 using 2000 as baseline. In line with the MDG Goal 6, the programme is aimed at halting malaria deaths by 2015. This study aimed to assess progress towards attainment of 2015 MDG/RBM malaria targets in Ghana. This study employed desk review of annual reports, policy and operational guidelines, reports of health facility and community based surveys including Multiple Indicator Survey (MICS), Demographic and Health Surveys (DHS) and Malaria Indicator Surveys between 2000 -2014. All cause under-five years mortality rate was reduced from 111/1000 in 2003 to 60/1000 in 2014. Malaria case fatality rate in under-five children reduced from 14.4% in 2001 to 0.51% in 2014. Ghana also achieved up to 50% reduction in parasite prevalence between 2002 and 2014 from a national average of over 50% to 26.7%. There was an increase in the use of ITNs among children under five and pregnant women, from 3.5% and 2.7% in 2003 to 58.8% and 56.3% in 2014 respectively. Proportion of pregnant women receiving at least 2 doses of IPT was 64.4% in 2011 against the target of 80% by 2015. Proportion of malaria cases treated with Artemisinin-based Combination Therapy (ACT) increase from 39% in 2011 to 82.5% in 2014. Ghana has made significant progress towards attainment of the MDGs goals of halting malaria deaths. There is however the need to sustain the gains and improve in areas such the coverage of IPTp and ITN use.

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ESTIMATING THE MOST RESOURCE-EFFICIENT MALARIA INTERVENTION PACKAGES AND SPATIAL SCALES TO ACHIEVE ELIMINATION ACROSS AFRICA

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Resource constraints mean that the elimination of malaria in much of Africa, if achievable, is likely to require available resources to be allocated carefully. Here we use a dynamical model of malaria transmission, capturing the heterogeneity in transmission of malaria which exists across sub-Saharan Africa, to identify the most efficient combination of interventions and spatial scale of implementation to achieve different elimination milestones. Our results demonstrate that, while bed-nets are a cost-effective step towards any control target, the choice of further interventions to deploy within a setting depends on the time scale over which a given target is to be achieved and whether disease reduction or elimination of infection is the priority. We found that while chemoprevention within risk groups such as children are the most cost-effective means by which to achieve rapid reductions in malaria burden, the changing age-distribution of disease as transmission falls and large asymptomatic reservoir means that sustaining low burden or other more ambitious elimination milestones requires interventions which target the general population such as indoor residual spraying or mass drug administration (MDA). At 90% population coverage, we estimate that packages including one, two or three annual rounds of

MDA would achieve pre-elimination levels of transmission (<0.001 case per person year) in 75%, 82% and 91% of the population at risk in mainland Africa respectively, with the latter achieving pre-elimination in 25 of the 42 countries with ongoing transmission. We then investigated how the resources needed to achieve this target depend upon whether interventions are deployed at the country or provincial (first administrative unit) level. We found that on average provincial level policies reduced the resources necessary to achieve pre-elimination by 40%, with countries with high levels of heterogeneity in transmission such as Tanzania, Senegal, and Angola benefiting most. In other countries such as Zambia, high within-province variation may suggest the need for even higher resolution local-level policies.

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SPATIAL AND TEMPORAL DYNAMICS OF MALARIA INCIDENCE FROM 2009-2013 IN BANDIAGARA, MALI

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Despite recent progress in reducing malaria morbidity and mortality in many places, empirical and theoretical evidence suggests that the current suite of interventions will not be sufficient to eliminate malaria from many areas in sub-Saharan Africa with historically high levels of malaria transmission. Careful description of the micro-epidemiology of malaria is essential for an accurate assessment of local transmission risk and hotspot localization in order to support malaria control programs, particularly in countries with limited resources. Our study aimed to measure malaria incidence and to investigate spatial and temporal dynamics of malaria in a single town in Mali from June 2009 to July 2013. Malaria incidence was measured in a cohort of 400 children through active and passive surveillance. Households were georeferenced using a handheld Global Position System (Garmin: Personal Navigator) with an accuracy goal of <15m and assigned to blocks of houses. A GIS map of Bandiagara was established from satellite photography and field surveys. Malaria cases were mapped using PHILCARTO (TM). SaTScan® software was used to look for intra-annual and inter-annual clusters of high or low risk. The incidence was stable from 2009-2013. The annual cumulative incidence varied from 1.3 to 2.0 clinical episodes per child from 2009-2013. The weekly clinical malaria episodes and rainfall were plotted. Three month phase shifts were detected between the beginning of seasonal rains and the peaks of clinical malaria episodes. During high malaria transmission periods, hotspots were stable and localized to the southwest part of the town, as well as in the northeast section near the Yamé river. The central and eastern parts of the town were relatively protected. No significant clustering was found during the low transmission period. Overall, there was a marked spatial heterogeneity of malaria cases with stable hotspots and stable incidence in Bandiagara from 2009 to 2013. This analysis of malaria microepidemiology may inform local malaria control and elimination strategies.

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COST-EFFECTIVE SCALE-UP OF CURRENT INTERVENTIONS AND RTS,S IN SUB-SAHARAN AFRICAN SETTINGS

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Despite continuing progress in the control of malaria, in many countries, especially in sub-Saharan Africa, the burden of disease remains high and the availability of interventions remains sub-optimal and subject to resource constraints. In light of this, the setting-specific cost-effectiveness of new tools to control malaria, such as the RTS,S vaccine, must be evaluated alongside other control options, including investing more resources in further scaling-up existing interventions already in place. We used an existing mathematical model of malaria to describe the effectiveness of LLINS, RTS,S and chemoprevention in children across the highly heterogeneous range of transmission settings that exist across Africa. Costs were estimated using a production function framework for a range of interventions, including LLINs and the RTS,S vaccine. The most efficient, step-wise approach to scaling up a combination of interventions was determined by comparing the incremental cost-effectiveness ratios (ICERs) associated with increasing the coverage of each intervention and choosing the most favourable option. This process was repeated until the coverage or usage of all interventions was maximised. Outcomes considered include the reduction over ten years of clinical incidence in infants (6 months to 5 years old) and clinical incidence in all age groups. LLINs are generally considered the most cost-effective malaria control method currently available. We found that, when including RTS,S with an assumed unit cost of production of $5 per dose as an option, providing LLINs to the majority of the population remains the most cost-effective control strategy across a wide range of transmission settings. However, under the realistic assumption that reaching individuals without a net becomes increasingly difficult as coverage increases, if there are sufficient remaining resources, strategies such as chemoprevention or RTS,S may be a more cost-effective alternative to achieving very high levels of LLIN coverage.

1565

COMPARING THE EPIDEMIOLOGY OF MALARIA INFECTION AND ILLNESS IN PNG CHILDREN BEFORE, DURING AND AFTER THE INTENSIFICATION OF CONTROL MEASURES

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In Papua New Guinea (PNG), children less than 5 years of age typically experience high levels of clinical illness with ***Plasmodium falciparum*** and ***P. vivax***. Children appear to acquire clinical immunity to ***P. vivax*** rapidly and clinical episodes are rare after 5 years of age, whilst clinical episodes of ***P. falciparum*** remain common. Perennial exposure as well as the high rates of relapses and clinical disease due to ***P. vivax*** in early childhood may contribute to the rapid acquisition of clinical immunity early in life. The incidence of clinical malaria with both species is comparable in children less than 5 years. However, the prevalence and incidence of ***P. falciparum*** infection and illness increases with age, with minimal signs of any acquisition of clinical immunity to ***P. falciparum*** in this age group. In the last decade, there has been a renewed focus on the implementation of an effective National Malaria Control Program and this is successfully reducing malaria transmission in the country. However, a detailed understanding of the impact on the burden of infection and clinical malaria, as well as the acquisition of clinical immunity in the most vulnerable age group, 1-5 year olds, is lacking. To assess this, a longitudinal cohort study of 1-5 year

olds was conducted in the same area of East Sepik Province as previous studies that were conducted prior to (Lin et al) and during (Betuela et al) the introduction of sustained control measures. Preliminary analysis shows a marked reduction in the prevalence of ***P. vivax*** infection at enrolment (by PCR) from 53.0% in 2006 to 14.0% in 2013 and ***P. falciparum*** infection from 49.6% to 14.0%. Similarly, prevalence of clinical malarial episodes has significantly declined from 20.5% to 4.8%. Detailed analysis of the incidence and risk of malaria in the 10 month follow-up period will be presented. In conclusion, preliminary data confirms a profound decline in the prevalence of malaria infection and episodes achieved through sustained control measures.

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EXPLORING THE RELATIONSHIP BETWEEN CLIMATIC FACTORS AND ITN USE IN 17 AFRICAN COUNTRIES

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While there is much anecdotal evidence of climatic factors such as rainfall and temperature affecting insecticide-treated net (ITN) use, there is little actual data demonstrating this association. Qualitative research has reported decreased ITN use during the dry season due to perceptions of being too hot, and increased use during the rains due to increases in perceived nuisance biting. This analysis uses data from national household surveys, as well as remotely-sensed climate data, to assess how factors such as ITN use is influenced in different ecological environments at different times of the year. The most recent national survey with available geographic location data was obtained for 17 African countries. Monthly rainfall estimates (mm) at a roughly 4 km resolution were acquired, and the mean rainfall estimate at each survey cluster location for the month in which the survey was conducted was merged with the national survey dataset. Logistic regression was run to assess whether there was a significant relationship between estimated rainfall quantile and ITN use in each of the study countries. Preliminary results suggest that in 10 of the 17 countries surveyed in this analysis, there is a significant association between estimated rainfall and ITN use. In some countries, higher quantiles of estimated rainfall increased the odds of net use significantly (Benin – OR: 1.45, p=0.001). However, in a few countries, higher quantiles were associated with decreased odds of using an ITN when controlling for access to an ITN in the household (Senegal – OR: 0.75, p=0.024). Further studies are necessary in order to understand what additional climatic factors, such as land surface temperature, nocturnal dew point, and relative humidity, play a role in ITN use, as well as the reasoning behind these associations and what they mean for in-country malaria prevention programs.

1567

THE EFFECTIVENESS OF A TARGETED INDOOR RESIDUAL SPRAY CAMPAIGN WITH PIRIMIPHOS-METHYL IN NCHELENGE DISTRICT, NORTHERN ZAMBIA

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The WHO has identified vector control as a key priority in reducing community malaria burden. In Nchelenge District, Luapula Province, Zambia, the main malaria vectors are An. gambiae s.s. and An. funestus, both of which are highly anthropophilic and endophilic, making indoor residual spraying (IRS) a potentially effective strategy. Nchelenge District has hyperendemic malaria, with greater than 50% parasite prevalence by rapid diagnostic test (RDT). Malaria vectors in Nchelenge District are susceptibile to the organophosphate insecticide pirimiphos-methyl, and this compound was used for the first time in Zambia in a large-scale IRS campaign in Nchelenge District conducted by the Ministry of Health, the President's Malaria Initiative (PMI), and NGO partners over 6 weeks in October-December 2014. Sub-district areas were targeted for spraying based on population density and health center case reports. This is the first use of a targeted IRS strategy in Zambia. Approximately 30 months of data from 425 households in active surveillance cohorts was used to establish baseline seasonal malaria prevalence in Nchelenge District. After the IRS campaign, six months of enhanced surveillance from 250 households will be used to evaluate the impact of this IRS strategy, both in targeted areas and district-wide. Parasite prevalence as measured by rapid tests and PCR in serial bimonthly cross-sectional data collections are compared pre- and post-intervention, and changes in time to reinfection in longitudinal cohorts and vector abundance using CDC light traps are quantified.

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SHIFTING EPIDEMIOLOGICAL PATTERN OF MALARIA IN NORTH CENTRAL AND SOUTHWESTERN NIGERIA

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Characterization of malariometric indices is a necessary precursor for planning malaria control activities. Resulting baseline values of these epidemiological parameters provide evidence basis for intervention purposes. A survey of four villages in Borgu, Niger State representing a Sudan savannah vegetation of North Central Nigeria and Ijede a semi urban town, in a forest vegetation belt of South West was conducted. Consenting participants were screened for malaria using mRDT and microscopy. Temperature, PCV, height and weight were measured. Of a total of 1648, 813 from Borgu and 835 from Ijede, malaria positivity by RDT was 19.4%, 95% CI = 17.42-21.53 (Ijede 9.6% and Borgu 32.5%) and 11.9 %, 95% CI = 10.33-13.67 by microscopy (Ijede 11.5% and Borgu 17.1%). Fever was significantly associated with malaria positivity [OR = 2.1417 (1.3554-3.3842), P=0.001] overall but not at individual sites [Ijede: OR=1.361 (0.4647 - 3.9865), P=0.573; Borgu: OR= 1.6869 (0.9812 - 2.90), P=0.057]. Malaria positivity rate was significantly lower in under 5 year than in 5-15 year (P = 0.043) in both study sites. Children under five years of age had significantly lower malaria positivity rate (16.3%) as well as a lower parasitemia level(382p/µl) when compared to 5-15 years age group with 41.3% and 490p/µl corresponding values (P<0.001). Overall anemia rate was 21.6%; 95% CI 19.46%-23.74%. Anemia rate and distribution was similar at both sites with children under the age of 5years 17.7% recording lower rates, compared to the rest of the population 22.3 %(P<0.001). This preliminary investigation revealed a lower malaria prevalence rate and parasitemia in under fives compared to children 5-10years. There should be a high index of suspicion for alternative causes of fever, other than malaria in children under the age of 5years in Nigeria.

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MULTIPLICITY OF INFECTION AND DISEASE SEVERITY IN *PLASMODIUM FALCIPARUM* AND *P. VIVAX* IN AREAS WITH SEASONAL TRANSMISSION OF COLOMBIA

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A common observation in malaria endemic areas is that there could be multiple lineages of the parasite concurrently infecting a patient; this is referred to as multiplicity of infection (MOI). MOI is the result of the co-transmission of parasite variants (co-infections) or the overlap of variants due to multiple infectious contacts (superinfections). Distinguishing between the two processes is laborious so MOI usually refers to a sample with multiple parasite genotypes. Previous studies have postulated a link between MOI and disease severity; association that has been tested in *Plasmodium falciparum* mostly in Africa with limited information on *P. vivax*. In this study, the association between MOI and disease severity is explored in seasonal malaria areas from Colombia where *P. vivax* and *P. falciparum* can be compared. Overall 422 *P. vivax* and 282 *P. falciparum* samples were collected between 2011 and 2013 from three distinct malaria endemic areas of Colombia. A total of 41 *P. vivax* and 27 *P. falciparum* cases were classified as clinically complicated cases. Samples were genotyped by using 8 microsatellite loci, any infection with two or more alleles at any locus was considered a multiple infection. *P. vivax* displayed more MOI (50.9%) than *P. falciparum* (21.6%). Uncomplicated cases were sampled in an interval of 5 to 8 days around each complicated case so both complicated/uncomplicated cases where matched in time as closely as possible. An analysis using a Fisher exact test yield a significant association ($p<<0.05$) between MOI and disease severity in *P. vivax*. In contrast, no association was observed in *P. falciparum*. Similar analyses were performed on all the samples regardless time of collection and the association observed in *P. vivax* remained whereas no association was still observed in *P. falciparum*. We observed that multiple infections with *P. falciparum* usually involved genetically related lineages whereas in *P. vivax* there were distinct genotypes circulating. However, the low number of complicated malaria cases did not allow to proper exploring the effect that this factor may have in the differences observed between the two parasites species.

1570

DECLINE IN THE BURDEN OF MALARIA IN PREGNANCY IN SOUTHERN MOZAMBIQUE: EVIDENCE FROM A 14-YEAR PERIOD AND IMPLICATIONS FOR MALARIA ELIMINATION

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Knowledge of the trends in malaria transmission is essential to evaluate the impact of elimination campaigns. Pregnant women are especially vulnerable to malaria and have been suggested as a potential reservoir of *Plasmodium falciparum* parasites. The objective of this study was to describe changes in the burden of malaria in pregnancy and its maternal and infant adverse consequences over a 14-year period in southern Mozambique. The study was designed as a retrospective pooled analysis of data collected in five studies that enrolled pregnant women of all gravidities between 1998 and 2012. Temporal changes of maternal *P. falciparum* infection and pregnancy outcomes, as well as the effect of gravidity and HIV infection on the changes on malaria burden were assessed over the period. 4973 pregnant women contributed to this analysis; 27.2% of them were primigravidae and 29.3% were HIV-infected. A significant drop in peripheral parasitemia prevalence was found between 1998 and 2010 (from 15.3 % to 0.5% $p<0.001$) with a slight but significant rebound in 2012 (to 3.5%, $p=0.003$). The mean parasite density in peripheral blood was higher in 2010 compared with that in 1998 (66683 versus 4667.9 parasites/μL, $p<0.001$). The prevalence of placental infection also significantly drop during the study years (from 50.7% in 2003 to 3.5% in 2012, $p<0.001$).Primigravidae had higher prevalence of *P. falciparum* infection than multigravidae across all studied periods ($p<0.001$). No significant differences were found in the prevalence of peripheral parasitemia between HIV- infected and uninfected pregnant women. Maternal anemia ranged between 57.4% and 37.4% with no specific pattern over the study years. The prevalence of low birth weight decreased during the study period (from 19.8% in 2003 to 6.1% in 2012, $p<0.001$).A significant decrease in the malaria burden in pregnancy was observed in this area of southern Mozambique over the period. Importantly, the increase in P falciparum infection found at the end of the period calls for the need of continuous monitoring and surveillance of malaria transmission in pregnant women.

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MALARIA EPIDEMIOLOGY IN LOW ENDEMICITY AREAS OF THE NORTHERN COAST OF ECUADOR

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The recent scale up in malaria control measures in Latin America has resulted in an impressive decrease in the number of reported cases in most countries of the region. In Ecuador the incidence decreased from more than 100 thousand cases in 2001 to 377 cases in 2013) with occasional outbreaks of both falciparum and vivax malaria particularly in the coastal and Amazonian regions. The success in control measures in recent years has led Ecuador to change its malaria policy from control to elimination, nevertheless, there is no evidence that current interventions alone particularly the passive case detection could lead to malaria elimination in the country as it has been reported that a large proportion of human malaria infections could be asymptomatic. We have studied the malaria prevalence in four communities of an endemic area in northwest Ecuador. A total of 650 blood samples were analyzed by microscopy, real time PCR as well as serology using ELISA and immunofluorescence. The total prevalence of malaria infections in the study area was 8% (98% asymptomatics). This number is comparable to the reported prevalence in a neighbor endemic area of Colombia (Tumaco) and it implies a much higher prevalence of malaria in the area than expected according to the official records. Our results infer that the transition from control to elimination strategies in a country like Ecuador will demand an improvement in malaria diagnostics to detect parasites in asymptomatic carriers and in infections with low parasite densities as well as a change in the treatment policy.

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CHANGING MALARIA EPIDEMIOLOGY IN UGANDA BETWEEN 2011 AND 2014: RETROSPECTIVE ANALYSIS OF KEY MALARIA INDICATORS AND SURVEILLANCE DATA

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The National Malaria Program has been implementing the malaria 2011-2015 strategic plan (MSP) since January 2011. A mid-term review was conducted to examine progress against the targets outlined in the MSP, identify key challenges and provide evidence to inform the next 2016-2020 strategic planning period. This paper summarizes key findings from the review and their implications on future malaria control plan. The Review was conducted between November 2013 and April 2014. Data to assess changes in intervention coverage, malaria burden and compliance to Uganda's malaria "testing guidelines" were primarily from the HMIS (DHIS2). Where available, sentinel surveillance data were used to validate HMIS findings and additional data were got from surveys and program reports. We used descriptive statistics to assess changes in intervention coverage and generate trends for key malaria indicators. To assess the changes in malaria burden, we analyzed the difference between the Test Positivity Rate in the baseline year (2011) and 2014. The proportion of households with 1 LLIN per 2 persons increased from 28% in 2011 to 59.6 % by end of 2013 while outpatient visits attributed to malaria in under-5s was recorded to have declined from 52% to 13.7% in the same period. In ten districts where IRS has been consistently deployed for 5 years, the test positivity rate declined by 25%. Malaria Case fatality rate dropped from 2% in 2010 to 0.72% in 2013. Proportion of cases receiving a parasitological test before treatment improved from 25% in 2010 to 59% in 2013.The MTR was used as an opportunity to identify,collate and analyze data from different sources to evaluate progress in malaria control. Although our results should be interpreted with caution because of the inherent data quality problems of routine surveillance systems and low malaria testing during the review period, the compilation of data from different sources suggests that Uganda has made progress in malaria control and is on track to achieve most of its 2015 intervention targets. Strategies contributing to these successes were evaluated and informed the next strategic planning period

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MALARIA PREVALENCE IN REPUBLIC DEMOCRATIC OF THE CONGO: PRELIMINARY RESULTS IN 13 HEALTH ZONES

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Malaria remains a health problem in the DRC despite interventions today conducted a large scale. Few evaluations were conducted in the DRC on malaria, the country has very little information on the basic indices of malaria. This makes it difficult to evaluate the interventions and monitor their impact. A community survey was conducted in 13 health zones in different regions with different epidemiological characteristics The prevalence ranged from 0.4% to 42% respectively at Musienene in North Kivu Province and Kapolowe in Katanga Province. The average prevalence was 12.5%. With a mean parasitaemia of 356 trophozoites per microliterd with the highest parasitemia at 27300 trophozoites per microliter These results show that the malaria profil in DRC is vaery varoius and the control effort must be considered differently.

1574

HOW INFECTIOUS IS THE ASYMPTOMATIC RESERVOIR?

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In a malaria endemic population, many individuals will have asymptomatic infections. These individuals do not seek treatment, so continue to infect mosquitoes and continue transmission. In a time where malaria elimination is back on the agenda, identifying and treating these asymptomatic individuals is likely to be a key part of any elimination strategy. An important question yet to be answered is: how infectious is the asymptomatic reservoir, and how much of this reservoir do we need to target to drive the reproductive number of malaria below one? In this study we use a range of data sources from the published literature to estimate how infectivity varies across different age groups, different transmission settings, and across the transmission season. We also consider how parasite densities of asymptomatic populations vary, and, for a given time, how many of these infections are detectable using a diagnostic such as microscopy or RDT. A study from Burkina Faso estimates that 53% of infected individuals in a population have sub-microscopic parasite densities, and that these individuals contribute 24% to the infectious reservoir. We also find that in areas with high transmission, children are more infectious to mosquitoes than adults, therefore identifying and treating children could be a relatively more impactful way to reduce the infectious reservoir. Finally, we use the available information to validate an existing mathematical model of malaria transmission and to explore the implications of age- and location-specific infection and infectivity profiles on malaria control interventions.

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DECLINING PARASITE PREVALENCE AND IDENTIFICATION OF NON-*PLASMODIUM FALCIPARUM* INFECTIONS IN MALARIA-ENDEMIC SOUTHWESTERN UGANDA

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Malaria transmission in Uganda is remarkably heterogeneous, and declines in prevalence have not been uniform. Previous surveys in southwestern Uganda have shown declines in parasite prevalence from 2004 to 2010. In order to assess the current status of malaria control, we conducted a multi-stage cluster sampling cross sectional survey for malaria infection using rapid diagnostic tests and microscopy. 631 children under five years of age were sampled from three districts, previously characterized as low (Mbarara), intermediate (Bushenyi), and high (Isingiro) transmission intensities. Blood samples were collected to determine the presence of parasitemia using the following: (1) a combined ***Plasmodium*** HRP-2/LDH rapid diagnostic test (RDT) (SD Bioline Malaria Ag P.f/Pan), (2) microscopy, and (3) PCR testing for species confirmation. Prevalence of parasitemia was higher by RDT compared to microscopy (6.2% (95% CI: 4.3-8.1) vs. 3.2% (95% CI: 1.8-4.5)). By district, parasitemia prevalence was 1.2% (3/242) in Mbarara, 3.2% (5/157) in Bushenyi, and 5.2% (12/232) in Isingiro. All 20 microscopy positive cases were detected by RDT. Of the 19 cases detected only by RDT, 7 (36.8%) reported having been treated for malaria within the past month. Notably, of the 20 microscopy positive children, 50% (10/20) were infected with ***P. falciparum***, 40% (8/20) with ***P. malariae***, and the remaining 2 children were ***P. vivax*** and ***P. ovale*** mono-infections.

Knowledge, attitudes, and practice regarding malaria prevention were also assessed, revealing a high proportion of households reporting bednet use (91.6%), but only a small fraction of households participating in indoor residual spraying (0.8%). Our preliminary findings indicate continued significant strides in malaria control over the past 10 years in southwestern Uganda. Combined HRP-2/LDH correctly identified all microscopy positive cases. Most notably, our survey reveals a striking shift in species prevalence in this region of Uganda, with nearly 50% of asymptomatic children infected with non-falciparum species. PCR confirmation of screening results is currently underway and will be presented.

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MALARIA INFECTION AND GAMETOCYTE CARRIAGE RATE, ASSESSED IN A COHORT OF ADULTS DURING MALARIA TRANSMISSION BLOCKING ASSAY DEVELOPMENT IN BANCOUMANA, MALI

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For malaria elimination/eradication, transmission blocking tools are key interventions to be integrated into existing control strategies. Epidemiological characterization of the transmission reservoirs in endemic countries are being evaluated in targeted populations prior to the start of phase 1 or 2 transmission blocking vaccine clinical trials in order to support study designs and analysis plans. From 2011 to 2014, we have followed a dynamic cohort of adults aged from 18 to 50 years, in Bancoumana, Mali, in order to improve assays that support trials of transmission blocking vaccines. After community permission, individual informed consent was obtained for each volunteer. Both National Institute of Allergy and Infectious Diseases/NIH in USA and FMPOS IRB in Mali approved the study protocol and informed consent documents. Volunteers were screened monthly for malaria parasites and gametocytes carriage. Passive surveillance and care were also provided to volunteers in cases of illness throughout the duration of the study. Malaria smears were stained using Giemsa and assessed by two different readers for the presence of malaria parasites. In November, a peak month for parasite carriage, the ***Plasmodium falciparum*** infection rates were: 22.58 (14/62), 25.79 (57/221), 32.94 (28/85), respectively in 2011, 2012 and 2014. Likewise, October, a peak month for gametocytes carriage, the gametocyte carriage rates were: 8.57 (6/70), 10.07 (30/298), 6.82 (6/88), respectively in 2011, 2012 and 2014. There were no statistically significant differences seen, in the ***Plasmodium falciparum*** infection rate or the gametocyte carriage rate between the different years of the study during the peak months of carriage. Malaria infection and gametocytes carriage are sufficiently prevalent in the adult population at the study area to conduct trials that assess the activity of transmission blocking vaccines.

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EPIDEMIOLOGICAL AND ENTOMOLOGICAL CHARACTERIZATION OF URBAN MALARIA TRANSMISSION IN QUIBDÓ-COLOMBIA

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It is suspected that anarchical urbanization, presence of mosquito vector with high adaptability to urban environments, arrival of infected individuals to communities with low levels of immunity and deficiency of sanity and infrastructure contributes to establishment of malaria transmission in urban and peri-urban areas. A number of malaria endemic municipalities in Colombia (n=17) have been reported by the National Surveillance System as having urban malaria transmission, although there is poor evidence for the presence of autochthonous urban cases. A pilot study is being conducted in Quibdó the municipality with highest amount of reported cases in order to ascertain urban malaria transmission. The study includes malaria active case detection (ACD) and passive case detection (PCD) both followed by a reactive case detection (RCD), to assess epidemiological and entomological dynamics in selected urban and peri-urban settings. Adult Anopheles mosquitos collected by human landing catches and larval habitats are being searched neighborhood of malaric houses in urban, peri-urban settings. Preliminary data has been collected from a total of 68 houses with 390 inhabitants in the peri-urban (Cabi and Casablanca) and urban neighborhoods by ACD. Two positive cases were detected by thick blood smear (1Pv, 1 Pf) whereas qPCR detected eight (3Pf 5Pv). So far only 1 case (Pv) has been recorded among urban patients who reported not having visited endemic areas. RCD of this urban case indicated that neither the family nor neighbors had malaria, in contrast, the RCD of the other seven positive cases confirmed by qPCR as index cases indicated that: In peri-urban areas, Casablanca none of the family members of one of the cases was infected, whereas a neighbor of a second case was positive (Pf). In Cabi, a total of 13 additional cases were found in index case homes and 11 more in neighbors' houses. Of 298 adult mosquitos collected 75% were from rural area, 23% peri-urban area and only 1 specimen (0.3%) urban area where no larvae habitats were found. This data clearly indicate that transmission in Quibdó is mainly peri-urban with so far no evidence for urban transmission.

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MALARIA CASES TRENDS IN RWANDA 2001-2014

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Rwanda has achieved signficant progress in scaling up malaria control interventions with reductions of more than 50% of malaria prevalence and incidence countrywide. Rwanda is now considered to be on target to achieve malaria pre-elimination by 2018. However, recently the NMCP Rwanda reported rise of malaria cases for the past 3 years which threatens to undermine these gains. The aim of our study was to investigate the trends in malaria over a 13-year period (2001-2014) and to evaluate the impact of malaria control interventions on malaria burden in Rwanda. Monthly health facility data on countrywide confirmed malaria cases from

the Rwanda Health information system were assembled for the period 2001-2014. Data were aggregated by district, province and national levels. Time series analaysis was undertaken to describe the trends in malaria cases, slide positivity rate, crude case incidence and the proportion of outpatients attendances that were due to malaria. These were matched with a timeline of changes in climate indices, policy and malaria control interventions to contextualise the observed trends. From 2001 there was a generally declining trend in malaria slide positivity rate while cases numbers were lower compared to the peak of 2005. However, compared to 2011, when the lowest case numbers and slide positivity rates were recorded, there was an increase in the malaria burden. The highest increase for the last 3 years was in the districts of Eastern and Southern provinces located in high malaria transmission zones bordering the high transmission countries . Some of the peaks appear to correspond to lagged rainfall and temperature changes as well as low malaria control interventions effective coverage. In conclusion, malaria cases have steadily increase in Rwanda in the last three years in Rwanda despite substantial scale up in malaria control. The increase in malaria control interventions coverage appear to result in a declining trend as was observed between 2005-2008 and 2010-2011. A detailed empirical analysis is required to determine the key drivers of the rising trend.

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TOOLS FOR IDENTIFYING MALARIA HIGH RISK POPULATIONS: PILOT OF A CASE-CONTROL METHODOLOGY IN ZAMBEZI REGION, NAMIBIA

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Targeting interventions to populations at high-risk for malaria infection remains a challenge in meeting Namibia's elimination target for 2020. To effectively target infections, it is crucial to better understand the demographic, occupational and geographic risk factors for malaria. A case-control methodology, termed the Malaria Elimination Risk Factor Assessment Tool (MERFAT), was piloted between February and May 2015 in Zambezi Region, in order to locally characterize high risk groups and inform intervention strategies. All malaria cases confirmed by RDT at six randomly selected health facilities were included in the study. Controls were age and gender-matched to cases using frequency matching within broad categories and allocated to health facilities based on probability proportional to size of the health facility catchment population. Following an incident case, the first eligible patient who (i) tested negative for malaria by RDT or microscopy and (ii) matched a site-specific control profile was recruited into the study, with an aim of matching all cases within a two-week period. Multivariate logistic regression was used to evaluate potential environmental, socio-demographic, intervention, behavioral and travel-related risk factors. Spatial patterns of malaria risk were also analyzed. We will present descriptive statistics of the case and control study populations and evaluate spatial clustering of infection over the study area. We will also present results from the risk factor analysis, including assessment of environmental, sociodemographic, intervention, behavioral and travel-related risk factors. Implications for targeting local intervention strategies by geographical location and to profiled high-risk characteristics will be discussed, as well as the use of these methods as a programmatic tool in elimination settings.

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THE ROLE OF MATERNALLY DERIVED ANTIBODIES TO *PLASMODIUM FALCIPARUM* SCHIZONT EGRESS ANTIGEN-1 (PFSEA-1) AND NATURAL EXPOSURE IN THE DEVELOPMENT OF INFANTS' ANTIBODIES TO PFSEA-1

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Recently, we demonstrated that antibodies (Abs) to a novel malarial vaccine candidate, ***Plasmodium falciparum*** schizont egress antigen-1 (PfSEA-1), are associated with protection from severe malaria in children. Few studies have evaluated the impact of passively transferred cord blood anti-malarial Abs on the development of antimalarial Abs following natural exposure in early childhood. The objectives of this study were to a) longitudinally assess the relationship between parasite densities and subsequent anti-PfSEA-1 Ab levels among young children and b) evaluate whether higher levels of maternal Abs modified the relationship between natural exposure and development of infant anti-PfSEA-1 Ab. A total of 646 Tanzanian infants born between 2002 and 2005 were monitored every 6 months for anti-PfSEA-1 Ab levels until 30 months of age. We used two-way repeated measures analysis of variance to evaluate the changes in anti-PfSEA-1 Ab levels across three levels of both cord blood anti-PfSEA-1 Ab and cumulative parasite density in the preceding six months. Anti-PfSEA-1 Ab levels decreased up to 6 months of age and then began to increase until 24 months. Parasitemia within 6 months after birth induced significantly higher anti-PfSEA-1 Abs at 6 and 12 months compared to the group without parasitemia ($P < 0.05$). At 6, 12, and 24 months of age, infants with the highest tertile of average parasite densities for 6 months prior had significantly higher anti-PfSEA-1 level than the non-parasitemic group ($P < 0.05$). Cord blood Ab level did not modify the relationship between parasite density in preceding six months and infant anti-PfSEA-1 Ab level, ($P < 0.05$) suggesting cord blood Ab endowment does not interfere with Ab development during natural exposure. Malaria exposure influenced anti-PfSEA-1 Ab levels in infants longitudinally, and cord blood anti-PfSEA-1 Ab levels did not dampen acquisition of anti-PfSEA-1 Abs during natural exposure, suggesting that vaccinating pregnant women is unlikely to interfere with naturally acquired immune responses to PfSEA-1.

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HETEROGENEOUS PREVALENCE OF ASYMPTOMATIC MALARIA AND LOW PREVALENCE OF HIV IN PREGNANT WOMEN IN MYANMAR

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As plans develop to eliminate malaria from Greater Mekong Subregion in hopes of stopping the dissemination of artemisinin-resistant ***Plasmodium falciparum***, malaria infection without clinically recognizable illness is being targeted for drug treatment. It has long been recognized that asymptomatic malaria is common in semi-immune people in parts of sub-

Saharan Africa where malaria transmission is moderate or high, and that asymptomatic malaria during pregnancy in these regions is associated with adverse pregnancy outcomes. However, studies of asymptomatic malaria are very limited in areas of low malaria transmission such as Myanmar, particularly in pregnant women. We conducted a prospective longitudinal cohort study of pregnant women in 12 villages in Shwe Kyin and Madaya, two rural townships in Bago and Mandalay Regions, respectively, in Myanmar, to estimate the prevalence of asymptomatic malaria over time. Co-infections including HIV and intestinal helminths were also diagnosed. A total of 699 pregnant women making their first antenatal visit to a rural health center were enrolled between August 2013 and October 2014, and followed monthly for clinical and laboratory evaluations. Blood was collected for hemoglobin measurement, microscopic and PCR analysis of ***P. falciparum*** and ***P. vivax*** malaria, and rapid diagnostic testing of HIV. Stool was collected for microscopic examination of ova and parasites and speciation of intestinal helminths. In preliminary analyses, the prevalence of falciparum malaria at enrollment ranged from 3-15% and the prevalence of vivax malaria was less than 1%, and only one participant tested positive for HIV. Results from laboratory analyses, including the sequence analysis of K13 molecular marker associated with artemisinin resistance, and longitudinal dynamics of falciparum and vivax malaria will be presented. Preliminary data suggest that the burden of asymptomatic malaria in pregnancy in Myanmar is higher than expected at some sites, and heterogeneous across the study sites.

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SEASONAL CHANGES IN THE EPIDEMIOLOGY OF MALARIA IN THE GAMBIA

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Recent reductions in the burden of malaria observed in many malaria endemic areas have mainly been due to improved coverage of malaria control interventions. To track the progress from these interventions and assess their impact, changes in malaria transmission and in the disease and immunity patterns needs to be monitored regularly in areas where these interventions are deployed. In a prospective cohort study, we enrolled 1402 individuals of all ages and of both genders from 168 households in Gambissara(GMB), Sare Bondo(SBND), Fula Morie Boche(FMB) and Sare Jawbe(SJWB) villages located within distance of 3 to 11 kilometres from each other in the upper river region (URR) of The Gambia. Over malaria transmission seasons of 2012, 2013 and 2014, we followed up the cohort via cross sectional surveys at beginning and at the end of each transmission season to determine parasite prevalence by microscopy. In addition, filter paper blood spots for later polymerase chain reaction analysis and blood samples for later haemoglobin typing and determination of prevalence of antibodies to selected malaria parasite antigens were collected at each survey. Results of 2012 surveys showed malaria prevalence was 1.6%(19/1147),10.9%(7/65), 18.1%(21/116) and 29.4%(20/68) at the beginning of transmission season and 10.7%(97/907), 14.3%(8/56), 35.2%(38/108), and 60.3%(35/58) at the end of the transmission season in GMB, SBND, FMB and SJWB villages respectively. In 2013, malaria prevalence was 2.6%(25/969), 8.1%(5/62), 20.0%(19/95) and 26.2%(16/61) at the beginning of transmission season and 4.6%(42/896), 5.4%(3/55), 20.3%(21/103), and 40.0%(21/53) at the end of the transmission season in GMB, SBND, FMB and SJWB villages respectively. In 2014, malaria prevalence was 3.4%(31/906), 6.4%(4/62), 14.0%(14/102) and 42.2%(21/54) at the beginning of transmission season in GMB, SBND, FMB and SJWB villages respectively. The wide differences in malaria prevalence consistently observed between our study villages over three transmission seasons suggests heterogeneity in malaria transmission in the URR of The Gambia.

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FOCAL TRANSMISSION OF MALARIA: PREVALENCE AND INCIDENCE OF MALARIA IN DANGASSA VS. BAMOGOLA IN MALI FROM 2012 TO 2014

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During the past decade, the Government of Mali has launched a number of interventions to improve the prevention and treatment of ***Plasmodium falciparum*** malaria among the most vulnerable subgroups within the population. The purpose of this study was to examine the prevalence and incidence of ***P. falciparum*** malaria in Dangassa, a village located in an area of Mali with intense seasonal transmission. In 2011 we performed a census to select a representative sample of 1412 persons from the general population. Because we used a household-based sampling approach, once a household was selected, all the members of the household were invited to participate in the study. We then performed five serial (longitudinal) surveys to estimate the prevalence of parasitemia (infection) in the study population at the beginning and the end of the transmission season and during the dry season. A clinician based in Dangassa throughout the year was responsible for passive case detection (PCD) during those two and a half years in order to assess the incidence of malarial disease. Standard methods such as rapid diagnostic tests and positive thick smears were used to identify ***P. falciparum*** infection and thus to estimate the prevalence of infection and the incidence of disease (based on PCD). The prevalence of parasitemia (infection) varied with the season from 10% in June (at the beginning of the transmission season) to 39% in October (at the peak of the transmission season). A similarly low prevalence of 9% was observed during the dry season in February. These variations in the prevalence of infection are consistent with the PCD data which indicate that 55% of malaria cases occurred during the four month period from August to November. In addition, 60% of the severe malaria cases occurred during October and November. Despite the use of multiple interventions such as insecticide-treated bed nets for malaria prevention, the prevalence of infection (parasitemia) remains high in Dangassa among all age groups during the transmission season. Interventions such larviciding and seasonal malaria chemoprevention may be required to further reduce the burdens of infection and disease in such communities.

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MALARIA IN GOLD-MINING AREAS IN COLOMBIA

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Despite significant decrease in recent years, malaria remains a major public health problem in Colombia. Among the multiple factors contributing to malaria maintenance, gold mining appears to play a major role. During the last decades, gold mining mostly illegal and associated to the military conflict, has significantly expanded in Colombia in areas with limited health care and disease prevention for the National Malaria Control Program, as well as their efficacy and generate a great negative impact on the socio-economic structure of the mining communities. In order to set up the bases for more effective malaria control strategies a descriptive study has been carried out to characterize the epidemiology and transmission of malaria in gold mining areas. The study focused on determining the prevalence of malaria cases in mining districts of Colombia. Approximately 31.6% of malaria cases from gold mining

district were reported from Nordeste Antioqueño (Antioquia), Montelibano (Córdoba) and from Buenaventura (Valle del Cauca) in the Pacific Coast. In Itsmina mining district (Chocó), it was found that 58.5% of malaria cases (165/282) displayed clinical symptoms some of them with (52/165) warning signs, and 52.8% in adolescents (< 18 years old). While on the study in Buenaventura was detected that 33% (162/492) of the cases occurred in miner worker specifically from Zaragoza, a rural site near the city which contributed 89% of total cases of the region; most of them (34%) asymptomatic only detectable by PCR. In conclusion, gold mining represents a specific epidemiological scenario of high risk for malaria transmission in communities engaged in this activity. The constant generation of small and temporary mines creates mosquito breeding sites which make it difficult to establish a profile of the vector species involved in transmission. Intervention strategies in the mining population have not been evaluated but are potentially useful to reduce the disease burden. The impact on the socio-economic structure of the mining communities help to maintain or increase malaria when mining populations migrate from or to malaria naïve communities.

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PARALLEL DECLINES IN MALARIAL AND NON-MALARIAL CHILDHOOD FEBRILE ILLNESS IN WESTERN KENYA

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Much of sub-Saharan Africa has recently seen substantial declines in malaria-related childhood morbidity due, in part, to effective control interventions. This has implications both for the etiologies of acute febrile illness (AFI) and demand for healthcare services. Overall childhood AFI may decline in parallel with malaria or, alternatively, non-malarial AFI may increase due to a reduction in asymptomatic parasitemia that was previously misclassified as malarial AFI. Current research has only described changes in rates of malaria rather than AFI overall. We used data from an area of high malaria transmission to examine this issue. We included 11,560 records of visits of children (<5 years) to an outpatient clinic in western Kenya from Jan. 1, 2009-Dec. 31, 2014. AFI was defined as temperature of ≥38.0°C from any cause and malaria by a positive smear result. We used quasi-Poisson regression to explore changes in counts of total, malarial, and non-malarial AFI, and quasi-binomial regression to examine the change in proportion of AFI that was malaria positive. We compared outpatient data with rates of self-reported AFI during regular household visits over the same period. There was marked seasonal variation in numbers of clinic visits for both malarial and non-malarial AFI, with some commonality to the patterns. We found similar rates of decline for numbers of total, malarial, and non-malarial AFI visits (0.76%, 0.79%, and 0.72% per month, respectively, P<0.01 for all three rates). The probability of a febrile child having malaria varied seasonally but remained constant across each year (P<0.748). Self-reported AFI from household surveillance declined at a slightly greater rate (0.94% per month, P<0.001). We found no evidence of change in the ratio of malarial to non-malarial AFI; both decreased at similar rates and may reflect a general improvement in the population's health. Non-malarial AFI declines may also stem from introduction of the pneumococcal conjugate vaccine or a link between malaria and risk of other AFI etiologies. Long-term reductions in outpatient AFI morbidity may be driven by factors other than malaria control programs.

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GENOME-WIDE ANALYSIS AND DIVERSITY OF *PLASMODIUM FALCIPARUM* IN SOUTHWESTERN NIGERIA

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The burden of malaria is especially high in sub-Saharan Africa. Varying selection caused by differences in host immunity and antimalarial drug pressure leads to evolutionary changes responsible for high level of genetic variations in the parasite. Effective control methods require population-specific genetic studies to survey for genes under polymorphisms as a result of the influence of drug pressure or host immunity. We performed a genome-wide analysis of Nigerian isolates of ***Plasmodium falciparum*** and used frequency based neutrality test (Tajima's D) and integrated haplotype score (iHS) to identify genes under selection. Fourteen shared iHS regions that had at least 2 SNPs with a score > 2.5 were identified. These regions contained genes that were likely to have been under strong directional selection. Two of such genes were chloroquine resistance transporter (CRT) located in chromosome 7 and multidrug resistance 1 (MDR1) located in chromosome 5. There was a weak signature of selection in dihydrofolate reductase (DHFR) in chromosome 4 and MDR5 genes in chromosome 13 with only 2 and 3 SNPs respectively identified within the iHS window. Although there was no evidence of recent directional selection in dihydropteroate synthase (DHPS) gene in chromosome 8, there exists strong selection pressure attributable to continued chloroquine and sulfadoxine-pyrimethamine use despite the official proscription; hence the re-introduction of the drug as was done in other endemic countries after a period of withdrawal may yet be inappropriate. There was also a major selective sweep on chromosome 6 which had 32 SNPs within the shared iHS region. Tajimas's D values were mostly negative with a mean value of -0.86. One hundred and twelve genes (3.59%) had positive values. Tajima's D values of Circumsporozoite Protein (CSP), Erythrocyte-Binding Antigen (EBA-175), Merozoite Surface Proteins - MSP3 and MSP7, Merozoite Surface Protein Duffy Binding-Like (MSPDBL2) and Serine Repeat Antigen (SERA-5) were 1.38, 1.29, 0.73, 0.84 and 0.21 respectively. These positive values suggest their candidacy as target antigens of host immunity.

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COMPLEXITY OF INFECTION, DIVERSITY AND DRUG RESISTANCE IN CAMBODIAN *PLASMODIUM VIVAX*

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In Cambodia, malaria is one of the foremost public health problems, with 2.5 of the estimated 13 million inhabitants living at risk of contracting the disease. Despite the implementation of extensive control efforts, the number of reported malaria cases attributed specifically to ***Plasmodium vivax*** has significantly increased since 1997 and treatment failure to chloroquine (CQ) has been reported. In absence of reliable long-term ***in vitro*** cultures, measures of genetic diversity by using neutral single nucleotide polymorphisms (SNPs) are useful to characterize ***P. vivax*** population structure, assess impacts of control efforts, and identify and track parasites lineages through space and time. We developed a genotyping technique to amplify and sequence 130 selected SNPs distributed throughout the ***P. vivax*** genome that are highly variable among Cambodian ***P. vivax*** isolates. We first applied this assay to 401 ***P. vivax***-infected patients recruited from nine study sites throughout Cambodia between 2004 and 2013. Our analyses revealed that most ***P. vivax*** infections in Cambodia (92%) are polyclonal and confirmed the

high genetic diversity of this population. Interestingly, our analyses showed that the proportion of monoclonal infections significantly increased between 2004 and 2013 suggesting that the control strategies against vivax malaria in Cambodia may be successfully affecting the parasite population. We then analyzed with the same method, blood samples from 23 ***P. vivax*** infected patients collected before treatment and every eight hours after CQ treatment to evaluate the relative susceptibility of each clone to CQ compared to the original infection. Combined with on-going whole genome sequencing of the parasites before treatment, these data will enable us to conduct a genome wide association study to hopefully identify polymorphisms underlying CQ susceptibility in ***P. vivax***.

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A SPATIAL GENETIC-EPIDEMIOLOGICAL MODEL OF MALARIA PARASITE TRANSMISSION

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A model of malaria transmission dynamics is presented that tracks the full genomes of individual ***Plasmodium falciparum*** infections including outcrossing of strains both within and between infections. We explore quantitatively the relationship between transmission intensity and genetic observations, for example the repeated observations of identical and closely related strains and their persistence across successive transmission seasons. Extending to a spatially connected network of human and parasite populations, we model the sensitivity of genetic sequencing to identify the relative contributions of local hotspots versus re-importation in sustaining transmission in pre-elimination settings. Finally, we model the effects of local transmission intensity and anti-malarial drug pressure on the population-level genetic signatures of emerging drug resistance.

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ROBUST SCORING OF MICROSATELLITE MARKERS FROM NEXT GENERATION SEQUENCING IN *PLASMODIUM FALCIPARUM*

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There are enormous numbers of microsatellites repeats (~1 microsatellite every 650bp) in the genome of the malaria parasite, ***Plasmodium falciparum***. These markers are multiallelic and therefore more informative than SNPs for many research questions, yet they are problematic to score using next generation sequence data, and remain an untapped source of genetic variation for population genomic analyses. We used three approaches to validate scoring of microsatellite loci from next generation sequence data: (1) we sequenced the progeny of a genetic cross (n=14), and examined microsatellite calling in genome regions inherited from each parent; (2) we sequenced (in duplicate) parasites from a mutation accumulation experiment (n=38), and measured reproducibility and accuracy of scoring in >36K microsatellite loci throughout the parasite genome. (3) We compared microsatellite genotypes in genome sequence data from 6 single clone patient samples that had previously been scored for 335 microsatellites using traditional capillary electrophoresis. These analyses define the robustness with which microsatellites bearing different sequence motifs can be scored, and provide a map of callable microsatellites for large scale analysis of malaria parasite populations. To examine the utility of these markers for population genomic analyses, we determined genome-wide patterns of microsatellite variation in sequence data from 50 isolates obtained from a focus of emerging drug resistance in South-East Asia. We identify loci under strong, recent, positive selection in these samples, including known drug resistance loci, and we recapture the kelch locus underpinning artemisinin resistance.

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PATTERNS OF *PLASMODIUM FALCIPARUM* GENOTYPES IN AN AREA OF LOW MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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Understanding the epidemiologic patterns of local malaria transmission and importation is crucial to achieving elimination and preventing re-introduction. A 24 single nucleotide polymorphism (SNP) ***Plasmodium falciparum*** molecular barcode has been used to identify and track circulating parasite populations. This molecular barcode was used to determine the genetic diversity and complexity of the circulating parasite population among malaria infected participants enrolled in a population-based, serial cross-sectional cohort study in Choma District, southern Zambia from 2008-2013. In this region the prevalence of malaria by rapid diagnostic test decreased from 8% in 2008 to <1% in 2013. Parasite DNA was extracted from dried blood spots from approximately 100 infected participants using the Chelex method. DNA pre-amplification specific to the target sites of each of the 24 SNPs was performed because of the low level of parasitemia. The molecular barcoding was performed using TaqMan genotyping assays on the Applied Biosystems StepOne and the Roche Lightcycler. Genotype results were assigned using each system's software. When the software was not able to identify the allele, the call was made manually. A subsample of duplicates was analyzed using both the Applied Biosystems and Roche platforms to validate the results. Analyses were conducted to determine the number of identical molecular barcodes within each year and persistent molecular barcodes between years. The proportions of mixed infections and polygenomic molecular barcodes were calculated for each year and compared across all years. No identical barcodes were identified within any year or between years. The proportion of samples with mixed infections was high for all years with an increasing trend as prevalence declined (90% in 2008, 94% in 2009, and 100% in years 2010-2013). The proportion of samples with polygenomic molecular barcodes decreased from 85% in 2008 to 73% in 2010, then increased to 92% in 2011 and 100% in 2012 and 2013. The results suggest that as malaria prevalence declined, local transmission ceased, and all infections were imported.

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COMBINATION OF HEMOGLOBIN-A2 PROMOTER VARIANTS AND THALASSEMIA-$A^{3.7}$ DELETION IS ASSOCIATED WITH *PLASMODIUM FALCIPARUM* ANEMIA

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Alpha (α)$^+$ thalassemia (α-thal) is a hemoglobinopathy induced by defective α-globin production which can be characterized by deletions in either or both hemoglobin (***HBA1*** and ***HBA2***) genes. Amongst populations in sub-Saharan Africa, a 3.7kb ($-\alpha^{3.7}$) deletion of the region between ***HBA1*** and ***HBA2*** results in phenotypes of heterozygous ($-\alpha^{3.7}/\alpha\alpha$) and homozygous ($-\alpha^{3.7}/-\alpha^{3.7}$) carriers. Within sub-Saharan African populations, severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dL with ***Plasmodium falciparum*** infection], is a leading cause of morbidity and mortality,

particularly in children. In the present study, we utilized our findings from high-throughput genotyping [Human BeadChip' (>2.45x10^6 markers)] and global gene expression arrays [HumanHT-12 v4 BeadChip (47,231 probes)] to identify novel genetic variants. Among the list of significant novel genes, ***HBA2*** emerged as an important gene associated with susceptibility to severe malaria in Kenya children (3-36mos). To validate the whole genome findings, we genotyped two functional promoter SNPs (rs1203833 and rs2974771) in ***HBA2*** (n=1,668 study participants). To examine the influence of the $\alpha^{3.7}$ deletion on SMA, this genotype was also generated. Binary logistic regression analysis, controlling for covariates (age, gender, G6PD, HIV-1, bacteremia, and HbAS status) revealed that carriage of the TA haplotype (-1789T/-4314A) together with the homozygous α-thalassemia deletion ($-\alpha^{3.7}/-\alpha^{3.7}$) increased susceptibility to SMA (OR: 3.2, 95%CI: 1.2-8.4, ***P***<0.05). These results demonstrate that variation in ***HBA2*** in combination with α-thalassemia variants influence susceptibility to SMA.

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HAPLOTYPES WITHIN THE NFKB1 PROMOTER ARE ASSOCIATED WITH RISK OF SEVERE MALARIAL ANEMIA

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Understanding the molecular mechanisms involved in pathogenesis of severe malaria anaemia [SMA, hemoglobin (Hb)<5.0g/dL and any density parasitemia)] in children is a crucial step in the design of therapeutics interventions. Transcription factors are important in modulating cellular processes, such as immunity, since they are the primary regulators of gene expression. For example, nuclear factor of kappa light enhancer in B-cells (NF-κB) plays an important role in infectious and autoimmune disease pathogenesis through regulation of molecular pathways that generate soluble immune modulators (e.g. cytokines). Imbalances in immune modulators, such as IL-17, are central to the pathogenesis of childhood SMA. As such, we examined if genetic variation within the ***NFKB1*** promoter affected susceptibility to SMA. Functional association between ***NFKB1*** variants (-3297 T>C, rs980455 and -8079A>G, rs747559), SMA, and production of IL-17 was, therefore, determined in children (n=1,065, aged 6-36 months) presenting with ***P. falciparum*** malaria in Siaya County, a holoendemic transmission area of Kenya. Bivariate regression analyses controlling for covariates revealed that carriage of the ***NFKB1*** -8079A/-3297C (AC) haplotype was associated with increased risk of SMA (OR 1.60, 95%CI 1.10-2.40, ***P***=0.012). The AC haplotype was also associated with reduced Hb levels (***P***=0.001) and low circulating IL-17 levels (***P***=0.018) determined by the Human Cytokine 25-plex Array (Invitrogen). Spearman correlation revealed that Hb and IL-17 levels were positively associated (ρ=0.151, ***P***=0.027). Taken together, these results demonstrate that variation in the promoter of ***NFKB1*** is associated with susceptibility to pediatric SMA, potentially through altering the downstream expression of inflammatory mediators, such as IL-17.

1593

ASSOCIATION BETWEEN INTERFERON GAMMA (IFN-Γ) PROMOTER HAPLOTYPES AND ERYTHROPOIESIS IN KENYAN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

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Plasmodium falciparum malaria is among the leading causes of morbidity and mortality among African children. In ***P. falciparum*** holoendemic transmission areas of western Kenya, severe malaria commonly manifests as severe malarial anemia [SMA; hemoglobin (Hb) <5.0 g/dL, any density parasitemia] in pediatric populations. Interferon-gamma (IFN-γ) is a pleotrophic cytokine associated susceptibility to severe malaria. Since the functional role of IFN-γ genetic variants in conditioning susceptibility to SMA remains largely unexplored, the association between single nucleotide polymorphisms (IFN-γ; -183 G/T, rs2069709 and -1616 A/G, rs2069705), their haplotypic structures, and SMA were investigated in parasitemic children (n=744, aged 3-36 mos.) presenting at a rural hospital in Siaya, western Kenya. Bivariate logistic regression analysis, controlling for age, gender, sickle-cell trait, bacteremia, and HIV-1 status, demonstrated that relative to homozygous -1616 A (wild-type) individuals, carriage of GG genotype was associated with protection against reduced erythropoiesis [reticulocyte production index (RPI)<2] (OR, 0.564; 95% CI, 0.323-0.983; ***P***=0.043). Additionally, carriage of the GA haplotype was associated with protection against reduced erythropoiesis (OR, 0.500; 95% CI, 0.393-0.637; ***P***<0.001). Conversely, GG (OR, 1.910; 95% CI, 1.504-2.427; ***P***<0.001) and TG (OR, 6.551; 95% CI, 1.399-30.679; ***P***=0.017) haplotypes were associated with increased risk of reduced erythropoiesis. Taken together, these results suggest the association between variation in ***IFNG*** and SMA may be mediated through the impact of the variants on erythropoiesis

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AUTOMATED METHODS FOR HIGH-THROUGHPUT ANALYSIS OF *PLASMODIUM* MICROSATELLITE GENOTYPING DATA

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Microsatellite genotyping of ***Plasmodium*** infections offers some advantages, in particular the relatively large diversity present at each locus and the resultant ability to obtain information from mixed infections. However, manual classification of electropherogram peaks as true alleles versus artifact can make this process subjective and time-consuming. To overcome these barriers, we have developed software to analyze microsatellite data in a high throughput and automated manner. These processes include automated processing of raw data files to carry out basic quality control and identify spectral pull-up, storage of run data in a centralized database, robust automated peak detection, contextual algorithms exploiting patterns identified in artifactual peak stutter on a per microsatellite basis that allow for true peak classification, and a web-based interface for manual curation of data. Using lab-cultured strains of ***Plasmodium falciparum*** (Pf) with known genotypes, we quantified segmental linear relationships between allele length and surrounding artifactual "stutter" peaks. With these relationships, we classified peaks as true alleles or artifact. Using 9 lab-cultured strains of Pf in 16 distinct double strain mixtures with relative parasite concentrations ranging from

2% to 98%, for a total 181 combinations, we identified true alleles for 9 microsatellite loci. Of these 9 loci, 2 were found to have complex artifact patterns, such as interactions between alleles, not well handled by a linear model and requiring manual curation. For the remaining 7, we identified true alleles with 94% specificity and 85% sensitivity. On a per microsatellite basis, specificity ranged from 83% to 98% and sensitivity ranged from 76% to 97%. Automated peak classification shortens time from experiment to results and reduces bias incurred by manual analysis and makes possible high-resolution genotyping.

1595

PRELIMINARY STUDY: DIFFERENT POPULATION STRUCTURE OF *PLASMODIUM VIVAX* BETWEEN SYMPTOMATIC AND ASYMPTOMATIC PATIENTS FROM PERUVIAN AMAZON

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Different diagnostic methods have revealed a high frequency of ***Plasmodium vivax*** infections that occur without clinical symptoms. Furthermore little is known about its implications in the transmission dynamics of these infections and their relationship with the parasite population structure. The aim of this study was to explore the parasite population structure of symptomatic and asymptomatic patients from Cahuide, a rural community located 60 km away from Iquitos city. To achieve our goal, 20 microsatellites were amplified (a new set of 11 markers and 9 previously reported) in a selected group of samples. In this way, 113 ***P. vivax*** positive field samples were collected in March (55 samples) and June 2013 (58 samples) by active case detection. 25 Samples were excluded due to low parasitemia levels and the 88 remaining samples were used for microsatellite analysis. Microsatellite PCRs were performed and subsequently analyzed using an ABI PRISM 3100 avant genetic analyzer. Out of 88 samples, 24 were polyclonal whereas 64 were monoclonal, but just 50 had an almost-complete allele profile (75% or higher). Our results showed an average genetic diversity of 0.47 and 39 haplotypes were found. Interestingly, the diversity found in June was greater than in March (0.58 and 0.38 respectively). Using Structure ver2.3, 2 sub-populations (A and B) were found arranging by month (March 2013 and June 2013) and by symptomatology (symptomatic and asymptomatic patients). In March sub-population A was mainly found in asymptomatic patients while sub-population B was predominant in the symptomatic ones. In contrast, in June sub-population A was predominant in the symptomatic patients and both sub-populations were equally distributed in the asymptomatic ones. This data suggests that the asymptomatic patients carrying sub-population A might have served as a reservoir for further infections of the symptomatic patients. This study provides a first approach of the role of asymptomatic patients in malaria transmission which should be considered within the malaria control strategies.

1596

A NEW ASSESSMENT OF THE TRANSCRIPTOMES OF PFSPZ AND THREE DAY LIVER STAGES

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Over the last 20 years, genomics, transcriptomics, and proteomics have provided fascinating insights into ***Plasmodium*** biology and pathogenesis, contributing to basic research and development of new diagnostics, drugs, and vaccines against malaria. The opportunity to understand the biology of sporozoites and exoerythrocytic stages has arisen due to the ability to produce large quantities of these stages for analysis. We assessed aseptic, purified, and viable ***P. falciparum*** salivary gland sporozoites, and sporozoites that were axenically transformed, morphologically similar to 3-day 'liver stage' parasites expressing new proteins. We used next generation sequencing to profile transcripts expressed in sporozoites, axenic 72-hr liver stages, and asexual stages in order to uncover the molecular, biochemical, and cell biological processes that occur across the parasite's life cycle.

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THE HUMAN PRENATAL ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM*

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In malaria endemic areas, the fetus may be exposed to malarial antigens ***in utero*** and produce sensitized B and T lymphocytes. Most of our knowledge of ***in utero*** responses to malaria comes from studies of full term newborns. Little is known about the timing of malaria-specific immune responses ***in utero***. This study investigated the timing of ***in utero*** antibody (Ab) responses to ***P. falciparum*** (Pf) antigens. Since B1-cells are functional during prenatal life, the possibility of innate B1-derived Ab to Pf was also explored. Plasma samples from 600 Cameroonian neonates were studied: 373 full-term deliveries (FTD, ≥37weeks) and 227 pre-term deliveries (PTD, <28 weeks [n=33], 28-33 weeks [n=100] and 34-36 weeks [n=94]). Since IgM does not cross the placenta, fetal IgM in cord plasma was quantified to a panel of 8 Pf antigens (PfAg) and to known B1 antigens phosphorylcholine (PC) and pneumococcal polysaccharides (PP). IgG to PfAg was also measured in culture supernatants of cord blood mononuclear cells (CB-MNC) (n=70). Placental malaria (PM) was assessed from placental impression smears. IgM to one or more PfAg was detected in 10.6% of the neonates, with similar prevalence and titers in PTD and FTD. IgM to PfAg was detected at 22 weeks of gestation and throughout the 2nd and 3rd trimesters. PfAg IgM was significantly associated with PM in PTD and FTD (p=0.01). The proportion of PC IgM responders was not influenced by PM (p=0.83) and 51% of PC IgM-positive newborns were Pf IgM negative, suggesting that ***in utero*** "innate" Ab were not directly induced by malaria. Overall, 65% of culture supernatants from 70 CB-MNC cultures had IgG to one or more PfAg. In summary, the data show that fetal B cells made Pf-specific IgM Ab from as early as 22weeks of pregnancy through birth. Many of the newborns also had Pf-specific IgG-producing cells at delivery. B1-derived Ab were not associated with malaria. Studies are in progress to determine if ***in utero*** exposure to Pf leads to the production of short-lived antibody secreting cells or memory B cells.

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IMMUNE PROFILING OF *PLASMODIUM FALCIPARUM* ENDEMIC SERA USING BESPOKE PROTEIN MICROARRAYS

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The complex nature of the ***Plasmodium falciparum*** life cycle coupled with the further complexities of antigenic polymorphism presents a significant challenge for the design of an effective vaccine against malaria. There is a strong body of evidence that supports antigens expressed by ***P. falciparum*** merozoites as important targets of human immunity and therefore promising vaccine candidates. However, the specific targets of protective immunity remain elusive. Therefore identifying candidate antigens that correlate with a protective immune response remains a priority. We have developed an antigen production pipeline based on E. coli for the expression of purified recombinant antigens. By applying a number of in silico tools intended to aid the design of each antigen construct, we have focused on key regions, where appropriate, of each target gene avoiding the wholesale expression of candidate open reading frames. Using this approach we have been able to avoid difficult to express regions (e.g. transmembrane domains) and achieve expression of soluble constructs with a >90% success rate. Each antigen is then screened by ELISA and validated for reactivity to a subset of positive and negative control sera. Using protein microarrays we have screened approximately 94 validated antigens, many of which represent new, previously undescribed targets across a range of lifecycle stages. So far this panel largely focusses on the merozoite stage (81%) but also includes proteins associated with the infected erythrocyte (18%) and sporozoite (1%) stages. Work is currently ongoing to address this unintended bias. Comparison of data between the protein microarray and ELISA platforms using key validatory antigens, PfMSP1-19 and PfAMA, showed a strong correlation between the platforms (r2=0.78 and 0.73, respectively). Antibody reactivities from 350 individuals assayed at three time points (1050 sera total) from a region of high endemicity in Northern Uganda were used to determine reactivity profiles for each antigen. Preliminary analysis has identified a number of novel immune-reactive targets for prioritisation as putative vaccine candidates.

1599

IMPACT OF MALARIA, HYPERGAMMAGLOBULINEMIA, AND IMMUNE COMPLEXES ON TRANSPLACENTAL TRANSFER OF MALARIA-SPECIFIC AND ANTI-TETANUS TOXOID ANTIBODIES IN PAPUA NEW GUINEA

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Malaria in pregnancy (MIP) can induce placental alterations (PM), malaria-specific immune complexes (IC), and/or maternal hypergammaglobulinemia (MHG) that may interfere with transplacental transfer of immunoglobulin G (IgG). To determine the relative roles of these factors in IgG transfer, 300 paired maternal and cord serum specimens were collected from two geographically similar cohorts in Papua New Guinea in 2005-08 (AXH) and after malaria control interventions 2011-13 (FIS). We measured IgG levels detected by bead array recognizing 20 ***P. falciparum*** (Pf) antigens and 14 ***P. vivax*** (Pv) antigens. ICs were measured using a C1q-based ELISA. Tetanus toxoid (TT) IgG levels served as controls. Placental malaria (PM) prevalence was 59%, MHG (≥1700mg/dL) was 54%, and high IC (≥0.85 mg/ml) was 30% in the AXH cohort; compared to 10%, 7% and 10% respectively in the FIS cohort. Differences were statistically significant with p values of <0.0001, 0.0034, and 0.0004. The cord to maternal transfer ratio (CMTR) was significantly lower in the AXH cohort for almost all anti-malaria antibodies (Ab), compared to the FIS cohort (P <0.001). Impaired transport of malaria-specific IgG (CMTR <1) ranged from 66% to 92% in AXH compared to 48% to 84% in FIS. By contrast, transplacental transport of TT-specific IgG was the same between the two cohorts and averaged of 35% impaired transport. A multivariate model showed a strong negative effect of MHG (P=0.0013), but no association of PM or IC, on transplacental transfer of TT Ab. By contrast, impaired transplacental transfer of Pf and Pv antibodies was associated with MHG, PM and IC with p values of <0.01, <0.001, and <0.001 respectively. There was an interaction between MHG, PM and IC for some antigens, but those factors were also independently associated with impaired transplacental transfer of malaria-specific IgG.The negative correlation of PM and IC only with Pf and Pv Ab but not with TT suggest receptor saturation and/or trapping of Abs rather than impaired placental function are the primary factors reducing transplacental Ab transfer.

1600

THE TIMING OF MALARIA INFECTION DURING PREGNANCY INFLUENCES THE TYPE OF FETAL IMMUNE RESPONSE

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Epidemiological studies show that infants born to mothers with ***Plasmodium falciparum*** (Pf) malaria during pregnancy have a higher risk of malaria infection in early childhood. This increased risk is associated with whether the fetus has been exposed to malaria ***in utero*** and the generation of an immunoregulatory phenotype characterized by high IL-10 and low IFNg/IL-13 production that persists into early childhood. Why some fetuses acquire this immunoregulatory phenotype and others do not may relate to when the fetus is exposed to malaria during gestation. To examine this hypothesis, mothers were randomly assigned to receive intensive or limited malaria chemoprophylaxis during pregnancy. Depending on the gestational age at the time of enrolment and study arm, mothers were protected from malaria during defined times during pregnancy. Cytokine levels in cord plasma and/or cord blood mononuclear cells from 407 newborns defined the type of immune response. Cytokine responses were measured in culture supernatants, by flow cytometry of lymphocyte subsets and transcript levels by quantitative PCR following stimulation with Pf schizont extract and/or recombinant PfMSP1. Preliminary results indicate malaria exposure during the second trimester compared to the third trimester is associated with elevated IFNg and low IL-10 levels. By contrast the offspring of women exposed to malaria during the third trimester was associated with higher IL-10 levels relative to IFNg levels. More complete results will be available by the time of the meeting. These results suggest that the timing of malaria infection during pregnancy differently affects the newborns' lymphocyte responses and thus implications for the timing and frequency of malaria chemoprophylaxis during pregnancy.

1601

RELATING ANTIBODIES TO THE SURFACE OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTE WITH CLINICAL OUTCOMES

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In areas of high malaria transmission, immunity to disease develops more rapidly than immunity to infection. Proteins expressed on the surface of infected erythrocytes (IE) have been described as the targets of immunity to malaria. Chan et al 2012, reported that the presence of antibodies to 3D7 parasite line is associated with a reduced risk of developing symptomatic malaria. Here, in the context of a longitudinal birth cohort we examined prospectively the association between antibodies to surface IE of parasites circulating among the children and protection from clinical malaria and reduction in parasite density. The study was conducted in Ouelessebougou Mali, where malaria transmission is highly seasonal. Antibodies to IE surface proteins in plasma were measured by flow cytometry. The analysis included samples from 125 children at the end of the transmission season and at the end of the dry season. Median (range) age of the children at the two time points were 17.9 (6-32) and 21 (9.3-39) months. IgG levels were defined as geometric MFI after subtracting MFI with uninfected erythrocytes and reactivity with plasma from naïve donors. On average children included in this analysis experienced 2.2 malaria infections (range 0-12) and 1.4 clinical malaria episodes (range 0-5). The proportion of parasite isolates recognized by the plasma samples and the level of reactivity did not predict protection from clinical malaria. Similarly, antibody reactivity with surface IE proteins was not associated with reduced parasite burden. Here, the children were younger than subjects included in previous studies that reported increased IE recognition with age that could account for the different results. Alternatively, we hypothesize that functional antibodies such as those that inhibit parasite adhesion may be more strongly associated with reduced risk of clinical malaria and parasite density, and these studies are ongoing. We will present data relating anti-adhesion antibodies with surface IE recognition, and the association between anti-adhesion antibodies to multiple receptors and the risk of malaria disease and parasite density.

1602

MALAWIAN NEWBORNS SHARE A VARIANT SURFACE ANTIGEN ANTIBODY REPERTOIRE WITH THEIR MOTHERS THAT IS AFFECTED BY MATERNAL MALARIA INFECTION IN PREGNANCY

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In malaria-endemic regions, severe malaria in infants is rare. Protection may be attributable to maternal antibody transfer during pregnancy. Parasite antigens expressed on the surface of infected erythrocytes are associated with severe malaria pathogenesis. We hypothesized that the repertoire of antibodies to these variant surface antigens (VSAs), particularly ***Plasmodium falciparum*** erythrocyte membrane protein-1 (PfEMP1) antigens, is transferred to newborns, potentially providing protection against severe malaria. A protein microarray was printed with 176 fragments of PfEMP1s based on the 3D7 reference genome, including both extracellular and intracellular fragments, along with three repetitive interspersed family proteins (RIFINs), and six sub-telomeric variable open reading frame proteins (STEVORs). Seroreactivity to these fragments was measured in the second trimester for 36 Malawian mothers in their first or second pregnancy and compared to seroreactivity at delivery and in cord blood samples of their newborns. Eleven mothers had evidence of malaria infection in the placental or peripheral blood during pregnancy. Reactivity to VSAs was higher in sera from women with evidence of malaria infection versus women who did not have malaria during pregnancy at both enrollment and delivery and extended to the cord blood of newborns. Overall, seroreactivity intensity to VSAs was similar in cord blood compared to mothers at delivery. However, for mothers who did not have malaria during pregnancy, seroreactivity for several VSAs was higher in the infant than in the mother. These findings indicate that malaria infection during pregnancy may interfere with antibody transfer and may lead to increased risk of malaria disease during infancy. Future work will examine malaria infection and disease vis-à-vis the rate of decline of VSA antibodies in this infant cohort over time.

1603

CYTOKINE PROFILE IN HELMINTH AND MALARIA INFECTIONS AMONG AFEBRILE AND FEBRILE CHILDREN IN IBADAN, SOUTHWEST NIGERIA

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Intestinal helminths and malaria are among the most prevalent infectious diseases in the tropics. The effect of coinfections on immune response is not clearly understood. We therefore investigated the immune response profile in children with and without symptoms. A total of 78 afebrile school children (20 helminth-malaria co-infected, 17 helminth infected, 19

malaria infected and 22 uninfected) and 75 febrile children (14 helminth-malaria co-infected, 16 helminth infected, 20 malaria infected and 25 uninfected) were recruited into the study. Helminths were screened using Kato Katz method while malaria parasite screening was done using Giemsa-stained thick blood films. Circulating TNF-α, IFN-γ, IL-1, IL-10 and IL-6 concentrations were assessed by ELISA from serum samples. Data were analysed using analysis of variance. Among the afebrile school children, IL-10 was significantly increased in helminth infected children compared with helminth-malaria co-infected, malaria infected and uninfected groups ($p<0.05$). IFN-γ was significantly elevated in malaria and malaria-helminth coinfection relative to helminth alone ($p<0.05$). IL-1 level was significantly higher in single infection of helminth and malaria relative to coinfection and the uninfected groups ($p<0.05$). An insignificant difference was observed for IL-6 and TNF-α concentrations across all the four groups while among febrile children, IL-6 was significantly increased among helminth alone and helminth-malaria coinfection relative to malaria infected group ($p<0.05$). IL-10 was significantly elevated in co-infected group compared with helminth or malaria infected group while TNF-α was significantly increased in helminth and helminth-malaria coinfection compared with uninfected or malaria infected group ($p<0.05$). IFN-γ level was insignificant in the infection groups relative to uninfected group ($p<0.05$). IL-1 level similar across the groups. Helminth infection seem to upregulate Th2 immune response among children with symptomatic uncomplicated malaria while there was no significant changes in Th immune response among afebrile children.

1604

BIOSIGNATURES OF NATURALLY ACQUIRED IMMUNITY UNDER CHANGING MALARIA TRANSMISSION IN MALARIA ENDEMIC AREA OF PAPUA NEW GUINEA

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A simple blood test that assesses levels of naturally acquired immunity (NAI) would provide a critical tool to monitor the impact of interventions to reduce malaria transmission and broaden our understanding of how NAI develops around the world as a function of age and exposure. Since antibodies (Abs) are important in mediating malaria immunity, we postulate when an individual acquires a certain threshold of Abs to panel of malaria antigens (Ags), they become clinically immune to malaria. To examine this hypothesis, we measured immunoglobulin G (IgG) Abs to a panel of 32 ***Plasmodium falciparum*** (Pf) and ***P. vivax*** (Pv) antigens, using a multiplex bead array assay. Samples were collected from two geographically similar cohorts of children aged 5-14 years. The first cohort was completed before malaria control measures (Mugil I, 2004, N=206) and the second cohort was completed following malaria control measures (Mugil II, 2012/13, N=433). Children who were treated with antimalarials at enrollment were observed prospectively for malaria reinfection. High IgG levels to Pf microneme, rhoptry and EBA proteins, which are involved in merozoite invasion of erythrocytes, were strongly associated with protection against clinical malaria, similar to previously published studies from the same cohort. High IgG levels to Pv microneme, rhoptry and some merozoite proteins were significantly associated with protection against Pv infection. IgG levels to almost all the proteins were significantly lower in subjects from the Mugil II versus Mugil I cohorts although some individuals retained high malaria-specific IgG levels associated with protection in the Mugil I cohort. Analysis is currently underway to assess whether individuals that sustained high Ab levels corresponding to the protective threshold found in the Mugil I cohort also confer protection in the Mugil II cohort. These analyses will be completed by the time of the meeting. This study identifies Ags that are possible targets of protective immunity and/or biosignatures of immunity in malaria surveillance and control.

1605

CHARACTERIZATION OF NATURALLY ACQUIRED BINDING-INHIBITORY ANTIBODIES AGAINST *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN IN RURAL AMAZONIA

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Plasmodium vivax (Pv) invades human reticulocytes requiring an interaction of the parasite ligand, Duffy Binding Protein (PvDBPII), with an erythrocyte membrane protein known as the Duffy antigen/receptor for chemokines or DARC. Some Pv exposed subjects acquire functional antibodies that block binding of PvDBPII to DARC (Fy), partially inhibit Pv invasion of reticulocytes ***in vitro***, and correlate with protection against Pv malaria. PvDBPII is highly polymorphic and DARC has a polymorphism which influences PvDBPII binding to DARC. The frequency, persistence, and strain-specificity of these naturally acquired binding-inhibitory antibodies (BIAb) under changing malaria transmission conditions have been poorly characterized. We measured BIAbs from 587 samples from 271 individuals seen 1-8 times in serial cross-sectional surveys, conducted in the Brazilian Amazon between 2010 and 2014, when malaria transmission dropped significantly. 82% of malaria infections were Pv**.** Binding inhibition was measured using an ELISA-based format to look at binding between DARC and multiple PvDBPII variants. Samples with greater than 80% binding inhibition relative to a North American control were defined as high blockers. We found 17% of unique individuals tested had high blocking activity and the proportion of individuals with high blocking activity increased with age. Once high levels of BIAb were obtained, the majority of individuals with repeated sampling retained high blocking activity even in the presence of declining Pv transmission. Some individuals retained high blocking activity with titers as high as 1:640. For most individuals, blocking activity was strain-specific - apparent when samples were titered. The presence of DARC polymorphisms affected acquisition of high BIAbs; 4% of the FyA/Fy- (reduced binding) compared to 16% of all other DARC phenotypes associated with higher binding ($p=0.065$). The presence of high BIAbs may represent a biomarker of naturally acquired immunity in some individuals and may suggest induction of high blocking activity following vaccination with rDBPII, predicting strain-specific protection against Pv.

1606

ANTIBODY SEROREACTIVITY TO *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN 1 DOMAINS AND CLINICAL PRESENTATION OF MALARIA IN MALIAN CHILDREN

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A strong correlate for protection from malaria disease has been largely elusive. ***Plasmodium falciparum*** erythrocyte membrane protein-1 (PfEMP1) is a parasite surface adhesion protein implicated in malaria pathogenesis, particularly for placental and cerebral malaria. We probed a diversity-reflecting protein microarray with pre-rainy season sera collected from 75 children one to six years of age enrolled in a malaria vaccine trial in Bandiagara, Mali. The array was populated with protein fragments representing coupled domains of all PfEMP1 proteins in the laboratory reference strain 3D7. Clinical malaria outcome was defined as parasitemia (>2500 parasites per ul) and presence of fever (>37.5C) during

unscheduled clinic visits during 240 days of follow up. Using a Kaplan-Meier survival analysis, children with above-mean PfEMP1 seroreactivity at baseline had a delayed time until first clinical malaria episode versus those with below-mean PfEMP1 seroreactivity. Lower pre-season seroreactivity to PfEMP1 domains was associated with an increased odds of having at least one clinical malaria episode, controlling for covariates. Pre-season PfEMP1 seroreactivity was significantly associated with time to clinical malaria episode in a multivariable Cox proportional hazards model; suggesting that antibodies directed at PfEMP1 may have an association with protection distinct from other malaria antigens. In addition, pre-season seroreactivity to CD36 binding PfEMP1 domains correlated with increased time until clinical malaria episode compared to non-CD36 binding domains. These results suggest the importance of PfEMP1 antibodies in the prevention or delay in the progression of more severe instances of symptomatic malaria, particularly when compared to antibodies directed at other malaria candidate vaccine antigens. Multivariable regression models are particularly powerful in using protein microarray results to identify antigens associated with natural protection against malaria.

1607

INTERFERON GAMMA AND INTERLEUKIN-10 PROFILE IN PATIENTS WITH *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN LAGOS, NIGERIA

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Malaria is the world's most prevalent parasitic disease and is a top public health concern although currently, its control and interventions has been scaled up in Nigeria. Challenges encountered in the design of a successful vaccine against ***Plasmodium*** are our dearth knowledge of protective immunity and how it can be induced. The immune response to ***Plasmodium falciparum*** infection involves interplay between different cell types and cytokines. Therefore, a greater appreciation of the mechanisms of protective immunity and of immunopathology would provide crucial clues as to how manipulation of the immune system may best be achieved. The study aimed to determine the plasma profile of IFN-γ and IL-10 in malaria patient drawn from a ***P. falciparum***-endemic area in Ikorodu LGA of Lagos State, Nigeria. A cross sectional study of 1650 participants who assessed malaria diagnostic services at our study sites (four health facilities at Ikorodu LGA, Lagos State) were screened for malaria by microscopic method using Giemsa stained thick and thin blood films. The plasma level of IL-10 and IFN- γ was assessed among 140 patients who were diagnosed as having ***P. falciparum*** infection and 8 malaria negative subjects, using indirect enzyme-linked immunosorbent assays (ELISA) (MABTECH AB, Sweden). The mean plasma concentration of IL-10 was found to be elevated (6966.8 ±5028.2 pg/ml) in malaria infected individuals compared to the aparasitemic control group 5747.6 ± 3861.7 pg/ml and this was statistically significant ($p<0.01$). In contrast IFN-γ levels were found to be lower in malaria patients (17277.6 ± 12055.8 pg/ml) compared to non-malaria patients tested (23362.4 ± 11341.3pg/ml). There was however a significant relationship between IL-10 and parasite density ($r=0.213$; p value= 0.009); negative correlation between age and parasite density ($r=-0.163$; $p=0.048$). In conclusion, levels of IFN- γ and IL-10 and the relative balance between the pro- and anti-inflammatory cytokines response, illustrates how populations residing in areas of varying disease endemicity may respond to ***P. falciparum***-induced immune challenge.

1608

LIVER STAGE MALARIA INDUCES INTERFERON-DEPENDENT GENE EXPRESSION IN HEPATOCYTES

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Malaria is a serious global health problem that causes substantial morbidity and mortality in developing countries. Infection is caused by ***Plasmodium*** sporozoites, which are transmitted to a mammalian host through a bite from an infected female ***Anopheles*** mosquito. Sporozoites then travel from the skin to the liver, invade hepatocytes, and quickly replicate to produce tens of thousands of first generation infectious merozoites. These merozoites initiate the blood stage infection that is responsible for symptomatic illness. Live-attenuated sporozoite vaccination can prevent the onset of disease by inducing sterile, adaptive immunity against liver stage infection. However, few studies have explored whether parasite liver infection engenders localized innate immune responses as well. Recent studies in our laboratory have shown that ***Plasmodium*** induces type I interferon (IFN) responses in the liver, and that these responses can protect from a secondary liver stage infection. We show here that these type I IFNs are produced by infected hepatocytes and coincide with the induction of pro-inflammatory Interferon Stimulated Genes (ISGs) such as the chemokine CXCL10 and the anti-viral protein Viperin. Furthermore, we demonstrate that ***Plasmodium*** infection of hepatocytes impacts expression of the JNK signaling pathway in a type I IFN-dependent manner. Future work will help uncover how these initial responses impact inflammatory gene expression, liver stage parasite growth, and the progression to blood stage infection. This knowledge will have significant impact on the selection and use of adjuvants in the future development of vaccines targeting the liver stage of malaria infection.

1609

ANALYSIS OF ANTIBODIES IN LYMPHOCYTE SECRETIONS (ALS) OF MALIAN CHILDREN WITH ACUTE MALARIA REVEALS PLASMABLASTS AND MEMORY B-CELL SUB-POPULATIONS OF DIVERSE ANTIGEN SPECIFICITY

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During a primary response to an acute infection, naïve B cells are activated in the presence of Follicular Helper T cells within the follicles of secondary lymphoid tissue leading to clonal expansion and affinity maturation to produce antigen-specific memory B cells and plasma cells in the germinal center (GC). Prior to homing into secondary lymphoid tissue, immature plasma cells (plasmablasts) circulate in the blood producing the initial protective antibodies against invading pathogens. After several days, plasmablasts die or differentiate into mature plasma cells which migrate to the bone marrow to become long lived plasma cells. Affinity-matured plasma cells and memory B cells provide the antibody response to subsequent infection by the same pathogen. To evaluate the plasmablast and memory B cell/plasma cell compartments generated after infection in the context of malaria, we employed the ALS assay and proteome microarray. PBMCs and serum samples were collected from Malian children with malaria at day 14 post infection and from malaria-naïve US adults. PBMCs were cultured in media containing stimulatory factors and after 3 days the culture media was removed and replaced with fresh media. PBMCs were then cultured for 3 additional days and final culture supernatants were collected at day 6. Sera and culture supernatants

containing lymphocyte secretions were probed on Pf1000 microarray containing 1000 proteins from ***Plasmodium falciparum*** strain 3D7. Our results show that the antibody profile of 3-day (plasmablast) cultures is a subset of the 6-day (memory B cell/plasma cell) cultures and serum antibody profiles. The results also reveal a sub-population of antibodies present only in the memory B cell/plasma cell compartment and in serum. Our approach successfully illustrates how combining ALS assay and proteome arrays can be used to rapidly profile serum antibody and antibodies in the plasmablast and memory B cell/plasma cell compartments after an acute infection.

1610

SERO-EPIDEMIOLOGICAL EVALUATION OF *PLASMODIUM FALCIPARUM* MALARIA IN SENEGAL

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In Senegal, significance decrease of malaria transmission intensity has been noted the last years. In such a context parasitaemia has become lower and therefore more difficult to be detected by microscopy. In the context of submicroscopic parasitaemia, it has become relevant to rely on relevant malaria surveillance tools to better document malaria epidemiology in such settings. Serological markers have been proposed as an essential tool for malaria surveillance. This study aimed to evaluate the sero-epidemiological situation of ***Plasmodium falciparum*** malaria in two sentinels sites in Senegal. Cross sectional surveys were carried out in Velingara (South of Senegal) and Keur Soce (Center of Senegal) between September and October 2010. Children less than 10 years living in these areas were enrolled using two level random sampling methods. ***P. falciparum*** infection was diagnosed using microscopy. Pf antibodies against Circum Sporozoït Protein (CSP), Apical Membrane Protein (AMA1) and Merozoït Surface Protein 1_19 (MSP1_42) were measured by ELISA method. A stepwise logistic regression analysis was done to asses factors associated with Pf antibodies carriage. A total of 1865 children less than 10 years were enrolled. The overall Pf malaria prevalence was 4.99% with high prevalence in Velingara 10.03% compared to Keur Soce 0.03%. Symptomatic malaria cases (fever associated with parasitaemia) represented 17.37%. Sero-prevalence of anti-AMA1, anti-MSP1 and anti-CSP antibody was 38.12%, 41.55% and 40.38% respectively. The sero-prevalence was more important in Velingara and increased with age, active malaria infection and area of residence. Conclusion: The use of serological markers can contribute to improve malaria surveillance in areas with declining malaria transmission. This study provided useful baseline information about the sero-epidemiological situation of malaria in Senegal and can contribute to the identification of malaria hot spots in order to concentrate intervention efforts.

1611

DEVELOPMENT OF A MICROSCALE INHIBITION OF LIVER STAGE DEVELOPMENT ASSAY FOR *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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Vaccine development targeting sporozoite and pre-erythrocytic stages of the ***Plasmodium*** parasite is one of most challenging areas in malaria research because biological complexities, such as inadequate methods for ***in vitro*** culture, result in the lack of a functional assay capable of visualizing and quantifying antibody inhibition. This study describes the development of a new type of Inhibition of Liver Stage Development Assay (ILSDA) utilizing cryopreserved sporozoites paired with an ***in vitro*** human liver model based on a microscale platform. Proof of concept is demonstrated in experiments targeting the ***P. vivax*** and ***P. falciparum*** circumsporozoite (CSP) protein with inhibitory concentrations of species-specific anti-CSP monoclonal antibodies. The bioassay was found to be sensitive and efficient, showing the blocking of pre-erythrocytic stages of both ***P. vivax*** and ***P. falciparum*** in a concentration dependent fashion. Additionally, the assay allows for further functional validation as immunofluorescence imaging revealed a potential anti-CSP mechanism involved in the post-invasion inhibition of sporozoite and liver stage development. This study provides an improved ILSDA that can be used to evaluate future drug and vaccine candidates targeting the pre-erythrocytic stages of ***P. falciparum*** and ***P. vivax***.

1612

IDENTIFYING CRITICAL BIOLOGICAL AND IMMUNOLOGICAL ROLES OF GLYCOSAMINOGLYCANS IN THE DEVELOPMENT OF PLACENTAL MALARIA

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During pregnancy, the developing fetus must be protected from the maternal immune system. The maternal immune system is considered to be in an altered or compromised state during pregnancy and this is achieved by factors released by the placenta, including hormones and cytokines. Placental malaria is characterised by the accumulation of ***Plasmodium falciparum***-infected erythrocytes (IE) in the placenta, and results in poor outcomes for both the mother and the fetus, including anaemia, low birth weight and miscarriage. Pregnancy-specific IE bind to the glycosaminoglycan chondroitin sulphate A within the placenta. We hypothesise that chondroitin sulphate containing proteoglycans act as immune system modulators during pregnancy and that the binding of IE to chondroitin sulphate A in the placenta allows the establishment of the initial parasite niche. Here, we show that bovine-derived chondroitin sulphate A dampens the immune response of human peripheral blood mononuclear cells (PBMCs) to IE and bacterial products. In the presence of chondroitin sulphate A, monocytes produced less tumour necrosis factor, had differential expression of pattern recognition receptors and were skewed towards a classical phenotype. We show that proteoglycans derived from human placental tissue also modulate the monocyte response to pathogen products and discuss the implications of the parasite interacting with the placental barrier.

1613

MEASURING ANTIBODY-MEDIATED PROTECTION AGAINST *PLASMODIUM FALCIPARUM* CHALLENGE IN LIVER CHIMERIC-HUMANIZED MICE

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Immunization with live-attenuated sporozoites has proven to be the most efficacious means of vaccination against malaria parasite infection, yielding complete sterilizing protection in controlled human malaria challenge trials. These encouraging clinical results not only provide a strong rational for development of a live-attenuated parasite vaccine, but enable exploration of immune correlates of protection which further accelerate malaria vaccine development and will inform the design of subunit malaria vaccine candidates. However, there are limited tools available to directly assess contribution of cellular and humoral immune responses to protection. Currently, functional analysis of anti-***Plasmodium falciparum*** sporozoite antibodies is largely limited to ***in vitro*** invasion assays using hepatoma cells, an environment which does not accurately mimic the complex journey of sporozoites from the dermis to the liver parenchyma. Here, we have optimized ***in vivo P. falciparum*** infection in a humanized liver-chimeric mouse model where sporozoite challenge is delivered by mosquito bite and liver stage parasite burden quantified by bioluminescent imaging. This model supports measurement of protection by passive transfer of polyclonal antibodies from individuals immunized with whole sporozoites as well as monoclonal antibodies to individual sporozoite proteins such as CSP. We found this method to be sensitive and reliable in directly detecting varying degrees of antibody efficacy against sporozoite liver infection while allowing for animal survival and simultaneous analysis of parasite progression to blood stage infection. We will present data on the inhibitory activity of serum and IgG elicited by different types of attenuated sporozoite immunizations as well as data for antibodies to specific parasite proteins. The liver-chimeric humanized mouse/challenge model thus constitutes the most physiologically relevant system for analysis of antibody-mediated protection against malaria infection. As such it will accelerate the identification of new antibody targets and the establishment of immune-correlates of protection.

1614

NOVEL SEROLOGIC BIOMARKERS PROVIDE ACCURATE ESTIMATES OF RECENT *PLASMODIUM FALCIPARUM* EXPOSURE FOR INDIVIDUALS AND COMMUNITIES

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Tools to reliably measure ***Plasmodium falciparum*** (Pf) exposure in populations are needed to guide and evaluate malaria control interventions. Serologic assays can potentially produce precise exposure estimates at low cost; however current approaches, based on responses to a few established antigens, are not designed to detect recent changes or estimate exposure in individuals. Pf-specific antibody responses differ by antigen, suggesting that selection of antigens with defined kinetic profiles will improve estimates of Pf exposure. We tested this hypothesis by evaluating responses to 856 Pf antigens via protein microarray in 186 Ugandan children, for whom detailed Pf exposure data in the prior year were available. Using data-adaptive statistical methods, we identified combinations of antibody responses which maximized information on exposure. Considering individual exposure, responses to three novel Pf antigens accurately classified whether an individual had been infected within the last 30, 90, or 365 days (cross-validated AUC: 0.86-0.93), while responses to another three antigens accurately estimated malaria incidence in the prior year. Considering community exposure, cross-validated incidence predictions provided accurate stratification of exposure between communities and suggest that precise estimates can be obtained from analysis of a single plasma sample from a small subset of the population. In addition, serologic incidence predictions accurately characterized heterogeneity within a community, performing nearly as well as one year of incidence data. Development of simple, ELISA-based assays derived from the successful selection strategy outlined here offers the potential to generate rich epidemiologic surveillance data that will be widely accessible to malaria control programs.

1615

HOUSE STRUCTURE AND SLEEPING ARRANGEMENT AFFECTS BED NET USE IN THE GAMBIA

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Although coverage of Long-Lasting Insecticide-treated Nets (LLIN) has steadily increased, a growing number of studies report gaps between net ownership and use. In order to achieve universal coverage and use of LLIN, there is a need to identify those population sub-groups that are least protected. Certain types of housing structures and inside-outside sleeping arrangements may make the attempt to use bed nets difficult. This study aimed to identify risk groups by understanding the relationship between

bed net use, housing structure and inside-outside sleeping patterns in the Gambia. An ethnographic study was carried out in the Gambia between April 2013 and November 2014 in two phases: a first explorative phase in 12 rural villages representing a variety of ethnic groups, and a second phase in which two villages with different traditional housing styles were selected as cases for in-depth analysis. In addition to net availability, net use is affected by housing structure and sleeping patterns inside and outside the house. Children, women and men often rest and sleep unprotected outside their houses during the early evenings. Boys leave the bed of their mother at a younger age compared to girls, and as such are less likely to sleep under LLIN. Boys and adolescent men are particularly at risk because they are not prioritized when allocating nets, are more likely to rest and sleep outdoor in public spaces, and often move between different sleeping spaces. In addition, particular subgroups are unlikely to use their bed nets due to late night activities such as the protection of farms, the herding of cattle, charcoal burning, fishing and hunting. Malaria risk varies substantially between different regions in the Gambia, which could partially be explained by the variable uptake of malaria control interventions such as LLINs. Designing bed nets adapted to the local context and taking into account people's net preference may increase the uptake of LLINs in particular risk groups.

1616

SUPPLY CHAIN MANAGEMENT CONSIDERATIONS FOR LONG-LASTING INSECTICIDE-TREATED BED NETS CONTINUOUS DISTRIBUTION SYSTEMS

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Many countries, as part of their national malaria strategy, use a combination of mass distribution of free long-lasting insecticide-treated bed nets (LLINs) and continuous distribution of LLINs through health facilities as a proven intervention to reduce malaria-related morbidity and mortality. LLINs have unique supply chain characteristics that must be considered when implementing continuous distribution systems. Generally, LLINs have long procurement lead times. Because they contain pesticides, their logistics management is often outside malaria medicines and tests. They are extremely bulky, which has implications for attendant storage and inventory management. Expert coordination is required because they may be managed at different points within a facility, and by different cadres of staff. LLINs can be a target for theft, underscoring the need for accountability; as they need to be replaced periodically, their demand is ongoing. The demand for LLINs for campaigns may take priority over LLINs earmarked for routine distribution. Program managers should conduct a logistics system design activity by describing how commodities and information moves through the different levels of the health system. As part of this design, a logistics management information system (LMIS) is included_managers should collect consumption data on LLINs and days out of stock. Reporting and resupply should be linked. To calculate resupply, consumption data and stock on hand data should be used. Accountability of LLINs throughout the supply chain is critical, and information detailing the movement and use of LLINs must be tracked and verified. To accommodate the bulkiness of LLINs, minimal buffer stock should be used to reduce the required storage space; if possible, smaller bale sizes should be procured. System designers should consider the advantages and disadvantages of longer review periods; they must also ensure sufficient and secure storage. The quantification of LLINs for continuous distribution and campaigns should be coordinated; program managers should routinely compare LMIS data and health management information systems data.

1617

INSECTICIDE-TREATED NET DISTRIBUTIONS IN SUB-SAHARAN AFRICA: AN ASSESSMENT OF MASS CAMPAIGNS FROM 2006-2014

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Insecticide-treated mosquito nets (ITNs) are one of the most cost-effective malaria interventions. This aim of this project is to compile and analyze data from ITN distribution campaigns in Sub-Saharan Africa from 2006-2014. A database of ITN distribution campaigns occurring from 2006-2014 was assembled by extracting data from weekly conference calls organized by the Alliance for Malaria Prevention, and supplementing campaign data through a systematic review of published literature. We identified 95 ITN campaigns occurring from 2006 to 2014. The defining characteristics of ITN distribution campaigns were determined to be geographic location, length, targeting, integration, allocation, and delivery system. There were campaigns across all geographic regions of Africa, with the majority in West Africa. The lengths of campaigns were found to be highly varied from a couple days to a few years. Several other common characteristics were noted: campaigns tended to target the general population rather than solely focusing on pregnant women and children under five years old; among campaigns aiming to achieve universal coverage, the most commonly used net allocation formula was one net per two people, although there were several other formulas utilized; and the majority of campaigns directly delivered the ITNs, while few campaigns used voucher schemes. Of the campaigns that reported integrating the distribution with other public health interventions, a majority were integrated with immunization campaigns. The most widely cited problems in planning and implementing campaigns were financial and availability of nets at the time of the campaign. The results of this analysis have highlighted a number of needs pertaining to the surveillance and monitoring of insecticide-treated net distribution for the prevention of malaria. With such a large amount of resources being invested in ITNs as a malaria intervention, there is a need for systematic tracking of campaigns. Assessments of ITN distribution mechanisms should continue, and the results should be incorporated into campaign planning processes.

1618

DEVELOPING NOVEL *IN VITRO* MODELS OF HEME-INDUCED DISRUPTION OF BLOOD-BRAIN BARRIER

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Background: The blood-brain barrier (BBB) breakdown is the major pathological change of cerebral malaria. The BBB is an active interface which contains cerebral endothelial cells sealed together by tight junctions (TJ), the basement membrane, and pericytes as well as astrocytes. In this study, we tried to develop an ***in vitro*** BBB model based on the culture of human brain endothelial cells (HBVEC) and C6 glioma cells to test the effects of heme on the TJs.Methods: Co-culture (Trans-well): C6 glioma cells were grown in the bottom of the lower chamber which possibly influence the other cells by secreted factors into the media. 24 h later, HBVECs were grown on a filter insert coated with attachment factor for 24 h. After starvation with serum-free medium for 24 h, HBVECs were then treated with 60μM heme for another 24 h. Staining by anti- occluding, claudin and ZO-1 antibodies were visualized using the Alexa-488 fluorescence system. Results: In mono-culture systems, phase contrast images show the spindle shaped morphology o HBVECs in culture. Proteins occludin, claudin-5 are located in the cell membrane, while ZO-1 is located in cytoplasmic region. However the cells are not

capable of forming a continuous line of tight junctions at the cell borders as detected by staining with antibodies against the TJ proteins occludin, ZO-1 and claudin-5. In co-culture systems, transmembrane protein occludin and cytoplasmic protein ZO-1 show a clear and continuous membrane staining, which reveals a line of tight junctions at the cell borders. However, the normal structure of tight junctions is significantly lost after heme treatment. In addition, heme also decreases expression of occludin, ZO-1 and claudin-5, and induces an irregular cell border staining.Conclusions: Data regarding baseline expression of TJ proteins and changes in expression of these proteins in response to heme stimuli can be obtained by using ***in vitro*** co-culture BBB models. These co-culture conditions provide a BBB model that is easy to use to perform histological analysis and permeability test induced by heme.

1619

COMPARISON OF MALARIA DIAGNOSTIC TESTING AND TREATMENT OUTCOMES AT OUTLETS PARTICIPATING IN A PROJECT TO SUPPORT PRIVATE SECTOR RDT ADOPTION IN MBEYA, MOROGORO AND TANGA, TANZANIA: RESULTS FROM CLIENT EXIT INTERVIEWS

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Tanzania's malaria treatment guidelines state that all suspected cases should be confirmed prior to treatment. By late 2012 RDTs had been rolled out to public facilities nationwide, supported by a comprehensive training plan. As part of an intervention to support increased RDT availability and correct use in the private sector in Tanzania, we conducted client exit interviews at 26 project (intervention) and 26 matched non-project (control) facilities 8 months into the project. The aim was to describe differences in testing and treatment outcomes between the two groups. Eligible outlets were those that offered any diagnostic testing services at the time of interview and eligible clients were adults seeking treatment for fever for themselves or on behalf of someone else. In total, 2,383 clients were screened and interviews were conducted with 1,118 eligible clients. Clients at intervention clinics were slightly less likely to be tested than those at control clinics (89.4% compared to 94.8%, p=0.08), and intervention clinics presented greater clinic-level variation in testing levels (min: 45.1%, max: 100%) than did control clinics (min: 73.3%, max: 100%). Overall, 46.1% of tested clients reported testing positive for malaria, with no significant difference between the groups. 86.8% of test-positive clients received ACT at intervention clinics, compared with 77.2% at control clinics (p=0.3), and less than 5% of test-negative clients received any antimalarial in both groups. The study provides some evidence that when both microscopy and RDTs were available RDT use was more common in intervention clinics (44.0%) than control clinics (25.4%, p=0.09), while microscopy was more common in the control clinics (65.4% compared to 41.5%, p=0.06). Preliminary modelling suggests clients tested by microscopy were twice as likely to receive a positive test result, and that treatment outcomes depended on both test result and type of test. Understanding variations between and within study groups is important to ensure efficient and cost-effective performance improvement steps are taken to improve malaria case management in these private facilities.

1620

QUINAZOLINDIONE SERIES IDENTIFIED FROM TCAMS: A NEW ANTIMALARIAL SERIES WITH POTENTIAL FOR BLOCKING TRANSMISSION OF THE DISEASE

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Nowadays, Malaria is still one of the major global health problems. ***Plasmodium*** has been able to adapt to the different treatments developed by humans along history. However there has not been such a wide knowledge of the illness as we currently have. This fact, joined to the urgent need for novel antimalarial drugs that can replace ACTs and the awareness of governments/health systems/funding agencies, makes the current time a unique opportunity to change the course of this disease and achieve the control and finally the eradication. Using a Phenotypic screening approach, in 2010 GSK published the Tres Cantos antimalarial set (TCAMS) which comprises over 13,533 hits derived from whole cell screening of 2M compounds from the GSK corporate collection against ***P. falciparum***. A clear strategy was required to rapidly identify those molecules that have both the best chance of being converted into differentiated antimalarial drugs and that are also likely to have the lowest risk of attrition in development. Identification of a new class of anti-malarial agents that possess dual activity and are able to inhibit the asexual blood-stage (schizonticidal, responsible of disease symptoms) as well as block transmission was initiated in our group. As a result, a new assay to screen compounds for their potential to inhibit late stage gametocytes was developed and used successfully to screen the output from TCAMS. Quinazolindione series was identified as a very promising family with dual activity (both schizonticidal and gametocytocidal). Initial weaknesses of the series were modest ***in vitro*** and ***in vivo*** potency as well as poor pharmacokinetic profile. After a Lead Optimisation program, Late Leads have been identified having excellent ***in vitro*** and ***in vivo*** potency, a very good developability profile and potential for targeting two different TCPs (Target Compound Profiles). Medicinal Chemistry strategy followed during the Lead Optimisation program was focused on improving the physicochemical and developability properties.

1621

"THERE IS NO FREE HERE, YOU HAVE TO PAY": ACTUAL AND PERCEIVED COSTS AS BARRIERS TO INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY IN MALI

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"There is no free here," the words of a Malian husband, illustrate how perceptions of cost can deter uptake of intermittent preventive treatment of malaria in pregnancy (IPTp). Following WHO recommendations, the Malian Ministry of Health (MOH) recommends three doses of IPTp at monthly intervals. However, despite a national policy that IPTp be provided free of charge, only 35% of pregnant women receive at least one dose and less than 20% receive two or more doses. We explored perceptions and experiences of IPTp cost in Mali, and their impact on uptake, using qualitative interviews and focus groups with pregnant women, husbands and mothers-in-law. We also interviewed and observed health workers at four health centers two in Sikasso Region and two in Koulikoro. Despite national-level policies, actual IPTp costs varied widely at our study sites - between regions, facilities, and visits. Pregnant women may pay for IPTp, receive it free, or both at different times. Health centers often charge a lump sum for ANC visits that include both some free and some fee-based drugs and services. This makes it difficult for women and families to decipher which services are free and which require payments. As a result, some forego even free care that, because it is not itemized, appears not

to be free. Varying costs also complicate household budgeting for health care, particularly as women often rely on their husbands or husbands' families for money. While health facilities operating under the cost-recovery model strive to provide free IPTp, their own financial constraints often make this impossible. Preventing malaria in pregnancy depends upon women receiving the recommended doses of IPTp. However, it is clear that both actual and perceived costs are currently barriers to IPTp uptake. Given the confusion around cost of services in the two study regions, more detailed national-level studies of both perceived and actual costs could help inform policy and program decisions promoting IPTp. These studies should evaluate both quantitatively and qualitatively the cost information provided by health facilities and pharmacies to pregnant women and their families.

1622

BENEFICIARY SATISFACTION ASSESSMENT TO GUIDE HEALTH PROGRAM COMMUNICATION AND INCREASE ACCEPTANCE OF INDOOR RESIDUAL SPRAYING

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Indoor Residual Spraying (IRS) is considered effective when 85 percent of a community's homes are sprayed. However, there are logistical and educational barriers that affect residents' decisions to accept IRS and a program's ability to fully protect a targeted area from malaria infection. Current behavioral change communication (BCC) efforts for an IRS campaign primarily require paying hundreds of mobilizers to walk long distances to reach residents during work hours, when people are typically not home. BCC efforts seek to highlight the dangers of malaria, the benefits of IRS, and how and when people can prepare their homes for spraying; but, these messages are not universally received. After the 2014 IRS campaign in Zimbabwe, the President's Malaria Initiative-supported (PMI) Africa Indoor Residual Spraying (AIRS) project randomly surveyed 485 households in four IRS districts to assess beneficiary IRS knowledge and satisfaction, and identify locally-preferred methods of IRS messaging. Two thirds of the respondents were females, roughly 43 years old, reporting a high level of IRS and malaria knowledge. However, preliminary findings show that the BCC campaign did not reach all target areas or publicize clearly the IRS schedule. It is crucial to finish an IRS campaign in the fewest number of days possible, since each additional day needed to revisit an unprepared village adds significant costs to the program budget. AIRS Zimbabwe is using the beneficiary feedback to reshape their BCC messaging and improve operational efficiency in 2015. I will present the full findings of the assessment and explain how this activity, a first for the project, can also improve spray coverage in the other AIRS countries.

1623

THE MASS DISTRIBUTION OF LONG-LASTING INSECTICIDE-TREATED NETS IN SOUTHERN PROVINCE, ZAMBIA: HOW MUCH DOES IT COST?

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The Zambian Ministry of Health and partners are implementing a package of interventions and surveillance systems in Southern Province in support of the national malaria control and elimination agenda. As part of this package, mass distribution of long-lasting, insecticide-treated nets (LLINs) was implemented in 2014 with the aim of achieving universal coverage of all household sleeping spaces and a net utilization rate of 80%. We estimated the cost of this mass distribution of LLINs in 183 health facility catchment areas (HFCAs) in ten districts in Southern Province. Various partners were engaged at different stages of implementation, which included importing LLINs with delivery to Lusaka, delivery from Lusaka to multiple staging locations in Southern Province, delivery from staging locations to specific HFCAs, and then delivery by community health workers (CHWs) to households in their catchment area. Primary data on unit costs of various resources used during implementation, quantities of resources used, and key programmatic outputs were obtained from programmatic records. The average implementation cost per HFCA in 2014 was US$28,892. Procurement of LLINs and delivery to Lusaka accounted for 54% of the total cost. Other costs included storage, loading, offloading (43%), and labor for CHWs, health facility, and district-based staff (3%). On average, 1,800 households_representing approximately 5,300 sleeping spaces_received LLINs in each HFCA during the distribution. A coverage rate of 80% was achieved. The average cost per sleeping space reached was US$5.45 (US$15 per household reached). These results are preliminary and do not yet include costs associated with training and supervision of implementation. Costs of LLIN distribution and other malaria interventions are useful for budgeting and planning, identifying opportunities to improve program efficiency, and informing additional analyses on the cost-effectiveness and budget impact of different approaches to malaria elimination.

1624

DETERMINING THE OPTIMAL MODE FOR DELIVERY OF SEASONAL MALARIA CHEMOPREVENTION IN MALI

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Seasonal malaria chemoprevention (SMC), the administration of complete therapeutic courses of antimalarials to children aged 3-59 months during the malaria transmission season, is a new strategy recommended by WHO for malaria control in Sahelian countries with seasonal transmission such as Mali. The strategy is a highly cost-effective approach to reduce malaria burden in these areas. Despite the huge benefit of SMC on malaria infection and disease, the optimal approach to deliver SMC remains to be determined. While fixed-point delivery (FPD) combined with non-directly observed treatment (NDOT) by community health workers is attractive for the SMC implementation, it needs to be evaluated and compared to other modes of delivery. To determine the optimal mode (fixed-point (FPD) vs door-to-door delivery (DDD); directly observed treatment (DOT) vs. non-DOT (NDOT)), 32 villages in four health sub-districts were randomized to receive three rounds of SMC with SP+AQ at monthly intervals using one of the following methods: FPD+DOT; FPD+NDOT; DDD+DOT; DDD+NDOT. The primary endpoint was SMC coverage assessed by cross-sectional survey in 2,132 children at the end of intervention period. Coverage was defined as the proportion of children who received all three days of SMC treatment during the three monthly rounds (primary endpoint). Coverage was significantly higher in children who received SMC using DDD compared to FPD (76.1% versus 62.2%), p = 0.0028). It was similar in children who received SMC using DOT or NDOT (68.2% versus 68.6%; p = 0.95). When the four arms are compared, coverage was highest in DDD+NDOT (77.7%), followed by DDD+DOT (74.5%), FPD+DOT (64.2%) and FPD+NDOT (60.0%), p =0.038. In summary, door-to-door delivery of SMC provides better coverage than FPD. Directly observed therapy, which requires more time and resources, did not improve coverage with SMC.

1625

MDA-A: AN ANDROID-BASED ODK TOOL TO SUPPORT FIELD DATA COLLECTION AND MANAGEMENT FOR A MALARIA MASS DRUG ADMINISTRATION TRIAL IN ZAMBIA

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Since 2011, the Zambia Ministry of Health (MoH) has developed time-limited antimalarial treatment campaigns conducted by trained community health workers (CHWs) to reduce the malaria parasite reservoir in an area of Southern Province with current high levels of malaria prevention coverage. These operational research activities focused on mass drug administration (MDA) or focal MDA (fMDA) targeting 60 health facility catchment areas (HFCAs) randomized for participation. Key to monitoring uptake of treatment interventions is collection of intervention data on a large scale across all HFCAs. Data collection tools were developed and have been used by CHWs and enumerators that visit households as part of a cross-sectional campaign or longitudinal cohort study. An application, MDA-a, was developed on an Android platform using ODK survey software. It is used to document data of all eligible household participants including their existing coverage and use of prevention interventions, travel histories, and results from malaria parasite tests. Adherence monitoring to taking DHAp (anti-malaria drug dihydroartemisinin-piperaquine) for mass and focal treatment administration is also done by in-field data exchange among adherence officers accompanying testing pairs. The application monitors the campaign process by the CHW-enumerator pair team and adherence to treatment administered by the testing pair team matched to individuals from a household. We will describe the development of MDA-a, using android-based smart phones at catchment level for large volume data handling, to effectively plan, implement, and monitor MDA activities. The implementation of MDA-a, its functionality, and field use experience during the two rounds of the MDA trial in Zambia will be discussed. Results show that in the first round a total of 35,996 households were involved in the MDA and fMDA arms, with 81% of household members tested for malaria in the fMDA arm, and 89% in the MDA arm. Malaria incidence in these areas was low to modest, with a rapid diagnostic test prevalence of 8.3%.

1626

MASS TESTING AND TREATMENT FOR MALARIA FOLLOWED BY WEEKLY VISITS TO SCREEN FOR FEVER CASES IN LOW TRANSMISSION AREAS IN MATAM AND LOUGA REGIONS, SENEGAL: A PILOT STUDY

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Population-wide interventions using antimalarial drugs to decrease the reservoir of ***Plasmodium falciparum*** infection are malaria elimination tools that need further evaluation in different transmission settings. A pilot quasi-experimental study of a malaria mass testing and treatment (MTAT) followed by weekly screening of fever cases was conducted in six health post catchment areas in the districts of Kanel, Linguère, and Ranérou (Senegal). Seven adjacent health posts with similar characteristics and malaria incidences were selected as controls. Villages within the catchment areas were stratified according to the 2013 incidences of passively detected malaria cases, and those with an incidence ≥15 cases/1000/year were targeted for an MTAT early in the 2014 malaria transmission season (September). All households were visited, all consenting individuals were tested with a rapid diagnostic test (RDT), and, if positive, treated with dihydroartemisinin-piperaquine. Following the MTAT, a weekly screening of fever cases, testing (only fever cases were tested), and treatment was conducted in all households throughout the transmission season until end of January 2015. To evaluate the impact of the interventions, the incidence of passively detected, RDT-confirmed malaria cases at the health posts will be compared before and after the intervention and between intervention and control villages. During the MTAT, 1954 (83%) of 2361 total households were visited, 16,583 (86%) out of 19,389 individuals were tested, and of those 1.5% had a positive RDT (ranging from 0.5% to 5.2% by health post). Overall, 44% of infections occurred in households with at least one other infection and 94% of households did not have any RDT positives; 82% of RDT-positive individuals were younger than 20 years old, 72% were asymptomatic (no fever or history of fever in the last 24 hours), and 85% completed treatment. All reported adverse events (9.5% vomited, 3.5% had fever and 0.4% had itching) were mild and tolerable. Results of the impact evaluation, assessment of geographical clustering of infections, and estimates of implementation costs will be available in mid-2015.

1627

REVIEW OF DATA STRENGTHENING PROCESS FOR MALARIA SURVEILLANCE SYSTEM, ZAMBIA

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A robust, high-quality surveillance system is crucial for the Zambia Ministry of Health to achieve its goal of at least five malaria free zones by 2015. Effective malaria control depends on timely acquisition of high-quality data to efficiently deploy supplies, plan interventions, and focus attention where most needed. To inform action and monitor progress towards this goal, MACEPA supported the Zambia National Malaria Control Program to establish a scalable Rapid Reporting system at health facility and community levels in 2011. This system is currently active in 36 districts and was designed to track malaria outpatient trends, malaria diagnostics, and treatment commodities at health facility and community levels using the DHIS2 mobile reporting platform with low cost mobile phones. Data quality standards to strengthen malaria surveillance are enforced at two levels. Within DHIS2, dashboard charts are used to identify outliers, data validation rules are checked to identify logical errors, and summary reports are run to review completeness and timeliness. At the facility and community levels, routine data quality audits (RDQA) compare data in source documents with reported data on DHIS2. The RDQA approach includes monitoring and technical support (MTS) to discuss key findings, develop data quality improvement action plans, and provide necessary field-level support. The RDQA and MTS approach identifies data to be corrected and reporting procedures to strengthen, provides feedback to community and health facility staff, and promotes capacity building and collaboration across multiple levels of the health system. Factors identified in 2015 that affect reporting completeness and timeliness include staff turnover with poor hand-over procedures, poor network coverage, and damaged or lost phones. Sources of most data quality problems included entering data incorrectly on mobile phones and incomplete aggregation of data from registers. Thus, integration of monitoring and technical support with data audits can provide instant on-site capacity building and promote collaboration between health facility staff and the local health teams.

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CASE INVESTIGATION WITH REACTIVE FOCAL TESTING AND TREATMENT FOR MALARIA IN A LOW-TRANSMISSION AREA IN AMHARA REGION, ETHIOPIA

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When malaria transmission is low, investigation of individual cases and neighboring households is a means of identifying and containing the source and spread of infections. Case investigation with testing and treatment for malaria in the case and neighboring households was implemented in ten intervention villages in Amhara Region, Ethiopia during the 2014 transmission season. Intervention villages were purposively selected and matched with control villages based on the incidence of passively detected ***Plasmodium falciparum*** (***Pf***) and mixed malaria cases during the 2013 malaria transmission season. In intervention villages, a passively detected ***Pf*** or mixed index case triggered an investigation that targeted the index case household and up to ten neighboring households in a 100-meter radius. All available household members received a rapid diagnostic test (RDT) and RDT-positive individuals received artemether-lumefantrine (***Pf***, mixed) or chloroquine (***P. vivax*** [***Pv***]). From October to November, 2014, 353 ***Pf*** or mixed index cases were passively detected in the intervention villages. Of these, 209 (59.2%) were investigated; 88.9% were male; 63.5% were aged 20-39 years; and 61.5% spent ≥1 night away from home in the last month, ranging from 0.0% to 96.6% by village. Among the 3,711 residents in the 838 households investigated, 2,923 (78.8%) received an RDT and 121 (4.1%) were RDT-positive (2.4% ***Pf***, 0.6% ***Pv***, and 1.2% mixed). Comparisons between intervention and control villages and estimates of implementation costs will be available in October 2015. In six of ten villages, few index cases and secondary cases were identified, suggesting little local transmission. In three of ten villages, many index cases were identified, most had a history of travel, and few secondary cases were identified, suggesting importation with little local transmission. In one of ten villages, no cases had a history of travel and many secondary cases were identified, suggesting substantial local transmission. To achieve malaria elimination in Amhara Region, intervention strategies targeting these patterns of transmission and population movement are required.

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MULTI-COUNTRY ROUTINE DATA QUALITY ASSESSMENT (RDQA) FOR MALARIA INFORMATION

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As National Malaria Control Programs increasingly focus on malaria elimination, real time, accurate, and actionable data are critical to target geographies and populations and to optimize the allocation of resources. The MACEPA project assessed the quality of weekly data captured through a mobile phone application built on DHIS2 in Ethiopia and Zambia over a 24-month period to understand how data quality improvement practices can be strengthened to support system needs for rapid and more focal data. Data was captured from 209 health facility catchment areas across four health zones in Amhara Region, Ethiopia, covering an estimated population of 1.6 million in Ethiopia and 220 facility catchments in Southern Province, Zambia, covering a population of nearly 2 million in Zambia. We used two methods for assessing data quality: applying data validation rules on reported data elements directly in DHIS2 to check for logical inconsistencies and produce performance indicators around reporting, completeness, timeliness, and validity; and applying a routine data quality assessment tool to describe the strengths and gaps in the data management and reporting systems, comparing source data from health facility registries with data reported through the mobile phone-based rapid reporting system. This multi-country approach has produced well documented, promising quality assessment practices and lessons learned along with an opportunity to compare the quality of data from rapid reporting systems in different contexts: varying malaria incidence profiles, different mobile technology, role of data reporters, and levels of support by partners and national health systems, providing insights into factors to strengthen and normalize data quality.

1630

SAIMIRI BOLIVIENSIS AS A MODEL TO STUDY MOSQUITO TRANSMISSION OF *PLASMODIUM VIVAX*

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Despite the global burden of malaria disease caused by ***Plasmodium vivax***, little advancement has been made to enable the study of this parasite's unique biology. Lack of an ***in vitro*** continuous culture system has made non-human primates (NHPs) a critical model to study ***P. vivax*** asexual and sexual stages, and mosquito transmission. It has also become the main source of intraerythrocytic stages and sporozoites to investigate drug efficacy, liver stage biology, dormant hypnozoite forms, and relapse. Despite the suitability of NHPs for ***P. vivax*** infection, efforts to produce a robust, reliable model of the complete life cycle have been limited, and this invaluable tool remains elusive. Using recombinant progeny from a genetic cross between subpopulations of a ***P. vivax*** line with chloroquine sensitive (VK210) and resistant (VK247) parasites (NIH-1993-S×NIH-1993-R) we are evaluating conditions to maximize production of sporozoites and select an adapted line to complete the parasite life cycle using ***Saimiri boliviensis*** monkeys. Infections initiated via intravenous inoculation of infected erythrocytes varied widely in time to patency and intensity, but parasitemias reaching ~1% were obtained. ***Anopheles freeborni*** and ***An. albimanus*** were found to be the most competent vectors among those tested. Oocyst and sporozoite counts indicated that artificial membrane feeding following serum replacement with human AB^+ serum produced the most intense infections. No correlation was found between the intensity of mosquito infection and parasitemia or gametocytemia. A more sensitive molecular assay to retrospectively correlate gametocytemia and the male:female sex ratio with transmission success is under development. Our findings will provide important information regarding the utility of this New World monkey as a model to study ***P. vivax*** biology, and to facilitate development of novel diagnostics, effective antimalarial drugs and vaccines, which will be indispensible for malaria eradication.

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PHASE 1, DOSE ESCALATION, RANDOMIZED CONTROLLED TRIAL TO EVALUATE THE SAFETY, IMMUNOGENICITY AND EFFICACY OF INTRAVENOUSLY ADMINISTERED ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE IN TANZANIAN ADULTS: PRELIMINARY RESULTS

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A Phase 1 study of PfSPZ Vaccine administered IV at doses ranging from 2,000 to 135,000 PfSPZ in USA showed the vaccine is safe, well-tolerated and immunogenic in malaria naïve population in the U.S. It is important to know early on if there are differences with endemic population. The present double blind placebo controlled trial was conducted at the Bagamoyo Clinical Trials Unit of the Ifakara Health Institute in Tanzania and aimed at demonstrating that doses of 135,000 and 270,000 PfSPZ are safe, immunogenic and effective in African population as in USA. A total of 73 healthy adult male subjects were enrolled according to pre-defined inclusion and exclusion criteria into five groups. The 1st group of 3 volunteers were given 3 ascending doses of 30,000, 135,000 and 270,000 PfSPZ each, at 4 weeks interval for demonstration of safety. The 2nd and 3rd groups had 20 out of 24 subjects per group given 5 vaccinations of 135,000 and 270,000 PfSPZ respectively while 4 out of 24 subjects in each group were blinded normal saline controls. After the 5th vaccination, all subjects in the 2nd and 3rd groups were challenged with controlled human malaria infection (CHMI) at 3 weeks and at 24 weeks. The 4th group had 6 subjects receiving 5 vaccinations of 270,000 PfSPZ and were challenged with CHMI at 24 weeks after the 5th vaccination. The fifth group had 16 subjects who were used as unblinded normal saline controls for 2nd and 3rd groups during the CHMI at 3 weeks (2 subjects per group) and at 24 (6 subjects per group) after the 5th vaccination. The primary outcomes were safety and tolerability after each vaccination as well as protective efficacy after CHMI with PfSPZ homologous Challenge (NF54). Other important outcomes were immune responses after PfSPZ vaccine. Preliminary results suggest that the vaccine given at 135,000 PfSPZ and 270,000 PfSPZ is as safe and well tolerated in Tanzanian adults as in naïve US population. The assessment of efficacy using controlled human malaria infection for the second, third and fourth groups is underway and will be presented. Potential future trials to establish effective dose in different age groups will be also be discussed.

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PROTECTIVE EFFICACY OF *PLASMODIUM VIVAX* RADIATION ATTENUATED SPOROZOITES IN HUMAN VOLUNTEERS

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Immunization of human volunteers with radiation-attenuated sporozoites (RAS) of ***Plasmodium falciparum*** has shown to be highly protective against infectious challenge with live sporozoites. Due to the lack of P.vivax ***in vitro*** cultures, development of a vaccine based on RAS to this species has lagged behind. A phase 1/2, controlled clinical trial was conducted with the aim of assessing the safety, protective efficacy, and immune correlates of protection of immunization with Pv RAS which may guide future vaccine development. Healthy adult volunteers were immunized with Pv RAS (150 ± 10 cGy) through mosquito bites. Duffy positive (Fy+) individuals (n=21) were randomly assigned to either experimental (Exp, n=14) or control group (Ctr, n=7), and received bites from irradiated ***P. vivax***-infected and non-infected mosquitoes, respectively. Additionally, Duffy negative (Fy-) volunteers (n=7) were assigned to a third group and received bites of infected non-irradiated mosquitoes in order to assess the response to non-attenuated parasites. After each immunization, patients were followed-up with clinical assessments and safety laboratory tests. After completing a total of seven immunizations, volunteers were subjected to a ***P. vivax*** sporozoite infectious challenge. A total of 20 volunteers completed the infectious challenge phase (12 Exp, 3 Ctr and 5 Fy-). Exp volunteers received a mean of 430 (362-497) infective Pv-irrad-spz mosquito bites, Ctr, 934 (895-963) non-infective bites and Fy- 442 (358-487) infected non-irradiated bites. All infected volunteers developed symptoms except for one, with a mean incubation period of 9.9 days (range 8-13), and a mean pre patent period 12.6 days (range 11-14). The immunization model with Pv RAS was safe and sterile immunity was achieved in five experimental volunteers (42%) with an average dose of 418 infectious bites and Fy- volunteers remained negative. The overall protection was lower than expected, probably because the planed minimal dose was not achieved in all the volunteers (1000 infective bites). High throughput immune analyses and correlates of protection will be presented.

1633

DIRECT VENOUS INOCULATION: AN INNOVATIVE METHOD FOR ADMINISTERING WHOLE SPOROZOITE MALARIA VACCINES THAT IS SAFE, WELL TOLERATED, IMMUNOGENIC AND HIGHLY PROTECTIVE

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The oral, intramuscular, subcutaneous (SC) and intradermal (ID) routes are often selected to inoculate humans with antigenic material for the purpose of inducing long term, protective immunity. The intravenous (IV) route, while standard for cellular or humoral immunotherapies and immunoprophylaxis (e.g., hepatitis B immune globulin), has not generally been used. Sanaria and collaborators are now testing the IV route for a vaccine immunogen – whole ***Plasmodium falciparum*** (Pf) sporozoites (SPZ) – to facilitate targeting the liver. Clinical grade vaccine product

was first manufactured in 2008, consisting of aseptic, purified, radiation attenuated cryopreserved PfSPZ meeting FDA standards for parenteral injection (PfSPZ Vaccine). Initial trials showed high-grade (100%) protection against Pf with IV injection through an indwelling catheter (IV line), as previously published, but not when injected by the ID or SC routes (Epstein et al ***Science***, 2011). Because it is impractical to consider immunizing large numbers of individuals by placing an IV line and then injecting through it, and to improve operability for mass administration campaigns to eliminate malaria, direct venous inoculation (DVI) has been developed. A small-bore (25 gauge) needle is inserted, blood "flashback" is documented, and the PfSPZ are injected rapidly. This often painless procedure has been performed 913 times to inject PfSPZ into 305 adults in the US, Germany, Spain, Mali, Tanzania, Gabon and Equatorial Guinea. When used for PfSPZ Vaccine, DVI induces high-grade protection after 3 doses. The injections cause limited local and essentially no systemic reactogenicity, and there have been no allergic reactions or disenrollments due to adverse events. Moreover, injection of infectious PfSPZ (PfSPZ Challenge) by DVI induces Pf infection consistently, and when study subjects receive concurrent oral chloroquine, PfSPZ Challenge given by DVI induces high levels of protective immunity. Based on the excellent safety, tolerability, infectivity and potency record in adults, the first trials of PfSPZ administered to infants and children by DVI will be initiated in 2015.

1634

USE OF FLUOROSPOT ASSAY TO ASSESS IFNG, IL2, AND IFNG PLUS IL2 RESPONSES AGAINST WHOLE *PLASMODIUM FALCIPARUM* SPOROZOITES AND SELECTED ANTIGENS AFTER IMMUNIZATION WITH RADIATION-ATTENUATED SPOROZOITES ADMINISTERED BY MOSQUITO BITES

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In vivo depletion experiments in animals have shown that protective immunity induced by radiation-attenuated sporozoites (RAS) is dependent on CD8+ T cells (mice and non-human primates) and IFNg (mice). Based on results from our previous clinical trial where 50% (5 of 10) of subjects immunized with RAS were protected after controlled human malaria infection (CHMI), the number of bites required to achieve the same level of 50% protection was calculated and used for the current study. Among the 11 subjects who received an average of 1,027 bites over 5 immunization-sessions, 6 were protected after CHMI (55%), allowing for the study of protective immune effector mechanisms and/or surrogate markers of protective immunity. Of the possible 5000 antigens predicted to be present in ***Plasmodium falciparum (Pf)***, the specific antigens involved in protective immunity induced by RAS vaccine is unknown. Using the highly robust FluoroSpot assay that assesses single cells simultaneously secreting two cytokines, IFNg and IL2, we measured T cell responses to purified aseptic sporozoites (SPZ) using freshly isolated peripheral blood mononuclear cells (PBMC) from subjects prior to each of the 5 immunizations, and before and after CHMI. We also assessed T cell responses against overlapping 15-mer peptide pools of four standard ***Pf*** proteins that have been evaluated in clinical trials, including CSP, AMA1, SSP2/TRAP, and CelTOS. All true immunized subjects, but none of the mock immunized, developed IFNg and IL2 T cell responses to SPZ. The highest magnitude and frequency of responses was detected at 28 days post first immunization. At pre-CHMI, there was a trend towards higher magnitude of responses and percent responders in protected subjects vs. non-protected subjects, but this did not reach statistical significance. As expected, responses against the four antigens tested were of lower magnitude and prevalence than the responses against whole SPZ. Interestingly, all pre-CHMI responses decreased in protected and increased in non-protected subjects post-CHMI. A possible hypothesis to explain this finding will be discussed.

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CHEMOPROPHYLAXIS VACCINATION (CVAC) OF RHESUS MACAQUES USING *PLASMODIUM KNOWLESI* SPOROZOITES AND THE ANTIMALARIAL DRUG PYRIMETHAMINE: ESTABLISHING A REGIMEN THAT COMPLETELY PREVENTS DEVELOPMENT OF BLOOD STAGES

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Chemoprophylaxis Vaccination (CVac) involves inoculation of ***Plasmodium*** sporozoites under antimalarial drug coverage, and CVac using chloroquine (CQ) has been shown to induce potent and long-lasting immunity in humans and animals. The contribution of blood stage immunity versus liver stage immunity to protection has been a point of controversy. We seek to induce highly effective immunity in humans with CVac regimens that kill parasites during liver stage development thus completely inhibiting exposure to blood stages. We have conducted a study of a CVac drug schedule in Rhesus monkeys. Rhesus macaques were inoculated with 12,500 purified, cryopreserved, ***P. knowlesi*** sporozoites (PkSPZ) by direct venous injection (DVI). Monkeys subsequently received oral pyrimethamine (PYR; 1 mg/kg) treatment, in an attempt to kill liver stage parasites and prevent emergence of blood stage infection. An Up/Down study design was implemented to evaluate the appropriate timing of PYR following PkSPZ DVI. Groups of macaques were dosed with PYR on either days 1, 2 (Group 2, n=4) or on days 2, 3 (Group 3, n=7) post PkSPZ. qRT-PCR and blood smears were performed from day 5 to day 27 post PkSPZ inoculation, to detect subpatent and patent parasitemia respectively. All macaques (n=11) in Groups 2 and 3 were blood smear and qRT-PCR negative at all time points after PkSPZ inoculation. All control macaques that did not receive PYR were qRT-PCR and blood smears positive by day 6 and day 8, respectively. Upon second and third vaccinations, administered 4 weeks apart, all macaques in Groups 2 and 3 remained negative for subpatent and patent parasitemia. Results from homologous challenge (2,500 PkSPZ DVI) to evaluate development of protective immunity will be presented. This study provides evidence that PYR following PkSPZ CVac can prevent blood stage infection in non-human primates, thus guiding the design for an upcoming human trial of CVac with PYR.

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SAFETY AND EFFICACY OF DIRECT VENOUS INOCULATION WITH RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* NF54 SPOROZOITES (PFSPZ VACCINE) IN HEALTHY MALIAN ADULTS

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A double blind, randomized Phase 1/2 clinical trial was conducted in Mali, West Africa to assess the safety, immunogenicity and protective efficacy of Sanaria® PfSPZ Vaccine administered via direct venous inoculation (DVI) against natural malaria exposure in healthy 18-35 year old adults. Twelve volunteers (safety group) received 2 doses of PfSPZ Vaccine (Day 0: $1.35x10^5$ and Day 14: $2.7x10^5$) and 88 volunteers received 5 doses of $2.7x10^5$ PfSPZ Vaccine or normal saline (Day 0, 28, 56, 84, 140; total dose of 13.5×10^5). The incidence and severity of local and systemic adverse events occurring within 7 days after each dose were solicited. During the malaria transmission season, volunteers were examined and blood smears obtained every 2 weeks for 20 weeks in total, with the primary efficacy endpoint being time to first positive blood smear. Injection site pain, headache, fatigue, myalgia, and pyrexia were the most frequent solicited adverse events in both vaccine groups. There was no significant difference in local site reactogenicity, solicited systemic adverse events, or laboratory abnormalities between PfSPZ Vaccine and control volunteers. In this adult population, malaria transmission was intense with 93% (41/44) of the control group having at least one positive blood smear. Vaccine efficacy beginning 28 days after the last dose of PfSPZ Vaccine and through the next 20 weeks by estimated by cox proportional hazard 48% (15 , 69) P-value: 0.01 and by proportional analysis was 29% [8, 47], p = 0.006. Immunogenicity, measured by PfCSP antibody responses by ELISA, was not predictive of individuals who remained blood smear negative throughout the transmission season. The study demonstrates that repeated DVI administration of the PfSPZ Vaccine in a healthy adult African population was easy to administer, safe, well tolerated and efficacious in an intense seasonal malaria transmission area of Mali. To our knowledge, this is the highest estimated protective efficacy against Pf parasitemia for 6 month follow-up vaccination of African adults. We are now working on altering the immunization regimen to increase immune responses and protective efficacy.

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A MODEL FOR DEPLOYMENT OF CRYOPRESERVED PFSPZ VACCINE FOR FOCAL ELIMINATION OF MALARIA ON BIOKO ISLAND

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PfSPZ Vaccine, composed of aseptic, purified, radiation-attenuated, cryopreserved, ***P. falciparum*** (Pf) sporozoites (SPZ) is being assessed in multiple clinical trials in the USA, Europe and Africa. The goal is to finalize a 3-dose regimen that induces high level (>80%), durable (>6 months) protection against controlled human malaria infection and natural exposure by heterologous/heterogeneous Pf parasites. The PfSPZ, being eukaryotic organisms, are cryopreserved and stored and distributed through a liquid nitrogen vapor phase (LNVP) cold chain. The clinical development plan has a goal of licensure by mid to late 2018 for non-immune travelers and early 2019 for use in targeted focal elimination campaigns, for which it will serve as an ideal tool alone or in combination with other control measures. The first campaign is planned for Bioko Island, Equatorial Guinea. Bioko is ~2,000 km^2 and has a population estimated for 2018 of ~300,000, concentrated in the capital, Malabo. The island is accessible by automobile, 95% of houses are currently mapped, and 85% of residents enumerated using a tablet-based Campaign Management Information System. Vaccine will be airfreighted to Malabo in large LNVP dry shippers each containing 5×10^4 cryovials and transferred at a central store to a LNVP cryobank or directly into small LNVP dry shippers for transport to the immunization clinics. Options for vaccinating Bioko's population include fixed clinics, mobile clinics, and door-to-door vaccination. Each will be tested in a trial of 3,000 subjects in 2017. The LNVP dry shippers (free-standing, independent of electricity) will serve both for delivery and for temporary storage on a rotating schedule to fixed or mobile clinics. Each dry shipper will hold up to 1,000 single dose vaccine vials. Plans include immunizing 15,000 people per day and completing each immunization round in 4 weeks. The assumptions and challenges of this and other approaches will be discussed. As the first such focal elimination campaign, Bioko Island will serve as a crucial test of the distribution logistics for PfSPZ Vaccine and as a model for subsequent campaigns.

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ASSESSING AND INCREASING THE POTENCY OF *PLASMODIUM FALCIPARUM* SPOROZOITES FOLLOWING CRYOPRESERVATION

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Sanaria has a unique capability to manufacture aseptic, purified ***Plasmodium falciparum*** (Pf) sporozoites (SPZ), which are stored in liquid nitrogen vapor phase until clinical use. The effectiveness of thermostabilization is shown by 100% protection of volunteers against controlled human malaria infection (CHMI) after immunization with radiation-attenuated PfSPZ (PfSPZ Vaccine); 100% infection following

injection of infectious PfSPZ (PfSPZ Challenge); and 100% protection against CHMI when chloroquine is given concurrently with PfSPZ Challenge (PfSPZ-CVac approach). These results using cryopreserved PfSPZ, unprecedented in clinical vaccinology, indicate that Sanaria's PfSPZ are potent and infective. They are also concordant with the results of 4 ***in vitro*** assays we use to monitor PfSPZ viability and potency: a membrane integrity assay, 3- and 6-day hepatocyte assays that assess PfSPZ infectivity in hepatocytes, and an axenic culture assay that measures PfSPZ transformation in cell-free culture. By these assays, cryopreservation leads to losses of ~10%, 5%, 5% and 40%, respectively, compared to freshly dissected PfSPZ, while the micropatterned cellular co-culture (MPCC) assay integrates multiple aspects of viability and shows cryopreservation losses of 85% to 90%. These reductions compare very favorably with those of other live vaccine agents after thermostabilization but also indicate that process improvements could enable more efficient manufacturing and lower cost of goods. To this aim, Sanaria has focused on improving 3 areas using Pf, ***P. vivax*** (Pv) and ***P. yoelii*** (Py) SPZ: cryoprotectant additive mixtures, freezing protocols, and thawing methods. Combining several approaches, we have doubled the infectivity and potency of cryopreserved PySPZ ***in vivo***. In the MPCC assay infectivity has been increased by 2- to 3-fold for both PfSPZ and PvSPZ. When integrated into manufacturing processes in the future, these improvements are expected to reduce by 2- to 3-fold the numbers of PfSPZ required to achieve high-level protective efficacy or infectivity after administration of PfSPZ Vaccine, PfSPZ-CVac, or PfSPZ Challenge, respectively.

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THEY SAID IT COULD NOT BE DONE: MANUFACTURING PFSPZ VACCINES AT SCALE FOR MALARIA ELIMINATION

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Sanaria® PfSPZ Vaccine and PfSPZ-CVac are highly protective vaccines targeting ***Plasmodium falciparum*** (Pf) that are currently in clinical trials in the US, Europe and multiple countries in Africa. The immunogen comprises aseptic, purified, cryopreserved, metabolically active whole Pf sporozoites (SPZ) attenuated by irradiation or an antimalarial drug. The PfSPZ consortium of international investigators met on March 11-12, 2015 in Germany to develop plans to urgently move these promising PfSPZ vaccines forward. Evidence that such radiation attenuated PfSPZ administered to humans by mosquito bite could confer sterile protection has been available since the 1970s. Why then was this approach not developed as a vaccine? In large part this was due to the misconception that such a vaccine that met regulatory and cost of good standards could not be manufactured. The idea that aseptic mosquitoes, highly infected with PfSPZ, could be reared and the PfSPZs harvested was considered an impossible dream. Compounding this notion was the thought that purifying these PfSPZs and stabilizing them for pharmaceutical use was not possible. Sanaria's manufacturing team has optimized the Pf life cycle in compliance with current Good Manufacturing Practices to obtain >1.5x10^5 infectious PfSPZ/ aseptic mosquito. Nonetheless, there continues to be disbelief that a mosquito can ever be raised as an aseptic organism. Sanaria's aseptic mosquitoes are quality controlled to pass USP<71> sterility tests that are mandated for aseptic manufacture. But how can PfSPZs be harvested from each mosquito salivary gland in a manner that is compatible with GMP at scale? Our operators harvest at average rates of 3 mosquitoes/minute. Thus a single lot of 1x10^3 vials of PfSPZ vialed at 1.5x10^5 PfSPZ per vial requires a team of 6 dissectors working for 2 hours; a yield of 3x10^3doses of PfSPZ-CVac. Our automated dissection intends on harvesting 1 mosquito/sec. Our cryopreservation methodology stabilizes PfSPZ for >4 years. This presentation will de-mystify our manufacturing process and explain the specifics of how we intend to manufacture for Phase 3 clinical trials and product launch.

1640

THE NF54-BASED WHOLE-ORGANISM PFSPZ VACCINE IN THE CONTEXT OF GLOBAL GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR SELECTION OF VACCINE AND CHALLENGE STRAINS

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Genetic diversity in ***Plasmodium falciparum*** (Pf) is an obstacle to broadly efficacious malaria vaccines. The attenuated whole-organism malaria vaccine PfSPZ Vaccine is based on the African strain NF54, the parental stock of the reference Pf isolate 3D7. Controlled human malaria infection (CHMI) trials are underway to replicate initial promising results from homologous CHMI, and to assess heterologous protection using the South American strain 7G8. The degree of genetic dissimilarity between NF54 and strains used in CHMI, as well as to circulating strains in natural populations, can assist in the interpretation of protective efficacy, in the identification of additional challenge strains, and in the determination of whether regional-based or multivalent whole-organism vaccines are needed. To this end, we utilized whole-genome sequencing data from 32 clinical isolates from Africa and southeast Asia (SEA), which we compared with NF54, 7G8, and NF135 (the latter an isolate from SEA and potential vaccine component). We generated SNP calls relative to 3D7 for a panel consisting of ~1 million validated variable positions in protein-coding genes. We identified an average of 4,200, 4,500 and 5,300 SNPs in samples from East and West Africa and SEA, respectively. Similarly to clinical samples from SEA, NF135 differs from 3D7 in 5,280 SNPs, while the difference between 7G8 and 3D7, at 4,722 SNPs, is intermediate between that of African and of SEA samples. With no SNPs called, NF54 is identical to 3D7 in all nucleotide positions in the panel. Principal coordinate analysis using genetic distances between samples showed a clear separation of strains from Asia and Africa according to the first two coordinates, with NF135 clustering with the former. NF54 clustered more closely with strains from West Africa, and 7G8 appears in the periphery of the African clade according to the first four components, suggesting clear differences but close ancestry between them. These results suggest that heterologous challenge studies demonstrating efficacy of PfSPZ in preventing infection against 7G8 would be very encouraging for the prospect of its efficacy in Africa.

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MECHANISTIC CHARACTERIZATION OF THE COMPLEMENT RECEPTOR 2 DERIVED PEPTIDE P28 AS A POTENT ADJUVANT FOR VACCINES

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Second generation malaria vaccines are currently being identified with the help of reverse vaccinology, which takes advantage of genome- and proteome-based antigen discovery. Targeting immune responses to antigens expressed on sporozoites ideally impacts the ability of parasites to migrate to the liver and/or infect hepatocytes. The ***Plasmodium*** Cell-Traversal protein for Ookinetes and Sporozoites (CelTOS) plays an essential role in parasite movement in both, mosquitoes and vertebrates, and is required for successful infection. We previously characterized the ability of a CelTOS-based protein vaccine to induce protective immunity in preclinical models using conventional adjuvants. To overcome the

shortcomings plaguing malaria vaccines, ***i.e.***, requirement for large antigen doses and short duration of protection, we employed a peptide adjuvant that has been shown to greatly enhance the immunogenicity of circumsporozoite protein (CSP). The peptide is based on the minimal binding site for complement receptor 2 (CD21) of complement factor C3d. Antigen uptake and activation studies with bone marrow derived dendritic cells revealed superiority of the PfCelTOS-p28 chimeric protein over the wildtype PfCelTOS. Mice were immunized with different doses, containing or lacking extraneous adjuvant (Montanide) to determine the optimal dose of the vaccine for a subsequent challenge with heterologous ***P. berghei*** sporozoites. The cellular analysis revealed a) that significant IFN-γ and IL-4 responses were induced even in the absence of extraneous adjuvant, and b) superiority in the magnitude of the responses compared to the wildtype PfCelTOS. Immunization with 100 fold lower dose of the chimeric protein (0.1 µg PfCelTOS-p28) had a higher efficacy (80%) than immunization with adjuvanted wildtype protein (10 µg). Studies are underway to determine the longevity of the protective response. In conclusion, targeting a protein vaccine to CD21 using this peptide adjuvant can greatly enhance immunity and can also bypass the need for extraneous adjuvant.

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THE COMBINED IMPACT OF TRANSMISSION-BLOCKING DRUGS AND RTS,S VACCINES IS SYNERGISTIC?

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The Malaria Vaccine Roadmap aims to develop a vaccine that provides 80% protective efficacy against ***Plasmodium falciparum*** by 2020. The most advanced malarial vaccine is currently the pre-erythrocytic vaccine RTS,S which targets the circumsporozoite protein (CSP) and has been shown in clinical trials to reduce the clinical incidence of malaria in children by 46% (range 40 - 77%). It is thought to be most efficacious in regions of low transmission. Methods to reduce mosquito-to-human transmission within a given region prior to vaccine application could enhance the vaccine's overall effectiveness. One approach could be the use of partially effective transmission blocking interventions (TBIs) which aim to reduce human-mosquito transmission. Using mosquito-vertebrate systems we estimate the combined impact of a TBI candidate (atovaquone) and a pre-erythrocytic vaccine (which targets CSP in a similar manner to RTS,S and has an estimated efficacy of approximately 50%) to determine whether the different interventions are antagonistic, additive or synergistic. A mathematical model is developed which captures the population dynamics of the parasite in the mosquito and mouse population and is used to estimate the mosquito-to-vertebrate and vertebrate-to-mosquito transmission probabilities and how they change following the introduction of vaccine. The functional relationship between the probability of blood-stage infection and quantity of oocysts in the mosquito mid-gut does not change with TBI treatment but the number of oocysts is reduced at lower biting rates (and eliminated at 1 or 2 bites after 4 successive generations). The estimated probability of transmission to the vertebrate is therefore lower with TBIs compared to control estimates. We present and discuss the potential benefits of using TBIs with RTS,S-like vaccines to reduce malarial transmission.

1643

A NEWLY ENGINEERED MUTANT OF THE *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN ENHANCES INDUCTION OF BROADLY NEUTRALIZING ANTIBODIES

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The vital role ***Plasmodium vivax*** Duffy binding protein (DBP) in blood-stage development justifies its consideration as a vaccine candidate. However, similar to other microbial vaccine candidates the polymorphic nature of this ligand represents a serious problem that may compromise efficacy of a DBP vaccine. In particular there is the tendency for immunity to be strain specific. Our hypothesis is that polymorphic dominant B cell epitopes of DBP represent an evasion mechanism to misdirect immune responses away from functional, conserved epitopes. Consistent with this interpretation, the dominant B cell epitopes in DBP are polymorphic surface-exposed motifs adjacent to the dimer interface but these variable residues are generally not important for binding the erythrocyte receptor DARC. To overcome the inherent bias towards strain immunity, we previously evaluated an engineered DBP mutant DEKnull that partially overcame strain-specific immunity. In the current study we have evaluated three additional novel engineered DBP immunogens that altered (1) variant epitopes, (2) functional residues, and (3) residues for dimerization. Each strategy altered the nature of functional antibodies elicited and importantly design #1 induced more broadly neutralizing antibodies against a range of diverse allelic variants. The successful results indicate a potential approach that can be used generally to improve efficacy of other malaria vaccine candidates. Structural studies into these novel antigens are currently underway.

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IDENTIFICATION AND CHARACTERIZATION OF NEW *PLASMODIUM VIVAX* ANTIGENS AS POTENTIAL TARGETS FOR PRE-ERYTHROCYTIC VACCINES AGAINST VIVAX MALARIA

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Despite the large global burden of ***Plasmodium vivax*** (Pv) infection, there is a dearth of promising vaccine candidates against vivax malaria. Four vaccines have progressed to clinical trials, three of which are based on the abundant sporozoite surface protein, the circumsporozoite protein. To identify additional promising pre-erythrocytic vaccine antigens, data from a high-throughput ***P. falciparum*** (Pf) antigen screening project was used. The most immunogenic proteins were selected based of their ability to induce antibodies and cytokine production in T cells from volunteers immunized with radiation attenuated Pf sporozoites. Two of the Pf proteins, falstatin, a cysteine protease inhibitor, and the gamete egress and sporozoite traversal protein (GEST) are expressed in the sporozoite and liver stages and are capable of inducing protection in a ***P. yoelii*** rodent model. We expressed full-length PvFalstatin and PvGEST using an optimized wheat germ cell-free expression system protocol. Antibodies to both falstatin and GEST were measured by ELISA in individuals naturally infected with ***P. vivax***. Additionally, we assessed the capacity of these proteins to induce cytokine production in T cells from ***P. vivax***-infected individuals living in endemic areas in the Peruvian Amazon. We will present data that indicate that lessons from ***P. falciparum*** antigen discovery may provide a promising and valid platform for identifying potential vivax vaccine candidates.

1645

IMPORTANCE OF CHOOSING THE RIGHT ANIMAL MODEL FOR THE DOWN-SELECTION OF SECOND GENERATION CSP BASED VACCINES

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Plasmodium falciparum Circumsporozoite protein (CSP) remains the most promising malaria vaccine candidate. We have expressed a near

full-length soluble recombinant CSP in ***E. coli***. The protein contains the conserved N-terminal region, NANP repeats and the C-terminal region of PfCSP. It is widely believed that adjuvanted soluble CSP would not be sufficiently immunogenic to confer protection against parasite challenge and that particulate delivery platforms are a way to overcome this hurdle. We investigated whether conjugating the soluble CSP to a nanoparticle would substantially augment its immunogenicity as compared to adjuvanted soluble CSP. A recombinant capsid protein of bacteriophage Qb that assembles into a 25 nm particle was used as a carrier. CSP was chemically conjugated to the Qb virus-like particle (VLP) and the immunogenicity of the resulting VLP vaccine was tested in C57Bl/6 mice. Alum adjuvanted Qb -CSP induced higher antibody titersthan soluble CSP. Upon challenge with a transgenic ***P. berghei*** parasite that expresses the full-length ***P. falciparum*** gene, the CSP and Qb-CSP vaccines showed high level of sterile protection. The differences between soluble and particulate CSP was most pronounced after the 1st vaccination and particulate presentation most strongly influenced the titers of the repeat region. Since immunogenicity in mice does not always translate to humans we also tested the Qb-CSP and soluble CSP vaccine in the Rhesus monkey model. Unlike mouse data, no significant difference in the immunogenicity of Alum adjuvanted CSP and Qb-CSP was observed in Rhesus. While potent immunogenicity and protection was observed in mice, Alum adjuvanted CSP and Qb-CSP were not highly immunogenic in the Rhesus model. The availability of transgenic parasite challenge strains allows us to use mice as a go-no-go model for vaccine progression, however our data shows that mice may be much more sensitive to Alum based formulations and particulate vaccines than higher primates. Hence Rhesus immunogenicity should remain as the final go-no-go criteria for down-selecting second generation CSP based vaccines for human trials.

1646

BREADTH OF MALARIA VACCINE TARGET ANTIGEN SEROREACTIVITY AND PLACENTAL TRANSFER AMONG MOTHER-NEWBORN PAIRS IN MALAWI

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In young infants, malaria vaccine efficacy may be inhibited by maternal antibody interference with potential vaccine targets. Little is known about the comparative efficiency of transplacental transfer of antibodies targeting different ***Plasmodium falciparum*** antigen variants and the effect of placental malaria (PM) infection. PM is one of several factors thought to inhibit antibody transfer. We used a protein microarray to assess breadth of seroreactivity to 314 target malaria vaccine antigens in 32 mother-newborn pairs in Malawi. The array included 264 apical membrane antigen 1 (AMA1), 20 merozoite surface proteins (MSP) 1 and 2, and 30 reticulocyte-binding protein homologue 5 (RH5) protein variants. At delivery, maternal peripheral and cord blood samples were obtained. Placentas were inspected for histological and molecular evidence of PM. The categorical definition of seroreactivity was median fluorescent intensity (MFI) 2 standard errors above the mean for 10 malaria-naïve controls for each antigen variant. Of all mothers, 80% (95%CI: 78-81) were seroreactive across AMA1 variants, 75% (95%CI: 70-81) to MSP, and 33% (95%CI: 26-40) to RH5 variants. Breadth of seroreactivity was greater among mothers with evidence of PM than without. Categorical mother-newborn outcomes were concordant in all three antigen groups by McNemar's test while Spearman correlation of continuous MFIs was 0.74, 0.83, and 0.35 for AMA1, MSP, and RH5 respectively. Maternal-newborn correlation results for each antigen group were similar stratifying by PM status. In this pilot study, seroreactivity to RH5 was less prevalent among mothers and less correlated between mothers and newborns than for AMA1 or MSP variants. PM may alter breadth of seroreactivity in mothers and their newborns. Protein microarrays may be a userful tool that will allow us to determine the influence of PM and maternal antibody transfer to the fetus on potential vaccine candidates.

1647

INDUCING BOTH PROTECTIVE ANTIBODIES AND CD8 T CELLS BY PRIME-BOOST WITH LIVE ATTENUATED VACCINE

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There are >2x10^8 cases and 6x10^5 deaths due to ***Plasmodium falciparum*** (Pf) annually, despite $2B/yr spent on malaria control. The malaria vaccine, RTS,S/AS01 is safe and delays clinical malaria onset by 30-50% depending on age group. Protection may be primarily mediated by antibodies against the repeat region and some CD4+ T cell responses against the C' terminus of PfCSP. The vaccine does not induce meaningful CD8+ T cell responses. A vaccine for the military and travellers needs to provide >80% protective immunity for at least 6 months. We hypothesize that by adding highly functional, protective CD8+ T cell responses to antibody responses against the PfCSP, such long lived protective immunity can be achieved. We use live-attenuated ***Listeria monocytogenes*** (Lm) as a vaccine platform and use its properties of effectively stimulating robust, multi-functional, cell-mediated immunity, as a result of its intracellular lifecycle and ability to infect, deliver antigen and stimulate DCs ***in vivo***. Our strategy for improving protective efficacy of our recombinant (r) PfCSP vaccine as compared to RTS,S/AS01 is to, 1) Include N'-terminus sequences containing neutralizing antibody and T cell epitopes to diversify and increase potency of the antibody and cellular responses to PfCSP; 2) Conjugate rPfCSP to a carrier protein to increase antibody titers; and 3) Boost with attenuated rLm expressing PfCSP (Lm-PfCSP) to enhance CD8+ T cell responses. We demonstrate that priming with rPfCSP in adjuvant and boosting with Lm-PfCSP induces high levels of PfCSP-specific inhibitory antibodies and CD8+ and CD4+ T cells. Conjugating rPfCSP to a carrier further enhances the antibody response to PfCSP by >8 fold as compared to unconjugated rPfCSP. Most importantly, 100% of mice immunized with adjuvanted rPfCSP and boosted with Lm-PfCSP were protected against sporozoite challenge with a transgenic rodent malaria parasite which express PfCSP in place of PbCSP. The high levels of inhibitory antibodies as assessed by inhibition of liver stage development, impressive ***in vivo*** protection and assessment of long term memory provides impetus for urgent development of this strategy.

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RECOMBINANT EXPRESSION, PURIFICATION AND IMMUNIZATION STUDIES OF NOVEL PRE-ERYTHROCYTIC VACCINE ANTIGENS IN THE *PLASMODIUM YOELII* MOUSE MALARIA MODEL

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The pre-erythrocytic (PE) stage is a metabolically highly active but symptomatically silent preparatory phase of the ***Plasmodium*** life cycle. Intervening to kill the parasite at this stage prevents the symptomatic blood stage of infection, and is an attractive vaccine target. Using microarray analysis and DNA vaccination, 8 genes were previously identified as up-regulated in Liver Stage parasites and able to induce partial protective immunity against PE infection. For further studies of these vaccine candidates, we prepared their ***P. yoelii*** orthologues as recombinant proteins. Five genes were expressed as recombinant protein with C terminal His tag in insect cells. Proteins Py370w and PyMIF4 were in soluble form when expressed in insect cells and could be purified under native conditions using Ni-NTA column. Proteins PyMAL7, PyLISP1 and PySHMT were initially insoluble and were purified under denaturing conditions in presence of 6M GuHCL and subsequently refolded using appropriate buffer conditions. Prior to formulation with adjuvants, the refolded PyMAL7 and PyLISP1 proteins were then passed over detergent removal column to remove detergent present in the refolding buffer, and detergent from refolded PySHMT was removed by passing over a second Ni-NTA column. Proteins PySAP1, Py1995c and Py305w were expressed and purified from ***E. coli*** under denaturing conditions and the proteins refolded using appropriate buffer conditions. Immunization studies of these proteins have been initiated in mice, and details of the results will be presented.

1649

ROLE FOR CHEMICAL CONJUGATION TO ENHANCE THE BREADTH AND QUANTITY OF *PLASMODIUM FALCIPARUM* RECOMBINANT CIRCUMSPOROZOITE PROTEIN-SPECIFIC ANTIBODIES

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The circumsporozoite protein (CSP) remains a significant vaccine target due to the success of RTS,S. The antigenic component of RTS,S is comprised of about two-thirds of the native CSP including the immunodominant NANP repeat region and the thrombospondin repeat-like (TSR) domain fused to the hepatitis B surface antigen. We and others have recently shown that the N-terminal region of the CSP is also a target for antibody blockade of hepatocyte invasion. Furthermore, we observed that in Anopheles mosquitoes, a fragment of approximately 70 amino acids is cleaved from the N-termini and then within the salivary gland the CSP changes its shape to protect both the N- and C-terminal regions against antibody recognition prior to hepatocyte invasion. Thus, our development efforts have focused on the evaluation of two recombinant forms of the CSP, one mimicking the unprocessed form or full-length protein (EcCSP) and the other mimicking the cleaved form (PpCSP) identified in salivary gland sporozoites. The N-terminus of CSP is poorly immunogenic compared to the NANP repeat region and the TSR domain. This was observed regardless of the adjuvant formulation tested (Alhydrogel, oil-in-water stable emulsion or liposome), as determined by ELISA against synthetic peptides or region-specific recombinant polypeptides using sera from two rhesus monkey trials and multiple mouse studies in which immunization with either monomeric forms or chemical conjugates to ExoProtein A or other carriers was examined. To date, chemical conjugation of a recombinant CSP to a carrier protein formulated in an adjuvant other than Alhydrogel has provided the best evidence for enhancement of the breadth of the antibody response to include the processed N-terminal region as well as a 5 - 100 fold increase in the overall CSP-specific response in mice. These preclinical results are significant in context to the rationale design of a second-generation CSP vaccine.

1650

LIMITED EFFICACY OF RTS,S VACCINE AGAINST MALARIA MAY BE ATTRIBUTED TO LACK OF T CELL EPITOPE CONSERVATION

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In a recent clinical trial, the RTS,S vaccine against malaria achieved efficacies of 55.8% and 31.3% for protection against the first clinical episode in children (5-17 months) and infants (6-12 weeks), respectively. While promising, these results leave much room for improvement. One reason for the limited success of RTS,S may be that the strain of circumsporozoite protein (csp) included in the vaccine is poorly representative of circulating strains in endemic areas. Using sequences from 57 unique csp variants collected in Liongwe, Malawi, we applied a novel informatics algorithm called EpiCC (Epitope Content Comparison) to determine relatedness among variants at the T cell epitope level. EpiCC uses HLA-DR binding predictions generated by the EpiMatrix tool to quantify the likelihood that a candidate vaccine strain will induce protection against other strains, based on the nature and similarity of their T cell epitopes. According to EpiCC, the csp strain included in RTS,S had a low degree of T cell epitope relatedness compared to the other variants. The relationships among the variants at the T cell epitope level differed from the phylogeny of the whole genome in many cases. If the csp T cell epitopes in the RTS,S vaccine are not well conserved among the strains found in malaria-endemic areas, the vaccine may only confer partial protection. Lack of epitope coverage may explain why RTS,S has not achieved better efficacy in clinical trials. This concept can be broadly expanded to sample data over multiple malaria-endemic areas. EpiCC may provide an objective approach to aid in strain selection for vaccine development.

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PRELIMINARY RESULTS OF A RANDOMIZED, CONTROLLED, DOUBLE-BLIND, SINGLE-CENTER PHASE 1 CLINICAL TRIAL TO EVALUATE SAFETY, TOLERABILITY, IMMUNOGENICITY AND EFFICACY OF CAF01 AND ALUMINUM HYDROXIDE AS ADJUVANTS FOR THE MALARIA VACCINE CANDIDATE GMZ2 IN HEALTHY ADULT AFRICAN VOLUNTEERS

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GMZ2 is a malaria vaccine candidate consisting of conserved domains of the two ***Plasmodium falciparum*** asexual blood-stage antigens merozoite

surface protein 3 (MSP3) and glutamate rich protein (GLURP). The antigens were selected based on sero-epidemiological and functional studies and showed excellent safety, tolerability, and immunogenicity in pre-clinical studies when adjuvanted with aluminium hydroxide. The vaccine candidate GMZ2/aluminum hydroxide has been clinically developed up to Phase 2. Results of a Phase 2 trial in African children are still pending but preliminary data support the very good safety and tolerability profile of GMZ2. In general, data from the four trials suggest that improved formulations may result in stronger efficacy and a vaccine with potential health benefits. Therefore, combination of GMZ2 with a more potent adjuvant is the rational next step in the clinical development. CAF01 is a novel adjuvant, which has demonstrated good safety, tolerability and improved immunogenicity profile when combined with various vaccine candidates. The current GMZ2/CAF01 study is performed to compare safety, tolerability, immunogenicity and efficacy of GMZ2 formulated with CAF01 to a control vaccine (Rabies) and 100 µg GMZ2 in aluminium hydroxide, the best studied GMZ2 regimen so far. The study has started in April 2015. A total of 50 participants were randomized and received vaccinations. Participants receive three times control vaccine (n = 8; Group A), 100 µg GMZ2 formulated in alum (n = 12; Group B), 30 µg GMZ2 formulated in CAF01 (n = 8; Group C) or 100 µg GMZ2 formulated in CAF01 (n = 22; Groups D and E). Five weeks after completion of the vaccination regimen participants of groups A, B, C and D will receive a direct venous inoculation of 3200 PfSPZ Challenge to test for the efficacy of the vaccine. Follow up of the study participants will end six months after the administration of the last vaccine injection. We will report the result of the preliminary analysis of the GMZ2CAF01 study. The safety profile, the tolerability as well as the efficacy of the GMZ2 candidate vaccine formulated with CAF01 will be discussed.

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SEASONAL MALARIA CHEMOPREVENTION IMPLEMENTATION IN CHILDREN FROM THREE TO 120 MONTHS EXPERIENCE IN THE FOUR SOUTHERN REGIONS IN SÉNÉGAL

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Because malaria still remains a major cause of disease and death in infants and children, NMCP strives to reduce drastically these morbidity and mortality among children. For this aim, Seasonal malaria chemoprevention (SMC) is adopted as a new intervention in malaria control policy after a long process launched in March 2012. According to WHO criteria the target areas for SMC are the four regions mentioned above. Drugs administration lays on door to door campaign strategy with community volunteers. On the first day, the volunteers, trained by health agents in health facilities, administer drugs to the children under surveillance of mothers or children guardians. For the 2 remaining days, mothers have to substitute to the volunteers. In 2014, SMC Campaign CPS was conducted in the four areas with three cycles covering the higher transmission season (August, September and November). The target was enlarged to the old children from 3 to 120 months (624 139). This enlargement of the target, compared with WHO recommendations (3 to 60 months,) was due to vulnerability sliding towards the ages from 60 to 120 months as shown by the epidemiologic data on malaria morbidity in Senegal. The campaign results were very satisfactory since the coverage rates for the 3 passages, are respectively 98, 63%, 97, 85% and 98, 04%. However, it should be noted that behind these beautiful coverage rates, many challenges have to be faced to achieve such results. Indeed, considering the innovation of the intervention, its and and the strong Community component, it was necessary to set up an operative system allowing an availability of the commodities, management tools supports and accessories on the sites and to prepare the actors to roll the strategy as recommended. In addition, regular coordination and a follow-up with an effective data collection and transmission system, was one of the key-success elements. Significant communication activities had accompanied all the process. In parallel, pharmacovigilance system strengthening was set up because of the characteristic of the drugs used and the relatively important quantity administered to children within such a short time.

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FIELD TESTING OF A PYRETHROID QUANTIFICATION KIT IN VILLAGES COVERED BY INDOOR RESIDUAL SPRAY IN MULEBA, TANZANIA

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Insecticide treated nets (ITN) and indoor residual spraying (IRS) are two of the primary methods of malaria prevention in Africa. In order for these methods to be effective it is essential that adequate concentrations of insecticide are present on nets and wall surfaces to kill mosquitoes. There is no easy assay to quantify insecticide levels without expensive laboratory equipment and procedures. To address this, LSHTM has developed a simple field-applicable kit for monitoring pyrethroid residues on insecticide-treated nets- the Pyrethroid Quanitification Kit (PQK)-which can be adapted to other types of treated surfaces. During the initial trial the PQK kit was calibrated against a variety of sprayed surfaces and with different concentrations of lambdacyhalothrin before being taken into the field. Mosquito cone bioassay was conducted to show whether the surface concentrations of insecticide detected by the PQK were sufficient to kill a susceptible strain of mosquitoes. Houses in six villages were visited 3 months after IRS had been conducted in Muleba, Tanzania. The samples were analysed in the field using a handheld spectrophotometer. In each house, five areas of the wall were examined to give an indication of insecticide distribution and within-wall variation. Results showed that the actual spraying results differed from expectation. Preliminary results showedthat only 28% of houses had all rooms sprayed, leaving 72% of houses partially sprayed, and insecticide concentration varied dramatically across sprayed walls. The PQK is an easy to use quality assurance tool for monitoring of pyrethroid application rates and improving the quality of IRS campaigns.

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MULTIPLE PYRETHROID INSECTICIDES RESISTANCE MECHANISMS IN *ANOPHELES GAMBIAE* S.S. FROM MALARIA SURVEILLANCE SITES IN NIGERIA

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Insecticide resistance in ***Anopheles gambiae*** sensu stricto is a major concern to malaria control. Resistance is mainly due to target-site insensitivity arising from a single point mutation, often referred to as knockdown resistance (kdr). Metabolic-based resistance mechanisms associated with elevated level of monooxygenase and Glutathione-S-Transferase (GST) have also been implicated but to a lesser extent. Here we report the co-existence of both resistance mechanisms in populations of ***An. gambiae*** s.s. from malaria surveillance sites in Nigeria. Anopheles larvae were collected from 12 malaria surveillance sites located in 3 ecological zones (north-central, south-west and south-south) in Nigeria. All ***Anopheles*** tested belonged to the ***An. gambiae*** complex. ***An. gambiae*** s.s. was predominant (82.5%) and found at the 12 sites. ***An. arabienis*** represented 17.2% but found at 3 sites. Mosquitoes from three sites located in the south-south and north-central were 100% susceptible to pyrethroid insecticides (permethrin and deltamethrin). Mosquito susceptibility level to pyrethroids was 55-70% at the remaining sites. Bioassay, synergist and biochemical analysis carried out on resistant and susceptible ***An. gambiae*** s.s. from the 12 sites revealed 35-60%

of the West African kdr mutation in the resistant mosquitoes. Resistant mosquitoes synergized using pyperonyl butoxide before insecticide exposure showed a significant increase in mortality compared with the non-synergized. Biochemical assays showed significant increased levels of monooxygenase activities in the resistant mosquito at six sites in addition to significant increased in GST level at two sites, indicating the presence of multiple pyrethroid resistance mechanisms at these malaria surveillance sites. This current survey of insecticide resistance in ***Anopheles*** provides baseline for resistance monitoring and highlights the need for routine resistance surveillance as an integral part of malaria control.

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INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY: INCREASING THE DOSES IN BURKINA FASO

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In Burkina Faso, Antenatal Care (ANC) is a national platform for malaria in pregnancy prevention and control. The 2010 Demographic and Health Survey showed a good initial ANC registration rate (95%), but over 56% of pregnant women in rural areas do not register until their second or third trimester. Thus they may have missed the full regimen of ANC services including Long Lasting Insecticide-treated nets and intermittent preventive treatment of malaria in pregnancy (IPTp). In 2010 only 10.6% of pregnant women nationally and 8.4% in rural areas received two doses of IPTp. The United States Agency for International Development-supported Improving Malaria Care (IMC) project in Burkina Faso has been providing technical assistance and training to health districts and their ANC staff on implementing updated (2012) WHO IPTp guidelines. The recommended provision of IPTp at every ANC visit from the 13th week of pregnancy onward leads to the possibility of 3 or more doses per woman. The new guidance was incorporated into the update of Burkina Faso's malaria strategy and has been disseminated since September 2014. Annual data from the Health Management and information System for 2014 from three districts (Batie, Po and Ouargaye) and 61 health clinics where IMC has been working were collected and summarized. A total of 26,909 women registered for ANC. Of these 89.7%, 73.2% and 39.8% attended ANC twice, three and four times respectively. Of those registered 84.1%, 73.2% and 18.8% received IPTp once, twice and thrice. Eleven (17.7%) had not started the updated IPTp guidance. The Ministry of Health also experienced stock-outs of sulfadoxine-pyrimethamine. Based on this slow implementation and uptake of IPTp3+, the IMC project in collaboration with the National Malaria Control Program is examining ways to strengthen antenatal malaria prevention including capacity building for ANC staff and community IPTp provision.

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USE OF LONG-LASTING INSECTICIDE-TREATED BEDNETS IN AKWA IBOM STATE NIGERIA AFTER A MAJOR DISTRIBUTION CAMPAIGN

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While long lasting insecticide-treated nets (LLINs) have made a major dent in the incidence of malaria in Africa, LLINs need to be replaced at intervals. Akwa Ibom State Ministry of Health conducted a mass net distribution in 2010 during which 1.8 million LLINs in the 31 local government areas (LGAs/Districts). An estimated 2.7 million nets were acquired with Global Fund support for replacement distribution in November and December 2014. To learn about the outcome of the exercise, the Ministry organized a follow-up household survey in all LGAs in January 2015. The state formed a technical working group which developed a checklist and interview guide to gather follow-up information on number of households that acquired nets, hung nets, slept under nets, their reasons for not using nets and sources of information about nets. Interviewers from each LGA were trained to use the checklist and recognize appropriate net hanging and use. Twelve interviewers were assigned to each Ward of each LGA. A total of 2,696,476 net cards were issued to households based on two nets per household, and 2,626,966 nets (97.4%) were redeemed. Retention rate in sampled households was 97.1%, while hanging rate of those retained was 71.8%%. Overall 69.6% household members reported that they slept under a net the previous night. A greater proportion of pregnant women (92.1%) reported using nets compared to children below 5 years of age (82.3%) and other household members (63.3%). Main reasons for not using nets included feeling hot (44.5%), inability to hang the net (19.7%) and concern about the chemical used to treat the net (11.4%). Akwa Ibom is located in Nigeria's highest malaria transmission zone. Hence there is need to use LLINs throughout the year. In contrast between 2013 DHS (14.1% residents slept under LLIN) and current results is stark and implies that net use may likely decline as nets age. Even 1-2 months out from a campaign there are people who are not hanging and using nets. Continuous systems for community level education and reinforcement and health system-based routine distribution for periods between campaigns are needed.

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LLIN DISTRIBUTION CAMPAIGN PROCESSES: LESSONS LEARNED AND CHALLENGES FROM AKWA IBOM STATE, NIGERIA

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Long Lasting Insecticide-Treated Nets (LLINs) protect users from malaria only if they reach the home. A smoothly functioning distribution is essential to ensure nets reach their end users. Routine distribution at clinics helps to maintain supplies, but mass campaigns are also needed to replace nets on a wide scale. The recent LLIN mass campaign in Akwa Ibom State Nigeria offers lessons and challenges on this process. A State support team was set-up and estimated the total nets needed on one net to 2 people. A total of 21,167 different cadres of personnel were recruited from supervisory to outreach jobs. One-day training was conducted in batches in each of the 31 Local Government Areas (LGAs). To begin household mobilizers issued net cards and registered household members Town announcers helped in demand creation. A private firm was hired bring 2,715,160 nets to 1,242 delivery points. A reporting tool tracked and monitored the distribution process. Reports flowed from the distribution points to the Ward supervisor, the LGA team leader and on to the state technical support team. The State team met at the end of each day to review activities and address challenges and re-strategize. The distribution lasted from 18-22 December, 2104. Overall, thirty-five thousand households were mobilized, and no settlement was reported omitted. 2,715,160 nets were distributed, and 88,049 nets remained in the LGAs, while 23,080 were left in the central store for mob-up. Unfortunately 145 50-net bales were missing. Mobilization led to active involvement of the faith-based leaders, traditional rulers and members of the national youth service corps scheme. Despite advocacy, state political officials focused more on upcoming elections that the net distribution. Although demand was created and short term need was met, more attention is needed to longer term use and supplies for routine services. The remaining supplies unfortunately were affected by security lapses and lost nets and may not serve the needs of complimentary routine distribution. The State needs to assess the long term costs and sustainability of such massive efforts in terms of meeting its malaria control needs.

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SUSCEPTIBILITY STATUS OF *ANOPHELES GAMBIAE S.L.* TO INDOOR RESIDUAL SPRAY INSECTICIDES FROM A SIX SITE BIENNIAL SURVEY PROJECT, UGANDA, 2009-2013

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Insecticide resistance threatens vector control interventions in Uganda, past studies having shown the development of resistance in Ugandan vector populations. We followed up on the 2009 national insecticide susceptibility study to investigate possible changes in susceptibility levels in ***Anopheles gambiae s.l.*** to DDT, six pyrethroid, two organophosphate and two carbamate insecticides collected from six sentinel surveillance sites across Uganda in 2011 and 2013 using the WHO bioassay test kit. Resistance to DDT (mortality <90%), occurred in Wakiso, Tororo and Kanungu Districts. In Apac and Kitgum Districts where IRS using carbamate insecticides occurred the prior three years, mortality to DDT was 91.5% and 94%, respectively. There was reduced mortality in ***An. gambiae s.s.*** to almost all pyrethroids tested from the six sites except for etofenprox in Kitgum District (92%). Mortality rates >98% were observed in ***An. gambiae s.s.*** populations to both organophosphate and carbamate insecticides across all test sites, except for propoxur in Tororo (81%). ***An. gambiae s.s.*** in Uganda is resistant to DDT and most pyrethroid insecticides but is susceptible to organophosphate and carbamate insecticides, while ***Anopheles arabiensis*** appears susceptible to DDT. ***An. arabiensis*** was found to predominate in the IRS districts of Apac and Kitgum following rotation to carbamate insecticides, suggesting ***that An. arabiensis*** is not resistant to DDT and indicating that IRS greatly reduced ***An. gambiae s.s.*** populations. Data from Tororo and Kanungu Districts indicate that there is reduced susceptibility to carbamates, strongly indicating the need for routine annual monitoring for insecticide resistance from around the country.

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LLIN USE IN THE PYGMY COMMUNITY IN THE HEALTH OF ZONE OF WAMBA, NORTHEAST OF THE DEMOCRATIC REPUBLIC OF CONGO

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Autochthones are one of the three groups of key populations in need of malaria control interventions. A key population is defined as a community that is highly affected by malaria and which has limited access to malaria control interventions. This study assessed LLIN access and use among pygmies, an autochthone population, in the health zone of Wamba, Province Orientale in DRC. A descriptive cross sectional study was conducted among pygmy populations in the health zone of Wamba from 17-22 February 2014, 4 years after the previous LLIN distribution campaign (2010) in order to establish a baseline data prior to the 2014 LLIN distribution campaign. Data was collected through interviews with heads of households using structured questionnaires. Data was entered using EPIDATA and analysed using SPSS for Windows. 602 heads of households in pygmy populations or their representatives were interviewed. The median household size was 4. The persons surveyed lived mostly in huts (57%) or small houses (43%). The proportion of households owning at least one LLIN, the use of LLIN by pregnant women and children under 5 years old were 18.3%, 9.9% and 10.1%, respectively. Heads of households mentioned other uses of LLIN such as for fishing and protecting crops. Despite substantial progress achieved over the last seven years in malaria prevention in DRC, the use of LLIN among the pygmy populations in the health zone of Wamba remains low. LLIN mass distribution planners should develop mechanisms to reach autochthone populations including a participatory process, inclusion of these key population representatives in decision making, targeted communication, and procurement of smaller size LLIN.

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USE OF GIS-BASED HOUSEHOLD MAPPING SYSTEM TO IMPROVE PLANNING, IMPLEMENTATION AND MONITORING OF MALARIA CONTROL STRATEGIES IN BIOKO ISLAND

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As it is in many malaria-endemic countries, the informal housing pattern in Equatorial Guinea without street names and building numbers makes malaria control difficult to effectively plan, implement and monitor. Beginning in 2012, the Bioko Island Malaria Control Project (BIMCP) created a geo-referenced mapping system that assigned each household a unique identifier similar to an address. The Island was divided into 1 km2 sequentially numbered map areas, which in turn were sub-divided into 100m2 sequentially numbered sectors. Using printed high resolution satellite images of each sector that clearly identified each building, a local field team assigned a sequential number to each household in each building in each sector. Buildings with more than one household were ascribed a unique number for each household. Households residing in multi-story buildings received a floor identifier. The concatenation of the map area, sector, household number, and floor level yielded a unique identifier, which was printed on a sticker glued to each household entryway, and subsequently recorded on all spray cards and LLIN distribution forms. Areas of the island where satellite images were not available were mapped using GPS by a team that drove every road, walked every footpath, and identified every building/household. The information from the field maps was then digitized in ArcGIS. Field teams updated the household database during IRS rounds in 2013 and 2014, and a mass LLIN distribution in 2015. This has enabled the BIMCP to effectively plan, implement and monitor vector control activities and respond to the substantial housing growth due to the economic boom on the Island. By being able to track IRS and LLIN supply to households and link this to annual survey data, the BIMCP has had an improved understanding of factors that contribute to increased parasite prevalence and to stratify the Island in terms of transmission characteristics and service coverage. In areas such as Bioko where informal housing patterns exist, the GIS-based mapping system instituted by the BIMCP allows for improved planning, implementation and monitoring of malaria control activities.

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DESIGN AND DEPLOYMENT OF A GIS-BASED CAMPAIGN INFORMATION MANAGEMENT SYSTEM FOR PLANNING, MANAGING AND MONITORING MALARIA CONTROL INCLUDING FUTURE VACCINATION ON BIOKO ISLAND

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Medical Care Development International (MCDI) and the University of Southern Maine (USM) are developing an Android-based tablet application for planning, managing and monitoring community-based malaria control on Bioko Island called the Campaign Information Management System (CIMS). The CIMS is currently deployed for IRS and LLIN distribution and keep-up and will later be used for managing rapid case detection and treatment, highly focused control around residual hotspots, and possibly mass vaccination with a transmission-blocking vaccine. The CIMS includes four modules, two of which leverage pre-existing open source applications developed by USM and other partners - OpenHDS, a health and demographic surveillance system, and MOTECH, a suite of mhealth tools that include technology for integrating with telcom systems to send voice or text messages to individuals/patients based on defined calling criteria. The CIMS modules include: (1) a geo-referenced household listing and associated member census based on OpenHDS; (2) an individual identification system that uniquely identifies all residents; (3) a set of campaign participation applications developed in ODK that track individual or household participation in various campaigns; and (4) a cell phone-based notification system using MOTECH which notifies households about impending campaign events, follows-up on missed contacts, and encourages participation in future events. When engaging households during campaigns, field workers select enumerated households from a hierarchical geographic selection system. Household information can be updated in real time, accounting for population movement or construction of new houses. Campaign participation information is then entered separately for IRS, LLINs, or any other type of campaign. These data are stored in distinct data sets on a central server (cloud or local). The CIMS provide an easy-to-use, adaptable and scalable solution for planning, managing and monitoring malaria control campaigns that is particularly well suited for countries heading towards elimination.

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UTILIZING IQKS AND A CAMPAIGN INFORMATION MANAGEMENT SYSTEM (CIMS) FOR IMPROVED QUALITY CONTROL OF INDOOR RESIDUAL SPRAYING

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IRS programs have traditionally faced challenges ensuring that: (1) sprayers do not falsify spray records; (2) rooms within houses are sprayed well (i.e. without coverage gaps and with a sufficient amount of insecticide applied); and, (3) effective insecticidal coverage has been achieved in each spray area. The Bioko Island Malaria Control Project (BIMCP) has instituted a quality control procedure to address these challenges using Insecticide Quantification Kits (IQKs) to test houses randomly selected from a Campaign Information Management System (CIMS) that maps in real time the houses reportedly sprayed each day. Sprayer performance is assessed at various intervals during a spray round by randomly selecting a house per sprayer from those recorded in the CIMS the previous day. IQKs are used to test samples from three spots arrayed from the top left to the bottom right of a sprayed wall. Six qualitative outcomes are measured ranging from "Well Sprayed" where all three samples comply with WHO concentration norms, to "Not Sprayed" where all three have no insecticide. Sprayers who do not satisfy the "Well Sprayed" outcome receive intensified training and supervision. Repeated poor performance can lead to dismissal. A first evaluation in 2014 revealed that 6% of sprayers achieved a "Well Sprayed" status and 4% a "Not Sprayed" status. Data from the current IRS round at various time intervals will be presented. Program performance is assessed using Lot Quality Assurance Sampling (LQAS) and IQKs to determine if effective insecticidal coverage has been achieved (i.e., that at least 80% of 19 randomly selected communities each in turn have at least 80% of 19 randomly selected houses sprayed with the recommended insecticide concentration). The samples from all lots are then pooled to derive an effective insecticidal coverage rate for the Island as a whole. These data for the current round will be presented. The ability to randomly select, locate and test via IQK houses reportedly sprayed the prior day has markedly improved the quality of IRS on Bioko Island, virtually eliminating falsification, and enabling the project to better evaluate its performance.

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PREVALENCE OF ACUTE GASTROENTERITIS AMONG U.S. MILITARY PERSONNEL DEPLOYED TO HONDURAS DURING 2014-2015

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Acute gastroenteritis (AGE) is a leading cause of lost duty days in military personnel, and vaccine candidates against common diarrhea pathogens are in phase II clinical trials. The need for field sites to evaluate vaccine efficacy in deployed personnel is a strategic need for the U.S. military and collaborating partners. Thus, we established Soto Cano Air Base in Honduras as a field site for clinical trials in Latin America including an on-site lab for stool cultures and reference capacity at NAMRU-6 in Peru. Additionally, post-deployment surveys were integrated into medical out-processing to capture unreported cases of AGE. To date, 36/168 (21.4%) personnel evaluated in the post-deployment surveys reported experiencing diarrhea during a 6-9 month deployment (mean 3.2 different episodes, range 1-10). The most common symptoms reported were cramps (43.9%), nausea (40.3%), fever (26.3%), vomiting (14.3%), with 2 (3.5%) persons reported experiencing bloody diarrhea, and the median number of loose stools in a 24 hour period was 4 (range 2-15). Eleven (19.3%) persons reported experiencing lost duty time (range 1-4 days), 29 (50.8%) experienced decreased performance (range 1-10 days), and 8 (14.0%) reporting being sick in quarters. Further, 22 (38.6%) persons reported to the clinic and 4 (7.0%) needed IV fluids. From passive surveillance, 85 persons reported to the clinic with diarrhea and 36 (42.9%) met the case definitions of moderate-severe diarrhea. From these 36 patients, 3 (8.3%) samples were positive by primary stool culture, with ***Shigella***, ***Salmonella***, and ***Aeromonas*** each identified. The remaining samples were positive for lactose-fermenting Gram-negative rods consistent with potential pathogenic ***E. coli*** subtypes. These samples are being analyzed at NAMRU-6 by multiplex PCR using the Luminex Gastrointestinal Pathogens Panel (GPP) as well as multiplex PCR detection of pathogenic ***E. coli*** virulence factors, norovirus RT-PCR, and stools ova and parasite exam. These findings highlight the impact of diarrhea on military operations and provide the information/logistics needed to establish Soto Cano as a site for phase I-IV clinical trials.

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AN ORAL CHOLERA VACCINATION CAMPAIGN COVERAGE SURVEY, HAITI 2014

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As part of a 10-year cholera elimination plan in response to the 2010 cholera outbreak in Haiti, the Haitian Ministry of Public Health and Population (MSPP) has proposed vaccinating 600,000 persons living in areas with high cholera attack rates and poor access to healthcare, clean water, and basic sanitation with a two dose oral cholera vaccine (OCV). During August –September 2014, MSPP conducted its second OCV campaign targeting 185,314 persons in certain areas of 3 departments: Artibonite, Centre and Ouest. This was the first time vaccine from the global OCV stockpile was used in Haiti. We conducted a multi-stage stratified cluster survey to assess OCV coverage by department. We sampled 80 enumeration areas (EAs) using stratified random sampling and 20 households were selected in each EA. In each selected household, a general and an individual interview were conducted; one person per age group (1-4 years, 5-14 years, and ≥ 15 years) was randomly selected for interview. Overall, 1,489 household and 3,201 individual interviews were conducted. Coverage estimates and 95% confidence intervals (CI) were calculated accounting for the design. Two-dose OCV coverage was 74% (95% CI: 64, 82), 64% (56, 72), and 48% (40, 56) in Artibonite, Centre, and Ouest, respectively, and drop-out after the first dose was 7%, 8%, and 2%, respectively. Children 1 to 14 years were more likely to be vaccinated than persons ≥15 years old ($p<0.01$); in Centre, females were more likely to be vaccinated than males ($p=0.03$). The most common reason for not receiving any OCV doses was being absent during the campaign in Centre and Artibonite and not hearing about vaccination activities in Ouest. The most common reason for receiving only one dose was being absent during the second round of campaign in all three departments. While coverage in Artibonite and Centre was comparable with that in other OCV campaigns in Haiti and elsewhere, outdated population estimates and inadequate social mobilization might have contributed to lower coverage estimates in Ouest. Future OCV campaigns in Haiti should be informed by more up to date population estimates and robust social mobilization activities.

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EXPLORING REG1B AS A POTENTIAL MARKER OF INTESTINAL HEALTH

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Environmental enteropathy (EE) is a gut disorder characterized by intestinal inflammation without overt diarrhea that occurs in individuals exposed over time to poor sanitation and hygiene. In children from low-income countries EE, is implicated as a cause of malnutrition, oral vaccine failure and impaired cognitive development. Unfortunately, a lack of direct and specific diagnostic tests has made it challenging to study EE. We have developed a fecal ELISA for Reg1 to test it as a potential biomarker of small intestinal health. Reg1 plays a role in gut health and is known to be up-regulated in a variety of enteric infections and inflammatory conditions. Our laboratory has previously found that fecal Reg1 predicted future stunting through the first two years of life when measured in 12-week-old children in birth cohorts from Bangladesh and Peru. We are currently testing Reg1 for its ability to independently predict gut damage in approximately 220 children from Mirpur, Bangladesh who were enrolled in the MAL-ED cohort study. We will test for fecal Reg1B in monthly and diarrheal stools for all children up to 12 months of age. We hope to assess the relationship between Reg1B and stunting in this cohort and determine a normal fecal Reg1B level in our population, an important step towards using this test in the clinical setting.

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CLIMATIC INFLUENCES ON ENDEMIC CHOLERA IN KALEMIE, DEMOCRATIC REPUBLIC OF THE CONGO

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We examined associations of environmental factors with cholera incidence in Kalemie, a city by Lake Tanganyika in the Democratic Republic of Congo. From January 2002 to February 2012 Kalemie had a total of 20,559 suspected cholera cases averaging 39 per week (IQR 12-52). Cases appear year-round with two annual peaks, atypical of Africa. The region has a single rainy season that peaks between November and January and a single dry season from May-August. Climatic factors were estimated through remote sensing and considered as lagged variables in analyses from 0 to 30 weeks before the week of cholera incidence. Factors included Lake Tanganyika height, temperature and chlorophyll a, maximum and minimum weekly air temperatures, precipitation, solar radiation, and water in the top meter of the soil column. In univariate models, cholera incidence was most associated with chlorophyll a levels (Pearson correlation = 0.23) and maximum and minimum weekly air temperatures one week prior (Pearson correlation = 0.26 and 0.27 respectively). Multivariable negative binomial regression models including climatic factors were compared based on Akakie Information Criteria and mean-squared prediction error using 'hold one year out' cross validation. The best model by both methods included: Lake Tanganyika water level, temperature and chlorophyll a, soil water content of top meter of soil, rainfall, net solar radiation, and minimum/maximum air temperature at varying time lags. 4.4% of weekly case counts were above and 21.9% below the cross validation predictive interval. These climate factors explain 36.3% of the week-to-week variation in cholera. Cholera incidence is clearly associated with climatic factors, however, models based on solely on climate fail to predict many major variations in cholera incidence. In Kalemie, 61% of the weeks when cases exceeded the prediction interval occurred in years with documented large-scale power outages and influxes of migrant populations. Improved predictive models using increased local knowledge of factors impacting the epidemic may allow us to better evaluate effective interventions such as vaccination campaigns.

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HARD BLOOD AGAR IN THE ISOLATION OF *ARCOBACTER SPP*

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Arcobacter spp., is currently considered emerging zoonotic pathogen; isolation by conventional methods in developing countries is difficult and expensive, sometimes by contamination with bacteria such as ***Proteus.*** Here, we present the hard blood agar (HBA) as a simple culture medium for the isolation of ***Arcobacter spp***. We worked with 100 human fecal samples and 100 animals (50 pigs and 50 cattle) for the isolation of ***Arcobacter spp.***; using the HBA and Columbia Blood Agar (CBA), both with the method of the filter (0.45um porosity), and microaerophilic

incubation with the method of ***Klebsiella*** and closing or sealing of the inoculated plate with rubber bands obtained from powder-free latex gloves. Incubation at 28 ° C and read after 24-48 hours. The identification of ***Arcobacter*** was only based on their phenotypic characteristics. ***Arcobacter*** was isolated 3/100 (3%) of human specimens in CBA, and 4/100 (4%) in HBA; in animals: in cattle 10/50 (20%) was isolated in CBA, and 28/50 (56%) in HBA; in 18/50 pigs (36%) was isolated in CBA, and 35/50 (70%) in HBA. Hard blood agar (HBA) is an effective, simple and inexpensive alternative culture medium for the isolation of ***Arcobacter spp.***, even in samples with ***Proteus*** passing through the filter and by swarming on cultures, masking and difficult isolation of ***Arcobacter***.

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VIRULENCE GENES AND ANTIMICROBIAL RESISTANCE IN *SHIGELLA* SPP. ISOLATED FROM CHILDREN WITH DIARRHEAL DISEASES REQUIRING MEDICAL CARE IN FORTALEZA, CEARA, BRAZIL

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Shigella spp. is one of the most prevalent etiological agents of enteric infection. This study aimed to determine circulating species, virulence genes and antimicrobial resistance profile of Shigella spp. obtained from a cross-sectional study of moderate to severe childhood diarrhea in Fortaleza, Ceara, Brazil. Fecal specimens and clinical data were collected from May 2008 to April 2009 and 63 Shigella strains were isolated by standard microbiological methods. Immunoagglutination assay was employed for species characterization and four multiplex-PCRs were developed to detect 14 sequences encoding virulence genes (ial, set, virF, sen, sigA, pic, sepA, ipaA, ipaB, ipaC, ipaD, icsB, Stx and virB). Antimicrobial susceptibility tests were performed using the Kirby-Bauer disk diffusion method with 13 antimicrobial discs commercially available. The most prevalent ***Shigella*** species were ***S. flexneri*** and ***S. sonnei*** (43%, 27/63 for both), followed by ***S. dysenteriae*** (8%, 5/63) and S. boydii (6%, 4/63). The protease associated with mucosal binding (pic), enterotoxin 1(set) and protein associated with cell invasion (sepA) genes were significantly associated with S. flexneri infection ($p=0.0002$, $p<0.0001$ and $p<0.0001$, respectively), while other virulence genes showed no difference among species. The concomitant presence of pic, set and sepA was correlated with intense abdominal pain ($p=0.0379$) and multi-drug resistance to at least three drugs ($p=0.0028$), being sulfamethoxazole/trimethoprim + tetracycline + ampicillin + chloramphenicol the most frequent pattern. This study suggests that more severe shigellosis is caused by S. flexneri carrying the virulence genes pic, set and sepA. These strains also present multi-drug resistant phenotype, indicating a link between virulence and antimicrobial resistance pressure selection and evolution.

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CLINICAL PRESENTATION AND VIRULENCE GENES PROFILE FROM *CAMPYLOBACTER JEJUNI* INFECTION ISOLATED FROM CHILDREN WITH MODERATE TO SEVERE DIARRHEA IN FORTALEZA, CEARÁ, BRAZIL

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Campylobacter spp. is considered the most common cause of bacterial gastroenteritis. This study characterized ***C. jejuni*** virulence-associated genes that may play a role in ***C. jejuni*** pathogenicity in children aged 0-36 months who required emergency medical care due to moderate to severe diarrheal disease. It is part of a project entitled "'Diarrhea Enteric Card (DEC)' aiming to develop PCR-based multiplex diagnose assay for bacterial enteric pathogens. The project was approved by the local and national ethical committees in Brazil (HIAS 80/06 and CONEPE 13523/2007, respectively). DNA was extracted directly from fecal samples collected from 226 children during May 2008 and April 2009, in Fortaleza, Ceará, Brazil. A questionnaire was applied to characterize clinical symptoms. Conventional PCR was performed for detection of ***C. jejuni*** strains and investigation of nine virulence genes. The identification of ***C. jejuni*** was performed using hipO gene, providing 19,5% (44/226) of positivity. The presence of ***C. jejuni***´s virulence-associated genes encoding proteins related to pathogenesis of the micro-organism were detected in the following proportions of ***C. jejuni***-positive DNA samples: racR, 97.7% (43/44), dnaJ, 88.6% (39/44), and flaA, 79.5% (35/44); - related to bacterial adhesion and colonization; ciaB, 97.7 % (43/44); pldA, 45.4% (20/44) and pVir 0% (0/44) - related to invasion, and cdtABC in 95.4% (42/44) related to cytolethal distending toxin (CDT). These data showed that ***C. jejuni*** were detected in a significant percentage of children aged 0-36 months with moderate to severe diarrhea. Virulence genes related to bacterial adhesion, colonization and cytotoxicity were detected in a great proportion of ***C. jejuni***-positive samples, while invasion-related virulence genes were detected in lower frequency. The distribution profile of virulence genes from ***C. jejuni*** was not correlated with the clinical presentation of the disease, suggesting that maybe other virulence genes or host factors such as nutritional status are important in defining the clinical severity of diarrheal diseases associated with ***C. jejuni***.

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EVIDENCE FOR GASTROINTESTINAL CARRIAGE OF *CLOSTRIDIUM DIFFICILE* AMONG CHILDREN IN THE PERUVIAN AMAZON RIVER BASIN

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Diarrheal disease is a leading cause of death and illness in developing countries and is responsible for nearly one in five child deaths worldwide each year. Unsafe water, poor sanitation, and inadequate hygiene are the major risk factors for the spread of disease. Belen is an impoverished river dwelling community without public sewage systems and trash disposal. There are few laboratory based studies on infectious gastroenteritis in this community and none on the prevalence of ***Clostridium difficile***. This study examines the prevalence of ***C. difficile*** in children ages 5 and under in Belen. With local IRB approval and informed consent from heads of households, 206 stool samples were collected from children 5 and under from May to October 2014. All samples were analyzed for ***C. difficile*** antigen (glutamate dehydrogenase) or toxin (A and B) using ***C. difficile*** QUIK CHEK (TechLab, Blacksburg, VA). All stool samples were accepted irrespective of consistency. Sixteen of 206 stool samples (7.77%) assayed showed antigenic (Ag) or toxigenic (Tox) evidence of ***C. difficile***; two samples (0.97%) for toxigenic strains (Ag+ / Tox+), 13 samples (6.31%) for non-toxigenic strains (Ag+ / Tox-), and one sample (0.49%) was positive for toxin only (Ag- / Tox+). This is the first laboratory based study assessing the prevalence of ***C. difficile*** in this community and provides strong evidence for childhood carriage of non-toxigenic, and more limited evidence for toxigenic, strains of ***C. difficile***. These studies will be augmented in the future by culture and/or nucleic acid amplification for genes coding for all virulence factors associated with ***C. difficile***. Unfortunately, symptomatic vs. asymptomatic data could not be consistently ascertained, but future studies associating symptoms and the presence of ***C. difficile*** could lead to a better understanding of the epidemiology of non-nosocomial, community-acquired ***C. difficile*** in the Amazon River Basin. To date, there is no data linking ***C. difficile*** carriage to the environment, hygiene practices or socioeconomic factors in Belen. Additional investigations of other etiologies of infectious gastroenteritis are also ongoing.

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INCORPORATING MODEL UNCERTAINTY INTO PREDICTIONS OF THE BURDEN OF TYPHOID FEVER

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Upcoming efforts in vaccination against typhoid fever require an assessment of the baseline burden of disease in countries at risk. Model-based estimates are a feasible alternative to costly and time-consuming field-based estimates of typhoid fever incidence. In most countries, typhoid incidence data is not collected, and therefore current estimates of incidence are based on interpolation of data from 32 studies. Recent estimates of typhoid burden are based on models that have not been assessed for predictive accuracy. Furthermore, estimates of age-specific incidence are based on assumptions about how the age distribution varies with incidence, rather than empirical estimates of age-specific heterogeneity. An understanding of the difference in incidence attributable to age is imperative to guide upcoming vaccine policies. Therefore, it is necessary to identify readily-available predictors of typhoid incidence, such as access to clean water, access to improved sanitation, country-level Gross Domestic Product, etc. We developed a random-intercept, mixed effects model fit to data from 32 population-based studies of typhoid incidence, 17 of which report incidence for different age groups. We used Bayesian model averaging to incorporate uncertainty around the estimates of the predictors as well as the uncertainty in model selection. The current re-analysis permits prediction of the overall as well as age-specific incidence of typhoid fever, as well as the associated uncertainty, in low- and middle-income countries.

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EPIDEMIOLOGIC FEATURES OF DIARRHEAL DISEASE DUE TO *AEROMONAS SPP.* AND *PLESIOMONAS SHIGELLOIDES* INFECTIONS IN KAMPONG CHAM, CAMBODIA

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To investigate the possible etiological agents of diarrheal disease in Cambodia, a cohort based study was conducted from August 2012 to March 2015. The US Naval Medical Research Unit Two (NAMRU-2) Detachment Phnom Penh in collaboration with the Cambodian National Institute of Public Health (NIPH) conducted active surveillance among four villages in Kampong Cham Province, Cambodia. Subjects self reported symptoms to study staff and were also followed on a weekly basis. Stool samples were collected from subjects if they presented with symptoms of acute diarrhea defined as 3 or more loose stools within 24 hours or 2 loose stools associated with gastrointestinal complaint within 24 hours. Study subjects were comprised of 2,921 adults and 1,749 children (aged < 18 years old). There were 258 ***Aeromonas spp.*** and 266 ***Plesiomonas shigelloides*** isolates identified from 2,603 specimens collected and tested between August 2012 and March 2015. Study data indicates that adults have a higher risk of being infected with ***Aeromonas spp.*** (OR=1.4, 95%CI: 1.1-1.9) or ***P. shigelloides*** (OR=1.9, 95%CI: 1.5-2.6). Subjects who worked in retail settings (shop owners or workers) also had a higher risk of being infected with ***P. shigelloides*** (OR=2.0, 95%CI: 1.2-3.3) whereas the participants who were students or worked at home tended to have lower risk (OR=0.7, 95%CI: 0.5-0.8). Abdominal pain and mucus laden stool are the two symptoms significantly associated with the infection of these two pathogens ($p< 0.05$). Lack of access to clean drinking water (filtered or boiled) or lack of sanitary toilet facilities were identified as increased risk factors for positive stools. 52.3% and 70.2% of ***Aeromonas spp.*** positive subjects and 55.6% and 70.3% of ***P. shigelloides*** positive subjects did not have access to clean water or toilet facilities, respectively. The prevalence of these pathogens appears to be correlated with inadequate access to sanity water and toilet facilities.

1673

ORAL CHOLERA VACCINE STUDIES IN CHOLERA ENDEMIC SETTINGS IN BANGLADESH

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Bangladesh has one of the world's highest burden of endemic cholera with an estimated 350,000 cholera cases and over 4,500 deaths annually. A serious need therefore exists for control of cholera using vaccines and other preventive measures. Over the last few years large scale vaccination studies are being conducted with the whole cell oral cholera vaccine (OCV) Shanchol in high risk urban as well as rural populations. The objective of such studies is to assess the feasibility of delivery as well as effectiveness of vaccination program utilizing the existing national immunization infrastructure of Bangladesh. Vaccine coverage was 65% and protection was 53%, which was evident in all age groups and sustained for 2 years. In a study in a rural setting in Bangladesh carried out in 65,000 participants and the OCV program was successful and 92% of the vaccinees receiving the first dose of vaccine also returned for the second dose. In addition we have recently conducted a single dose oral cholera vaccine study to determine if only one dose can be protective, a strategy very important for use in epidemics and outbreaks. The oral cholera vaccines need to be stored at 2-8 °C. This is one of the limiting factors for delivery of vaccines in resource poor settings globally. We therefore studied, if storage of vaccine at higher temperatures (25°C, 37°C and 42°C) resulted in similar safety and immunogenicity compared to vaccine stored in the cold. The vaccine is safe, well tolerated and satisfactory immune response is elicited. The OCV studies were feasible and effective using government facilities. These studies demonstrate that the Shanchol can be delivered to all age groups using resources already available in the country. Strategies to plan and implement OCV uptake in national immunization programmes in high risk hotspots is needed for Bangladesh. Studies to assess vaccination strategies, target age groups for vaccination, uptake, feasibility as well as cost-effectiveness analyses need to be made using the same approach as used for other immunisation programmes which have been successful in Bangladesh and such studies are being planned.

1674

IFN-γ; HAS DIRECT ANTI-ENTEROAGGREGATIVE *E. COLI* PROPERTIES AND IS NECESSARY FOR RESILIENCE TO SEVERE EAEC-INDUCED ENTEROPATHY IN ZINC-DEFICIENT MICE

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Enteroaggregative E. coli is a major cause of early childhood diarrhea and growth impairment. Descriptions of EAEC-diarrhea are typically mucoid with occasional leukocyte products. While increased fecal IL-8 has been seen in symptomatic patients with EAEC infection, and mutations in the IL-8 promoter that lead to increased IL-8 expression also associate with symptomatic disease, other inflammatory mucosal pathways such as the IL23-IL17 and IFN-γ; axis relevant to other enteropathy-inducing pathogens are less well understood, and could contribute to disease during EAEC infection. In a murine model of EAEC-induced enteropathy we have previously shown that zinc deficiency both up-regulates aggR-regulated EAEC virulence genes and alters host inflammatory responses leading to increased Il8 and Mcp1 expression, mucus production, Muc2 expression, and Cftr expression, increased Il17, but decreased Il23, Il6, Tnfalpha, and Il1beta. To interrogate the role of the IL17-IL23 axis we infected zinc-deficient IL17RKO mice with the virulent EAEC042 strain. IL17RKO mice were protected from weight loss, more rapidly cleared EAEC, and demonstrated a 5-fold increased expression of IFN-γ;. Neutralization of IFN-γ; reverted the resilient IL17RKO mice to a susceptible wild-type phenotype. IFN-γ; demonstrated direct effects ***in vitro***, inhibiting EAEC growth in a dose-dependent manner. Further studies are planned to

confirm this new recognition of anti-EAEC properties of IFN-γ;, and whether genetically determined alterations in mucosal inflammatory responses may promote host resiliency to enteropathogens despite the disadvantage of severe micronutrient deficiency.

1675

ISOLATION AND SPECIES IDENTIFICATION OF GRAM-POSITIVE BACTERIA FROM THE CANNED FOOD BY SEQUENCE CHARACTERIZATION AT *GYR*B LOCUS: A PUBLIC HEALTH IMPORTANCE

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The primary mission of FDA is to enforce the Food, Drug and Cosmetic Act and regulate food, drug and cosmetic products. FDA uses presence of pathogenic microorganisms in these commodities as one of the regulatory action criteria and to ensure that the goods are safe for human consumption. This study was conducted to assess the effectiveness of pathogen control in a canned food facility located in the United States. A total of 9 unopened recalled canned food jars from the same lot containing Black Bean Corn Poblano Salsa were initially examined by conventional microbiologic protocols. Of these, while analyzing 8 subsamples for each sample, all subsamples of one of the containers was found positive for the presence of slow growing rod-shaped, Gram-positive facultative anaerobic bacteria. Species identification of 8 recovered Gram-positive bacterial isolates from the positive unopened jar was accomplished by our recently developed protocol based on DNA sequencing of PCR amplified ***gyr***B gene products. Multiple alignments confirmed all 8 generated ***gyr***B gene sequences to be identical. These sequences matched 99% (2-point-mutation) with the published sequence of ***Lactobacillus fermentum*** sequence available in public domain (GenBank Accession No. AP008937). Thus, the ***gyr***B-based molecular diagnostic protocol can be used as a suitable genetic marker for rapid detection of ***Lactobacillus*** in the canned food monitoring program of public health importance.

1676

STRINGENT QUALITY CONTROL MEASURES ALLOWS PROMPT IDENTIFICATION OF CRUCIAL PROBLEMS TO OPTIMIZE THE RELIABILITY OF MENINGOCOCCAL CARRIAGE SURVEYS

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We conducted a cross-sectional study to identify carriage of ***Neisseria meningitidis*** among 1000 schoolchildren in Fajara, The Gambia, to evaluate improved detection methods. We conducted this study using quality control (QC) standards applied to clinical trials. In addition to developing Standard Operating Procedures, Study Operations and Laboratory Operations Manuals and ensuring documented training of staff, we conducted regular internal audits of field and laboratory activities. We verified the ability of the laboratory team to perform the tests through enrolment in External Quality Assessment (EQA). We performed regular monitoring of positive controls used for qualitative PCR and of standard curves used for quantitative PCR through Levey-Jennings charts (LJC). We used non-template negative controls every twenty tested samples to ensure that there was no PCR contamination. The internal audits allowed identification of several minor challenges and a major one: non-collection of critical samples, a situation that was promptly corrected. Further laboratory audits did not identify other major findings. Monitoring of positive controls through LJC allowed identification of problems with primers and probes used for qualitative PCR, triggered reagents replacement and minimized inter-assay variation. Monitoring of standard curves through LJC led to exclusion of the results from one plate out of 72, where key values of the standard curve were found to be out of the defined acceptable range. EQA results identified a risk of PCR contamination in the sodC gene and promoted development of stricter rules to be applied in our molecular biology laboratories. No non-template negative control was positive during the entire course of the study. All staff working on this cross-sectional study were trained to apply these high standards. Large-scale epidemiological studies should be conducted with QC standards approaching the ones required in clinical trials. Adopting these standards allowed us to identify critical issues during the course of a study of meningococcal carriage and to correct them promptly.

1677

DETECTION OF *NEISSERIA GONORRHEA* USING CULTURE AND NUCLEIC ACID AMPLIFICATION TEST IN FIVE HEALTH FACILITIES IN GHANA

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Neisseria gonorrhea is one of the major etiologies of sexually transmitted infections (STIs) and when left untreated can lead to serious complications such as pelvic inflammatory disease, ectopic pregnancy, infertility in women and sterility in men. Microscopic examination and microbial culture are common techniques used in gonorrhea diagnostics; however both are insufficiently sensitive and/or specific. Noninvasive techniques utilizing newer technology such as antigen detection by immunoassay and nucleic acid amplification tests (NAATs) have become more acceptable because of their precision, suitability and time-saving aspects. The objective of this study is to determine the percentage of ***N. gonorrhea*** positive cases occurring among Ghanaian military members using culture and NAAT. Urethral or endocervical swabs and urine samples were obtained from consenting patients presenting with STI symptoms at five health facilities. Swabs were cultured on Modified Thayer-Martin agar and colonies subcultured on chocolate agar. ***N. gonorrhea*** isolates were identified by gram staining, catalase as well as oxidase tests. Purified colonies were confirmed using the API-NH. Urine samples were tested by NAAT. Of 679 specimen screened, 25.4% was positive for gonorrhea by NAAT and 6.9% by culture. All specimens positive by culture were also positive by NAAT. Compared to culture, NAAT performed better in identifying gonorrhea in STI-symptomatic patients presenting to health facilities in Ghana. Our finding demonstrates that NAAT could be beneficial in testing high-risk groups or patients who are culture negative but have persistent symptoms.

1678

DETECTION OF *LEPTOSPIRA*-SPECIFIC ANTIBODIES USING RECOMBINANT LIPL32, LIPL41, LIGA, AND LIGB IN ENZYME-LINKED IMMUNOSORBENT ASSAY

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Leptospirosis is caused by the infection of spirochetes of the genus ***Leptospira***. This zoonotic disease has become widespread in developing nations, particularly those located in tropical areas. In the early stage of the

bacterial infection, the disease can be treated with antibiotics. However leptospirosis is often misdiagnosed due to its nonspecific symptoms and lack of good diagnostic tests which can be easily performed at the clinical site. The current standard method for diagnosis of leptospirosis is the microscopic agglutination test (MAT). This assay requires live cultures, not only technically complex but also time-consuming. In an effort to replace MAT with a test that does not require live cultures, we produced highly purified recombinant antigens, rLipL32, rLipL41, rLigA, and rLigB, and evaluated their performance individually and a cocktail of 1:1:1:1 combination in ELISA to detect IgG and IgM antibodies specific for ***Leptospira*** using a panel of 429 human sera (337 MAT positive and 92 MAT negative sera). The positive samples had MAT titers greater than 100 against one or several of 18 serovars from 5 major pathogenic ***Leptospira*** species (***L. interrogans, L. kirschneri, L. borgpetersenii, L. noguchii, and L. weilii***). There were 145 (43%) samples that showed either detectable IgG or IgM against rLipL32; 177 (53%) samples against rLipL41; 216 (64%) samples against rLigA; and 228 (68%) samples against rLigB. There were 5, 15, 16, and 23 patient sera that had specific antibodies against only one antigen rLipL32, rLipL41, rLigA, and rLigB, respectively. Combining the detection results of IgG and IgM from these four individual antigens, the overall sensitivity is close to 90% but the specificity is only 65%, based on the MAT reference method. The overall sensitivity and specificity of the cocktail of 1:1:1:1 combination was 82% and 86%, respectively. These results showed that an ELISA using the combination of recombinant antigens has the potential to replace the reference method MAT. Further optimization of the relative amount of each antigen in the final formulation to increase the overall sensitivity by ELISA is underway.

1679

LEPROSY NEW CASE DETECTION TRENDS AND THE EFFECT OF PREVENTIVE INTERVENTIONS IN PARÁ STATE, BRAZIL: A MODELING STUDY

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Leprosy is still a public health problem in Brazil. Although the overall number of new cases is declining, there are still areas with a high disease burden, in particular Pará State. We aim to predict future trends in new case detection rate (NCDR) and explore the potential impact of contact tracing and chemoprophylaxis with single-dose rifampicin on NCDR in Pará State. We used SIMCOLEP, an existing individual-based model for the transmission and control of M. leprae in a population structured by households. The model was quantified to mimic the NCDR trend of leprosy in Pará state between 1990 and 2012. The baseline scenario (i.e. continuation of current control) includes multidrug therapy, passive case detection and BCG vaccination of infants. Leprosy data was obtained from the SINAN databases. We also investigated the impact of two interventions: 1) contact tracing, and 2) contact tracing in combination with administering chemoprophylaxis to contacts. All interventions start in 2015 with predictions made until 2050. The modelled trend in Pará State after 2012 shows a continuous downward trend, reaching the official elimination target of 10 per 100,000 annual new cases by 2028. Systematic contact tracing in combination with chemoprophylaxis to household contacts would bring the achievement of elimination forward to 2026. Contact tracing would increase the number of detected cases in the first 9 years, but in the long run would drop below the number in the baseline scenario. Administering chemoprophylaxis would prevent almost 10% of new detected cases since the start of the intervention in the long run. Our study indicates that the leprosy incidence will further decrease in Brazil. Elimination of leprosy as a public health problem can possibly be achieved around 2028 in Pará state. This moment could be brought forward by two years through systematic contact tracing in combination with chemoprophylaxis.

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DEVELOPMENT OF A LUMINEX TREPONEMAL ANTIBODY ASSAY FOR LARGE-SCALE SURVEILLANCE AND IMPACT EVALUATION OF GLOBAL YAWS ERADICATION PROGRAM

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Yaws, a non-venereal treponemal infection, is characterized by papillomatous and ulcerative skin lesions. Mid 20th-century mass-treatment campaigns resulted in a significant reduction in yaws cases, but resurgence of the disease occurred after the campaigns were dismantled. Recently, interest in yaws eradication was renewed after demonstration that a single oral dose of azithromycin is efficacious treatment for yaws. Serological diagnosis of yaws requires detection of two distinct antibodies: one against a treponemal antigen that indicates exposure to infection (such as the ***Treponema pallidum*** particle agglutination [TPPA] or hemagglutination [TPHA] assay) and one against a non-treponemal antigen (such as the rapid plasma reagin [RPR]) that can discriminate active from past infections. Unfortunately, the RPR test cannot be automated for use in large-scale, integrated serosurveillance platforms. We therefore sought to assess the quantitative correlation of individual treponemal antigens on a Luminex® multiplex bead-based immunoassay platform against standard serological tests for yaws. A total of 847 serum and dried blood spot specimens were collected as part of yaws surveillance projects in Ghana, Vanuatu, and Papua New Guinea (PNG). Specimens were run at 1:400 dilutions on a Luminex assay to test for IgG antibodies directed against the recombinant treponemal antigens p17 (rp17) and TmpA. Results were compared to those obtained using TPPA/TPHA assays and quantitative RPR test. Compared to the TPPA/TPHA, reactive concordance of rp17 was 98.8% (331/335), while reactive concordance of TmpA was only 84.8% (284/335). When comparing anti-TmpA reactivity against RPR titers we observed a strong correlation that was not seen with rp17 responses. A significant increase was noted in TmpA reactivity with increasing RPR titers (R^2 =0.975 for Ghana and Vanuatu and 0.94 for PNG). Our results suggest that TmpA could be used as a treponemal antigen marker for recent or active infection. Further validation will assist in development of a tool that could useful to help yaws surveillance and impact monitoring of global yaws eradication programs.

1681

LARGE THROUGHPUT SCREENING AND COMPUTATIONAL APPROACHES FOR IDENTIFYING COMPOUNDS EFFICACIOUS AGAINST THE OBLIGATE INTRACELLULAR PATHOGEN, *RICKETTSIA*

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Rickettsia is a genus of arthropod vectored, obligate intracellular bacteria that is the etiological agent of multiple spotted fevers and typhus diseases, most notably Rocky Mountain Spotted Fever and epidemic typhus. Various historical and novel Rickettsial diseases are constantly re-emerging as major human pathogens such as ***R. parkerii*** causing Tidewater Spotted Fever that is spreading with climate change up the Eastern coast of the United States. In recent years, antibiotic resistance has been documented in clinical cases requiring physicians to resort to more toxic treatments and therefore new drug compounds are needed for anti-Rickettsial development. Rickettsia's obligate intracellular nature presents challenges not usually present in drug screening protocols, which we aimed to overcome here. By transforming ***R. canadensis*** with pRMA18dRGA (Wood et al) expressing Green Fluorescent Protein we are able to utilize

fluorescence to measure inhibition of growth of ***R. canadensis*** in the Vero cell line when exposed to drug-like compounds. After validation and optimization of the conditions for compound screening, we have begun to screen 2,000 compounds from the Microsource Compound Library. Toxicity for each compound was also assessed as a factor in the obligate intracellular nature of ***Rickettsia***. The compounds that are active ***in vitro*** against ***Rickettsia*** will be further analyzed through a Bayesian algorithm to calculate predictive values for future compounds' activity or lack thereof. This Bayesian algorithm has been used previously by our and collaborating laboratories to identify compounds active against Mycobacterium tuberculosis and Staphylococcus aureus. By adapting methods for large throughput screening and combining data produced with the Bayesian algorithm we aim to find novel compounds active against ***Rickettsia*** spp, and produce a baseline database for future drug discovery.

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IN VITRO ANTIMICROBIAL ACTIVITIES OF "ANTIBACT", AN HERBAL MEDICINAL PRODUCT AGAINST CLINICAL BACTERIAL ISOLATES

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In vitro antimicrobial activities of ethanol and aqueous "Antibact", herbal products consisting of a combination of the leaves and branches of four different plants were evaluated against twenty one pathogenic bacteria. Saponins, reducing sugars, phenolics, polyuronides, and triterpenes were the major phyto-constituents of both the aqueous and ethanol "Antibact". The LD50 analysis revealed the products were safe (LD50>5000 mg/kg bodyweight) for ***in vivo*** use. All the isolates (100%) were resistant to at least five of the 12 antibiotics used in the study. In total, the aqueous "Antibact" inhibited the growth of 5 out of the 21 (23.81%) microbes used with an average zone of inhibition of 9.73 ± 0.35 mm while thirteen (61.90%) out of the 21 microbes used were susceptible to the ethanol "Antibact", with an average inhibition zone of 10.80 ± 0.18 mm. The minimum inhibitory concentration (MIC) of the aqueous "Antibact" ranged from 4.00 to 32.00 mg/ml and 0.50 to 8.00 mg/ml for the wild and standard strains respectively. In the case of the ethanol "Antibact" the MIC ranged from 2.00 to 8.00 and 1.00 to 2.00 mg/ml for the wild and standard strains, respectively. The average minimum bactericidal concentration (MBC) for the aqueous "Antibact" was 32.00 mg/ml while thatof the ethanol "Antibact" was ranged from 4.00 to 16.00 mg/ml and 4.00 to 8.00 mg/ml for the wild and standard strains, respectively. Thus, both aqueous and ethanol "Antibact" are safe for human use and also effective against some pathogenic bacteriaevaluated ***in vitro*** with the ethanol "Antibact" showing better antimicrobial activity.

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MEETING THE CHALLENGES OF BURULI ULCER IN ANAMBRA STATE NIGERIA

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Buruli Ulcer remains a great public health problem in riverine areas of Anambra State, Nigeria. It is a debilitating disease affecting the social and economic life of the people who are essentially poor peasant farmers. It is shrouded with myths, superstitions, stigma and discrimination which affect early and prompt health seeking behaviour. Yet it is neglected and no ongoing robust programme is targeted to control the disease. Using in-depth interviews, key informant interviews and focus group discussion, the perceptions of the people were ascertained. Clinical presentation of the ulcers were also gathered from infected persons. This lasted from January to March 2015. Male and female are equally affected in the distribution of the ulcer with the lower limb mostly affected, late presentation results in complications such as chronic ulcer, contractures and disability. Myths associated with Buruli ulcer includes that it is caused by witches and wizards, enemies and offended deities. it is believed that only native juju doctors can cure the disease. The ulcer perpetuates poverty cycle as affected people are not able to farm thereby reducing the income in addition to the treatment bills spent in appeasing the deities and paying the juju doctors. Women are mostly affected by stigma which reduces the chances of marriage of affected young women. The key challenges are, the area is inaccessible because of poor terrain, the educational status is low, medical facilities with qualified personnels are limited. There is predominance of native juju doctors and sorcerers who perpetuates the superstition and myths. Poverty also affects their health seeking behaviour. There is need for health education and enlightenment in the area to make the people understand the aetiology of the disease, remove myths and superstition and thereby encourage early health seeking, diagnosis and treatment. There is need for research in the area to know further the determinants and distribution of the disease in the area. Poverty alleviation and empowerment programmes should be provided in addition to improved Orthodox health facilities.

1684

EXAMINATION OF TETANUS VACCINATION AND SEROPREVALENCE DATA BY PROVINCE IN CHILDREN UNDER 5 YEARS OF AGE FROM 2007 AND 2013 IN THE DEMOCRATIC REPUBLIC OF CONGO

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Critical evaluation of an immunization program is key to identifying areas of need and to ensuring efficacious and efficient resource allocation. We compared tetanus vaccination rates (determined by either maternal recall or vaccination card) by province for children 12-23 months of age in the Democratic Republic of Congo (DRC) from the 2007 (n=1,585) and 2013 (n=3,366) Demographics and Health (DHS) Surveys. A seroprevalence survey was also completed in tandem with the 2013 DHS Survey in which Dried Blood Spots (DBS) were collected from participants to determine IgG antibodies against tetanus for 8,116 children 6-59 months of age. Overall tetanus vaccination coverage (doses 1-3) in the 2007 DHS study for children 12-23 months of age was 70.6%, 59.1%, and 45.0%, respectively. In the 2013 study, overall tetanus vaccination coverage (doses 1-3) of children 12-23 months of age was 81.2%, 73.8%, and 60.5%, respectively. Tetanus seroprevalence was lowest in Maniema (20.1%) and Katanga (27.8%) provinces. In 2007, DTCoq vaccine coverage for children 12-23 months of age in Maniema was 51.9%, 38.3%, and 17.4%; and in Katanga was 60.4%, 51.6%, and 38.6%. In 2013, DTC/Pentavalent vaccine coverage for children 12-23 months of age in Maniema was 73.8%, 66.7%, and 47.2%; and in Katanga was 67.3%, 60.1%, and 51.3%. Tetanus seroprevalence was highest in North Kivu (51.8%) and Kinshasa (51.3%) provinces. In 2007, DTCoq vaccine coverage for children 12-23 months of age in North Kivu was 93.4%, 91.1%, and 83.3%; and in Kinshasa was 94.6%, 87.9%, and 81.1%. In 2013, DTC/Pentavalent vaccine coverage for children 12-23 months of age in North Kivu was 94.4%, 91.1%, and 87.0%; and in Kinshasa was 97.8%, 95.8%, and 83.7%. Although reported tetanus vaccination coverage has improved in the DRC, the low seroprevalence of tetanus antibodies, as well as the

divergence between reported coverage and seroprevalence warrants re-examination of current immunization and surveillance strategies. This data suggests that geographical disparities exist that may require novel or intensive strategies for effective immunization programs in certain provinces.

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DO TRACHOMA ASSESSMENTS PREDICT DISTRICTS ENDEMIC FOR TRACHOMA? COMPARISONS OF RESULTS FROM TANZANIA, UGANDA, BENIN AND DEMOCRATIC REPUBLIC OF CONGO

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Prior to SAFE implementation, baseline population based prevalence surveys (PBPS) of trachoma must be undertaken to facilitate planning. Trachoma assessments (TA) are a rational approach for prioritizing resources for PBPS. We compared: methods of TA, results of TA and results of PBPS undertaken in four countries. In Tanzania, TA in 56 districts included: review of health facility TT data; and assessment of TF and TT in two villages. PBPS were prioritized where villages had ≥ 5% TT or ≥15% TF, and districts that reported ≥30 TT cases over 5 years. In Uganda, key informant interviews and review of clinical records were done in 8 districts. PBPS criteria included: ≥10 TT cases for 2 consecutive years; >40% of health workers identified TT as a problem; and districts bordering areas with TF prevalence ≥10%. In Benin, Communes in 4 northern districts were assessed and criteria for PBPS were: bordering districts in neighboring countries with TF ≥10%; and reported TT surgery data. In Democratic Republic of Congo (DRC), TA included: investigation of TT cases seen at health facilities in Orientale, Equateur and Katanga; and assessment of TT cases seen in the community in North and South Kivu Provinces. Criteria for PBPS were: ≥10 TT cases seen at health facility; ≥10 TT cases seen in the community; and heath zones bordering countries where TF prevalence was ≥10%. PBPS were done using GTMP methods. In Tanzania, of 19 (out of 56) districts prioritized for PBPS, two had TF prevalence ≥10% and none had TT prevalence ≥1%. PBPS in four (out of 8) districts in Uganda showed TF prevalence and TT prevalence <10% and <1%, respectively. In Benin, 11 (out of 27) communes had PBPS of which two had TF prevalence ≥10% and 3 had TT prevalence ≥1%. In DRC, 32 (out of 99) health zones were prioritized for PBPS. PBPS have been completed in 10 health Zones where TF prevalence ≥10% and TT prevalence ≥1% was recorded in 4 and 8 the health zones, respectively. Results suggest that TA have been beneficial in identifying priority areas for PBPS. The PBPS provide important data for SAFE implementation; however, trachoma endemicity was low in most of the areas surveyed.

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WHICH DISTRICTS ARE ENDEMIC FOR TRACHOMA: METHODS AND RESULTS OF TRACHOMA ASSESSMENT IN TANZANIA

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By January 2014, baseline surveys of trachoma have been completed in 76 of 161 districts in Tanzania. The remaining 85 districts had unknown trachoma prevalence although most were suspected to be endemic. Establishing trachoma endemicity was needed to plan for implementation of the SAFE (surgery, antibiotics, facial cleanliness, environmental change) strategy in order to meet GET 2020 goals. We describe trachoma assessments undertaken to prioritize districts for population based prevalence surveys. The trachoma assessment was undertaken in 54 un-surveyed rural districts and two urban districts contiguous to highly trachoma endemic districts. The assessment comprised three activities: 1) key informant interviews with the district medical officers (or district eye-care coordinator or district NTD coordinator); 2) desk reviews to document data on trachomatous trichiasis over the last five years (2009 to 2013); and 3) spot checks in 2 villages per district to examine 50 children aged 1-9 years and 50 people aged 15 years and above, in each village, for active trachoma signs (trachomatous inflammation follicular [TF] and trachomatous inflammation intense [TI]), and trachomatous trichiasis (TT), respectively. Key informant interviews revealed that majority (85.7%) of the 56 districts perceived that there were areas in their district with trichiasis; however, 15 (27.8%) districts did not have any data on trichiasis. Based on the proposed criteria (proportion of TT ≥5% among 15 years and above, or active trachoma ≥15% in children aged 1-9 year, or at least 30 TT cases reported over previous five years) a total of 19 (33.9%) districts were found to be eligible for population based surveys of trachoma. The trachoma assessment provides a rational approach for deciding areas where resources for population based surveys and subsequent implementation of the SAFE strategy will be prioritized. Baseline surveys in the 19 districts will enhance Tanzania's progression toward elimination of trachoma by the year 2020.

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THE SHRINKING TRACHOMA MAP IN TANZANIA: RESULTS OF BASELINE SURVEYS IN 31 DISTRICTS

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Blinding trachoma is prevented through the surgery, antibiotics, facial cleanliness and environmental improvement (SAFE) strategy. Prior to SAFE implementation, baseline surveys of trachoma are recommended. As of 2012, only 61 out of 161 districts had been mapped from 2004

to 2006. Trachoma was suspected to be more widespread therefore further surveys were required for planning SAFE implementation. Between 2012 and 2014, trachoma baseline surveys were undertaken in 31 un-surveyed districts. In 2012 and 2013, 12 at risk districts were selected based on proximity to known trachoma endemic districts, while in 2014, trachoma assessments were undertaken and 19 districts prioritized for baseline surveys. A multi-stage cluster random survey sampling was applied whereby 20 villages (clusters) and 36 households per cluster were surveyed. Eligible participants, children aged 1-9 year and people aged 15 years and above, were examined for trachoma using the WHO simplified grading system. A total of 23,199 households were surveyed and 105,092 participants (4.3% of those enumerated) examined for trachoma signs. A total of 44,516 children aged 1-9 years were examined for trachomatous inflammation-follicular (TF) while 65,256 people aged 15 years and above were examined for trachomatous trichiasis (TT). Prevalence of TF varied by district ranging from 0.1%; 95% confidence interval [CI] (0.02-0.4) in Serengeti to 12.8%; 95% CI (8.3-19.3) in Chunya. TT prevalence was lowest in Misungwi [0.04; 95%CI (0.01-0.2)] and highest in Kibaha [2.5%; 95%CI (1.8-3.4). Two districts (Ngara and Chunya) had TF ≥10% while 3 districts (Chunya, Korogwe and Kibaha) had TT prevalence ≥1%. Based on the survey findings only two districts qualify for implementation of the full SAFE strategy. Trichiasis is still a public health problem in three districts and community based TT surgery services should be considered to prevent blindness due to trachoma. Although prevalence of trachoma was lower than previously suspected, in most districts, the findings will facilitate implementation of SAFE in endemic districts.

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PROGRESS TOWARDS ELIMINATION OF TRACHOMA IN TANZANIA: RESULTS OF IMPACT SURVEYS IN 28 DISTRICTS

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The World Health Organization recommends evaluation of the surgery, antibiotics, facial cleanliness and environmental change (SAFE) strategy after at least three years of implementation. We investigated the prevalence of trachomatous inflammation-follicular (TF) in children aged 1-9 years and trachomatous trichiasis (TT) in people aged 15 years and above in 28 districts. Surveys were conducted in districts where SAFE had been implemented for 3 years or more. Cluster random survey design was used to select the sample. Districts were stratified into three to four sub-districts and 10 to13 villages (clusters) randomly selected in each sub-district. Households were selected in using systematic random sampling. In sampled households, children aged 1-9 years were examined for TF and persons aged 15 years and above were examined for TT using World Health Organization (WHO) simplified grading system. A total of 41,641 households were surveyed. A total of 77,133 children aged 1-9 years and 110,763 people aged 15 years and above were examined for trachoma signs. District prevalence of TF ranged from 0.5%; 95% confidence interval [CI] (0.5-1.3) in Karagwe to 14.1%; 95%CI (10.9-18.1) in Kilindi. District prevalence of TF was below the 5% threshold for stopping mass drug administration (MDA) in 19 districts. Prevalence of TF was ≥10%, and between 5% and 9.9% in five and four districts, respectively. Prevalence of TT by district ranged from 0.1%; 95% CI (0.0-0.2) in Kondoa to 2.9%; 95% CI (2.2-3.9) in Lindi with 11 districts with TT prevalence of ≥1%. Survey findings suggest that the ultimate intervention goal for TF of <5%TF has been attained in 19 districts therefore MDA with Zithromax® should be stopped and surveillance surveys undertaken after 2 years. However, in the remaining districts at least 3 rounds of MDA and a single round of MDA are recommended in 5 and 4 districts, respectively; before further impact surveys are done. However, in many districts TT still remains a serious public health problem and therefore trichiasis surgery is required to clear the TT backlog.

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IMPACT OF MASS DRUG ADMINISTRATION ON TRACHOMA PREVALENCE IN 7 DISTRICTS OF THE FAR NORTH REGION IN CAMEROON

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Cameroon is known to be endemic with trachoma in the northern regions. Disease mapping in 2010-2011 in the Far North region of the country identified 13 health districts (HDs) with prevalence of trachomatous inflammation, follicular (TF) of more than 10% in children aged 1-9 years. These HDs were therefore qualified for district-level mass antibiotic treatments as well as intensive implementation of other components of the World Health Organization (WHO) endorsed SAFE (Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement) strategy. Of the 13 HDs, 7 completed 3 rounds of mass drug administration with good coverage and qualified for impact assessment. A cross-sectional, descriptive, cluster randomized survey was conducted in May-July 2014 in the 7 HDs to estimate the prevalence of TF in order to determine whether the criteria for stopping mass treatment with azithromycin had been achieved. A sample of 12,377 children aged 1-9 years was surveyed. The WHO simplified trachoma grading system was used for the recording of cases. Findings showed that average prevalence of TF in 7 HDs decreased from 20.58% (95% confidence interval: 19.9% - 21.2% in 2010 to 2.04% (95% confidence interval: 1.79 - 2.30%) in 2014. The TF prevalence in each HDs was 6.49% (Meri), 5.75% (Pette), 0.37% (Bourha), 0.90% (Hina), 0.28% (Koza), 0.37% (Mogode), and 0.29% (Roua). Five out of 7 HDs surveyed reached TF prevalence of <5% and met the criteria of stopping mass antibiotic treatment. Two HDs, Meri and Pette, recorded TF prevalence from 5-9.9% and will receive one additional round of treatment before conducting an additional impact assessment survey, according to the WHO recommendations.

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FILARIAL/*LEISHMANIA* CO-ENDEMNICITY IN TWO DISTINCT REGIONS OF MALI

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Filarial infections are a major public health problem that can lead to debilitating outcome such as the hydroceles and elephantiasis seen in lymphatic filariasis (LF). Phlebotomus duboscqi, the sand fly vector for Leishmania major (Lm) parasites, is found in areas endemic for LF in Mali. To determine whether there may be potential interactions between the presence of filarial infections and of Lm, the prevalence of the two major

filarial infection found in Mali (*W. bancrofti* [Wb] and M. perstans [Mp]) in two ecologically distinct regions - Tieneguebougou/Bougoudiana in Kolokani district (North Sudan savannah area) and Boundioba in the district of Kolondieba (South Sudan Savannah area) -- was assessed as was the prevalence of coincident delayed type hypersensitivity (DTH) responses to leishmanin as measured by skin test (LST). A total of 930 volunteers aged from 18 to 65 years were included from the two endemic areas. Prevalence rates for the Wb-specific circulating filarial antigen (CFA) were 7.69% (15/195), 3.04% (7/230) and 10.30% (52/505) while the Mp microfilaremia prevalence rates were 11.79% (23/195), 4.35% (10/230) and 9.50% (48/505), for Tieneguebougou, Bougoudiana and Boundioba, respectively. Interestingly, the prevalence rates based on LST for exposure to Lm infection were 25.12% (49/195), 8.88% (15/169) and 2.04% (8/393), respectively, in each of these villages. Single filarial infection was more common in the study population 15.16% (141/930) than DTH response to Lm within the filarial infected subjects 9.03% (14/155) (Chi2=4.076, p=0.044). Microfilaremia prevalence was comparable in Kolokani (7.76 %) and Kolondieba (9.50 %) (p=0.35) while CFA prevalence was significantly higher in Kolondieba (p=0.0040) as well as DTH response in Kolokani (p<0.0001). These findings establish the existence of co-endemnicity of filarial infections and Lm infections in regions in Mali. Additionally, because of the potential interaction between differing immune-mediated responses seen in chronic filarial infections and in Lm infection, more detailed and integrated approach to these infections is needed in co-endemic regions.

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THE EFFECTIVENESS OF AN ENHANCED ANTENATAL CARE PACKAGE FOR THE CONTROL OF MALARIA AND ANAEMIA IN PREGNANCY IN GHANA

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The prevalence of malaria and anaemia in pregnancy remains high despite efficacious antenatal care (ANC) interventions being available for more than two decades. Sub-optimal uptake of the interventions due to non-participation of pregnant women in ANC may be a contributory factor. We hypothesized that participation of pregnant women in their ANC would improve adherence to ANC recommendations and lead to better health outcomes. A cluster randomized trial was conducted to assess the effect of pregnant women's participation on the risk of anaemia and malaria parasitaemia during pregnancy. The intervention consisted of ANC staff showing pregnant women their rapid malaria test (RDT) and haemoglobin (Hb) colour scale (HCS) results to encourage their participation. The control group had routine care. We also assessed the feasibility and acceptability of the intervention. The overall mean age, gestational age and Hb at baseline were 26.4yrs, 17.3 weeks and 110 g/l, respectively, and similar in both groups; 10.7% had asymptomatic parasitaemia; 74.6% owned ITN of whom 48.8% slept under it the night prior to enrolment. The adjusted risk ratio by 8 weeks of follow up and at 36-40 weeks gestation in the intervention versus the control was 0.97 (95% CI: 0.78-1.22) and 0.92 (95% CI: 0.63-1.34) for anaemia and 1.17 (95% CI: 0.68-2.04) and 0.83 (95% CI: 0.27-2.57) for parasitaemia. The adjusted risk ratio for low birth weight was 0.93 (95% CI: 0.44-1.97) and for sub-optimal pregnancies (abortions, intra uterine foetal deaths and still births) was 0.77 (95% CI: 0.17-3.52) in the intervention group versus the control. Integration of the HCS and the RDT into ANC was feasible and acceptable. Both ANC staff and pregnant women perceived some improvement in pregnant women's adherence to ANC recommendations although clear evidence of a biological effect was not found. The effect may have been diluted out by the concurrent introduction of RDTs into routine ANC during the time of the study and some implementation challenges of the enhanced ANC package at the ANC clinics.

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PROVIDING SURGICAL CARE IN RURAL HAITI: LESSONS LEARNED AND PROGRESS MADE

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Surgical care in undeveloped poor countries is often lacking, only occurring at large regional hospitals. Rural residents may not have the financial means for care or the ability to travel. For the past 12 years, a team of medical professionals have been performing surgical procedures in a remote Haitian village in communication with the local Minister of Health. The College of Medicine has established a short-term global health elective for medical students and surgery residents. The purpose of this study was to review our experience providing surgical care to a profoundly underserved region. From 2003-2015, surgical procedures have been performed solely under local anesthesia. Early on, procedures were performed in a large room with separate areas for pre and postoperative care. Later, an on-site multi-room clinic was constructed with sinks, improving sanitary conditions. One surgical faculty, 4 circulating nurses, 3 nursing students, 6 surgical residents (elective began 2009), and 2 medical students (elective began 2013) have participated. Utilities are limited and alternative sterilization techniques employed. The procedures performed have included ventral and inguinal hernias (198), lipoma excisions (31), abscess drainages (31), thyroglossal duct cystectomy (2), skin cancer and large keloid excisions (15), and other (58). There have been no deaths and only 5 complications. Two hypertensive patients developed hematomas requiring evacuation and 2 patients had recurrence of their inguinal hernias prompting the change to a mesh repair in 2005. One hernia repair was aborted due to extensive scar tissue and the patient sent to a regional hospital. The global burden of surgical disease needs to be addressed on numerous fronts, including the most underserved and remote rural populations. The local conditions and volunteer expertise need to be matched so that excellent, appropriate, and safe care is provided. Trainees will benefit from the experience as well as on the addition of components of cultural education and global surgery ethics. Above all, participants must demand "Primum non nocere" or "First, Do No Harm".

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CHILD MALNUTRITION IN ECUADOR: RESULTS FROM THE ECUADORIAN NATIONAL HEALTH AND NUTRITION SURVEY (ENSANUT-ECU)

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The burden of child malnutrition in Latin America is high, although there is limited data from Ecuador. The recent Ecuadorian National Health and Nutrition Survey is the first national level nutrition survey since 1987, and provides a unique opportunity to examine the burden of malnutrition in children in Ecuador. Socio-demographic and anthropometric data (n=9,145) and venous blood samples (n=2,049) were collected. The prevalence of anemia, micronutrient deficiencies (zinc, vitamin A, iron, vitamin B12, folate), and stunting (LAZ<-2), underweight (WAZ<-2), wasting (WLZ1), and obesity (BMIZ>2) were calculated and mapped using ArcGIS. Binomial and linear regression models were used to examine the associations of anemia, micronutrient deficiencies, and WHO anthropometric indicators and socio-demographic characteristics. The prevalence of child stunting (25.0%), underweight (5.0%), wasting (2.3%), and overweight (30.0%) was high. Micronutrient deficiencies were common in children, particularly zinc deficiency (Zn<65.0 µg/dL; 25.0%), anemia (Hb<11.0 g/dL; 16.0%), and vitamin A deficiency (retinol0.05); however, the prevalence of anemia was higher in Coastal

Ecuador (22%; RR: 1.65, 95% CI: 1.36-2.00, p<0.01), compared to all other regions. Lower maternal education predicted increased prevalence of child anemia (19%; RR: 1.40, 95%: CI: 1.10-1.77, p<0.01) and vitamin A deficiency (19%; RR: 1.41, 95% CI: 1.11-1.79, p<0.01). The prevalence of child stunting was significantly higher in rural compared to urban areas (30% vs. 21%; p<0.01), and in the Sierra (29%, RR 1.32, CI 1.22-1.44, p<0.01). Indigenous children had the highest prevalence of stunting (39%; RR: 2.17, 95% CI: 1.97-2.39, p<0.01). Findings suggest that the burden of child malnutrition is high in Ecuador, including anemia, micronutrient deficiencies, and stunting, particularly in urban and coastal settings. Further understanding of the burden and distribution of child malnutrition will inform the design of targeted interventions.

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USE OF INTERFERON-Γ RELEASE ASSAYS FOR THE DIAGNOSIS OF TUBERCULOSIS IN PATIENTS WITH CONCURRENT MALARIA INFECTION IN TANZANIA

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Latent tuberculosis can be diagnosed with interferon-γ release assays (IGRAs). However, several conditions have been shown to interfere with IGRA performance, including concomitant glucocorticoid treatment, smoking, and human immunodeficiency virus (HIV) and helminth co-infection. It remains yet unknown whether concurrent malaria infection also affects IGRA performance; however, the well-established immune-modulating effects of malaria suggest this may be the case. We aimed in this clinical study in Tanzania to assess the effect of acute ***Plasmodium falciparum*** infection on the performance of two different IGRA tests: QuantiFERON-TB Gold in-tube (QFT-GIT) assay and the IFN-gamma-inducable protein 10 (IP-10) release assay. Study participants included adults with confirmed uncomplicated ***P. falciparum*** malaria attending Muheza District Hospital in north-eastern Tanzania as part of a larger trial looking at malaria treatment efficacy and safety in patients co-infected with HIV. A total of 241 adults were included: 184 patients with malaria (88 co-infected with HIV) and 57 patients with HIV infection only. Patients with malaria received the standard six-dose treatment with artemether-lumefantrine, and QFT-GIT and IP-10 release assays were performed prior to treatment on day 0 and again on day 7 and day 42 after treatment. Independently of HIV, significantly lower INF- and IP-10 responses to mitogen-stimulation were observed in patients with concurrent malaria compared to patients without malaria, with negative correlations observed between parasite density and INF- and IP-10 levels. These alterations reverted after malaria treatment. Malaria infection did not influence QFT-GIT testing results as such, while a higher proportion of IP-10 release assays showed indeterminate results during acute malaria. Our findings suggest that malaria infection interferes with IGRA performance through impairment of INF-γ and IP-10 responses. We therefore recommend being cautious when interpreting IGRA results in patients with malaria and that testing for tuberculosis be postponed until after completion of malaria treatment.

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DEVELOPMENT OF A REAL-TIME PCR DIAGNOSTIC FOR PATHOGENIC *LEPTOSPIRA*

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The current gold standard for the direct detection of ***Leptospira*** in clinical specimens is culture in semi-solid media, which requires up to 26 weeks of growth. In addition, leptospires lose viability in urine, a common sample type, within hours after collection. To address these drawbacks, we have developed a real-time PCR assay for the detection of pathogenic strains of ***Leptospira***. Here, we evaluated the ***Leptospira*** Assay for analytical sensitivity and specificity as well as the stability of organism in various specimen types. Patient samples were spiked into common sample types, urine, whole blood, and cerebrospinal fluid (CSF), and stored for varying times and temperatures. Samples were then extracted using the MagNA Pure LC automated platform, and subjected to Real-Time PCR using primers and probes specific to the 16S gene of pathogenic ***Leptospira***. The ***Leptospira*** Assay, which could be completed in 5 hours, detected ***Leptospira interrogans***, ***L. weilii***, ***L. santarosai***, ***L. borgpetersenii***, ***L. kirschneri***, and ***L. noguchii***, but did not detect nonpathogenic strains ***Leptospira biflexa***, ***L. meyeri***, and ***L. wolbachii.*** The ***Leptospira*** Assay detected as little as 600 cells/ml from blood, 150 cells/ml from urine, and 75 cells/ml from CSF. Several stability studies were performed, which demonstrated that, even for molecular testing, ***Leptospira*** were stable in urine for only 1 hour after spiking at room temperature, and for less than 1 day at 4°C. However, ***Leptospira*** were stable in urine for 30 days at -20°C and stable in blood and CSF for 2 days, 7 days, and 30 days at room temperature, 4°C, and -20°C, respectively. Therefore, it is highly recommended that urine samples are shipped frozen. With a turn-around time of 24 hours and increased analytical sensitivity, especially in frozen samples, the ***Leptospira*** real-time PCR assay is an improvement over culture.

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ULTRASOUND FINDINGS OF TROPICAL CONDITIONS IN A COHORT OF SOUTH ASIAN IMMIGRANTS SEEN IN VICENZA, ITALY

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Immigrants from developing countries are a potential source of imported tropical conditions, including transmissible diseases. While illegal immigrants mostly come from sub-Saharan Africa crossing the Mediterranean sea, Asian immigrants generally travel legally to Italy and receive less medical attention. We describe the epidemiological, clinical and sonographic findings in a cohort of 145 Asian immigrants admitted to our Infectious Diseases and Tropical Medicine Department in Vicenza, Italy, from 2000 to 2014. Their countries of origin were India (45 patients, 31%),Bangladesh (72 p., 49.6%) and Pakistan (28 p., 19.3%).In all patients, point of care ultrasound (PoC-US) was performed in the first week of admission and during the hospital stay.The main diagnosis at discharge was: tuberculosis (TB) in 31 cases (21.3 %, 13 pulmonary TB, 18 extrapulmonary TB), acute viral hepatitis in 29 cases (20 %, 10 HEV, 2 HAV, 4 HBV, 2 of unknown origin); 12 patients had acute-on-chronic viral or alcoholic liver damage, in another three cases acute hepatitis was associated with Dengue Fever. 15 patients (10.4%) had typhoid or paratyphoid fever, 15 (10.4%) had pneumonia, 8 meningitis (5.51%), and 11 soft tissue infections (7.58%). Parasitic diseases (malaria, liver amebiasis, trichuriasis, toxoplasmosis and scabies) accounted for 8 cases (5.51 %). Only two patients (1.37 %) tested positive for HIV. The main ultrasonic findings were: hepatomegaly (52, 35.8%), fatty liver (48 , 33.1 %), splenomegaly (39, 26.8 %), pleural, pericardial and/or abdominal effusions (42, 28.9%), lymph nodal abscesses, both superficial and deep (12, 8.27%), coarse liver pattern (8, 5.5%) hepatic and splenic abscesses (7, 4.8%).Most patients (120, 82.75 %) had recently traveled to their country of origin or were recently immigrated and had an imported infectious condition. Immigrants from South Asia visiting friends and relatives (VFR) need more clinical attention from the ID specialist. PoC-US is helpful in diagnosing and managing tropical conditions in this population, as it allows identification of relevant, and sometimes pathognomonic findings in these conditions.

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CLINICAL, LABORATORY AND EPIDEMIOLOGICAL PROFILE AND TREATMENT OF SEVERE SCHISTOSOMIASIS MANSONI IN A TEACHING HOSPITAL IN SÃO PAULO CITY, BRAZIL

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Schistosomiasis remains a public health problem in Brazil. The aim of this study was to evaluate the clinical, epidemiological and laboratory profile of patients with severe forms of schistosomiasis assisted at the Schistosomiasis Outpatient Clinic of the Hospital das Clinicas of the University of São Paulo. This is a case series study evaluating 42 outpatients' records. We developed a data collection questionnaire from patients' medical records and described their clinical and laboratory data. In addition, we made a comparison between patients with and without splenomegaly. The clinical, epidemiological and laboratory profile showed the following: predominance of males 57.1% (24/42); 100% (42/42) had the hepatosplenic form of the disease; 68.3% of patients (28/42) had just elementary school education and mean age of 48.24 years. Among the symptoms that led the patient to seek medical attention, presence of an enlargement in any organ was more prevalent: 55.3% (12/36). There was a statistically significant association between splenomegaly and thrombocytopenia ($p < 0.004$) between splenomegaly and leukopenia ($p < 0.046$), and only 2.5% (1/22) of patients presented with bleeding. Regarding specific treatment, 54.2% (13/24) of patients used Praziquantel and 45.8% (11/24), Oxaminiquine. Patients used the following non-specific drug therapy: 65% (26/40) Propranolol, 90% (36/40) Omeprazole, and 43.6% (39/42) Aluminum Hydroxide 92.9% (39/42) of patients required an upper endoscopy, 85% (39/40), sclerotherapy, 62.5% (25/15), elastic bandages, and 28.2% (11/39) underwent surgery. This preliminary study allowed us to observe the clinical, epidemiological and laboratory profile of patients treated in an outpatient clinic far from endemic areas for schistosomiasis, while pointing out that medical and endoscopic treatment, in combination with outpatient treatment can be effective in preventing bleeding and surgical treatment. The severe forms of this helminthiasis require an interdisciplinary outpatient follow-up.

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CHIKUNGUNYA RHEUMATISM DURING THE SUBACUTE PHASE OF THE DISEASE AND ITS CORRELATION WITH IGG ANTIBODIES: FINDINGS FROM AN OUTBREAK IN COLOMBIA

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Chikungunya rheumatism (CHIK-r) includes a broad spectrum of disorders ranging from mere pain to frank arthritis with severe incapacity, however, the burden of this complication, its clinical features, and its relationship with the immunological response have not been fully characterized, especially in areas where the disease has been recently introduced. We evaluated 78 adults with clinical diagnosis of Chikungunya (CHIK) fever and persistent musculoskeletal symptoms after 15 days of disease's onset, in the context of an outbreak in Capitanejo, Colombia. CHIK infection was confirmed in 73 patients (mean age: 59 years; 23% male; median disease's duration: 46 days) using micro-capture ELISA for IgG. Antibody levels were expressed in NovaTec units (NTU). Patients underwent a clinical evaluation focused on the determination of rheumatic symptoms and a physical exam that included the squeeze test (in hands and feet) and the handgrip maneuver (Jamar dynamometer). The association between antibody levels and clinical signs of rheumatic involvement was evaluated using multiple logistic regression, adjusting for age, sex, and disease's duration. Arthralgia was the most frequently reported symptom at disease's onset (97%) and the time of evaluation (90%). Myalgia was also highly prevalent at both moments (90% and 48%, respectively). Squeeze test was positive in 34 (50%) and 30 (44%) patients when performed in hands and feet, respectively (kappa: 0.82, $p<0.01$). Overall, patients had low grip-strength, especially those who had positive as compared to negative squeeze tests (12.5 kg vs. 16.3 kg, $p<0.05$; respectively). A positive squeeze test (in hands), but not the grip-strength, was more likely among patients with higher antibody levels: 6% higher probability of positivity per 1 NTU increase in antibody levels (OR=1.06, 95%CI: 1.01 - 1.12). The handgrip maneuver and the squeeze test may contribute to a better characterization of rheumatic complications and correlate with the antibody's response. Whether these tools are useful to assess prognosis deserves further evaluation

1699

IMPACT OF MALARIA RAPID DIAGNOSTIC TESTS ON PATIENTS' SUBSEQUENT TREATMENT-SEEKING, COSTS AND HEALTH OUTCOMES: RESULTS FROM THE ACT CONSORTIUM

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Malaria rapid diagnostic tests (RDTs) are intended to have a beneficial impact on management of suspected malaria, and on health outcomes and other patient-related outcomes. The ACT Consortium includes several studies designed to test operational strategies for artemisinin combination therapy (ACT) and RDT implementation in various settings, providing an opportunity to draw on data from multiple projects that have introduced RDTs across a range of clinical, social, and epidemiological contexts, and in public, private retail, and community health service sectors. The impact of RDTs on case management is presented in a separate abstract; this analysis focuses on events after the clinical consultation, including subsequent treatment seeking, patient costs, and self-reported health outcomes. Data were examined from eight studies comparing scenarios where RDTs were made available to control scenarios where RDTs were not made available. Preliminary analysis of three data sets shows that where RDTs were introduced, the proportion of patients seeking further care after the initial consultation increased in one high-transmission setting where community health workers offered only ACT; this was not the case in another setting in retail drug shops which offered non-ACT alternatives for RDT-negative patients. The same data sets indicate that patient or caregiver report of symptomatic recovery is approximately equivalent whether or not RDTs are available. Full results will include analysis by health care sector, epidemiology, patient age, routine and reference diagnostic results, and treatment prescribed at the initial consultation. This ongoing analysis aims to elucidate features associated with variation in post-consultation outcomes in order offer tailored guidance for policy and program development for RDT introduction in other areas.

1700

ASSESSMENT OF RISK FACTORS FOR PRETERM BIRTH AND CONTRIBUTIONS OF PREMATURITY TO FUTURE RISK OF ADVERSE OUTCOMES IN A BANGLADESHI BIRTH COHORT

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Preterm birth has been identified by the WHO as a major global public health problem and accounts for more infant deaths in the first month of life than any other cause. South Asia has the highest rates of preterm birth. We followed a birth cohort of 700 healthy infants from the urban slums of Dhaka, Bangladesh for 2 years. We examined factors contributing

to preterm birth and whether prematurity led to increased risk for adverse outcomes related to rotavirus vaccine efficacy and any diarrheal illness in the first year of life. In our cohort, gestational age assessments using the Dubowitz-Ballard scale were completed on a subset of 381 children at enrollment (0-7 days after birth) by a trained medical officer. Among 381 children, 32% were born preterm (32-36 weeks gestation). We examined predictors of prematurity including child's gender, birth order, mother's age and BMI, and delivery status (cesarean vs vaginal). No association was found between preterm birth and infant or maternal characteristics, nor with cesarean delivery. To survey associations between prematurity and increased risk for adverse outcomes, we used univariate and logistic regression analysis to examine incidence and duration of rotavirus and all-cause diarrhea in the first year of life; infant rotavirus-specific IgA at 6 and 18 weeks of age; enteric co-pathogen burden at 10 weeks; serum zinc at 6 and 18 weeks; and incidence of diarrhea-related serious adverse events in the first year. Prematurity was not found to predict increased risk for any of these outcomes. In a population with high prevalence of childhood malnutrition, prematurity notably may explain a portion of described malnutrition in our cohort: 59% of stunted children (HAZ<-2SD) at enrollment were also assessed as premature, as were 50% of children with WAZ<-2SD and 39% with WHZ<-2SD at enrollment. Preterm birth was not associated with increased risk of malnutrition by one year of age. Future analyses will include assessment of prematurity and cognitive function outcomes up to age 2 years which may indicate clear targets for public health interventions to prevent preterm births.

1701

SAFETY EVALUATION OF FIXED-DOSE DIHYDROARTEMISININ-PIPERAQUINE COMBINATION IN A REAL LIFE SETTINGS

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Uncommon and rare reactions with delayed onset may not be detected before new medicines are licensed. Post-licensure surveillances are carried out to expand the evidence base of products characteristics for which marketing authorization were granted. Using the established INDEPTH-network safety monitoring platform the study evaluated safety of dihydroartemisinin-piperaquine as first-line treatment for malaria across different settings in Ghana. Key questions answered were the safety of the drug when used under usual conditions and assessment of the occurrence of adverse events. Health and Demographic Surveillance areas of Navrongo, Kintampo and Dodowa research centres located in the northern, middle and coastal belts of Ghana were used. From September 2013 to June 2014 a prospective, observational, study was carried out. Participants were males and females, age >6 months, weighing ≥ 5kg, ability to take oral medications, acute febrile illness and informed consent. Detailed clinical enquiry was conducted. Data analysis involved descriptive characteristics and adverse events coded using MedDRA® System Organ Class classification. The protocol received approval from the Ghana Health Service Ethics Review Committee, Ghana Food and Drugs Authority and was registered with Clinicaltrials.gov (NCT02199951). Approximately 95.5% (4563/4777) patients comprising 52% females and 48% children < 6 years of age. Overall 347 adverse with incidence rate of 76/10000 population. Characteristics associated with adverse effects were body mass index and parasite density. The commonest events according the MedDRA® Coding per 10000 population were infestations (465), gastrointestinal disorder (103), Respiratory conditions(46), thoracic disorders(44), Nervous system disorders (26) and Skin disorders (26). In conclusion, fixed-dose dihydroartemisinin-piperaquine combination is very safe in African population with malaria in real life settings

1702

NATIONAL SEROPREVALENCE OF MEASLES, TETANUS AND RUBELLA AMONG CHILDREN AGED 6-59 MONTHS IN THE DEMOCRATIC REPUBLIC OF CONGO

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Serosurveys are a direct and accurate method to assess population level immunity and can provide critical insight into immunity gaps and operational program efficiency. In collaboration with the Demographics and Health Survey (DHS) we assessed population immunity to vaccine-preventable diseases in the Democratic Republic of Congo. We screened 8,117 children aged 6 to 59 months for IgG antibodies against measles, rubella and tetanus using dried blood spots (DBS) collected during the 2013 DHS. DBS were tested using the Dynex Multiplex M2 platform, an automated enzyme-linked immunosorbant assay at the National Laboratory for Biomedical Research in Kinshasa. Seropositivity rates for children in three age categories were determined for measles, tetanus, and rubella antibodies, respectively. Children aged 6 to 8 months had rates of 18.3, 46.7, and 14.0%; children 9 to 11 months had rates of 40.1, 43.6, and 23.9%; and children 12 to 59 months had rates of 73.1, 31.8 and 39.3% seropositivity for the three diseases described. VPD seroprevalence varied by province as well, ranging from 51.4-81.6% for measles, 20.1-51.8% for tetanus, and 19.3-52.5% for rubella. The lowest seropositivity rates for tetanus and measles were seen in Katanga and Maniema provinces. The prevalence of rubella was highest in Bandundu. The low seroprevalence of measles and tetanus antibodies indicate that large-scale immunity gaps exist across the DRC. The large percentage of children exposed to rubella in the absence of a vaccine, suggests widespread circulation of the virus throughout the country. In DRC, there is an immediate need to re-evaluate immunization strategies for these vaccine preventable diseases.

1703

HOSPITALIZATIONS IN IMMIGRANTS AND NON-IMMIGRANTS WITH CHRONIC HEPATITIS C INFECTION IN QUEBEC

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Chronic hepatitis C (HCV) causes considerable morbidity and mortality in Canada due to liver cirrhosis, liver failure and liver cancer that could be prevented through early screening and treatment. Immigrants are an underappreciated group at risk for HCV, often originating from high

prevalence countries. This study characterized all-cause and liver-related hospitalizations in HCV-infected immigrants compared to non-immigrants. We performed a retrospective population-based cohort study of all HCV cases in the Quebec mandatory disease reporting database from 1998 to 2007. Cases were deterministically linked to hospitalization and outpatient services databases. Liver-related hospitalizations were identified through discharge diagnoses and procedure codes. A total of 20,139 linked cases of chronic HCV were reported from 1998-2007, 9% (N=1,821) of whom were immigrants. At HCV diagnosis, immigrants were older (47.6 vs. 43.2y, p<0.05), more likely to be female (46.7 vs. 31.9%, p<0.05), and to have liver cancer (0.93 vs. 0.31%, p<0.05). The mean time to HCV diagnosis after arrival was 9.8 ± 6.9 years. Non-immigrants had a 3-10 fold higher prevalence of HCV-related risk factors, including drug or alcohol abuse, and HIV. Non-immigrants were more likely to be hospitalized at least once during follow-up compared to immigrants (49.3 vs. 35.8%, p<0.05 with hospitalization rates of 31.3 vs 18.2 /100 PY). Immigrants were as likely to have at least one liver-related hospitalization (7.8% vs. 7.1%), however in-hospital deaths were more likely to be liver related among immigrants as compared to non-immigrants (57.9% vs 41.8%, p<0.01). The most common primary discharge diagnoses for non-immigrants were mental disorders (20.5%) and injury/poisoning (10.3%) whereas for immigrants they were liver-related (11.6%) and neurologic disorders (10.2%). Higher all-cause hospitalizations in non-immigrants likely reflects more prevalent lifestyle comorbidities. The higher prevalence of liver cancer and in-hospital deaths due to liver disease in immigrants highlights the importance of early HCV screening and treatment in this population.

1704

INCIDENCE OF TROPICAL DISEASES IN THE US MILITARY 2004-2013

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Understanding the impact of tropical disease on health is important to the US military, because many service-members travel overseas to areas where there is a high risk of acquiring these illnesses. To understand the scope of this risk, we quantified the incidence of the five most important tropical diseases in the military from 2004 to 2013 using the Defense Medical Surveillance System (DMSS), a continuously expanding relational data base that documents military and medical experiences of service members throughout their careers. Standard ICD9 codes for the following diseases were included: Dengue, Leptospirosis, Leishmaniasis, Malaria, and Helminths. Descriptive techniques were used to calculate the incidence rates in the military over the period of time between 2004 and 2013. Overall, there were a total of 10,941 cases of the five tropical diseases, and an incidence rate of 60.4 cases per 10,000 person-years. Most of those cases were Helminths, which had a total of 8,398 cases over the ten years, and an incidence rate of 46.4 cases per 10,000 person-years. Leishmaniasis was the next most common illness, at 1,327, or an incidence rate of 4.7 cases per 10,000 person-years. Malaria and leptospirosis were less common, with 860 and 95 cases respectively, yielding incidence rates of 4.8 and 0.5 cases per 10,000 person-years. In all the diseases, there seems to be a general downward trend from 2004 to 2013, possibly a result of decreasing deployments to tropical areas or better conditions encountered in those areas where travel occurred.

1705

ACUTE FEBRILE SYNDROME IN COLOMBIAN NORTHWEST

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Fever is one of the most characteristic symptoms of infectious diseases, sometimes the only one or the most important. A few time of fever, usually due to self-limited viral processes or noninfectious diseases. When fever persists more than two weeks, it is considered prolonged as a result of an infectious disease in many cases, but also in malignancy, autoimmune or hematologic diseases, metabolic disorders and vascular disorders. The febrile syndrome is the most common and nonspecific, It is defined as sudden onset of fever and less than seven days duration in patients who have not been identified signs or symptoms related to an infectious focus. Infectious disease to discard in the course of a febrile syndrome are diverse, such as tuberculosis, typhoid fever, brucellosis, sepsis, endocarditis, localized infections, urinary tract infections, rickettsia and viral processes. On this study, were evaluate five infectious agents (dengue virus, ***Plasmodium*** sp., Brucella spp., Leptospira sp., and Salmonella sp.) that are cause of febrile illness at the Córdoba department in Colombia, using molecular and immunological techniques to identified. Dengue contributes 40% of all cases, followed by malaria with 20%. Was not possible to determine the etiology of 36% of patients and 2% of leptospirosis. Only one patient showed co-infection with dengue and malaria; brucellosis and salmonellosis is not evident as the etiologic agent of febrile syndrome in this study.

1706

EPIDEMIOLOGIC CHARACTERISTICS OF ACUTE NEUROLOGIC DEFICIT IN ELDERLY PEOPLE FROM A DEVELOPING COUNTRY

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Elderly patients are vulnerable people and neurologic disorders are a very important public health problem which carries a risk of loss of autonomy. In developing countries like Haiti, few data exist about neurologic disorders. The aim of this study was to describe epidemiologic patterns of elderly people with acute neurologic deficit from Haiti. It was a retrospective cohort study conduct in the internal medicine ward from a big teaching hospital in Port-au-Prince: La Paix Hospital. The study period was 1st January 2012 to 31 October 2014. Criteria of inclusion: all adults patients admitted in the internal medicine ward with acute neurologic deficit and older than 65 years old. The criteria of exclusion were: charts non available. 525 elderly patients were hospitalized during this period and 124 had acute neurologic disorder. A systematic sampling was applied over the registry of the site. One patient over two was included in the study. Data from 62 patients were analyzed. Mean age was 75 years old, 32 (51.6%) patients were female. 45 (72.5%) had previously hypertension; 7 (11.2%) had diabetes; 8 (13%) were at their second acute episode. Only 16 (25.8%) patients came in less than 6 hours at the hospital. 18 (29%) had a blood systolic pressure > 220mnHg or a diastolic pressure > 120 mmHg. 25(40.32%) patients died and 50% of them before 3 days. 12 patients (19.35%) had a coma; 20 (32.25%) had obnubilation, 13 (20.9%) had seizure and 24 (38.70%) had speech disorders. 15 (24.19%) patients had facial palsy; 27(43.54%) had a left motor neurologic deficit, 3 (4.83%) had a neurologic deficit located at right inferior limb and 14(22.58%) had neurologic deficit at right superior and inferior limbs. All of the 17(27.41%) patients who realized a CT scan done it after 24 hours of hospitalization. 12 (70.58%) of them were ischemic stroke while the 5 (29.41%) others were hemorrhagic stroke. In our cohort, 25 (40.32%) patients died. 50% of those who survived had a length of stay of 8 days

while it was only for those who died of 3 days. It is very important to develop Point of Care technologies for neurologic emergencies at low economic cost for developing countries.

1707

PREVALENCE AND DETERMINANTS OF *TRYPANOSOMA CRUZI* INFECTION AMONG CITIZENS OF BOLIVIAN DESCENT LIVING IN MUNICH, GERMANY

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Chagas disease (CD) affects 7 million humans worldwide and is responsible for 10,000 estimated deaths annually. Due to increased population mobility, CD has become an international health issue. Migration of Latin-American migrants from Spain (most CD-affected country in Europe) to other European countries is increasing lately. Germany lacks surveillance data. A cross-sectional, descriptive study was started in 2013 as a pilot project to determine the prevalence and determinants of CD among citizens of Bolivian descent living in Munich, Germany. Participants completed a questionnaire in order to collect socio-economic and medical data. Peripheral blood was drawn and specific antibodies against ***Trypanosoma cruzi*** antigens were determined by ELISA and IFAT. If positive, PCR, clinical staging and management of CD was initiated. A qualitative research was conducted through 2 interviews (one women T.cruzi+, one women with unknown serological status) and a focus group (3 subjects ***T. cruzi***-), all in Spanish language, in order to assess the impact of CD for individuals and the Bolivian community. Between June 2013 and June 2014, 43 citizens of Bolivian descent living in Munich could be enrolled. Four participants (9.3 %) were ***T. cruzi*** +, two of these also in PCR. Two of them were treated with benznidazole. Two of the ***T. cruzi*** + subjects had a mother with CD. A total of 55.8% of all participants (2 of the 4 ***T. cruzi*** +) had no knowledge about symptoms of CD and 30.2% (1/4 ***T. cruzi*** +) about ways of transmission. A total of 27.9% (0/4 ***T. cruzi*** +) had donated blood in the past without prior serological tests on CD, 62.8% (3/4 ***T. cruzi*** +) were motivated to donate blood and 53.5% (3/4 ***T. cruzi*** +) to donate organs. Regarding qualitative research, lack of knowledge about CD, stigma and fears associated with CD were identified. Regarding the lack of epidemiological data about CD in Germany and the absence of measures controlling non-vectorial transmission, the prevalence in this pilot study is alarming. Prevalence and determinants of CD in Germany have to be investigated further in a nationwide study and sensible control strategies with information campaigns should be put in place.

1708

SINGLE NUCLEOTIDE POLYMORPHISMS IN ANGIOGENIC AND INFLAMMATION PATHWAY GENES ARE ASSOCIATED WITH PATENT LYMPHATIC FILARIASIS

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Two-thirds of people with bancroftian lymphatic filariasis (LF) have sub-clinical disease or are asymptomatic and present as microfilaremic individuals (patent infections) or amicrofilaremic individuals who are antigen-positive (latent infections). These sub-groups are crucial to the continued transmission of ***Wuchereria bancrofti*** and have a direct bearing on the individual and overall public health burden of endemic communities. Although a clear distinction in immunogenetic profiles in response to LF is known to occur between overt clinical manifestations such as pathology and sub-clinical disease, it is unclear whether patent infections are distinct from latent states in sub-clinical disease. In a Ghanaian study, 101 single nucleotide polymorphisms (SNPs) and 1 insertion/deletion polymorphism from 48 candidate genes were selected for a case-control study. Genotyping was done for 302 patent and 389 latent unrelated individuals. SNPs in 9 genes [Caveolin-1 rs926198 (PATT = 0.03), Desmoplakin rs2076299 (PATT = 0.01), Endothelin-1 rs5370 (PATT = 0.04), GJA4 rs1764391 (PATT = 0.01), IFN-gamma rs2069707 (PATT = 0.049), IGF-1 rs2946834 (PATT = 0.002), NOD-1 rs736781 (PATT = 0.047), NOD2/Card15 rs1000331 (PATT = 0.005), TLR-4 rs5030725 (PATT = 9E-4)] and the TLR-2 gene insertion/deletion (-196 to -173 base pairs) (PATT = 0.04) were associated with patent infection. Haplotype analysis yielded haplotypes of SNPs in two genes that were significantly associated with infection status. AT in the IGF-1 gene was significantly associated with patent infection (Monte-Carlo method with 200,000 simulations), while the haplotype GT was significantly associated with latent infection (Global P = 1.5E-5). The haplotype CG in the IFN-γ gene was also associated with latent infection (Global P = 0.04). Our findings suggest that SNPs in angiogenic and inflammation genes are associated with patent infection and that the two states of sub-clinical disease are distinct chronic states in LF disease pathogenesis.

1709

MEDIATORS OF VASCULAR PERMEABILITY AND LYMPH/ANGIOGENESIS ARE MARKERS FOR HYDROCELES CAUSED BY *WUCHERERIA BANCROFTI* INFECTION

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Lymphatic filariasis, caused by the filarial nematode ***Wuchereria bancrofti***, is a disease that affects over 120 million people. Most individuals have no or just mild pathology, whereas others have severe pathology including lymphedema (~7% of infected individuals) or hydrocele (50% of infected men). Hydrocele is a painful swelling of the scrotum caused by accumulation of lymph fluid. The aim of this study was to identify biomarkers of hydrocele due to filarial infection. Hydrocele fluid was collected as part of a Volkswagen Foundation funded study investigating the use of doxycycline therapy in hydrocele patients. Eight hydrocele samples were analyzed: 3 treated, 3 control and 2 European idiopathic hydrocele samples. Using Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) the relative protein content of the 8 samples was measured simultaneously. Placebo samples were analyzed against doxycycline and idiopathic samples. From the iTRAQ results we identified ~1000 proteins that were up- or down-regulated in the placebo samples. Prioritization of the results identified 9 proteins that were significantly higher in lymphatic filariasis hydrocele fluid (placebo): the androgen receptor, angiopoietin 2, cystatin C, fibronectin, heparan sulfate extracellular region, IGFBP-2 and -6, osteopontin and VCAM-1. These proteins are mediators or interact with mediators of vascular permeability and lymph/angiogenesis. Androgen receptor, fibronectin, IGFBP-2 and -6, osteopontin, and down-stream pathway factors are being analyzed in hydrocele fluid and plasma as markers of lymphatic filariasis hydrocele that could aid in early diagnosis and interventions to prevent or reverse disease.

1710

UTILITY THICK SMEAR OF LARGER VOLUME FOR THE DIAGNOSIS OF *MANSONELLA OZZARDI* IN THE PERUVIAN AMAZON

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Although filariasis ***Mansonella ozzardi*** is very common in some regions of the Peruvian Amazon, little is known about its clinical presentations, its actual distribution in the Amazon Basin and its role in aggravation of other diseases. This filariasis is considered a neglected disease in the world. One of the major constraints to learning more about this condition is the difficulty of diagnosis. The Knott test, which is the gold standard for the diagnosis of this disease, is difficult to perform, and not done routinely in rural setting. For these reasons, we conducted a study to determine the sensitivity of the thick smear for malaria and a thick smear modified with increased blood volume compared to the Knott test for the diagnosis of this disease. The study was conducted in a rural community in the Peruvian Amazon of approximately 500 inhabitants where we knew there was a high prevalence of ***M. ozzardi***. Nearby and accessible houses were selected and after obtaining consent, samples of 5 ml of blood were taken from the arm of participants to perform a thick smear in a 6 µL volume of blood, similar to that performed for the diagnosis of malaria, and 2 thick smears of 60 µL, each of which were stained with giensa. A Knott test was also performed. The sensitivity and specificity were calculated. Samples were taken from 91 persons over 5 years. 56/91 (61.5%) of the samples were ***M. ozzardi***-positive by Knott test. The sensitivity of the thick smear was only 58.9% (CI 45.8-70.8) while the sensitivity of a thick film of 60 µL was 75.0% (CI 62.3- 84.4) and the sensitivity of two thick smear of 60 uL was 82.4% (CI 70.1-90.0). The specificities of all smears were 100%. In conclusion, the sensitivity, simplicity, and practicality of two thick smears of 60 uL would allow for sufficiently accurate diagnosis of filariasis by ***M. ozzardi*** in field studies and in rural health facilities.

1711

CHEMOKINE PROFILE OF HUMAN INTESTINAL HELMINTHIASIS IN AGBOR NIGERIA

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The study provides information on chemokine 5(CXCL-5) and chemokine 11(CXCL-11)profile in human intestinal helminthisis in Agbor Delta State, Nigeria.Feaces were examined using formo-ethol concentration method and Karto-Katz techniques. Sera from 72 volunteers infected with helminthes and 8 uninfected controls were subjected to chemokine evaluation using ELISA techniques.Eggs of intestinal helminthes identified were those of ***Ascaris lumbricoides*** (26.6%), ***Trichuris*** (5.8%), Hookworm (3.6%); Tapeworm and larvae of ***Strongyloides*** had 0.9% and 0.4% respectively. Plathyhelminthes had ***Schstosoma mansoni***, 7.6%; ***S. intercalatum*** 6.3% and Fasciola, 1.3%. CXCL-11 concentration showed no significant difference in expression between males and females (3500.00±42.08)pg/ml and their control subjects (2891±11.31)pg/ml. In CXCL-5, infected males (3839±2190.20)pg/ml showed no significant difference relative to male control (3549±3123.49)pg/ml ($p>0.05$). In the contrary, CXCL-5 concentration was high in infected females (7015.20±2190.20) pg/ml than in female control (1107.0±7391.77)pg/ml ($x^2=3796.20$, $p<0.001=124.84$). In conclusion, elevated concentration of CXCL-5 in serum of volunteers infected with intestinal helminthes implicates this chemokine as an important biomarker in the immunology of intestinal helminthiasis especially in subclinical cases.

1712

CHRONIC INFECTION WITH *LITOMOSOIDES SIGMODONTIS* PROTECTS AGAINST ANAPHYLAXIS IN ORALLY SENSITIZED MICE

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Anaphylaxis is a life-threatening condition that can result from IgE-mediated allergy. It is becoming increasingly prevalent in western countries and results in approximately 1,500 deaths a year in the United States. In this study we investigated the therapeutic potential of helminths on systemic anaphylaxis in orally sensitized mice. BALB/c mice were sensitized by weekly oral gavage with ovalbumin (OVA) plus Staphylococcal enterotoxin B (SEB) or mock sensitized with PBS for a period of two months. Two weeks after completion of sensitization mice were infected with ***Litomosoides sigmodontis***, a rodent filarial parasite, or mock infected with PBS. Ten weeks post-infection mice were challenge by intraperitoneal injection of 0.4 mg of OVA. Basal temperature was evaluated every 5 minutes for 30 minutes following OVA-challenge. OVA-sensitized and mock-infected mice dropped an average of 2.6 degrees Centigrade by 30 minutes. In contrast, OVA-sensitized mice that were infected with ***L. sigmodontis*** experienced no change in core body temperature. As expected, PBS-sensitized mice that were infected with ***L. sigmodontis*** or mock infected exhibited no change in core body temperature during the 30 minutes post-challenge. Thus, chronic infection with ***L. sigmodontis*** appears to prevent anaphylaxis in mice with pre-existing allergy. Identifying the mechanisms underlying this phenotype could be important in developing novel therapeutics to treat allergic disease.

1713

PHARMACOKINETICS OF ORALLY ADMINISTERED DIETHYLCARBAMAZINE AND ALBENDAZOLE IN FERRETS (*MUSTELA PUTORIUS FURO*)

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A major obstacle to eliminating lymphatic filariasis is a lack of effective macrofilaricidal drugs, medications that are active against the adult stage of these parasites. Drug development has been hindered by the lack of a small mammal model of lymphatic infection that develops clinical disease. We have established a previously developed ferret model of LF. In our model, 150 ***Brugia malayi*** L3's are injected subcutaneously in the right hind limb of ferrets. The adult worms reside in the lymphatic vessels, cause histologic and clinical lymphangitis, and develop permissive infections with the production of circulating L1 stage microfilarial worms. The objective of this study is to describe the pharmacokinetics of known anthelmintic drugs in our model in order to evaluate the suitability of ferrets for filarial drug testing. To achieve this, we administered a single oral dose of diethylcarbamazine (DEC) or albendazole (ALB) by gavage to healthy adult ferrets. In our experiment, 3 ferrets were given doses of: DEC 50mg/kg, DEC 250mg/kg, ALB 40mg/kg, or ALB 200mg/kg. The ferrets underwent four blood draws at baseline, 1 hour, 4 hours, and 24 hours after treatment. Plasma samples were analyzed by LC-MS/MS. The peak drug concentration for the low dose DEC was 1010 ng/ml and occurred at the 1 hour timepoint. Peak concentration for high dose DEC was 6200 ng/ml and occurred at 4 hours. Peak drug concentrations for low and high dose albendazole were 270 and 80 ng/ml, respectively. For both low and high dose albendazole the peak drug concentration was measured at 1 hour. In both DEC and ALB groups, the drugs reached the lower limit of detection by 24 hours. Based on our limited timepoints to date, we can estimate that the half-life for low dose DEC is 3-5 hours and the high dose is 12-15 hours. In humans, the half-life of DEC is 8 hours. The estimated half-life of ALB in ferrets is 2-3 hours for the high dose. In humans the half-life

of ALB is 10 hours. These findings give a foundation for determining antifilarial drug pharmacokinetics in ferrets. Future studies will utilize more timepoints and include other agents.

1714

INVESTIGATING EARLY INFECTION STATUS OF THE FILARIAL PARASITE *BRUGIA MALAYI* IN LABORATORY ANIMAL MODELS

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Lymphatic filariasis (LF) is a mosquito-borne disease caused by the parasitic nematodes ***Wuchereria bancrofti*** and ***Brugia malayi***. These parasites are a major cause of morbidity globally, with an estimated 120 million people infected. ***Brugia malayi*** is the preferred laboratory model for LF due to the fact that non-primate hosts can become infected. For over 30 years, the domestic cat has been utilized as the primary non-rodent experimental model for ***B. malayi***. However, only approximately 25% of felines become patent and maintain a microfilaremia of >1000 mf/ml, which is necessary for life cycle maintenance. The primary test to determine infection status is the presence of circulating microfilariae (mf), which are detectable as early as 4 months. The cost of maintaining this animal model is relatively high, and it would be beneficial to be able to detect a biomarker to indicate infection status before mf are observed. In order to compare host susceptibility in another experimental model, we also examined ***B. malayi***-infected canines, which have been implicated as a reservoir in endemic areas. Ten male domestic cats and eight adult male and three adult female, heartworm-negative, beagle dogs were infected by subcutaneous injection of 400 (cat) or 500-1,000 (dog) ***B. malayi*** third-stage larvae. We monitored complete blood counts (CBCs), lymphedema, and microfilaremia. Filarial infections are known to induce a strong Th2 response. Therefore, we examined eosinophil levels in the blood, with the hypothesis that animals with high eosinophilia would become infected, but would not develop a patent infection. However, sequential CBCs monitored over the period of one year showed that cats maintained a high eosinophilia regardless of their infection status. Canine eosinophil levels were all within normal established reference ranges, even though 50% would become patent. These results may indicate that eosinophils do not play a role in the initial response to infection in the dog and cat and provide further insight into the role of LF pathogenesis in these animal models.

1715

HOW CAN ONCHOCERCIASIS ELIMINATION IN AFRICA BE ACCELERATED? MODELING THE IMPACT OF STRATEGY ADJUSTMENTS

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Great progress has been made towards elimination of onchocerciasis in Africa by annual ivermectin mass treatment. Some countries are already close to elimination and might stop interventions before 2020. Other countries are lagging behind, e.g. due to a late start, very high pre-control endemicity level, implementation problems or contraindications for the implementation of ivermectin mass treatment. To eliminate onchocerciasis in these countries before 2025, interventions must be intensified or alternative treatment strategies must be implemented. We used the established ONCHOSIM model to investigate how much the time-to-elimination can be reduced by various strategy adjustments for different settings. Our results show that doubling the frequency of treatment from yearly to 6-monthly reduces remaining program duration by about 40%. Similar reductions can be achieved by continuing annual mass treatment with a more effective drug like moxidectin, or - if the achieved coverage is too low - by increasing treatment coverage to usual levels. The relative reductions in time-to-elimination do not depend much on the pre-control endemicity level and number of treatment rounds provided thus far. Test and treat approaches may be needed for ***Loa loa*** co-endemic areas where ivermectin mass treatment is contraindicated or compliance is poor due to perceived risk of side effects. We recommend that measures are taken to improve treatment coverage where needed. Further, biannual treatment should be considered when interventions are not yet fully scaled-up, or where pre-control levels are exceptionally high. Where these adjustments are expected to still be insufficient for achieving elimination by 2025, other strategies could be considered, including 3-monthly mass treatment, the addition of vector control through ground larviciding, or the use of more efficacious drugs for mass treatment or selective treatment of carriers. The cost-effectiveness, feasibility, and acceptability of different policy options should be taken into account.

1716

FIELD COMPARISON OF THE BINAX FILARIASIS NOW CARD TEST AND THE ALERE FILARIASIS TEST STRIP FOR DETECTION OF *WUCHERERIA BANCROFTI* CIRCULATING FILARIAL ANTIGENS

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The Global Program to Eliminate Lymphatic Filariasis (GPELF) recommends use of tests for circulating filarial antigens (CFAs) to identify areas that require mass drug administration (MDA), to assess the impact of MDA, and for post-MDA surveillance. We compared the Binax Now Filariasis card test (ICT) with the recently released Alere Filariasis Test Strip (FTS). Comparisons were conducted in two sites that were endemic for LF: (1) a pre-MDA village in the Democratic Republic of Congo (DRC), and (2) a village in the Republic of Congo after two years of semiannual MDA with albendazole. The FTS detected 44.8% more subjects with filarial antigenemia (all subjects positive by FTS and negative by ICT were amicrofilaraemic) than the ICT (42/187 vs 29/187) in the DRC study site. The difference in the sensitivity of the tests was much higher in the community under MDA, where the FTS detected 93.2% more infected subjects (only one subject amongst the 41 who were positive by FTS and negative by ICT was microfilaraemic) than the ICT (85/697 vs 44/697); one subject (amicrofilaremic) was positive by ICT and negative by FTS. When tests were scored with a semiquantitative scale 0-3, mean scores were about 1 unit lower with the ICT compared to the FTS. A portable densitometer (Konica FD-5) was used in DRC to measure the intensity of the T line in a sub-sample of 124 FTS (from which 16, 28, 24 and 56 were visually scored 0, 1, 2 and 3, respectively). We found a significant correlation between the semi-quantitative visual scores and the intensities of the T lines assessed on the spot by the densitometer (rho = 0.93, P-value < 0.001). Intensities of the T lines were also reassessed at different intervals to assess the stability of test results. Post hoc intensity measurements showed a high correlation with the original values up to 8 days. These results confirm that the FTS is more sensitive than the ICT, especially with lower levels of infection. Densitometry can be used to objectively read FTS as a means of quality control for visual readings.

1717

EFFECT OF AN INJECTABLE LONG-ACTING FORMULATION OF IVERMECTIN ON *ONCHOCERCA OCHENGI*

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Additional tools and strategies are needed to reach the objective of onchocerciasis elimination set at 2025, and the most urgent requirement is the availability of a safe macrofilaricidal drug. In this context, a pre-clinical trial was conducted in Cameroon to evaluate the effects of an injectable long-acting (12 months) formulation of ivermectin on the microfilariae (mf) and adult stages of ***Onchocerca ochengi***. Ten female Gudali zebu cattle (age: 3 years) naturally infected with the parasite were injected subcutaneously with either 500 mg ivermectin (group 1, N=4), 1000 mg ivermectin (group 2, N=4) or the vehicle (group 3, N=2). Four subcutaneous nodules and a skin sample were collected from each animal at each time-point (before and 6 and 12 months after treatment). Before treatment, the average ***O. ochengi*** microfilaridermia was similar in the 3 groups (471.6, 222.4 and 263.8 mf per gram of skin, respectively). Six months after treatment, all treated animals were free of skin mf whereas the two controls (vehicle group) still showed high mf densities (mean: 319.3 mf/g). Twelve months post-dose, the same trend in the reduction of microfiladermia was observed, except for one treated animal which had one microfilaria in its skin sample. This animal had the highest mf density before treatment (1336.0 mf/g) and received the lower dose of ivermectin (500 mg). The two animals of the control group showed an average mf density of 850.4 mf/g. None of the animals showed any local or general adverse effect after injection and during the 12-month period. The viability and fecundity of adult worms will be assessed by histology and by embryograms and the plasma concentrations of ivermectin will be assessed by liquid chromatography-mass spectrometry. All these results will be presented at the meeting. Should the long-lasting effect observed on the mf densities be due to a macrofilaricidal effect, the new sustained-release formulation of ivermectin might be an additional tool to accelerate the elimination of human onchocerciasis in specific settings.

1718

ANTI-*WOLBACHIA* (A-WOL) DRUG DISCOVERY: LIGAND BASED VIRTUAL SCREENING COMBINED WITH HTS

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Filariasis inflicts serious health problems throughout tropical communities causing lymphatic filariasis (elephantiasis) and onchocerciasis (river blindness). These diseases infect 120 million people worldwide, ranking filariasis as one of the leading causes of global morbidity.1 Wolbachia is a bacteria that lives inside the cells of the filarial worms. As the filaria are dependent on Wolbachia bacteria survival, eliminating the bacteria with novel-antibiotic drugs would kill the filaria and deliver a new and practical solution for eradicating these debilitating diseases. Through targeting the Wolbachia bacteria, we aim to discover novel antibiotic anti-Wolbachia (A-WOL) therapy which will deliver safe macrofilaricidal activity with superior therapeutic outcomes compared with the current the current gold standard, doxycycline. Our present hit identification activities involves an iterative combination of HTS and ligand based virtual screening, with the results of each screen (active and inactive compounds) informing computational models which are utilised to select the next set of compounds for screening with the aim of improving both hit rate and diversity of the hits. We will present the background, methodology and successes of our approach in our iterative compound selection and screening approach. Through our approach we will show i) an increased hit rate, ii) expansion of SAR around hits, iii) discovery of novel hit chemotypes, iv) scaffold hopping, and v) probing new areas of chemical space. We will also present an analysis of the physicochemical properties of A-AWOL active compounds.

1719

A FIELD COMPARISON BETWEEN TWO RAPID DIAGNOSTIC TESTS FOR DETECTING *WUCHERERIA BANCROFTI* ANTIGEN IN HUMAN WHOLE BLOOD

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Seventy-two countries are currently endemic for lymphatic filariasis. The Global Program to Eliminate Lymphatic Filariasis uses rapid filarial antigen testing to identify endemic areas where mass drug administration (MDA) is needed and to monitor these areas in post-MDA surveillance. Two filarial antigen tests are currently available: the Alere Filariasis Test Strip and the BinaxNOW Filariasis card test. Because of lower cost and greater stability, the test strip will likely replace the card test in a few years. In only one published study from Liberia, the strip test is about 20-30% more sensitive compared to the card test. Whether the test strip compares similarly to the card test in other filarial endemic areas has not been examined. We compared the two tests using 216 samples collected from Papua New Guinean communities. Capillary blood was collected by finger prick during the night. The test strips were run using 75 µL of blood at the point of collection. The card tests were run the following morning using 100 µL of blood collected in 500 µL, EDTA-coated BD Microtainer tubes. Both test strip and card test were accessed at 10 minutes after the application of their required amounts of blood. Seventy-one of 216 subjects (32.9%) were test strip positive compared to twenty-three of 216 (10.6%) that were card test positive. For the positive test strips, 18.3% had 1+ reactivity, 36.6% had 2+ reactivity, and 45.1% had 3+ reactivity compared to 95.7%, 4.3%, and 0% respectfully for the card tests. Only individuals with strong reactivity in the test strips showed reactivity in the card tests. Microfilarial counts show that card tests may have had decreased sensitivity as three of ten microfilarial positive blood smears had negative card test readings. Although further investigation is needed for the suspected decreased sensitivity of the card tests, these initial results show that the Alere Filariasis Test Strip has much greater sensitivity compared to the BinaxNOW Filariasis card test. These could have important implications for determining cut-off thresholds for when an area is considered filariasis free or for when transmission interruption has occur.

1720

A NOVEL APPROACH TO IMPROVE THE BIOAVAILABILITY OF FLUBENDAZOLE BY USING A SPRAY DRIED FORMULATION IN JIRDS, RATS AND DOGS

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Flubendazole (FBZ) is an anthelmintic with low oral bioavailability in its currently marketed formulations indicated for the treatment of Soil Transmitted Helminths. In view of the potential development of FBZ for the treatment of filariasis (onchocerciasis and lymphatic filariasis), aiming at systemic exposure, a new formulation with increased bioavailability has been developed. This new formulation of amorphous solid dispersion of FBZ, is manufactured using a modified high temperature spray dried technology (HSDT). It was administered at 1, 5, 10, 20 and 40 mg/kg as a

single dose and at 5 and 20 mg/kg for 5 days in jirds, at 20 mg/kg in rats and 35 mg/kg in dogs. Flubendazole and its 2 metabolites (Hydrolyzed and Reduced FBZ) were measured in jird, rat and dog plasma and in jird tissues (liver, lymph nodes, spleen and skin). After oral administration in jirds, C_{max} and AUC values increased less than dose proportionally, from 1 to 5 mg/kg/day, and increased dose proportionally, from 5 to 40 mg/kg/day. After repeated administration, the exposure was similar at 5 mg/kg and was lower at 20 mg/kg/day compared to the equivalent single dose. The plasma concentrations of its 2 metabolites were lower compared to FBZ. In tissues, 0.5 h after administration, FBZ was largely distributed in the liver, evenly in spleen and lymph nodes and less in skin compared to FBZ plasma concentration. Same pattern was seen in all doses and after repeated administration. The distribution of its 2 metabolites was slightly different with much higher liver concentrations than for FBZ. In rats and dogs, after oral administration, exposure was drastically increased compared to the marketed formulation. The plasma exposure of its 2 metabolites ranged between 0.55 to 0.67 compared to the FBZ exposure in rats and between 1.8 and 2.9 in dogs. In conclusion, the new FBZ formulation, using HSDT, improved drastically the bioavailability of FBZ after oral administration in jirds, rats and dogs. These encouraging data allowed to design efficacy studies in jirds with a broad range of FBZ doses and to start the toxicology program in rats and dogs.

1721

MODEL-BASED GEOSTATISTICAL MAPPING OF THE PRE-CONTROL PREVALENCE OF *ONCHOCERCA VOLVULUS* IN WEST AFRICA

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The initial endemicity (pre-control prevalence) of onchocerciasis has been shown to be an important determinant of the feasibility of elimination by mass ivermectin distribution. We present the first geostatistical map of microfilarial prevalence in the former Onchocerciasis Control Programme in West Africa (OCP) before commencement of antivectorial and antiparasitic interventions. Pre-control microfilarial prevalence data from 737 villages across the 11 constituent countries in the OCP epidemiological database were used as ground-truth data. These 737 data points, plus a set of statistically selected environmental covariates, were used in a Bayesian model-based geostatistical (B-MBG) approach to generate a continuous surface (at pixel resolution of 5 km x 5km) of microfilarial prevalence in West Africa prior to the commencement of the OCP. Uncertainty in model predictions was measured using a suite of validation statistics, performed on bootstrap samples of held-out validation data. The mean Pearson's correlation between observed and estimated prevalence at validation locations was 0.693; the mean prediction error (average difference between observed and estimated values) was 0.77%, and the mean absolute prediction error (average magnitude of difference between observed and estimated values) was 12.2%. Within OCP boundaries, 17.8 million people were deemed to have been at risk, 7.55 million to have been infected, and mean microfilarial prevalence to have been 45% (range: 2-90%) in 1975. This is the first map of initial onchocerciasis prevalence in West Africa using B-MBG. Important environmental predictors of infection prevalence were identified and used in a model out-performing those without spatial random effects or environmental covariates. Results may be compared with recent epidemiological mapping efforts to find areas of persisting transmission. These methods may be extended to areas where data are sparse, and may be used to help inform the feasibility of elimination with current and novel tools.

1722

STRENGTHENING LOCAL HEALTH SYSTEMS TO ADDRESS LYMPHATIC FILARIASIS MORBIDITY IN A RESOURCE-LIMITED SETTING: A MORBIDITY MANAGEMENT PROJECT IN THE LINDI AND MTWARA REGIONS OF TANZANIA

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In Tanzania, lymphatic filariasis (LF) affects over 6 million people leading to a high burden of LF associated hydrocele: a fluid-filled enlargement of the tunica vaginalis sac around the testes. The economic, physical, and psychosocial impact of hydrocele is devastating not only for the individual, but also for the family and community. In the Lindi and Mtwara regions alone, hydrocele backlog was estimated at 8000 men awaiting hydrocele surgery in 2011. However, most patients did not access surgeries due to the following: 1) inability to pay as almost 90% of patients depend on out of pocket payment; 2) an inadequate number of skilled clinicians to perform surgeries; 3) limited medical supplies; and 4) patients' fear of undergoing a non-emergency surgery under general anesthesia. To address these obstacles, the project focused on strengthening the local health system by pooling resources from stakeholders (donor, MOH, national referral hospital, regional/district hospitals, and communities) to allow for surgeries free of charge and to provide required supplies. Experienced surgeons mentored 40 local doctors and nurses on hydrocelectomy using sac partial excision technique under local anesthesia. Also, clinicians were provided a small incentive to conduct surgeries over weekends to avoid interfering with regular weekly duties. By strengthening the local health workforce, pooling resources, and increasing availability of supplies, over 2000 patients accessed high quality, safe hydrocelectomy over 24 months; almost 6 times the annual average of hydrocelectomy surgeries performed regionally before the intervention. This intervention can be sustained through increased local capacity and ownership of the hydrocele surgery methodology within district level staff.

1723

NOVEL ANTHELMINTICS FROM FILAMENTOUS FUNGI ACTIVE AGAINST FILARIAL WORMS AND SOIL TRANSMITTED HELMINTHS

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Mycosynthetix, Inc. owns one of the world's largest (55,000), diverse and unique collections of filamentous fungi which were sourced from a variety of ecosystems worldwide. We reported previously that a number of natural product compounds and a few fungus extracts active against many nematodes which cause serious neglected diseases in humans as well infections in animals. We continue to exploit the medicinal and pharmaceutical potential of this large library. The targets of the current work include ***Brugia malayi*** microfilaria (BmMF) representing filariasis, and ***Haemonchus contortus*** L1 stage larvae (HcL1) and Strongyloides stercoralis L3 stage larvae (SsL3) representing soil transmitted helminth (STH) infections. We screened 4,048 crude fungal extracts and 90 pure compounds against these three parasites in our 96-well plate based systems. Following fungus re-growth and confirmation of activity of original extract hits, 12 (BmMF), 16 (HcL1) and 9 (SsL3) fully active extract hits were identified. Many of these hits were produced by filamentous fungi not previously shown to produce anthelmintic metabolites, including

Polyporales spp. and others. De-replication procedures to identify the chemical structures of the active metabolites are ongoing, but data thus far indicates that many are novel compounds. Of the 90 pure compounds tested, 22 were fully active against HcL1, 63 were fully active against BmMF and 11 were fully active against SsL3. The most potent compounds were active to 12.5 µM vs HcL1, 0.39 µM against BmMF and 6.25 µM vs SsL3. One of the active pure compounds we identified was enniatin D which has been shown previously to be active against parasites, thus validating our natural product de-replication/screening paradigm. We will continue to evaluate extracts and pure compounds against these three targets over the next few months and will provide an update at the meeting. In conclusion, from our fungal collection we continue to find interesting, novel and potent compounds against nematode targets of importance.

1724

EFFECTS OF FLUBENDAZOLE ON FILARIAL NEMATODES: A TRANSCRIPTOMIC APPROACH

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The use of microfilaricial drugs for the control of onchocerciasis and lymphatic filariasis necessitates prolonged yearly dosing. Prospects for elimination or eradication of these diseases would be enhanced by availability of a macrofilaricidal drug. Flubendazole (FLBZ), a benzimidazole anthelmintic, is an appealing candidate macrofilaricide. FLBZ has demonstrated profound and potent macrofilaricidal effects in a number of experimental filarial rodent models and one human trial. Unfortunately, FLBZ was deemed unsatisfactory for use in mass drug administration (MDA) campaigns due to its markedly limited oral bioavailability. However, a new formulation that provided sufficient bioavailability following oral administration could render FLBZ an effective treatment for onchocerciasis and LF. Identification of drug derived effects is important in ascertaining a dosage range which is detrimental to the worm. Evaluation of drug-induced damage to tissues is challenging. In previous studies, histochemical analysis of morphological damage following exposure to FLBZ indicated that damage to tissues required for reproduction and survival can be achieved at pharmacologically relevant concentrations. However, histological damage is difficult to assess and there are individual differences in scoring of severity. The current study addressed this issue by taking a transcriptomic approach to confirm effects observed histologically and identify genes which were differentially expressed in FLBZ treated adult females worms (100 nM - 5 µM). Comparative analysis across treatment levels and time provided an overview of the processes which are affected by FLBZ exposure. The list of regulated genes correlates with reproductive effects observed via histology in previous studies. This study revealed transcriptional changes in genes involved in embryo and larval development, as well as cuticular synthesis. This data supports the indication that flubendazole acts predominantly on rapidly dividing cells, and provides a basis for selecting markers of drug-induced damage.

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DECLINING HIV RATES AT A VOLUNTARY COUNSELING AND TREATMENT CENTER IN ADDIS ABABA, ETHIOPIA 2009-2014

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Voluntary counseling and testing (VCT) plays a key role in increasing access to HIV diagnosis and linkage to care. In Addis Ababa, Ethiopia's capital, where the rate of HIV is estimated to be 5%, there has been little published in the peer-reviewed literature regarding HIV epidemiologic, and behavioral trends at VCT centers. This paper reports on findings from five years of data from a VCT and treatment center in Yeka neighborhood in Addis Ababa. A preliminary analysis of intake forms from 13,192 clients demonstrated an overall rate of positive HIV tests of 7.6% with a decrease from 9.8% in 2009 to 6% in 2014. Demographic variables significantly associated with a positive HIV test included female gender, pregnancy, increasing age, presenting as an individual for testing (as opposed to with a partner), marriage or widowhood, unemployment, and lower income groups. The majority of clients reported their primary reason for testing was to plan for the future. This study adds to the currently scant peer-reviewed literature on VCT in Addis Ababa, suggests a trend of declining rates of HIV at the VCT center, and identifies demographic characteristics associated with higher HIV rates. If the proportion of people testing at VCT centers is truly declining in the area this center is located, this study may help target testing outreach efforts towards higher-risk populations.

1726

LEPROMATOUS LEPROSY AND HIV CO-INFECTION ASSOCIATED WITH PHENOMENON OF LUCIO VERSUS IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME

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Diffuse lepromatous leprosy (DLL) is a severe clinical outcome of lepromatous leprosy. A new species, ***Mycobacterium lepromatosis***, was identified from a group of Mexican patients with DLL, and severe leprosy reactional state type 3 (phenomenon of Lucio). Clinically, the immune hypersensitivity triggered by bacterial antigens is associated with constitutional symptoms, necrotizing vasculitis, sepsis, and in some cases death. In addition, the advent of HIV and routine use of highly effective antiretroviral therapy (HAART) in patients with Hansen's disease, may relate to other events as immune reconstitution inflammatory syndrome (IRIS), which may occur in 40% of patients with HIV and HAART. In patients with HIV-leprosy, IRIS has been generally associated with inflammatory processes such leprosy-reactions type1, in contrast current clinical case shows a leprosy reaction type 3 or phenomenon of Lucio: Female, 37 years old, on 07/13 consulted by one month of recurrent febrile episodes and multiple skin ulcers in lower limbs. On physical examination patient has signs as pinna edema, loss of the bilateral external third of eyebrows, chronic indurated lesions in abdomen, and pigmented scarring lesions in lower limbs. In addition, patient has skin ulcers with burning pain associated with local edema and serous-hematic and purulent discharge. Patient refers weight loss of 4 kg in 6 months. She relates that seven days ago her spouse died by AIDS. Laboratory exams confirm HIV infection and leprosy. Multidrug therapy (MDT-MB) initiated with dapsone + rifampicin + clofazimine. HIV treatment abacavir/lamivudine plus lopinavir/ritonavir. After three weeks of treatment for HIV and leprosy, patient consulted with multiple skin blisters, with reticular pattern in upper limbs and proximal third of the lower limbs. Ulcer lesions in multiples sizes and different stages, dirty bottom, some of them necrotic with erythematous borders, numbness in legs and hands. DNA extracted from skin biopsy tested by Sanger sequencing technique ruling out the infection caused by M. lepromatosis. Results reported M. leprae European genotype 3-I as the cause of DLL.

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PERINATAL HIV INFECTION AND HEALTH RELATED QUALITY OF LIFE IN SCHOOL AGED UGANDAN CHILDREN

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This study was undertaken to evaluate the impact of perinatal human immunodeficiency virus (HIV) infection on health-related quality of life (QOL) among 166 Ugandan children 6 -18 years old. We implemented a clinic-based retrospective cohort study among perinatally HIV infected and exposed children of HIV-positive women and HIV-negative perinatally unexposed children from Kampala, Uganda. Perinatal HIV infection was diagnosed by the end of breastfeeding via DNA PCR. Current negative HIV-status was confirmed by HIV rapid-diagnostic test. Child QOL was assessed with self-report version of pediatric QOL inventory. Four QOL domains (physical, emotional, social and school functioning) each with scores ranging from 0 (least QOL) to 100 (highest QOL) were defined. Descriptive analyses estimated means, standard deviations (SD), numbers and percentages by perinatal HIV status. Multivariable linear regression models were used to estimate HIV-related differences (β) in QOL scores and 95% confidence intervals (CI). HIV-infected (n=56), exposed negative (n=56) and unexposed children (n=54) were enrolled. Mean age was 10.8 (SD 3.48) years and 77(45.8%) were females. QOL scores in the social functioning domain were similar by child HIV status (p=0.9385). However, perinatally HIV-infected children performed worse than HIV-exposed negative and HIV-unexposed children in the physical (p=0.0006), emotional (p=0.0253) and school (p<0.0001) functioning domains. Perinatally HIV infected children scored significantly lower on Global QOL compared to HIV-exposed negative (β=-5.7, 95%CI: -10.7, -0.7) and HIV-unexposed (β=-6.6, 95%CI: -11.6, -1.6) after adjusting for child's age, sex, nutritional status, relationship to caregiver, and caregiver's age and educational level. QOL scores were similar for HIV exposed negative and unexposed children. In conclusion, perinatal HIV-infection is a significant predictor of low QOL. Specific interventions to mitigate HIV related disadvantage in the physical, emotional and school functioning QOL domains may enhance functional survival in children living with HIV.

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HIV AND TROPICAL COFACTORS: LESSONS FROM THE STI-TREATMENT TRIALS IN SUB-SAHARAN AFRICA

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Substantial evidence indicates that sexually transmitted infections (STIs) promote HIV transmission by producing genital ulcers, inflammation, and viral shedding. The burden of untreated STIs is far higher in sub-Saharan Africa (SSA) than in any other region. Ten randomized controlled trials in SSA examined the effect of STI control on HIV incidence. Only the first trial in Mwanza produced statistically significant results. Consequently, support for STI treatment for HIV prevention faded. The 9 post-Mwanza trials suffered from insufficient exposure contrast due to ethical considerations that required essential STI treatments for controls. The small differences in interventions in treatment and control arms led to small differences in STI outcomes between arms. Substantial treatments for controls also explain their reduced risky sexual behavior that in turn led to similar STIs outcomes between arms. The trials tested alternative treatments of bacterial or viral STIs but not both and were thus subject to confounding from STIs not considered in the trial design. None of the trials examined genital morbidity from infections other than STIs that could enhance HIV transmission or acquisition. Thus none of the trials addressed potential effect modification from biological interactions in the genital microbial community. None of the trials considered genital ulceration and inflammation from non-sexually transmitted pathogens, most importantly Schistosoma hematobium, which is highly prevalent in SSA. None considered ulcers infected with streptococci, staphylococci, or fungi, common in the region. Treating one genital infection may have little effect on HIV incidence when other infections are untreated. Flaws in the design of the post-Mwanza trials render them unsuitable to inform HIV-prevention policy for treatment of cofactor infections. Given the evidence that STIs and other genital infections, including urogenital schistosomiasis, promote HIV transmission and acquisition, cofactor treatment should be considered an important method for reducing HIV incidence in SSA and elsewhere.

1729

CD31 EXPRESSION ON CD4+ CELLS: A POSSIBLE METHOD FOR QUANTITATION OF RECENT THYMUS EMIGRANT CD4 CELLS IN RESOURCE-LIMITED REGIONS

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HIV infected individuals with combined antiretroviral therapy (cART) with long term viremia suppression may remain clinically stable or progressively deteriorate. For cART suppressed patients, conventional clinical status measurements such as CD4% or HIV viral load are poor predictors of long term clinical outcome. Measurements of recent thymus emigrant T-cells (RTE) have been shown to have predictive value. However, established methods for measurement of RTE (T-cell receptor excision circles or complex FACS) are difficult to deploy to regions where most HIV infections occur and where cART is becoming widely available. Literature review suggests that direct measurement of CD31 expression on CD4+ cells provides a simple measure of RTE CD4+ cells. To test this hypothesis, we reviewed records of 69 perinatally infected HIV+ [median (IQR) 13.0 (8.6) years, 54% female, 71% black] and 51 HIV- children [1.6 (5.6) years, 51% female, 63% black] attending UTHealth, Houston clinics between January 2010 and September 2012 for whom CD4+CD31+ were measured. HIV disease was well controlled by cART [2.2 (1.5) log10 HIV RNA copies/ml, 33% (12%) CD4+]. In HIV- and HIV+ groups CD4+CD31+% correlated negatively with age [Spearman's ρ=-0.525, p<0.001 and ρ=-0.475 (p<0.001), respectively] and values were slightly higher in females; expected patterns for RTE. And, CD4+CD31+% values fell within ranges of normal values established using conventional methodology. Our results show that direct measurement of CD4+CD31+ cells in peripheral blood gives results with numerical and biological characteristics consistent with those expected for CD4+ RTE. This technique can be easily performed in technical and budget limited settings. The capacity to measure RTE in the absence of sophisticated laboratory support should prove useful for both clinical management and research.

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EVALUATION OF THE OUTCOME OF CHILDREN ON ANTIRETROVIRAL THERAPY IN CAMEROON: A FOUR YEAR RETROSPECTIVE COHORT STUDY

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Although Antiretroviral Therapy (ART) has significantly improved the lives of those infected with Human Immunodeficiency Virus (HIV), access for children still remains low. The goal of our study was to assess 12 month outcome of HIV-infected children on ART in Cameroon. A retrospective cohort study was carried out in 25 treatment centers in 3 of 10 regions

(North West, South West and Center), harboring over 60% of pediatric patients nationwide. Children who initiated ART between January 2007 and December 2010 were included. De-identified socio-demographic, clinical and biological were extracted from hospital records and analyzed using SPSS version 16. Survival was estimated by Kaplan Meier method. $\chi 2$ and Cox proportional hazards were used to determine predictors of mortality. P values < 0.05 were considered significant and ethical clearance was obtained from the Institutional Review Board of the School of Health Sciences, Catholic University of Central Africa. A total of 1,867 children <15 years were included in the study. 50.2% were females, mean age was 62.2 ± 4.8 months. Over 50% were <60 months old, 20.7% had one or both parents dead and 63.7% had severe clinical disease (WHO clinical stage III or IV) at ART initiation. Of the 7.1% (110/1558) who had tuberculosis (TB) at baseline, only 48.2% were on TB treatment. Over half (57.8%) were on Cotrimoxazole at baseline. Complete data for outcome analysis was available for 328/1867. After 12 months, 71.3% (234/328) were still alive and in care, 9.8% (32/328) were dead and 17.4% (57/328) were lost to follow up. Severe clinical disease (Chi^2=104, p<0.001), age < 12 months (Chi^2=16.4, p=0.001), presence of TB (Chi^2=5.9, p=0.022) and hemoglobin level <8g/dL (Chi^2=13, p=0.001) were all significantly associated to death. Predictors of mortality were severe clinical disease (aHR=16.6, p<0.001), TB presence (aHR=4.1, p=0.007) and age < 12 months (aHR=5.6, p=0.009). Outcome of children on ART in Cameroon is poor. Improving retention in care, strengthening community engagement and building capacity of service providers are priorities if outcome needs to be improved.

1731

HIV, TUBERCULOSIS AND HIV/TB CO-INFECTION IN MACHALA, ECUADOR

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Tuberculosis (TB) is a leading cause of death among HIV-infected individuals worldwide. The incidence of HIV in Ecuador has increased considerably in the past 15 years, and Ecuador has one of the highest burdens of HIV/TB co-infection in the Americas. We examined the burden of HIV, tuberculosis, and HIV/TB co-infection in Machala, Ecuador, a coastal city in southern Ecuador near the Peruvian border. A total of 398,919 records were analyzed from a citywide database of clinic visits in Machala, Ecuador in 2014. Binomial regression was used to examine the associations of HIV, tuberculosis, and HIV/TB co-infection with socio-demographic factors. There were 321 HIV cases, 1,210 tuberculosis cases, and 60 HIV/TB co-infection cases in 2014. The burden of HIV (RR: 3.16, 95% CI: 2.54-3.94, p<0.0001) and HIV/TB co-infection (RR: 4.91, 95% CI: 2.91-8.30, p<0.0001) was highest in adults aged 19 to 34 years, compared to all other age groups. In contrast, the risk of tuberculosis was highest among older adults (50-64y: RR: 3.27, 95% CI: 2.87-3.73, p<0.0001; ≥65y: RR: 2.21, 95% CI: 1.87-2.60, p<0.0001), compared to other age groups. Men had a greater risk of being infected with HIV (RR: 3.10, 95% CI: 2.48-3.89, p<0.0001), tuberculosis (RR: 3.53, 95% CI: 3.14-3.97, p<0.0001), and HIV/TB co-infection (RR: 8.70, 95% CI: 4.52-16.73, p<0.0001), compared to women. The burden of tuberculosis was higher in HIV-infected men, compared to HIV-infected women (24.9% vs. 8.9%; p<0.05). In summary, the burden of HIV, tuberculosis, and HIV/TB co-infection was relatively high in coastal Ecuador, with nearly one in five HIV-infected patients presenting with TB co-infection. Active surveillance efforts are needed to screen for HIV/TB co-infection and drug resistance in this setting.

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OLDER AND FORGOTTEN: MENOPAUSE SYMPTOM EXPERIENCE OF HIV POSITIVE AND NEGATIVE WOMEN IN IBADAN, NIGERIA

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Globally,not only are people living longer with HIV/AIDS, but there is also a significant increase in older individuals becoming infected .As the human immunodeficiency virus (HIV) epidemic enters its third decade, a high percentage of women with HIV will also be entering menopause their lives extended by improvements in antiretroviral therapies.This study aims to determine and compare the menopause symptom experience and perceived health status among HIV positive and negative women. A comparative, hospital based study was conducted among HIV positive and negative menopausal women. Focus group discussions were conducted among menopausal women attending the ARV and General Outpatient clinics at the University College Hospital Ibadan, Nigeria with the use of a focus group discussion guide. Opinions of discussants on knowledge and experience of menopausal symptoms, perceptions about the menopause and the different coping strategies were explored. Six focus group discussions were conducted among women aged 40 and 60 years in each of the two groups.Data was analysed thematically. The majority of the discussants had adequate knowledge of menopausal symptoms with most of them reporting vasomotor symptoms and musculoskeletal symptoms. In both groups, perceptions about the menopause were generally positive as most of them opined that the menopause means freedom from sexual activity and child birth. Concurrent health conditions mentioned include: hypertension, osteoporosis, depression and reduced libido. Older women with HIV infection reported higher occurrences of these conditions. Coping strategies reported include belonging to a support group and seeking information from health care workers. In conclusion, menopause may induce many of the same metabolic changes that are being observed with HIV infection, and this may complicate the health and quality of life of aging women with HIV infection. There's a need for the formulation of methods and interventions that will help these women in coping with the double burden of HIV infection and symptoms of menopause.

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LOW NUMBER OF NERVOUS SYSTEM INFECTIONS AMONG 4012 ADMISSIONS IN SHELUI TANZANIA WITH STABLE/LOW PREVALENCE OF HIV

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In sub-Saharan Africa, several countries did substantial progress in decreasing HIV prevalence, such as Coastof Kenya, Central Tanzania and North Uganda, when HIV prevalence dropped from 20-25% in 2000-2010 to 5-10% in 2010-2015. The aim of this study has to asses prevalence/ occurrence of opportunistic HIV related in central and peripheral nervous system infections in post Highly active antiretroviral therapy (HAART), in area of central Tanzania with stable HIV transmission. Shelui region is located in central Tanzania between Arusha and Mwanza in a dry climate area in altitude of 1000 metres. HIV prevalence in 2013 was 4,3% and in 2014 was 5,6%. A decade ago, according to the local statistic of Health Dispenzary in Shinquida (50km) in 2003 was 14,7%. We separated from the 4012 admissions from January 2013 to December 2014 all neuroinfections and double checked in monthly reports their occurence in

2013 - 2014. Within12 months, 4012 patients was treated on out patients basis, in Shelui St. Francis Dispenzary, and any 79 neuro-infections (less than 1%) were repeated (majority of them 24 (61%) was herpes zoster infection (HZV) with local manifestation along the peripheral only 5 (22%) were HIV positive. 15 cases have had severe malaria, with central nervous symptomatology (coma or serious neuropsychic event (seizures)). No one case of TBC neuritis, cryptoccocal meningitis or toxoplasma encephalitis were observed among HIV positive cases. In conclusion, in time of HAART and decreasing of HIV prevalence, only minimum number of central nervous opportunistic infections appeared and therefore prophylaxis of opportunistic neuroinfections is not indicated, as it has been considered in pre/or early HAART era.

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DEVELOPMENT AND EVALUATION OF THE NON-INSTRUMENTED NUCLEIC ACID AMPLIFICATION (NINA) ELECTRICITY-FREE PLATFORM FOR ACUTE AND EARLY INFANT HIV-1 DETECTION IN LOW-RESOURCE SETTINGS

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In low-resource and resource compromised settings (LRS), limited access to centralized medical facilities presents a critical barrier to timely diagnosis and treatment, of infectious diseases. Inadequate diagnostic laboratory infrastructure results in inaccurate diagnoses, lost test results, delays to treatment, and loss to follow-up associated with specimen collection and transportation to centralized health centers and subsequent response. The most accurate molecular diagnostic tests with low limits of detection (LODs) and high clinical sensitivity and specificity are only available through laboratory-based testing and more recently through portable nucleic acid amplification tests (NAATs). To ensure accurate diagnostics are available to everyone, PATH has developed a non-instrumented nucleic acid amplification (NINA) device that enables disposable isothermal amplification by thermally coupling an exothermic chemical reaction to an engineered phase change material (PCM) in an easy-to-use, low-cost heater. This patented, electricity-free heating platform approach can be used to provide highly sensitive isothermal molecular diagnostics away from the traditional laboratory setting. The US Centers for Disease Control and Prevention (CDC) has developed a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for HIV-1 that amplifies nucleic acid from a prepared whole-blood specimen. As nucleic acid amplification is the preferred method for acute HIV infection and early infant detection (EID), the introduction of this point-of-contact NAAT diagnostic will reduce the time to detection for HIV-1 infection by up to three weeks as compared to the currently used serology-based rapid diagnostic tests (RDTs). We present data on the design and manufacturing of 150 miniaturized single-use disposable NINA heaters, the lyophilization and stabilization of RT-LAMP reagents, and a qualitative HIV-1 assay. These data demonstrate our current status toward the goal of an inexpensive, easy-to-use molecular RDT for acute and early infant HIV infection detection.

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DEVELOPMENT AND DESIGN OF AN OBSERVATIONAL IMPACT EVALUATION OF IMMEDIATE ANTIRETROVIRAL THERAPY ON HIV INCIDENCE AMONG HIV-POSITIVE MEN WHO HAVE SEX WITH MEN IN SHANGHAI, CHINA

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For people living with HIV and AIDS, antiretroviral therapy (ART) provides life-saving clinical benefits. Immediate ART initiation after HIV diagnosis has also been shown to be highly effective at reducing HIV transmission risk within heterosexual HIV-serodiscordant couples; however, equivalent evidence for men who have sex with men (MSM) has been limited to a few observational studies in high-income settings. Although immediate treatment after HIV diagnosis (i.e., treatment as prevention), regardless of CD4 count, is now considered best practice for key populations including MSM, the evidence in support of this policy in most real-world settings is inadequate. In China, where HIV incidence is increasing among the general population and especially among MSM, immediate ART following diagnosis is being considered by public health authorities as a possible strategy to reduce transmission. We have proposed a strategic international collaboration between the University of Toronto and the Shanghai Centers for Disease Control and Prevention (Shanghai CDC) to conduct a three-phase observational impact evaluation that will investigate: (1) background HIV care indicators (i.e., the cascade of HIV care including linkage to care, ART initiation, retention in care, adherence, and viral suppression) drawing on routinely collected surveillance data (n=9,284); (2) HIV care outcomes among HIV-positive MSM who select immediate ART (CD4>=500 cells/μL) or the current standard of care (CD4<500 cells/μL) (n=236); and (3) transmission risk among the HIV-negative partners (n=360). Methods will include a retrospective study of the Shanghai CDC database and a prospective cohort study among HIV-positive MSM and their HIV-negative partners. The present work outlines the scope of this international collaboration, elaborates upon key local and global research gaps, and presents the proposed study design, anticipated challenges, and limitations. This study is poised to make significant scientific contributions to the global evidence-base for HIV prevention and to strengthen HIV care outcomes in China and globally.

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ASSESSMENT OF VECTOR SURVEILLANCE CAPACITY IN THE US DEPARTMENT OF DEFENSE FOR EMERGING VECTOR-BORNE DISEASE THREATS

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With the emergence of vector-borne diseases, such as chikungunya virus (CHIKV), it is necessary to assess the state of preparedness for the US Department of Defense (DoD) to detect and respond to vector-borne disease (VBD) threats. Assessments are required for vector surveillance activities and prevention strategies for DoD personnel in areas at risk. A risk assessment was needed to evaluate the threat of CHIKV to the US, measure DoD capabilities for rapid detection of cases, and capacity for vector surveillance. The evaluation identified gaps in current capabilities for responding to emerging vector-borne disease threats, and strategies for capacity-building to apply to current surveillance programs. The four-tiered data collection and analysis strategy employed was 1) a review of DoD policy; 2) a workshop on strengthening DoD surveillance and detection for CHIKV and Dengue; 3) semi-structured interviews and observations; and, 4) a review of DoD vector surveillance programs.

Qualitative analyses of interview and field notes data included analytical coding and deductive reasoning to determine and translate themes for identifying gaps. The resultant themes were: 1) variation in capabilities; 2) a demand for resources; and, 3) a need for communication and collaboration. Major gaps identified were in operational surveillance and organizational structure. Laboratory capabilities varied by region and service, which caused ***'Reactionary Surveillance'*** instead of prevention. Moreover, military-civilian collaboration varied by need and program. The workshop resulted in six recommendations for preparedness; policy development, long-term research, improved risk assessments and improved collaboration. DoD vector surveillance capabilities for emerging threats are high; however, resources were lacking and efforts, often unstructured and disparate. Improved civilian-military collaboration can improve efficiency and effectiveness of national VBD preparedness by using an integrated approach to surveillance among biosurveillance partners.

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COMPARISON OF VIROLOGICAL OUTCOMES IN HIV INFECTED CHILDREN AND ADOLESCENTS ON COMBINATION ANTIRETROVIRAL THERAPY, WITH AND WITHOUT HISTORY OF TUBERCULOSIS DURING ELEVEN YEARS OF FOLLOW UP IN CAMBODIA

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The immunological interaction between human immunodeficiency virus (HIV) and Mycobacterium tuberculosis (MTB) is bi-directional, with MTB increasing HIV viral replication possibly by inducing T-cell activation. But the impact of MTB disease on long-term virological response to combined antiretroviral therapy (cART) in MTB/HIV co-infected children and adolescents is unknown. We retrospectively analyzed a longitudinal cohort of 107 Cambodian HIV positive children (vertically infected) on cART continuously enrolled into 2 pediatric programs between 2003 and 2014. Children resided in a unique living setting that ensured a high degree of medication adherence. Median follow-up was 86 months (11-134). Median age was 7.0 years (1-15) at entry and 7.5 years (1.4-15.6) at cART initiation. Most children (n=60, 53%) had CD4 count ≤ 15% at the time of enrollment. Based on symptoms and radiographic evidence, 47 (44%) of 107 were diagnosed with active MTB infection. TB prevalence was 29.9%; the TB incidence rate was 61.5/100 children-years. From 47 co-infected children, 18 (38%) had virological failure of the first line (NNRTI-based) regimen, compared with 11 (18%) with no history of MTB (p=0.02). Total of 7 (21%) developed a recurrent episode of active MTB while on cART, 4 with evidence of virological failure (p=0.41). In a Cox proportional hazards model, children with co-infection were more likely to experience virological failure, as compared to children without MTB (HR 2.2, [95% CI 1.1, 4.2], p=0.02). After adjusting for age and using a left-truncated analysis (180 days post-cART), HR was 1.85, [95% CI 0.95, 3.8]. Active MTB infection, even if treated, appears to influence long-term virological outcomes of cART among HIV positive children and adolescents. The most apparent impact on virological outcomes was seen after 24 months of cART. High clinical recurrence of MTB among HIV positive children in both groups, with and without virological failure, suggests that standard MTB treatment was suboptimal at preventing recurrence or cART was unable to restore optimal MTB responsiveness.

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PEER LED DISCUSSIONS: A KEY ELEMENT OF CARE FOR YOUNG ADULTS WITH HIV

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In the USA the incidence of HIV infection in young people (13 to 24 years old) continues to increase, accounting for 1 in 4 new infections. These newly infected young people are at a critical juncture in the management of their chronic disease while transitioning into adulthood. To support this population, we sponsored an environment for Houston's young adults with HIV that supports personal capacity building through peer support and learnt coping mechanisms. A weekly advocacy and wellness group was established in May 2014. The typical meeting agenda encompassed dinner, social activities, peer-led discussion of personal challenges and a short didactic session. On a monthly basis, individual interviews and ongoing needs assessment were conducted. Over the past 30 group meetings, the attendance ranged from 1 to 5 participants, with an age range of 18 to 27 years old. The group consists of individuals from multiple ethnicities and different education levels, sexual orientations, and sero-concordant/discordant relationships. Of the participants, 66% have attended over 5 meetings. During the meetings clients speak about their health, and specifically how HIV has affected their lives. Topics discussed include medication adherence, disclosure, grief, substance abuse, and life skills such as interview techniques and budgeting. When some feel they have nowhere else to go, the group has provided an outlet for participants to share their dreams and goals while also unloading some emotional burden. The wellness group is an opportunity for individual young adults with HIV to engage peers outside the clinical setting, empowering them to overcome personal obstacles, achieve career goals, and be an active citizen of the community.

1739

NOVEL APPROACHES IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS

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Current methods to diagnose leishmaniasis have limitations due to the fact that they lack sufficient sensitivity and/or specificity, are difficult to conduct and often require invasive sample methods. Furthermore, variation in clinical accuracy of molecular diagnostic methods for leishmaniasis is commonly observed depending on sample source, method of DNA recovery and molecular test and few attempts have been made to compare these variables. Our research collaboration is seeking new methods in sampling and molecular methods that ultimately can be employed near patient. Swab and aspirate samples from lesions of patients with suspected cutaneous leishmaniasis (CL) were evaluated alongside standard diagnosis by microscopy or culture of parasites from lesion material. Three DNA extraction methods were compared: Qiagen on swab and aspirate specimens, Isohelix on swabs, and boil/spin of lesion aspirates. Recovery of ***Leishmania*** DNA was evaluated for each sample type by real-time PCR (18S rDNA). Swab sampling combined with Qiagen DNA extraction was the most efficient recovery method for ***Leishmania*** DNA , and the most sensitive (97%; 95% CI:91%-100%) and specific (84%; 95% CI:64%-95%) approach. Swab sampling of lesions was painless, simple to perform and coupled with standardized DNA extraction enhances the feasibility of molecular diagnosis of CL. To allow for near patient molecular testing we have developed an isothermal Pan-Leishmania LAMP assay for diagnosis of CL, which was evaluated

in a prospective cohort trial of 105 clinical CL suspects in South-West Colombia. Swab samples were processed for DNA extraction using the Qiagen Blood and Tissue kit. Microscopy was performed on skin scrapings of ulcers, aspirate samples were cultured, LAMP and qPCR performed on extracted DNA. A composite gold standard comprising of microscopy AND/OR culture positivity was used to calculate the diagnostic accuracy of LAMP and qPCR. LAMP was 95% sensitive (95% CI: 87.22 % to 98.53 %) and 86% specific(95% CI: 67.32 % to 95.88 %). This molecular test is more sensitive and specific than microscopy and culture alone.

1740

TOOLS FOR IMPROVED MANAGEMENT OF VISCERAL LEISHMANIASIS: CLINICAL EVALUATION OF THE RK28 RAPID DIAGNOSTIC TEST AND ASSESSMENT OF AN ANTIGEN-DETECTION TEST OF CURE

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Prompt diagnosis and treatment of visceral leishmaniasis (VL) provide the best clinical outcome. The rK39 Rapid Diagnostic Test (RDT) has multiple advantages over invasive tissue biopsies and microscopy for the confirmation of clinical diagnosis, but low sensitivity (~80%) has been reported in Africa. We conducted a clinical study to validate our previous observation that an RDT based on the rK28 antigen can improve the confirmation of VL in Africa. VL was diagnosed in 206 enrolled Sudanese patients by ***Leishmania*** parasite detection in lymph node or bone marrow aspirates, while another 79 enrolled patients were negative and considered as controls. rK28 RDTs were conducted with both whole blood and sera, then evaluated by blinded interpretation. The rK28 RDT had a high specificity when developed with whole blood (WB; 100%) and serum (97.7%) and exhibited good sensitivity (WB, 92.5%; serum, 94.5%). Performance was favorable when compared with a Direct Agglutination Test performed on the same sera (92.9% specificity and 83.5% sensitivity). Two blinded readers scored a given WB or serum sample the same on the rK28 RDT 99.2% and 100% of the time, respectively. A reader scored one individual's paired WB and serum RDT results the same ("reproducibility") 93.8% of the time. As a means to monitor parasite burden and response to drug treatment, we have also developed a test, the ***Leishmania*** Antigen Detect™ ELISA (LADE), to directly detect ***L. donovani*** antigens in VL patient urine. LADE had >90% sensitivity on VL urine from Sudan (N=64), Bangladesh (N=13), and Ethiopia (N=46) and 88% sensitivity on samples from Brazil (N=43). The test had 100% specificity (N=88). To confirm LADE's utility in monitoring treatment, urine samples were collected from Ethiopian VL patients on a drug treatment plan. ELISA ODs were highest at time of diagnosis and decreased during treatment: A 95% positivity by LADE at day 0 fell to 21% at day 30, and to zero by day 180. Thus, LADE provides a non-invasive, semi-quantitative tool to detect parasite products during acute infection. Taken together, the rK28 RDT and LADE represent tools that can fill existing gaps in VL management.

1741

DEFINING DRUG MECHANISM OF ACTION: LEVERAGING PHENOTYPIC HITS AGAINST KINETOPLASTIDS

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The development of new drugs for the treatment of kinetoplastid diseases remains a challenge, hampered by few validated drug targets and an insufficient understanding of basic kinetoplastid biology. These and other challenges have left drug discovery programs heavily reliant upon phenotypic screening of compound collections against parasites in culture. However, in the absence of understanding the mode of action (MoA) or the specific targets of phenotypically active compounds, chemical optimization to improve pharmacokinetics or avoid toxicity is difficult to achieve, leading to unacceptably high attrition rates. To address these issues, we have established a multiplex platform for the determination of MoA. Several hundred anti-kinetoplastid compounds have been identified through phenotypic screening of libraries, providing well-validated cell-active chemical matter encompassing a diverse range of chemical scaffolds. The diversity of the cell-actives suggests that multiple undefined MoAs are in play. Our MoA-platform incorporates a battery of chemical, biological and genetic tools, and the orthogonal basis of the platform provides particular strength in determination of the interactions between actives and the parasite. This should yield high value insights into MoA, mechanisms of drug resistance and molecular targets. The utility of this approach will be discussed in terms of developing compounds as potential therapies and as tools for the dissection of kinetoplastid biology.

1742

ESTIMATING THE COSTS OF IDENTIFYING HUMAN AFRICAN TRYPANOSOMIASIS CASES USING A NEW DIAGNOSTIC FRAMEWORK IN UGANDA

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Human African trypanosomiasis (HAT) cases in Uganda that are caused by the ***gambiense*** subspecies of the protozoan parasite ***Trypanosoma brucei*** have been declining in recent years. To maintain this decline, HAT cases must be identified early in their infection, but prior to 2013 only four facilities were equipped with HAT screening tests. In 2013 a program was launched to strengthen the passive surveillance infrastructure, here we evaluate the cost-effectiveness of this program. The program equipped all 197 public healthcare facilities in the focus with new Rapid Diagnostic Tests (RDTs). To confirm presence of the pathogen, positives by RDT were referred to one of 12 facilities that were equipped with enhanced microscopy. For those not confirmed by microscopy, a blood sample stored on filter paper was transported by motorcycle to one of three facilities equipped with loop mediated isothermal amplification of DNA (LAMP) equipment. To estimate the costs of running the program and costs of identifying each case we use an epidemiological model of the surveillance system and incorporate costs in this model. Data on costs (incurred by the program and by those screened) and epidemiological parameters were collected during the program. By June 2014, 5,036 RDTs had been performed and had identified 7 cases from 200 positive RDTs. Facilities had been enrolled in the program gradually from August 2013 until February 2014. We estimate that over one calendar year, an average 11,162 people would be screened, identifying 16 cases per year (at contemporaneous prevalence), at a total cost of 129,700 USD which is 8,360 USD per case identified or 658 USD per facility per year. We have demonstrated that large scale passive screening can be implemented in a cost-effective manner across an entire HAT focus. This model of easy access to screening through the nearest healthcare facility is a framework that can be used in other countries. Following a shrinkage in the HAT focus, this project was streamlined by reducing the extent of coverage of RDT facilities in July 2014. These results are being updated accordingly and will be modelled to estimate costs as prevalence declines.

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COMPARISON OF THE PERFORMANCE OF THE SD BIOLINE HAT RAPID TEST AND CATT IN VARIOUS DIAGNOSTIC ALGORITHMS FOR GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS

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Early diagnosis of human African trypanosomiasis (HAT) relies on screening large numbers of at-risk individuals. Screening for HAT has been carried out with the card agglutination test for trypanosomiasis (CATT), which is limited by its requirement for cold storage, electricity and its multi-test packaging format. Recently, Standard Diagnostics (SD)/Alere developed an individually formatted and thermostable rapid diagnostic test (RDT). Evaluation studies found that the SD BIOLINE HAT RDT prototype was less sensitive and specific than CATT. The prototype was optimized and we present a study to evaluate the performance of CATT and the optimized RDT in the Democratic Republic of the Congo. The study followed a clinical trial format. Participants were enrolled actively by four mobile teams, and passively at four healthcare facilities in three provinces. Each participant was tested with the RDT and CATT, participants positive by CATT were retested with CATT on 1:8 diluted plasma (CATT 1:8), and positives to CATT or RDT were tested by visual inspection for motile parasites in body fluids. Cases were those with visible parasites. Results were analysed in four algorithms - RDT and CATT as standalone tests and each test followed by CATT 1:8. During five months 131 cases and 13,527 controls were enrolled. The sensitivity of the RDT was 92.0% (95% confidence intervals (CIs) = 86.1, 95.5), and was significantly higher than CATT, which was 69.1% (95% CIs = 60.7, 76.4). The sensitivity of all the algorithms decreased when followed by CATT 1:8, to 52.8% (95% CIs = 44.1, 61.3) for the RDT and 59.2% (95% CIs = 50.4, 67.4) for CATT. The specificity of the RDT was 97.1% (95% CIs = 96.8 - 97.4), significantly lower than CATT which was 98.0% (95% CIs = 97.8, 98.2). Specificity was over 99.5% when a positive screening test was retested with CATT 1:8. Agreement between readers was very good. This study has demonstrated that an algorithm in which the SD BIOLINE HAT RDT is used for screening is optimal for case detection in both passive and active screening settings. However, its lower specificity will increase the burden on screening teams by generating more false positives.

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ARE WE NEARLY THERE YET? MODELLING SLEEPING SICKNESS ELIMINATION

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The number of cases of Gambian human African trypanosomiasis (HAT) has declined in recent years, however it has previously remained unclear if elimination of the disease as a public health problem can and will be achieved by 2020. Despite being targeted by the WHO in the London 2012 declaration, no detailed quantitative assessment of the impact of existing and proposed control strategies had been carried out in relation to this aim. Here, mathematical modelling techniques are utilised to address the feasibility the WHO goals, in particular focusing on answering this question for two high-endemic health zones in the DRC. Traditional assumptions such as considering Gambian HAT to be solely anthroponotic and supposing that tsetse cannot ever acquire infection after their first blood-meal are challenged by fitting the model to human incidence data from the DRC. Using a framework which incorporates the multiple disease stages as well as current screening, diagnostics and treatment practices, this predictive model uses our current understanding of the biological processes to determine the likely incidence of HAT by 2020. Formerly these health zones have relied on detection and treatment of cases to reduce disease burden, however introducing vector control may be necessary to reach and sustain elimination of HAT. The impact of active screening campaigns as a control strategy is analysed by investigating the effect of screening frequency and proportion of the population screened upon disease prevalence. Likewise the benefit of vector control as an additional strategy is assessed. Due to the highly heterogeneous nature of disease and control strategies in different geographic ***foci*** it is key to use robust models in conjunction with high quality regional data to ensure resources are effectively used to achieve elimination.

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DISSEMINATED LEISHMANIASIS: AN EMERGING, SEVERE AND DIFFICULT TO TREAT DISEASE IN BRAZIL

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Disseminated leishmaniasis (DL) has been characterized by multiple and pleomorphic cutaneous lesions, including papules, nodules and ulcerations, distributed in more than two noncontiguous parts of the body. Besides the great number of cutaneous lesions ranging from ten to hundreds, the severity of the disease is revealed by the presence of systemic symptoms like fever and chills during the dissemination phase, as well as nasal mucosal involvement in up to 44% of the cases. We have documented an increased frequency in the number of DL caused by L. braziliensis in the endemic area of Corte de Pedra, Bahia, Brazil. DL was a rare disease, responsible for 0.2% of all cutaneous leishmaniasis (CL) cases identified from 1978 to 1984. DL accounted for 1.9% and 3.9% of cases of CL from 1992 to 1998 and from 2004-2008 respectively, indicating that it has become an emerging form of leishmaniasis. Although the reasons for the development of DL have not been established, it involves a complex and poorly understood network involving L. braziliensis polymorphism, host immune response, and the environment. We have evaluated the peripheral and in situ production of cytokines and chemokines in DL, where a decrease in the type 1 immune response in the peripheral blood appears to be caused by the attraction of Leishmania-activated T cells to the multiple cutaneous lesions where few parasites may be found. Interestingly, DL patients were HIV negative and without other systemic immunosuppression. DL is a hard to treat disease and the majority of DL patients need more than three courses of meglumine antimoniate, or the use of 1.5 to 2 gr of amphotericin B deoxycholate for 45 to 60 days to cure. We have treated twenty DL patients with liposomal amphotericin B with a total dosage ranging from 22 to 35 mg/kg administered in 7 to 14 days and found 65% of cure rate, compared with 28% of cure rate using 30 days of meglumine antimoniate in its highest dosage of 20mg/kg/day. Our data show that DL in Brazil is an emerging and severe form of CL that represents a therapeutic challenge with an important socio-economic impact.

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DEVELOPMENT OF A HIGH SENSITIVITY LATERAL FLOW ASSAY FOR DETECTION OF *TRYPANOSOMA CRUZI* INFECTION IN LOW COMPLEXITY TESTING ENVIRONMENTS

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Chagas disease, despite global control efforts, still is a major health challenge in several countries of Latin America, with an estimated 8-10 million of acute or chronically infected people. Control programs have decreased vector-transmitted Chagas disease yet blood transfusion, organ transplant and principally congenital disease which accounts for >14,000 cases annually, are of major concern. Serology is frequently

difficult to interpret and of little value for congenital infections. Parasitological methods used to detect ***Trypanosoma cruzi*** infections have suboptimal sensitivity (microscopy, culture, and xenodiagnosis) or require infrastructure, equipment and trained personnel unattainable in endemic areas. PCR has shown to be more sensitive than other parasitological methods or serology to detect early infections. This is a critical aspect of Chagas management since early treatment is associated with greater therapeutic efficacy. We developed an innovative point of care molecular test to diagnose ***Trypanosoma cruzi*** infection that does not require expensive equipment and the results are read in < 1 hour. We used the kinetoplast DNA minicircle as target for designing primers and probes. ***Trypanosoma*** DNA was extracted with the Qiagen® kit and parasite detection was achieved using isothermal Recombinase Polymerase Amplification coupled with Lateral Flow (RPA-LF) assay for visual analysis of the amplification product. The test sensitivity was similar to real time PCR (reference test), detecting 0.1 ***T. cruzi*** per reaction. Preliminary results using a small number of ***T. cruzi*** (n= 10) and ***T. rangeli*** (n= 5) strains showed that the RPA-LF detected both parasite species. However, ***T. rangeli*** could be distinguished from ***T. cruzi*** when the RPA products were run in a 1% agarose gel, giving 300 bp or 190 bp band sizes, respectively. This could be relevant in regions where both parasite species are being transmitted. The RPA-LF test could be a practical tool to manage Chagas disease in endemic areas with resource-limited health infrastructure.

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NON-INVASIVE FUNCTIONAL CARDIAC MONITORING IN A MOUSE MODEL OF CHAGASIC CARDIOMYOPATHY TO EVALUATE A NEW THERAPEUTIC VACCINE

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New estimates from the World Health Organization indicate that 1.17 million people suffer from chagasic cardiomyopathy in Latin America. Initially, cardiac dysfunction is characterized by conduction disorders, which can then progresses to cardiomyopathy and even sudden cardiac death. We have utilized state-of-the-art non-invasive functional cardiac monitoring offered by a core facility at Baylor College of Medicine as an innovative approach to evaluate new therapeutics in a mouse model of chagasic cardiomyopathy. Current pharmacological treatments are plagued by significant side effects and poor efficacy. There is an urgent need for new treatment modalities. A therapeutic vaccine has potential advantages that include reduced adverse effects, cost savings, and the potential to be used as a replacement for current therapies or when paired with chemotherapy. Our laboratory has previously shown promising cell-mediated protective immunity and therapeutic efficacy of a nanoparticle vaccine in an acute mouse model of Chagas disease. In order to test new treatment modalities in a model that mimics key aspects of human disease, we have optimized a mouse model of chagasic cardiomyopathy. When female ICR mice (Taconic Biosciences, Inc) are infected with 500 trypomastigotes of a H1 strain originally isolated from a patient in Yucatán, Mexico, 70% of the mice survive through the acute phase of the disease and enter into the chronic stage. Of these mice, 22% have evidence of ECG abnormalities in the early chronic stage of disease including altered basal heart rates, ectopic activity, and conduction blocks. As these mice progress further into chronic infection 17% show severe conduction blocks by echocardiography. Currently, we are evaluating our therapeutic vaccine in this mouse model of chagasic cardiomyopathy. This work optimizes a mouse model of chagasic heart disease, and utilizes non-invasive functional cardiac monitoring as a novel translational technique to evaluate new therapeutic vaccines against Chagas disease.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF MILTEFOSINE IN CHILDREN AND ADULTS WITH CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA VIANNIA*

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A population pharmacokinetics phase IV clinical trial was conducted to comparatively analyze the pharmacokinetics and pharmacodynamics of miltefosine in children and adults. The protocol was approved and monitored by the institutional ethical committee. Sixty patients presenting with cutaneous leishmaniasis, 30 children of 2 to 12 years of age, and 30 adults of 18 to 60 years of age, participated this study. Plasma and intracellular drug concentrations were measured by mass spectrometry in samples obtained during treatment and six months following initiation of treatment, at which time therapeutic outcome was defined. Fifty-two patients cured, failure occurred in 5 children, and 3 patients were lost to follow-up. Plasma and intracellular miltefosine concentrations were overall, lower in children compared to adults. Exposure to miltefosine, estimated by the area under the curve and C_{max}, was significantly lower in children ($p<0.01$). Molecular evidence of Leishmania persistence was obtained in 43% and 28% of the study participants respectively, at the end of treatment and 90 days after beginning of treatment. The dynamics of ***in vivo*** parasite clearance were not predictive of the outcome of treatment. Significant and strong inverse correlations between end of treatment (EoT) parasite loads and intracellular EoT and C_{max} concentrations were established. ***Leishmania*** strains were isolated from 76.6% of patients. Strains were isolated from 3 of 5 patients who failed treatment, of which two presented low ***in vitro*** susceptibility to miltefosine, potentially contributing to treatment failure. Our results revealed fundamental pharmacokinetic differences for miltefosine in children and adults, which evidently influence the outcome of treatment. The results include the first intracellular pharmacokinetic profiles of an antileishmanial drug in adult and pediatric patients and their relevance in the therapeutic efficacy of drugs against intracellular parasites. These findings provide the knowledge base for optimizing the therapeutic regimen in the pediatric population, while reducing the risk of loss of drug susceptibility.

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ANTIPARASITIC POTENTIAL OF EXTRACELLULAR METABOLITES FROM MARINE ACTINOMYCETES AGAINST *LEISHMANIA (VIANNIA) PERUVIANA* AND *L. (V.) BRAZILIENSIS*

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Actinomycetes are recognized as producers of pharmacological and industrial compunds. In the last decades, the discovery of new metabolites isolated from the terrestrial actinomycetes has decreased and the researches have focused on searching unexplored habitats, like the marine environment. The vast majority of compounds produced by actinomycetes are antimicrobial and few have antiparasitic activity. This study aims at determining the antiparasitic potential of actinomycetal extracellular metabolites isolated from marine actinomycetes against ***Leishmania (Viannia) peruviana*** and ***L. (V.) braziliensis***. In order to carry out this study, a preliminary evaluation of antiparasitic activity ***in vitro*** with 13A1, EIIIA, EIIB and EIIIC strains against promastigotes of two species of ***Leishmania*** has been made; the results of this test showed that EIIB strain had the higher inhibitory activity. The EIIB strain was identified as a member of the genus Streptomyces using rRNA method. The minimum concentration of butanolic extract of actinomycete EIIB who filed the

anti-***Leishmania*** activity was 15 000 µg/mL for ***L. (Viannia) peruviana*** and ***L. (V.) braziliensis***. The preliminary chemical characterization of the extract confirmed the presence of coumarins. In conclusion, the results demonstrated the production of metabolites from marine actinomycetes with antiparasitic activity, which have a great potential in the biomedical and pharmacological field.

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EFFECT OF THE ADDITION OF PENTOXIFYLLINE ON THE THERAPEUTIC AND INFLAMMATORY RESPONSE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS: A RANDOMIZED PLACEBO CONTROLLED TRIAL

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The inflammatory response is an important factor in the therapeutic response of cutaneous leishmaniasis (CL). Co-adjuvant use of immunomodulators may improve the efficacy or outcome of treatment. This study sought to determine whether the addition of pentoxifylline (PTX) to meglumine antimonite (MA) treatment improves therapeutic response in CL patients and to determine the effect of PTX on the inflammatory response. A randomized, double-blind, "Add on" placebo-controlled trial was conducted in 2 centers in Colombia. The protocol was approved and monitored by the institutional ethical committee. Seventy-five parasitologically diagnosed patients were randomly allocated by computer. Inclusion criteria: age 18-65 years, lesion >1 month evolution, multiple lesions or single lesion ≥ 3 cm. Intervention: intramuscular MA (20 mg/ kg /day x 20 days) plus oral PTX 400 mg thrice daily (n=36). Control: MA plus placebo (n=37). Expression of inflammatory genes cxcl10,cxcl5,ccl2, IL1b, ptgs2 and csf1 was evaluated by qRT-PCR in peripheral blood mononuclear cells from CL patients, before and after antimonial treatment, in combination with either PTX (n=12) or placebo (n=12). Therapeutic response and adverse events were assessed at the end of treatment 5, 7, 13 and 26 weeks. Seventy participants (93%) were analyzed by intention to treat (ITT) and forty-eight (64%) per protocol (PP). Treatment failure was 12/34 (35.3%) for PTX vs. 9/36 25% placebo; (OR: 1.63; 95% CI: 0.58 - 4.5). PP failure rate was 6/20 (30%) for PTX and 5/28 (17.8%) for placebo (OR: 1.97; 95% CI: 0.50 - 7.68). No differences between overall frequency and severity of adverse events were found (PTX=142 vs. placebo=140). Expression of inflammatory mediators at the end of treatment was not altered by addition of PTX to MA. However, therapeutic failure was associated with significant overexpression of IL1β and Ptgs2 ($p<0.05$) irrespective of the study group. Addition of PTX to standard treatment of CL did not modify the therapeutic response in this population with early mild to moderate CL, or alter the gene expression of the evaluated inflammatory mediators.

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ANTIBODY DEVELOPMENT FOR *TRYPANOSOMA CRUZI* ANTIGEN DETECTION

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Chagas disease is an infection caused by the protozoan parasite ***Trypanosoma cruzi***. According to World Health Organization, between 8 to 10 million people in the world are infected. Currently, routine diagnosis is based on antibody detection but antibodies remain detectable even after a successful therapy and eradication of parasites. Confirmation of the presence of ***T. cruzi*** by detecting circulating antigens presents an undeniable advantage and an indication of an active infection. The present study was intended to produce polyclonal antibodies that could be further used for ***T. cruzi*** antigen detection. Three two-year old female alpacas (***Vicugna pacos***) were immunized with three different ***Trypanosoma cruzi*** antigens: TESA-antigen, somatic proteins and membrane proteins. Each animal was immunized with 300 ug of each antigen by subcutaneous route, employing Freud complete and incomplete adjuvant (50:50). Blood samples were taken before and after immunization. Sera obtained after centrifugation were stored at -20 ºC until use. Presence of specific antibodies was followed by Western-Blot using antigen obtained from ***T. cruzi*** Y strain. The antibodies showed a specific reaction against their respective antigen. The highest antibody titer was observed after the three immunization. When human urine spiked with parasite TESA antigen was tested against these whole alpaca sera, antibodies from alpacas immunized with membrane proteins performed the best. Additionally, this polyclonal antibody did not react with other human urine proteins. Antibodies induced in alpaca would represent an alternative diagnostic tool for the detection of parasite antigens in urine.

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USE OF IGY EGG YOLK ANTIBODIES FOR THE DETECTION OF *TRYPANOSOMA CRUZI* ANTIGENS

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Chagas disease, an infection caused by ***Trypanosoma cruzi***, remains one of the most important health problem especially in Latin America. Diagnosis of the disease is based on antibody detection; for a correct diagnosis the World Health Organization recommends that at least two of the so-called conventional test (indirect hemagglutination (IHA), indirect immunofluorescence (IIF) and the ELISA immunoenzymatic test) should be performed. However antibody detection is not always an indicative of current or active infection. Detection of ***T. cruzi*** circulating antigens in body fluids might be an important diagnostic tool. Circulating antigens are present only if parasites are present. The objective of the present study was to develop IgY (egg yolk antibodies) against different parasite fractions, the developed antibodies will be further tested as a tool for antigen detection in infected individuals. Eight New Hampshire strain hens were subcutaneously immunized every 14 days for two months with three different ***T. cruzi*** antigens: 1F8 (commercial flagellar antigen), TESA, and membrane proteins. Eggs were collected before and after each immunization. The IgY was extracted using chloroform or polyethylene glycol techniques. IgY yield was compared by protein concentration using Bradford. Presence of anti-***T. cruzi*** IgY was corroborated by indirect immunofluorescence using cultured ***T. cruzi*** Y strain epimastigotes. The chloroform showed to be the more efficient than the polyethylene glycol for purification of IgY from egg yolk. IgY antibodies show to be specific for their respective antigens as showed by immunofluorescence. Further studies will be oriented to determine if those antigens are useful for antigen detection in body fluids of infected individuals.

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NADPH-OXIDASE AN ESSENTIAL ENZYME FOR THE EXPANSION AND ACTIVATION OF CD8+ T CELLS DURING *TRYPANOSOMA CRUZI* INFECTION

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We investigated the significance of NADPH oxidase in the development of protective immunity to an intracellular pathogen ***Trypanosoma cruzi*** that causes Chagas disease. C57BL/6 p47phox-/- mice, as compared to wild-type (wt) mice succumbed within 30 days post-infection (pi) to low doses of ***T. cruzi*** and exhibited inability to control tissue parasites. P47phox-/- bone-marrow and splenic monocytes were not compromised in maturation, phagocytosis and parasite uptake capacity. The deficiency of NOX2-mediated oxidative burst was compensated by higher levels of iNOS/NO. activity and inflammatory cytokine (TNF-α, IFN-γ, IL-1β) release by p47phox-/- macrophages as compared to wt controls infected by ***T. cruzi***. The splenic activation of Th1 CD4+T cells and tissue infiltration of immune cells in T.cruzi-infected p47phox-/- mice was comparable or higher than that noted in infected/wt mice. However, generation and activation of type 1 CD8+T cells was severely compromised in p47phox-/- mice. In comparison, wt mice exhibited a robust ***T. cruzi***-specific CD8+T cell response with type 1 (IFN-γ+TNF-α>IL-4+IL-10), cytolytic effector (CD8+CD107a+IFN-γ+) phenotype. Macrophage activation of NOX2 and oxidative burst is suggested to kill ***T. cruzi*** that causes Chagas disease. However, the role of NOX2 in generation of protective immunity and whether these mechanisms are deregulated in the event of NOX2 deficiency are not known, and examined in this study. NOX2/ROS activity in macrophages signals the development of antigen-specific CD8+T cell response. In the event of NOX2 deficiency, a severely compromised CD8+T cell response was generated leading to increased parasite burden, tissue pathogenesis and mortality.

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SURVIVORSHIP OF *TRYPANOSOMA CRUZI*-INFECTED BED BUGS (*CIMEX LECTULARIUS*)

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The resurgence of the common bedbug (***Cimex lectularius***) has created a difficult public health problem around the world. Recently, we showed the vectorial competence of ***C. lectularius*** into laboratory conditions, proving that the common bedbug can transmit ***Trypanosoma cruzi*** to mice and can get infected when fed on ***T. cruzi***-carrying mice. ***T. cruzi*** is the causative agent of Chagas disease, a vector-borne disease transmitted by triatomine bugs and an increasing problem in many countries of the Americas. Specialized literature has reported that the infection with ***T. cruzi*** in triatomines, their natural vector, affects negatively their development, survival and reproductive index. Being the vector survival an important component of vectorial capacity, we studied the effect of the ***T. cruzi*** infection on ***C. lectularius*** survival. A cohort study of 280 ***T. cruzi***-infected bedbugs and 240 uninfected bedbugs was carried out in Arequipa – Peru, in the Zoonotic Disease Research Group lab. To get infected first instar bedbugs were fed on 4 female BALB/c mice (***Mus musculus***) previously infected by intraperitoneal inoculation with 2 x 10^3 trypomasgotes (***T. cruzi*** Arequipa strain TC35) in 100 ul. We fed them weekly for a month on mice and after that they were fed on uninfected guinea pigs until death. The same conditions were applied to the uninfected bedbug's cohorts. We evaluated weekly vector survival, nymphal development, fecundity and fertility and ***T. cruzi*** presence in the infected cohorts. The statistical results showed no difference in survivorship between the infected and uninfected bedbugs. Fecundity and fertility indexes were moderately higher in the infected cohort. The prevalence of the infection at time of death was 95% (N=274). These results suggest that ***T. cruzi*** infection does not affect negatively either the survival rate, or the fecundity and fertility indexes in bedbugs. The implications of these findings on the vectorial capacity of ***C. lectularius*** and its possible role as a vector of Chagas disease are discussed.

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A BAYESIAN MODEL FOR IDENTIFYING AND PREDICTING THE DYNAMICS OF URBAN INSECT INFESTATIONS

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Analyses of epidemics are complicated by several factors, including the fact that the true dispersal mechanism of disease agents and the precise infection times of patients are often unobserved. Instead, we often observe the infection state of each unit at discrete time intervals. For example, consider a recent study of the Chagas disease vector Triatoma infestans in Arequipa, Peru. The data are limited to observed insect presence at each household at three time points over several years. In addition, we observe the number of insects at each household, although with measurement error. To address these challenges, we propose a novel susceptible-infected-detected-removed model that incorporates the counts of vectors at each house and complex spatial dispersal dynamics observed in Arequipa. The fully Bayesian method is used to augment the data, estimate the dispersal parameters, and determine posterior infestation risk probabilities of households for future treatment. We investigate the properties of the model with simulation studies and analyze the Chagas disease vector data to create an informed control strategy.

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BED BUGS AS VECTORS OF *TRYPANOSOMA CRUZI*

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Through a series of experiments we show that bed bugs (***Cimex lectularius***) can acquire ***Trypanosoma cruzi*** by feeding on infected mice, transmit it back to susceptible animals during cohabitation (in which the mice ate them) and by the usual route (through their feces). Whether bed bugs are, or will become, epidemiologically important vectors of the parasite remains unclear. To address the issue we develop a modified Ross-MacDonald model of vector-borne ***T. cruzi*** transmission and apply it to both a traditional vector, ***Triatoma infestans***, and ***Cimex lectularius***. In relation to some key parameters of the model, such as the ratio of vectors to hosts and the biting rate of the vector, bed bugs are more worrying than ***T. infestans***. We consider the death rate of the vector, and run experiments to assess whether ***T. cruzi*** might increase mortality among bed bugs or triatomines. It does not. We consider the range of hosts and feeding preferences of each insect. We show that triatomines can only support sustained ***T. cruzi*** transmission when exposed to a rather complex host community; we discuss whether such conditions exist in houses, hotels, and other environments affected by bed bugs in the US and abroad.

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A CASE: CONTROL STUDY OF POSSIBLE RISK FACTORS FOR CUTANEOUS LEISHMANIASIS IN SOUTHERN SRI LANKA

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Leishmaniasis is a tropical disease that has not been given due priority, in spite of its importance as a parasitic disease associated with high morbidity and mortality. Cutaneous leishmaniasis (CL) in Sri Lanka is caused by ***L. donovani***. Transmission patterns were different in Southern and Northern Sri Lanka according to previous studies indicating the need for large scale studies. Therefore, current study examined the prevalence and risk factors of CL in Matara District, Southern Sri Lanka. Total of 2260 individuals from 4 District Secretariat divisions (DSDs) were screened by house to house survey using an interviewer administered questionnaire. Patients with skin lesions were investigated by light microscopy/culture/PCR. Prevalence was calculated and a case control study was conducted to identify risk factors. The study population had an age ranged between 1-90yrs (median=43 ±17.31), low monthly income (<20, 000 LKR, 52.8%) and male to female ratio of 1:2(males 31.7%, females 68.3%).Out of 80 clinically suggestive cases only 38 was laboratory confirmed. District prevalence was 0.03%. Over 50% of the patients were identified from the DS of Dickwella (55.3%), while lowest number of cases was from Devinuwara (2.6%). Case clustering nature was observed as significant in GIS map (Nearest neighbor ratio 0.46, p=0.000). The risk factors identified were un-plastered brick walls (P<0.05, OR 41.4), abysmal usage of mosquito repellents (p<0.05, OR 10.3), low income (p<0.05, OR 33.4), excessive time (>4 hrs/day) spent outdoors (p<0.05, OR 22.5) and unawareness of leishmaniasis (p<0.05, OR 10.76). Improper clothing while outdoors, common water source as the mode of water supply and having animal shelters in their gardens within 200m were not associated with the risk of acquiring the disease. Both peri-domestic and outdoor factors seem to be associated with leishmaniasis transmission in the study area. Spatial clustering of cases was observed. This may be due to the behavior of host coinciding with that of the vector, sand fly. Awareness regarding the disease and appropriate control measures are urgently required to minimize further spread.

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QUEUEING ANALYSIS OF A CHAGAS DISEASE CONTROL CAMPAIGN

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This work investigates the economic impact of public health intervention in an ongoing Chagas disease control campaign in Arequipa, Peru. A critical component of preventing and studying the spread of disease are door-to-door campaigns of public health inspectors, followed by insecticide application by exterminators to eradicate the vector infestation of households. The success of the campaign depends on adequate household participation as well as on the success of identifying infested households. We apply queueing theory to evaluate the rate of treatment and associated costs over time. In our queueing model, a household that is infested enters the queue of houses that will require treatment. Unlike conventional queueing systems, however, the customers (houses) joining the queue are invisible and need to be identified before being scheduled for treatment. An infested house becomes visible either via notification by its inhabitants or through a successful search by a team of public health inspectors. A multi-armed bandit procedure is being applied to optimize the search for invisible (infested) houses, based on available GIS data and findings in previous campaigns. We assume that houses in the queue will continue to spread the disease to noninfected houses at a rate that is a function of the time spent in queue. Households are being removed from the queue after successful identification and subsequent treatment with insecticides. Using stochastic queueing models, we project the rate at which household infestations can be successfully eliminated, allowing us to evaluate the cost of eliminating infestation in a neighborhood as a function of participation rates in blanket residual insecticide campaigns and the efficacy of subsequent community-based surveillance programs.

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TRYPANOSOMA CRUZI IN PATIENTS WITH ESOPHAGEAL ACHALASIA IN A NON-ENDEMIC AREA OF COLOMBIA

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Esophageal achalasia is a motility disorder of the esophagus, which can be secundary to chronic ***Trypanosoma cruzi*** infection. Few studies have been conducted in Latin America regarding the prevalence of esophageal achalasia as a manifestation of Chagas Disease and none in Colombia. The main purpose of this ongoing project is to determine the prevalence of anti-***T.cruzi*** antibodies in patients diagnosed with esophageal achalasia and to detect the parasite by polymerase chain reaction (PCR) in individuals found to be seropositive who attended to the Gastroenterology section in a referral hospital in Bogotá, Colombia; a non-endemic area. This cross-sectional study was carried out in adult patients (18-65 years) diagnosed with impaired esophageal motility. Blood samples were taken to assess antibodies against ***T. cruzi*** by IFAT and presence of ***T. cruzi's*** DNA by conventional PCR and qPCR. In total 30 patients have been evaluated with an average age of 47 (± 15.8 SD) years, including 14 men and 16 women; 75% of volunteers live in Bogotá, yet only half of them were born there. Most denied to having lived in rural housing and currently all reside in urban areas. Near 90% referred no family history of Chagas disease and 70% had never undergone blood transfusion. Four out of the 30 patients were positive for anti-***T. cruzi*** by IFAT (current seroprevalence is 13.3%), and, only one of these patients had parasite DNA detected by conventional PCR and detection by qPCR was positive, but below DNA equivalent to 1x10³ parasites. The parasite genotype was identified as TcI. This is the first study that associates esophageal achalasia and ***T. cruzi*** in Colombia. ELISA test will be carried out to confirm the antibodies results.

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MAPPING OF HUMAN AFRICAN TRYPANOSOMIASIS RISK IN DRC AND MODELING OF POTENTIAL PATHS TO ELIMINATION

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While surveillance suggests Human African Trypanosomiasis (HAT) incidence has decreased markedly over the past 15 years_from 26,318 recorded cases in 1998 to 5,983 cases in 2012_transmission remains widespread in the Democratic Republic of the Congo (DRC), which accounted for 84% of HAT cases globally in 2012. New tools - rapid diagnostic tests (RDT), low-cost vector control, and the oral drug fexinidazole - will hopefully make control of HAT far more effective

and enable diagnostic and treatment coverage of a much greater population. In preparation for renewed discussions on elimination, the geographic distribution of HAT risk and associated uncertainties must be well understood. We develop a spatial statistical model using both active and passive surveillance data for Trypanosoma brucei gambiense collected between 2000 and 2014 in DRC to assess the distribution of risk and identify important risk factors. Key uncertainties include how to appropriately address areas with limited records of functional surveillance and the impact of human migration patterns. By fitting the model only to areas reporting cases, we estimate that a substantial number of HAT infections may go undetected in remote areas. Preliminary mechanistic modeling suggests elimination will only be feasible in areas with high quality active and passive surveillance. The number of visits required by the active surveillance mobile teams to clear the parasite from the local population depends on the sensitivity of the RDT used and, as interventions reduce prevalence even further, overtreatment considerations will have to be weighed. Preventing patient drop-out (patients who fail to receive their confirmatory diagnosis or to complete treatment) will also be highly important; in that regard, newly implemented mobile technologies for data collection should enable better tracking of HAT patients through encounters with the healthcare system.

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EVALUATION OF INSECTICIDE-BASED CONTROL FOR CHAGAS DISEASE VECTORS IN SOUTHERN ECUADOR HIGHLIGHTS THE NEED TO FIND NEW STRATEGIES FOR LONG-TERM RISK REDUCTION OF *TRYPANOSOMA CRUZI* TRANSMISSION

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Prevention of Chagas disease depends mainly on control of the triatomine insects that transmit infection. Developing an optimal spraying strategy requires a detailed knowledge of the vector dynamics as well as the effect of the application of this strategy in the field. A prospective study to assess the long-term impact of spraying was conducted between 2008 and 2012 in three rural communities in Loja province, southern Ecuador. Entomological searches to detect the infestation of houses with triatomines and its natural infection with trypanosomes was conducted after the application of two different intervention strategies: 1) selective insecticide spraying (2008 and 2010) and 2) community-wide insecticide spraying (2011). Out of 53 houses searched at baseline (2008), 22 (41.5%) were infested with the vector species ***Rhodnius ecuadoriensis*** and ***Panstrongylus chinai***. The overall entomological data showed a density of 12.4; crowding of 29.8 and colonization index 68.2%. Houses infested at baseline were less likely to be infested after the first round of selective insecticide spraying (POR 0.38, CI 95% 0.17-0.89). However, an increased risk was observed after two rounds of selective spraying (2011) (POR 1.40, 95% CI 0.61-3.21). Although the association as not statistically significant, our data showed that one round of community-wide intervention strategy had a protective effect on the probability of infestation (POR 0.55, CI 95% 0.25-1.25). Persistent infestations were detected in >20% of houses after both interventions. Lower infection rates with ***Trypanosoma cruzi*** were found at the baseline visit in domicile and peridomicile, 27.3% and 6.3%, respectively. However, these rates dramatically increased during following visits, especially in the peridomicile that reached > 80% in 2011 and 2012. Full coverage spraying seems to be more effective than selective spraying; however both methods failed to confer long-term protection. These results remark the need to implement long-term control strategies, such as housing improvement and frequent monitoring of domestic and peridomestic environments.

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MOLECULAR CHARACTERIZATION OF AVIAN INFLUENZA A/H9N2 ISOLATED FROM HUMAN RESPIRATORY SPECIMEN IN EGYPT

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Since the mid-1990s, two lineages of Low Pathogenic A/H9N2 influenza A viruses have circulated in domestic poultry in China and South East Asia. A/H9N2 (G1-like) lineage has circulated in Egypt since 2011. To date, few human cases had been reported in China and SE Asia and no human infections have been reported in the Middle East. In this report we characterize the A/H9N2 virus isolated from a nasopharyngeal and oropharyngeal swabs combined in 2ml viral transport medium VTM, from human case with severe acute respiratory infection (SARI), who has been enrolled in sentinel SARI surveillance in Egypt. The total nucleic acid (TNA) extracted from VTM tested positive for the matrix (M) gene of influenza A by reverse transcriptase RT-PCR, but negative for the commonly circulating subtypes in Egypt; seasonal H3 and H1, PdmH1 and avian H5 as well as to other viral etiologies. TNA was further tested for H7 and H9 by RT-PCR and was found positive for H9. MDCK cell monolayers in culture tubes were inoculated with 100µl of the combined swab-VTM and monitored for CPE every 24hrs. On day two CPE was observed indicating viral replication. TNA was used for the sequencing of the haemagglutinin (HA) while viral RNA extracted from the cell culture lysate was used to sequence the neuramidase (NA) gene by Sanger technique. HA and NA gene sequencing demonstrated a virus with 97% and 98% similarity, respectively, to those of the H9N2 G1-like lineage, the predominant lineage circulating in the Middle East. Phylogenetic analysis showed close homology to previous A/H9N2 viruses from Egyptian poultry and those from neighboring Middle-East countries. Co-circulation of A/H5N1 and A/H9N2 has been documented in Egyptian poultry since 2011. Given the risk to human population from zoonotic influenza, in addition to the specter of antigenic shift in co-infected hosts, intensified control measures within the poultry production sector and increased bird and human surveillance is warranted.

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AN OPERATIONAL EVALUATION OF A SOCIOECONOMIC INTERVENTION TO PREVENT TB IN IMPOVERISHED PERUVIAN COMMUNITIES

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The World Health Organisation's post-2015 global TB strategy identifies social protection interventions (including cash transfers) as key pillars of global TB control but minimal rigorous evidence exists with which to guide their implementation. We designed, implemented, and refined a novel TB-specific social protection intervention that included cash transfers, which aimed to support TB prevention and cure in resource-constrained shantytowns in Lima, Peru. The design of the study was an household-randomized controlled: newly-diagnosed TB patients from 32 impoverished shantytowns of north Lima were eligible to be randomized (1:1) to receive the social protection intervention consisting of economic support and social activities. Economic support was provided to patient

households completing conditional cash transfers: screening for TB in household contacts and MDR-TB in patients; adhering to TB treatment and chemoprophylaxis; and engaging with CRESIPT social activities (household visits and community meetings). Throughout treatment, CRESIPT research nurses applied a questionnaire concerning health, socioeconomic position, and direct (out-of-pocket), indirect (lost income), and total TB-related costs. 282 patients were recruited to the study. Lost income contributed half of households' total costs. The burden of total costs was greater in poorer than less poor households (3.5 versus 2 months of that household's income respectively, p=0.0007). The 135 patients randomized to the supported arm of the intervention achieved 890 conditional cash transfers (80% of potential cash transfers), with an average total of 520 Peruvian Soles (173 US Dollars) received per household. The conditional cash transfers offset 22% of total costs and 43% of direct costs. Conditional cash transfers offset total costs to a greater extent in poorer households (23% versus 20%) and female patients (26% versus 20%, p=0.02). A novel TB-specific social protection intervention has been designed, implemented, refined, and was found to be both acceptable and equitable in a challenging, impoverished setting.

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COMBINATORIAL APPROACH TO PATHOGEN DETECTION IN RESPIRATORY SAMPLES OF UNKNOWN ETIOLOGY

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Emerging pathogens are a continuous threat to public health around the world. Respiratory pathogens in particular, such as those causing influenza-like illnesses and severe acute respiratory infections, are easily transmissible person-to-person and primarily affect children, the elderly, and confined populations, such as those in prisons or military bases. The U.S. Naval Medical Research Unit No. 6, Lima, Peru, conducts routine surveillance for respiratory pathogens throughout Latin America. About 60% of the 5,000 respiratory samples collected annually remain without an identified etiological agent, either because known pathogens are present at sub-threshold levels that render them undetectable to traditional PCR-based diagnostics or because pathogens are novel or divergent enough that they cannot be detected using existing methodologies. We used a combination of both classical (cell culture) and advanced laboratory methodologies (MassTag PCR and next-generation sequencing) to screen over 600 samples that showed cytopathic effect on cell culture but for which no pathogen could be identified. Initial screening using a highly multiplexed MassTag PCR-based approach identified potential etiologies in 41% of the samples tested, including enterovirus (39%); human parainfluenza virus 1 (2%), 2 (32%), 3 (2%) and 4 (1%); human metapneumovirus (10%); respiratory syncytial virus A (5%) and B (4%); coronavirus OC43 (3%) and 229E (1%); and influenza A virus (1%). We then conducted unbiased next-generation sequencing on selected samples and identified additional etiologies, including viruses that had never before been detected in Latin America, such as Saffold virus. Here, we describe our integrated approach to pathogen detection and report our complete findings for over 600 samples collected since 2011. This new combinatorial approach to pathogen detection enables rapid identification of known and novel pathogens present in respiratory samples in a modern and cost effective manner that should facilitate global respiratory disease surveillance efforts.

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COMPARISON OF THE AKONNI BIOSYSTEMS MDR-TB MICROARRAY TEST TO THE HAIN LIFESCIENCE GENOTYPE MTBDRPLUS FOR RESISTANCE TO RIFAMPIN AND ISONIAZID IN KAZAKH M. TUBERCULOSIS ISOLATES

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Early case detection and rapid treatment reduce ***Mycobacterium tuberculosis*** (MTB) transmission. MTB is considered multidrug-resistant TB (MDR-TB) when resistant to rifampin and isoniazid, and a test which simultaneously diagnoses MTB and multi-drug resistance (MDR) is urgently needed. The Akonni Biosystems MDR-TB microarray test (Akonni MDR-TB) (Frederick, MD USA) specifically reports on the presence of ***rpoB*** (30 mutations), and ***katG*** (2 mutations) and ***inhA*** (4 mutations) mutations known to confer resistance to rifampin and isoniazid, respectively. The assay contains markers for the ***M. avium*** complex (MAC), ***M. tuberculosis*** complex, and an internal amplification and hybridization control. Similarly, the Hain LifeScience GenoType MTBDRplus (Hain MTBDRplus) (Nehren, Germany) detects the presence of mutation in ***rpoB*** (4 mutations), and ***katG*** (2 mutations) and ***inhA*** (4 mutations). MRIGlobal partnered with the M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry (IMBB) to test 411 extracted isolates from the National Center of Tuberculosis Problems in Kazakhstan. Isolates indicating a mixed genotypes (26) or presence of MAC (8) were removed from the performance evaluation. The Akonni MDR-TB was compared to the Hain LifeScience GenoType MTBDRplus (377 isolates) for rifampin and isoniazid resistance mutations: percent agreement (95% Confidence Interval) rifampin 93.6% (90.7-95.9), isoniazid 97.9% (95.9-99.1); sensitivity (95% CI) rifampin 92.7% (88.9-95.6), isoniazid 98.7% (96.6-99.6); and specificity (95% CI) rifampin 95.7% (90.2-98.6), isoniazid 95.4% (88.6-99.2). Isoniazid testing on the Akonni MDR-TB resulted in 1 indeterminate result. Discrepant analysis between assays is ongoing with culture to adjudicate. The performance characteristics of these assays to detect specific resistance markers is further defined, while highlighting the advantages of using a practical and quick multiplexed microarray platform in a field forward setting for global surveillance.

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HIGH PREVALENCE OF *BORDETELLA PERTUSSIS* IN SEVERE ACUTE RESPIRATORY INFECTIONS IN HOSPITALIZED CHILDREN UNDER FIVE YEARS IN LIMA, PERU

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Acute respiratory infections (ARI) are the main cause of morbidity and mortality in children under 5 years worldwide. ***Bordetella pertussis*** is a highly contagious bacterium that can cause serious illness, and approximately half of infected infants less than 1 year old are hospitalized. Also, pertussis immunization series is not completed until six months of age, leaving young infants vulnerable to pertussis. In Peru, pertussis is an increasing health problem despite immunization efforts, and the role of ***B. pertussis*** in ARI is unknown. We determined the prevalence of ***B. pertussis*** among children under 5 years old admitted to Hospital Nacional Cayetano Heredia in Lima with diagnosis of ARI between Jan-2009 and Dec 2010. Epidemiological and clinical features were collected, and presence of ***B. pertussis*** was determined by PCR (pertussis toxin and IS481 gene). A total of 596 nasopharyngeal samples among children

under 5 years were analyzed. In 114 (19.1%) samples were positive for ***B. pertussis***. 32.5% of sample positive to ***B. pertussis*** were diagnosed as viral pneumonia at diagnosis. Importantly, 71.9% of cases were under 12 months of age and 58.8% have been contact with other ARI infected people. Significant differences in clinical symptoms between the total ARI cases and ***B. pertussis*** cases were not found. The most frequent symptoms in ***B. pertussis*** cases were fever (100%), rhinorrhea 78%, cough 71.9% and respiratory distress 60.5%. One child died due to the infection. ***B. pertussis*** cases showed a seasonal distribution with peaks during the months March June and November. This study shows the high prevalence of ***B. pertussis*** in infants who were hospitalized due to severe acute respiratory infections in Lima, Peru. Epidemiologic surveillance programs for ***B. pertussis*** are essential in the future in Peru.

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NOSOCOMIAL TUBERCULOSIS TRANSMISSION: IS AIR EXCHANGE ADEQUATE IN PATIENT CARE AREAS IN GULU AND MAKERERE TEACHING HOSPITALS, UGANDA

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In Uganda like similar developing countries, tuberculosis (TB) infection rates in medical students have been found to higher than that of comparable groups including students in other University programs and adults living in suburbs of the city where the medical school is located. In addition, a steep increase in prevalence of TB infection from year one (35%) to year five (55%) in a relatively short period suggests that exposure and infection may be related to clinical work, that is exposure and infection due to nosocomial TB. Thus we undertook the study to assess the adequacy of airborne ventilation in patient areas as per WHO recommendations. We conducted a study in Gulu and Makerere University teaching hospitals. The patient areas assessed included: causality/ emergency ward, bronchoscopy rooms, sputum induction rooms, inpatient wards, laboratories, out-patient departments, and the mortuary. Using an airflow alnor velometer/anemometer placed approximately 15 centimeters form the window or door opening, we measured the air speed of each patient-care area at different times of the day in the usual working situation, when all openings are fully open, when all openings half open and all openings closed for 4 measurements per opening per day for three day. In addition data on intensity of use of the different patient areas were classified into minimum use, moderate use and maximum use. Data were computed to determine the Air changes per hour (ACH) for patient-care areas for different intensity of use of the patient care areas. Preliminary data indicate that the ACH range to as low as 5 to as high as 14 ACH. Patient care areas of old building constructed 50 or more years were more likely to have adequate ACH as per recommendation of WHO even at maximum intensity of use of patient-care areas. Inadequate ACH in patient-care settings may be responsible for nosocomial transmission of TB and may explain the high TB infection rates among medical students. Precautionary measures to prevent transmission of airborne infections are recommended. (Abstract changes to be made)

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PREVALENCE OF PATHOGENS AS A CAUSE OF PNEUMONIA ASSOCIATED WITH MECHANICAL VENTILATION IN THE ADULT ICU OF A HEALTH PROVIDER MONTERÍA- ENTITY IN CORDOBA, DURING THE YEARS 2011 TO 2014

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Pneumonia associated with mechanical ventilation (VAP) is one of the most serious infections associated with health care, not only for the prolongation of hospital stay of the patient, but also because it results in increased mortality and increases in the costs of patient care. This pulmonary complication develops after 48 to 72 hours of endotracheal intubation. To determine the prevalence of disease as a cause of pneumonia associated with mechanical ventilation in adult ICU of a health institution in Montería, Córdoba. A retrospective study in the ICU of a hospital in Montería- Córdoba. Patients with ICU stay and VM for more than 48 hours period that meet the criteria of the American Thoracic Society / Infectious Diseases Society of America (ATS / IDSA). for mechanical ventilation associated pneumonia (VAP) and which has been carried them bronchial secretion cultures or tracheal aspirate were included. For data collection 1474 medical records of patients admitted to the ICU on study time. The information was processed with statistical software SPAD, making a multivariate correspondence analysis (MCA). ***Pseudomonas aeruginosa*** and ***Klebsiella pneumoniae***, were greatest number of isolates with rates equivalent to 39.34% and 27.87%, respectively, following the order of frequency ***Acinetobacter baumannii*** with 16.39%, ***Escherichia coli*** and ***Staphylococcus aureus*** with 4.92% of isolates, the less common bacteria found correspond to ***Enterobacter aerogenes*** with 3.28% and ***Burkholderia cepacia*** and ***Serratia marcescens*** with percentages corresponding to 1.64% respectively. VAP affects up to 38.36 % (61 of 159) of patients admitted to the ICU of the institution where the study was conducted, complicating their evolution , prolonging their stay in the ICU and increasing costs of health services. For this reason, more studies should be done to better understand the epidemiology, pathophysiology and etiology to improve the chances of survival of patients.

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CHALLENGES IN DIAGNOSIS OF PEDIATRIC TUBERCULOSIS: A CASE STUDY IN SIAYA COUNTY REFERRAL HOSPITAL, WESTERN KENYA

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Tuberculosis (TB) has a substantial global prevalence and results in high rates of morbidity and mortality. Kenya ranks among the top five TB-burden countries in sub-Saharan Africa. Although pediatric TB is estimated at 10.72% for all cases, this figure is likely an underestimation due to the challenges associated diagnosis of pediatric TB. Currently, pediatric TB diagnosis in Kenya relies (primarily) on patient history, physical examination, chest x-ray, and sputum smear microscopy (if available). However, challenges are substantial when TB diagnosis is based largely on clinical parameters since a number of frequent co-morbid endemic diseases present with common clinical signs and symptoms. For example, we enrolled a cohort of children (n=1,704, aged 3-36 months) at the Siaya County Hospital, western Kenya (2003-2014), who were followed longitudinally for 36 months. This particular region has holoendemic ***Plasmodium falciparum*** transmission and the highest prevalence of HIV in Kenya. Although diagnosis of malaria, HIV, and bacteremia were robust due to utilization of state-of-the-art diagnostic platforms, diagnosis of pediatric TB presented substantial challenges based on the use of clinical algorithms. In the cohort, 55 children (3.2%) presented with suspected TB. However, based on the WHO TB pediatric score chart, only 15 of the 55 suspected cases (27.3%) were confirmed as TB cases (0.88% of the cohort). Based on these challenges, we initiated development of diagnostic assays that utilize blood and/or urine samples for TB diagnosis. Towards

this end, we are currently investigating the use of novel technologies that employ detection of pathogen biomarkers coupled with sensitive measurement platforms to facilitate rapid and specific diagnosis of TB from blood and/or urine samples.

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IMPACT OF PCV7 ON NASOPHARYNGEAL DENSITY, SEROTYPE DISTRIBUTION AND ANTIBIOTIC RESISTANCE OF PNEUMOCOCCAL STRAINS ISOLATED FROM PERUVIAN CHILDREN

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Pneumococcal conjugate vaccines (PCV) have reduced the burden of pneumococcal disease (PD), and decreased nasopharyngeal carriage of vaccine-types. Few data exist from remote rural areas, so here, we investigated the serotype distribution and antibiotic resistance of carriage strains isolated from young children, before and two years after PCV7 was introduced, in a prospective cohort study conducted in the remote Peruvian Andes. Effects of PCV7 on pneumococcal nasopharyngeal density, obtained using qPCR reactions, were also assessed. Methods. Nasopharyngeal swabs were collected from a sample of children <3 years of age in both 2009 and 2011. Pneumococcal strains were isolated, quellung-serotyped and tested for susceptibility to antibiotics. Results. Nasopharyngeal prevalence of PCV7 strains decreased from 48% in 2009 to 28.8% in 2011, whereas non-PCV7 types increased from 52% to 71.2% (p=.002). Vaccination with PCV7 did not affect overall pneumococcal density in children colonized by a PCV7 type but did increase median nasopharyngeal density in those colonized with a non-PCV7 type [2009, 1.9x105 cfu/ml vs 2011, 5.5x105 cfu/ml; (p=0.046)]. The most prevalent serotypes were 19F (13.6%), 23F (12.8%), and 6B (12%) in 2009; and 19F (11.2%), 6C (11.2%), and 11A (9.6%) in 2011. A substantial 3.5-fold increase in carriage of serotype 6C was observed after PCV7 introduction (p=0.026). Overall antibiotic resistance did not change after vaccine introduction. Most pneumococcal strains were non-susceptible to tetracycline (97.2%), and to a lesser extent, to trimethoprim-sulfamethoxazole (56.4%), penicillin (34%), erythromycin (22.4%), chloramphenicol (18.8%) or clindamycin (12.4%). Conclusion. PCV7 impacted carriage of those serotypes in rural Peru. Replacement serotypes had increased density when less PCV7 serotypes were present. Peru should consider switching to the PCV13 vaccine to cover the emerging serotype 6C strain.

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COMMUNITY CASE MANAGEMENT OF PNEUMONIA IN ABIA STATE, NIGERIA

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This study aims at assessing community management of Pneumonia in children aged 2-59 months in Abia State using the Integrated Community Case Management (iCCM) Model. Community Resource Persons (CORPs) in the iCCM model are trained in diagnosis and treatment of Pneumonia; referring of severe cases to the health facilities; following up such cases; and advising all caregivers on protection against pneumonia by immunization. Pneumonia is defined as a cough or cold with fast breathing (counted with respiratory timers); while having chest indrawing additionally, defines Severe Pneumonia. Between November 2014 and February 2015, in 7 iCCM-eligible LGAs of Abia State: 6,455 under-fives were seen of which 2,505 had cough/difficulty breathing and 1,230 had fast breathing (Pneumonia: 19% of all children seen). Of these, 1004 cases were promptly treated with Dispersible Amoxicillin in the community; and 226 cases were referred to health facilities. Pneumonia is the leading infectious cause of death in children worldwide, and constitute 19% of the estimated 756,000 under-five deaths annually in Nigeria. iCCM is an equity-focused strategy of delivering curative treatment of childhood illnesses in the communities outside of health facilities; and is designed such that more than the hitherto estimated one-third of under-fives can access interventions and antibiotics for Pneumonia. In its 4 months of implementation in 7 LGAs in Abia State, 1004 under-fives who may not have reached the health facilities were managed in the community with antibiotics and preventable deaths averted. iCCM of Pneumonia is a veritable strategy for reducing the alarming rates of under-five mortality, as well as achievement of Millennium Development Goals in Abia State, Nigeria.

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CHILDHOOD PNEUMONIA IN RURAL MOUNTAINOUS PAKISTAN: CLINICAL FEATURES AND NASOPHARYNGEAL CARRIAGE OF VIRAL PATHOGENS

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Childhood pneumonia causes high morbidity/mortality in developing countries, including Pakistan; the role of viral pathogens is not clear. Two cohort studies of children under 5 years of age were conducted in rural Oshikhandass village by trained community research workers using weekly active surveillance and WHO defined criteria for pneumonia diagnosis. Pneumonia incidence declined from 44 episodes/100 child years (CY) in 1992-1996 to approximately 15/100 CY in 2012-2014. From 2012-2014, NP data were available for 184/207 (88.9%) pneumonia cases; 132/184 (71.7%) of these had NP viral carriage detected using Luminex® and Taqman® PCR methods, including RSV (19.6%), and influenza (5.4%). A subset of 155 cases tested with Luminex® had entero/rhinovirus (53.5%), coronavirus (7.7%) parainfluenza (7.1%), adenovirus (6.5%), metapneumovirus (5.2%) and bocavirus (1.9%). Of 207 pneumonia cases, 7 (3.4%) had severe pneumonia. Three children were <2 months of age, 50 (24.2%) were 2-11 months, and 154 (74.4%) were between 12-59 months; median age at diagnosis was 19 months. At presentation (Day 1), average duration of illness was 2.1 days, 29.0% had wheezing by exam, and 24.2% had axillary temperature>=100.4 F. Children were followed on Days 3, 5/6 and 14. Total duration of illness, defined as initial duration plus days until respiratory rate returned to normal as checked by health worker, was available for 152 cases (73.4%); mean total duration of illness was 5 days (range 3-17). Where respiratory rate was taken at each visit (84.5% of cases), most children (63.3%) recovered within 3 days of initiating antimicrobial therapy; 16.4% needed 5 days. Nine children (4.4%) needed referral, mostly on Day 1 (6/66%); 3 were hospitalized and none died. Community based pneumonia is primarily non-severe and with early detection, referral, and treatment, has low mortality. Enterovirus/rhinovirus and RSV were the main viruses detected, though multiple other viruses were found; influenza detection was relatively low. Clinical correlation with viral carriage is underway.

1773

BODY COMPOSITION AND VITAMIN D STATUS AMONG PATIENTS WITH TUBERCULOSIS IN RURAL SOUTH INDIA: THE NEED FOR INTEGRATING NUTRITIONAL MANAGEMENT IN CARE AND TREATMENT PROGRAMS

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The evidence base for modifiable nutritional risk factors in patients with Tuberculosis (TB) remains inadequate and very little is known about TB's association with body composition and micronutrient status in populations with high prevalence of undernutrition. The objective of this study was to describe and assess the association between body composition, vitamin D, and active TB among patients at a rural center in South India. Whole and segmental body composition indicators (body fat [%, kg], fat free mass [kg], total body water [kg], impedance) were measured by 8-electrode bioelectrical impedance analysis. 25-hydroxyvitamin D serum concentration was determined by a chemiluminescence immunoassay, and < 50 nmol/L was defined as deficient. Active TB was defined by acid-fast bacilli sputum smear microscopy, per standard of care, and HIV infection was determined by TriDot rapid test. The associations between body composition indicators with vitamin D and TB were assessed in multivariate linear regressions, adjusting for other covariates (including demographic [sex, age], hemoglobin, immunological and rheological indicators). 11.6% of patients had confirmed active TB disease, and 1.5% had HIV infection. Among study participants, mean body weight was 48.7 kg (±13.5). Nearly 25% of patients had 25 kg/m2. Mean vitamin D status was 51.4 nmol/L (±26.7), and 54.7% of patients were vitamin D deficient. Average fat mass was 7.2 kg (±4.3) among individuals with confirmed TB, compared to 11.2 kg (±7.2) among patients with probable TB. Vitamin D deficiency was associated with increased body fat (%) and fat mass (kg; all $p<0.05$), after controlling for other covariates. The results highlight the rampant undernutrition among patients with TB at our clinic in South India and underscore the need for integrating nutritional assessment and counseling in TB care and treatment programs. Further, future laboratory and longitudinal studies are needed to understand the direction of linkages between vitamin D, body composition, and TB along with their relevance for long-term health.

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ACCURACY OF SELECTED DEVICES FOR THE DETECTION OF PNEUMONIA SYMPTOMS - RESULTS FROM LABORATORY TESTING AND FIELD EVALUATIONS OF RESPIRATORY RATE COUNTERS AND PULSE OXIMETERS WITH FRONTLINE HEALTH WORKERS IN SUB-SAHARAN AFRICA AND SOUTHEAST ASIA

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Globally, pneumonia remains the leading infectious cause of death in children under five years. Diagnosis of pneumonia symptoms by community health workers (CHWs) and first level health facility workers (FLHFWs) is based on counting respiratory rate (RR), which is challenging and misclassification of the observed RR is common. Malaria Consortium's Pneumonia Diagnostics project is identifying the most accurate and acceptable devices to support CHWs and FLHFWs in the detection of pneumonia symptoms in children aged 0 to 59 months in Cambodia, Ethiopia, South Sudan and Uganda. Eight pulse oximeters and four respiratory rate counters were selected for evaluation based on formative research, including focus group discussions with 24 CHWs in each country and pile sorting exercises with government stakeholders and NGO partners. CHWs in all countries expressed a 'felt' need for new pneumonia diagnostic devices and automation was a preferred device characteristic. The pile sorting exercises favoured the fingertip pulse oximeters due to ease of use and affordability.The pulse oximeters, having not been used under field conditions, underwent laboratory testing for accuracy and robustness (prototypes excluded). All laboratory tested devices passed, except one fingertip pulse oximeter. Nine devices (5 pulse oximeters and 4 respiratory rate counters) were selected overall for further accuracy evaluation. Health facility accuracy evaluations in the project countries assessed accurate use of each device by CHWs/FLHFWs against reference standards. In each country 430 children aged 0-59 months were enrolled in the study. Children were presented randomly to the CHW/FLHFW, who was blinded to their condition and true RR. The accuracy evaluation is anticipated to finish in June 2015. Preliminary results are encouraging with health workers able to use all devices being trialled. It is hoped that by identifying devices that can be used by frontline health workers to accurately detect pneumonia symptoms in children aged 0-59 months, this will increase appropriate treatment for pneumonia while improving referral rates for patients with severe pneumonia.

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ACCEPTABILITY OF SELECTED RESPIRATORY RATE COUNTERS AND PULSE OXIMETERS FOR USE BY FRONTLINE HEALTH WORKERS IN THE DETECTION OF PNEUMONIA SYMPTOMS IN CHILDREN IN SUB-SAHARAN AFRICA AND SOUTHEAST ASIA

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Diagnosis of pneumonia, the leading infectious killer of children under the age of five, by community health workers (CHWs) and first level health facility workers (FLHFWs) is presumptive and based on counting respiratory rate (RR). This process can be challenging for frontline health workers in low resource settings and misclassification of the observed RR is common. Malaria Consortium's Pneumonia Diagnostics project is identifying the most accurate and acceptable respiratory rate counters and pulse oximeters to support CHWs and FLHFWs in the detection of pneumonia symptoms in children aged 0 to 59 months in Cambodia, Ethiopia, South Sudan and Uganda. In each country a diverse sample of 16 CHWs and 6 FLHFWs are provided with a respiratory rate counter or pulse oximeter. These devices will have been selected based on results of earlier accuracy evaluations and pile sorting exercises with CHWs/FLHFWs who have previously tried the devices. The CHWs/FLHWs will use the device in routine clinical practice for two months between July and October 2015, and filmed using them. The recordings will be analysed for device procedure accuracy, and reactions of the caregiver and their child. Additionally the CHWs and FLHFWs, as well as a diverse sample of 24 parents in each country, will be asked to participate in a semi-structured interview on their perceptions of the devices. Anticipated results will give insight into the acceptability of different diagnostic devices for frontline health workers in their daily work, as well as the reactions of caregivers and their children. It is hoped that this unique study will recommend accurate and acceptable devices for detection of childhood pneumonia symptoms by frontline health workers in Sub-Saharan Africa and Southeast Asia.

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SCHISTOSOMA MANSONI: ASSESSING THE FUNCTION OF MAPK PATHWAY AND OF HISTONE MODIFYING ENZYMES

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In most organisms, Mitogen-activated protein kinases (MAPKs) and Histone Modifying Enzymes (HMEs) influence a number of important tissue-specific biological activities such as cell proliferation, survival and, differentiation. Those enzymes are increasingly approved as targets for drug development with a growing amount of inhibitors under development. However, to date, the function of HMEs and MAPKs are uncertain in the parasite Schistosoma. Here, we employed RNA interference to elucidate the roles of 16 HMEs and 5 genes involved in MAPK signaling pathway in ***S, mansoni***. First, the selected genes were chosen based in their putative function and/or for being unique members of their subfamily (ePK) in the parasite. Among the targets are included: SmERK1, SmERK2, SmJNK, SmCaMK2, and Smp38 and 1 histone deacetylases (SmHDAC8), 10 methyltranferases (HMTs), 5 demethylases (HDM), from those, 8 were chosen for experimental validation. Pharmacological inhibition and RNAi were used to elucidate the functional role of MAPK signaling pathway proteins and HMEs in ***S, mansoni***. After RNAi, mice were injected with treated schistosomula and evidenced that SmHDAC8 and SmJNK are important to the parasite transformation and survival, whereas HDAC8, PRMT3, KDM1/KDM2, SmERK, and Smp38 seems to be associated in egg production as infected mice had significantly lower egg burdens and female worms presented underdeveloped ovaries. Moreover, worms depleted in SmJNK and Smp38 exhibited large damage in the tegument and, the later enzyme, seems to be involved in the activation of detoxification enzymes. Recently, RNAseq analysis of MAPKs knockdown parasites elucidated the genes which might be under the control of this signaling pathway. Our results help characterize the importance of MAPK pathway and HMEs in the maintenance and survival of the schistosome and propose some of these enzymes as valuable drug targets to prevent schistosomiasis progress.

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PHARMACOLOGICAL SENSITIVITIES OF TRP CHANNELS IN *SCHISTOSOMA MANSONI*

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There is an urgent need for new or repurposed antischistosomal agents, as praziquantel is effectively the only drug available for treatment and control of schistosomiasis, a disease that affects hundreds of millions. Ion channels are validated and common targets for current anthelmintics. Transient receptor potential (TRP) channels are a diverse family of channels essential for sensory transduction and other functions. However, the roles and pharmacological sensitivities of these channels are essentially unexplored in schistosomes and other parasitic helminths. We are using TRP channel modulators and RNAi to examine the pharmacological sensitivities of TRP channels in schistosomes. The ***S. mansoni*** genome predicts representatives of most TRP channel subfamilies, with the notable exception of TRPV, the vanilloid receptor family. Nonetheless, both adult and larval schistosomes exhibit dramatic hyperactivity in response to capsaicin, a selective activator of mammalian TRPV1. Surprisingly, the majority of this response is attenuated by knockdown of TRPA expression; TRPM7 also contributes to the capsaicin response. Knockdown has no effect on 5-HT-induced hyperactivity. These results indicate that some TRPV-mediated sensory functions may be fulfilled by schistosome TRP channels from other subfamilies, and that schistosome TRP channels likely have novel pharmacological properties. We have also been exploring the role of ***S. mansoni*** TRPM8 channels, which in mammals are cold sensitive and activated by icilin. Like capsaicin, icilin induces dramatic hyperactivity in adult and larval schistosomes. Suppression of TRPM8 expression strongly attenuates this increase in icilin-induced motility, but does not affect 5-HT-induced hyperactivity. This is the first demonstration of a TRPM8 agonist-induced pharmacological effect in schistosomes and provides a novel sensitive point for exploring the pharmacology and function of this channel. Targeted disruption of these channels may provide clues about the roles they play in the parasite and possible strategies for the development of new antischistosomal therapeutics.

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MOLECULAR DETECTION OF SCHISTOSOME AND OTHER PARASITIC HELMINTH INFECTIONS ON A DISPOSABLE MICROFLUIDIC CASSETTE

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Current classical and serological methods for diagnosis of parasitic helminth infections have limitations on sensitivity, specificity, and reliability, and are often labor intensive and require specialized training. Nucleic acid-based methods for detection of free parasite DNA in host blood, urine, feces, or tissues hold the promise of dramatic increases in diagnostic sensitivity and specificity, but often require infrastructure not available in the field. Self-contained, point-of-care molecular detection devices hold the promise of more extensive and accurate monitoring of infection dynamics and treatment efficacy. Here, we describe a simple, small, fully-integrated, inexpensive, and disposable microfluidic cassette adapted for molecular detection of parasitic helminth infections. The cassette, or chip, features an array of reaction chambers that house nucleic acid binding membranes. Nucleic acid capture, washing, amplification, and detection are all combined in a single multifunctional reaction chamber. The cassette is endowed with a concentration function since nucleic acids captured on a flow through membrane serve directly as templates for amplification without elution. This cassette is compatible with isothermal amplification methods such as loop-mediated isothermal amplification (LAMP) and reverse transcription (RT)-LAMP. Both monitoring the reaction and thermal control is low-cost and flexible. By adapting LAMP protocols with this cassette, we show that ***Schistosoma mansoni*** DNA is readily detectable on the chip using serum taken from mice as early as one-week post infection, several weeks prior to the onset of parasite egg production. Furthermore, we show that Dirofilaria immitis DNA is readily detected in plasma samples from infected dogs. These results provide a strategy for simple, inexpensive, and highly accurate diagnostic monitoring of schistosome and other parasitic helminth infections on site and in the clinic.

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BIOMPHALARIA GLABRATA EMBRYONIC (BGE) CELL SUPEROXIDE DISMUTASE (SOD) EXPRESSION PRE-AND POST-EXPOSURE TO *SCHISTOSOMA MANSONI* LARVAL TRANSFORMATION PROTEINS

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Schistosoma mansoni is one of several species of human blood flukes that causes schistosomiasis, which continues to afflict over 200 million people worldwide. In resistant strains of the snail intermediate host, ***Biomphalaria glabrata***, primary sporocysts are rapidly encapsulated by circulating hemocytes and killed by the release of reactive oxygen species. Superoxide dismutase (SOD) in hemocytes is responsible for the production of H_2O_2 and the destruction of encapsulated parasites. An increase in Cu/Zn SOD activity has been linked to the resistant snail strains and further

characterization has shown allele specific differences between strains. Cells of the ***B. glabrata*** embryonic (Bge) cell-line encapsulate larval ***S. mansoni*** under ***in vitro*** conditions, similar to hemocyte-sporocyst encapsulation reactions. In the present study we investigated if Bge cells, like hemocytes, possessed SOD activity, and whether exposure of cells to larval transformation proteins (LTP) affected its activity. Molecular, proteomic, and enzyme activity assays were used to investigate SOD in Bge cells and the effects of exposure to parasite proteins on its expression. Results of Cu/Zn SOD allele-specific PCR analysis in Bge cells showed that the A/C alleles, previously found to be associated with susceptible ***B. glabrata*** snails, were expressed. A quantitative real-time PCR assay showed an increase in Bge cell Cu/Zn SOD transcript levels after exposure to ***S. mansoni*** LTP although changes were not statistically significant. However, nanoLC-MS/MS mass spectrometry analysis of Bge cell proteins expressed pre- and post-exposure to LTP showed a significant increase in Cu/Zn SOD in the LTP exposed group. Finally, consistent with proteomic data, Bge cell lysates possess SOD activity, although whether or not this activity is affected by ***S. mansoni*** LTP remains to be investigated. Results of this study demonstrate the expression of Cu/Zn SOD in Bge cells and further validate the use of this cell line as a model for studying schistosome-snail immune interactions.

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GENETIC ANALYSIS OF TRANSMISSION-RELATED TRAITS IN SCHISTOSOME PARASITES

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Parasite traits associated with transmission success, such as the number of infective stages released, are expected to be optimized by natural selection. However, in the trematode parasite ***Schistosoma mansoni***, such transmission traits vary significantly within and between different parasite populations and selection experiments demonstrate that this variation has a strong genetic basis. We are using two approaches to determine the genetic architecture of this important transmission related life-history trait. First, we used deep sequencing of pooled exomes to compare allele frequencies across the 363Mb parasite genome in low and high shedding parasites from a single outbred laboratory schistosome population (SmLE). This experiment was conducted in triplicate using large parasite populations to minimize sampling issues and inbred snail lines to minimize the impact of host genetics. Second, we are conducting three generation genetic crosses between two laboratory parasite lines (SmBRE and SmLE) that differ >10 fold in numbers of cercariae shed from snails (mean(±se) cercariae per shedding, 2929±156 vs. 253±39) for a classical QTL analysis. The exomes from parents, F1s and F2 progeny from these genetic crosses will be sequenced and the genotype and phenotype data analyzed using linkage-based methods. The combination of these two approaches is expected to reveal the genetic architecture of a parasite trait that is critical for transmission in an important human pathogen.

1781

INDEPENDENT ORIGINS OF ALLELES CONFERRING OXAMNIQUINE RESISTANCE IN BRAZILIAN SCHISTOSOME PARASITES

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The evolution of drug and pesticide resistance provides a valuable opportunity to examine adaptation in natural populations. Oxamniquine was used to treat schistosomiasis patients in the 1970s to the late 1990s and several cases of parasite infections resistant to treatment were recorded. Our group has identified the gene and mutations involved in oxamniquine resistance. The gene involved encodes a sulfotransferase, SmSULT, required for intracellular drug activation. Resistance has a recessive basis and occurs when both SmSULT alleles encode for defective proteins. Here we examine SmSULT sequence variation in a natural schistosome population in Brazil after 30 years of treatment. Our central aim was to determine (i) the frequency of resistance alleles and (ii) the number of independent origins of resistance to this drug in a single parasite population. We collected fecal material containing ***S, mansoni*** eggs, hatched miracidia and stored these on FTA cards. Following whole genome amplification of DNA, we sequenced the two exons of SmSULT from each miracidia. We amplified miracidial DNA from 189/228 samples (83% success). These included one to 11 miracidia larvae from each of 50 patients. We found four mutations that are known or predicted to impair protein function from examination of their position in the SmSULT crystal structure and/or by biochemical assays. These were (i) the amino acid deletion (Δ142) (ii) a single bp insertion in exon 1 predicted to make a truncated protein product, and (iii) two substitutions (N44H, G206V), which are predicted to impair binding of the oxamniquine and the co-factor needed for oxamniquine activation respectively. One other mutation (N215Y) is not predicted to affect drug activation. Three results are of particular interest: (i) we recovered the Δ142 mutation, responsible for resistance in our genetic cross, in field collected parasite populations (i) allele frequencies of known or predicted resistance alleles are low (0.04-0.29%), perhaps consistent with fitness costs associated with carriage of these alleles (ii) we observed 2-4 independent origins of known or predicted resistant alleles.

1782

POPULATION "EXOMICS" OF *SCHISTOSOMA MANSONI*

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We have developed a robust, inexpensive approach for capture and sequencing of the ~17Mb ***Schistosoma mansoni*** exome that can be used for single larval parasites isolated from feces. The approach uses whole genome amplification of miracidia larvae preserved on FTA cards, solution-based capture of exome sequences using 120bp RNA baits, and Illumina sequencing, and can be extensively multiplexed to reduce costs and simplify sequence library preparation. Here we describe population genomic analysis of exome sequence data from 146 miracidia (one from each patient sampled) from Brazil, Tanzania and two West African countries (Senegal and Niger). All the African samples come from the Schistosome Collection At the Natural History Museum (SCAN collection). We detail patterns of genetic variation in autosomal and mtDNA, we characterize SNP variation at candidate vaccine and drug resistance loci, and examine geographic differentiation in allele frequencies to identify genome regions under strong directional and balancing selection. Exome sequencing has multiple applications for investigating the population biology and evolutionary genomics of schistosomes. This approach provides several advantages over other "genome reduction" approaches (eg RADseq) because the sequence captured can be unambiguously aligned to the parasite genome, coding sequences of central interest to most biologists are determined, and unwanted contaminating DNA is removed.

1783

EFFECT OF PARASITISM ON THE DISTRIBUTION OF SEROTONIN IN THE NERVOUS TISSUES OF *BIOMPHALARIA ALEXANDRINA*

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Schistosomiasis, or bilharzia, is estimated to affect more than 200 million people worldwide. The digenetic trematode worm ***Schistosoma mansoni*** that causes schistosomiasis employs the freshwater snail genus ***Biomphalaria*** as its primary intermediate host. It has been proposed that the transition from the free-living ***S. mansoni*** miracidium to parasitic mother sporocyst depends upon uptake of biogenic amines, e.g. serotonin (5HT), from the snail host. However, little is known about potential sources of serotonin in ***Biomphalaria alexandrina*** tissues and its potential changes during the course of infection. The purpose of this investigation was to localize serotonin-like immunoreactivity (5HTli) in the central nervous system (CNS) of ***B. alexandrina*** and examine 5HTli at critical points of the host-parasite interaction. 5HTli fibers were observed innervating the cephalopedal integument, the major site of ***S. mansoni*** miracidium penetration and transformation. However, no peripheral 5HTli neurons were detected. Clusters of 5HTli neurons were observed in the cerebral, pedal, left parietal, left pleural and visceral ganglia, suggesting that the peripheral serotonergic fibers originate from the CNS (see also Delgado et al. 2012). Specimens infected with ***S. mansoni*** were examined at 10 days post infection (10 dpi) and during their shedding stage. The total number of central 5HTli neurons decreased from 162.2 ± 40.0 (n = 5) under control conditions to 118.8 ± 11.9 10 dpi and 130.4 ± 6.7 at the shedding stage (one-way ANOVA, $p < 0.05$). Reductions of 5HTli were most evident in the pedal ganglion (control: 33.6 ± 8.8; 18.0 ± 3.7, 10 dpi; 25.6 ± 5, shedding; $p < 0.05$) and the left pleural ganglion (3.2 ± 2.8, control; 0 ± 0, 10 dpi; 0 ± 0, shedding; $p < 0.05$). The changes in 5HTli observed following infection by ***S. mansoni*** indicate that reductions in serotonin levels can occur in specific central neurons in parasitized snails and that these changes might contribute to the modifications in several behaviors that are observed during the course of infection.

1784

SCHISTOSOMIASIS IN REPRODUCTIVE-AGE WOMEN IN RURAL ZIMBABWE

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Schistosomiasis, which is highly prevalent in Zimbabwe, has well-recognised complications. However, the epidemiology of schistosomiasis in reproductive-age women and the impact of infection during pregnancy remain poorly understood. In this study we describe the epidemiology of schistosomiasis in reproductive-age women in rural Zimbabwe. The prevalence and risk factors for infection will be described, using the rich baseline dataset that includes demographic and socioeconomic variables, together with extensive data on sanitation coverage, water collection and exposure practices. Secondly, we explore associations between maternal schistosomiasis and adverse birth outcomes, including i) prematurity ii) low birth weight iii) miscarriage iv) stillbirth and v) neonatal death, stratified by maternal HIV status. Regression analysis for each birth outcome, using schistosomiasis as the exposure variable and including a range of covariates known to be associated with adverse birth outcomes (e.g. maternal age, parity, education, hypertension, smoking and alcohol use) will be presented. Thirdly, we present the relationship between the presence of inflammatory biomarkers (C-reactive protein, interleukin-6) in the plasma of 400 pregnant women with and without schistosomiasis.

1785

PATTERNS OF REACTIVITY TO *SCHISTOSOMA MANSONI* EGG GLYCAN ANTIGENS IN A POPULATION OF TREATMENT-NAÏVE KENYAN SCHOOL CHILDREN

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Children in schistosomiasis-endemic areas develop partial resistance to infection as they age. This resistance is associated with immune responses, including IgE and IgG, to parasite antigens. Anti-glycan antibodies are abundant in schistosome-infected hosts, but their significance in human disease resistance is still unclear. The aim of this study was to identify glycan epitopes targeted by children in ***S, mansoni*** endemic areas, and examine trends of reactivity with age in a population of egg-positive, treatment-naïve Kenyan school children. Plasmas from both egg-positive and -negative children demonstrate reactivity with several epitopes including core xylose/core α3 fucose, F-LDNF, LDN and LDNF on schistosome glycan microarrays. To explore age-related trends of such antibodies, we measured IgG and IgM to mock- and periodate-treated ***S, mansoni*** soluble egg antigen (SEA), and two parasite cross-reactive glycoproteins, keyhole limpet hemocyanin (KLH) and horseradish peroxidase (HRP). The ratio of periodate-resistant (primarily non-glycan) versus total IgG and IgM reactivity to SEA increased with age. IgG to HRP and IgM to KLH decreased with age, while IgG to KLH increased. These trends were especially pronounced throughout adolescence. The anti-glycan antibodies detected included a mix of IgG1, G3 and G4. Our results suggest that immune recognition of some glycan epitopes is negatively correlated, and others, positively correlated with age. Future studies on the function of such anti-glycan antibodies and their pattern of expression in human infection could be informative for the improvement of diagnostics and vaccine development.

1786

GENE REGULATION OF IMMUNE RESPONSES IN *BIOMPHALARIA GLABRATA*

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Biomphalaria glabrata snails act as the intermediate host to the trematode parasite, ***Schistosoma mansoni***. Following ***S. mansoni*** penetration of resistant ***B. glabrata,*** parasites are encapsulated by blood cells (hemocytes) and following are killed most likely via host-derived reactive oxygen and/or nitrogen intermediates. An immune response such as this is assumed to depend on altered gene expression to some degree. Moreover several studies have shown that the transcriptional profile of ***B. glabrata*** changes in response to stressors such as gram negative and gram positive bacteria, mechanical wounding, and metazoan parasites, as published previously. To date however, only a few transcription factors have been described in ***B. glabrata*** and an understanding of gene regulation in this species is lacking. The NF-kappaB family of transcription factors has been shown to regulate gene expression in a wide variety of biological processes, including immune and inflammatory responses. As a result, the NF-kappaB pathway is a prospective candidate for the regulation of immune-related genes in ***B. glabrata.*** NF-kappaB homologues have previously been identified in the snail and we have discovered putative kappa-binding sites in the regulatory regions upstream of immune-related genes such as p38 MAPK and Mpeg. Consequently we have examined whether these putative kappa-binding sites are recognized and bound by a ***B. glabrata*** NF-kappaB protein through electrophoretic

mobility shift assays (EMSAs). Furthermore the ability of NF-kappaB to translocate to the nucleus following exposure to PAMPS (pathogen associated molecular patterns) including ***S. mansoni*** larval transformation products was studied. Results support that NF-kappaB can function as a transcription factor and suggest its associated signaling pathway plays a role in the immune system in ***B. glabrata***.

1787

CHARACTERIZATION OF THE HUMORAL AND CELLULAR IMMUNE RESPONSES ELICITED BY THE IMMUNIZATION OF MICE WITH *SCHISTOSOMA MANSONI* CATHEPSIN B IN THE PRESENCE OF CPG OLIGODEOXYNUCLEOTIDES OR MONTANIDE ISA 720 VG

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Schistosomiasis is the most important human helminth infection due to its impact on public health. A vaccine could contribute to a long-lasting decrease in disease spectrum and transmission. Our previous vaccine study using ***Schistosoma mansoni*** Cathepsin B (SmCB) resulted in 59% and 60% worm burden reduction with CpG oligodeoxynucleotides and Montanide ISA720 VG as adjuvants, respectively. Furthermore, antibody production was significantly augmented in the vaccinated mice; both formulations elicited SmCB-specific total IgG endpoint titers > 120,000. In this study, the role of antibody-dependent cell-mediated cytotoxicity in SmCB-mediated protection was evaluated by incubating the parasite in the presence of pre-immune or immune sera with isolated lung CD45+ cells. These cells were obtained from mice immunized with recombinant SmCB adjuvanted with either CpG oligodeoxynucleotides or Montanide ISA 720 VG. Cells and sera from adjuvant and saline control animals were also included. SmCB + Montanide induced the highest killing when immune serum was present; suggesting the contribution of antibodies in cell-mediated parasite killing. In contrast, SmCB + CpG induced constant increased parasite killing which was independent of the addition of immune sera; implying that only cellular effects were elicited. To further investigate the immunological mechanisms, different cell populations were isolated and analyzed. CD8+ cells prominently contributed to SmCB + Montanide-induced parasite killing (67%) while CD4+ cells were the main effectors in SmCB + CpG-induced parasite killing (64%). Our results elucidate the importance of the adjuvants in influencing the immune mechanisms involved in parasite killing and protection against schistosomiasis.

1788

CHIENGI DISTRICT, ZAMBIA OPEN DEFECATION FREE!

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Open defecation is a leading risk factor for the transmission of diarrheal disease and soil-transmitted helminths. To become open defecation free (ODF) households must have access to a latrine with various adequacy parameters including a smooth, cleanable floor, a lid and a hand-washing station with soap or ash. Zambia has adopted community-led total sanitation (CLTS) as country policy in rural areas. CLTS is a behavior change communication campaign where communities are "triggered" through a discussion about open defecation and consequences of the behavior. Communities then construct their own latrines. With support from UNICEF, the Ministry of Local Government and Housing in Zambia has scaled CLTS throughout half the country. Included in the CLTS is a mobile-to-web real-time monitoring tool of ODF progress at the village level supported by DHIS2 that provides feedback to the community, district and traditional leaders. After 18 months of implementation of CLTS with the real-time monitoring the entirety of Chiengi District, Zambia has now certified as ODF despite challenges at the onset of the program. Chiengi District is the first in Zambia, and likely one of the first in all of sub-Saharan Africa to achieve ODF.

1789

THE ASSOCIATION BETWEEN SEASONALITY AND LEPTOSPIROSIS IN SOUTHERN THAILAND

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Introduction: Leptospirosis is a worldwide zoonosis with incidence rates of between 0.86 and 5.50 per 100,000 populations per year in Songkhla Province in southern Thailand. The incidence rate per month during the monsoon season was 3-5 times higher and up to 10-15 times higher during floods. Objectives: To study the association between the number of leptospirosis cases in Songkhla Province to the seasonal pattern and climate factors. Methods: We retrospectively reviewed the number of leptospirosis cases and climate factors including rainfall, temperature, relative humidity, light, wind and documentation of flooding from 1995-2015 and used time series analysis and logistic regression to study the association between them. The time series of monthly number of leptospirosis cases and climate information were taken and put into analytic model. Results: From 1995 to 2015, there were 1023 cases of leptospirosis in Songkhla Province. We found the documentation of flooding and 1-month lag of average rainfall were associated with number of leptospirosis cases with odds ratio (95% confidence interval) of 5.3(2.8-7.8, ***p***-value =0.003) and 1.4(1.1-2.2, ***p***-value=0.04). We also found the lack of association between chronological month, temperature, light and wind. For time series analysis there was no association between any more than 2-month lag climate factors and number of leptospirosis cases. Conclusion: The trend of leptospirosis in southern Thailand was associated with flooding and rainfall within a month. This model was purposed to predict the number of leptospirosis cases accurately.

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CAN DIARRHEAL DISEASES BE PREDICTED IN ADVANCE?

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Diarrheal diseases continues to pose a severe threat in tropical regions where WASH facilities remain marginal and are prone to destruction. With limited efficacy of vaccines, it is important to device alternate methods to determine environmental conditions favorable for diarrheal diseases. Outbreaks of several vibrio (V. cholerae., V. vulnificus, V. parahaemolyticus) have characteristic signatures that are associated with large scale climatic processes. Here, using cholera as one of the signature diarrheal disease, we present a framework coupling hydrological and microbiological understanding with satellite remote sensing data to predict environmental conditions for outbreak in several regions of sub-Saharan Africa. Hydroclimatic processes, primarily precipitation and temperature are found to be strongly associated with epidemic and episodic outbreak of cholera. Using spatial land surface temperature (LST) data from satellites along with water accessibility data and population data, we have developed an algorithm to classify regions at risk to cholera. A case study has been piloted to implement the algorithm in five epidemic regions e.g. Mozambique, Central African Republic, Cameroon, South Sudan and

Rwanda. Conditions for occurrence of cholera were detectable at least one month in advance. Additionally, we will present framework for prediction of V. vulnificus, V. parahaemolyticus in several coastal regions of US.

1791

POPULATION-LEVEL INDOOR PM2.5 EXPOSURE ASSOCIATED WITH HOUSEHOLD SOLID FUEL USE FOR THE ESTIMATION OF THE GLOBAL BURDEN OF HOUSEHOLD AIR POLLUTION (GBD 2013)

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Published evidence suggests that there is notable heterogeneity in PM2.5 exposure distribution across geographies due to various socioeconomic and environmental factors. However, there has been limited effort to quantify average PM2.5 exposure associated with household solid fuel use while accounting for this variation. To address this evidence gap, we estimated the population-level PM2.5 exposure associated with household solid fuel use with uncertainty for 138 developing countries from 1980 to 2013.We updated the Global Household Air Pollution Database by conducting a systematic review to include studies published as of January 2015. The final database comprised of 67 studies conducted in 16 countries from 8 regions. First, mean 24-hour kitchen PM2.5 concentration was estimated using a linear mixed effect model with maternal education as a covariate and nested random effect for GBD super region, region, and country. Then, ratios of personal exposure to average 24-hour kitchen PM2.5 concentration for men, women, and children were calculated separately. Finally, average personal exposure was calculated for men, women, and children by applying the ratio of personal exposure and kitchen concentration to the average 24-hour kitchen PM2.5 concentration for all 138 countries.Our analysis showed that mean PM2.5 exposure associated with household solid fuel varies geographically. Maternal education was a significant predictor of mean 24-hour PM2.5 kitchen concentration (p-value: 0.016). The estimated mean 24-hour PM2.5 kitchen concentration was highest in Ethiopia 691 µg/m3 (95% CI: 226-1750) and lowest in Costa Rica 103 µg/m3 (95% CI: 14.5-396) in 2013. The pooled ratio of personal to kitchen area PM2.5 concentration was highest for women 0.73 (95% CI: 0.66-0.81) and lowest for men 0.43 (95% CI: 0.37-0.50).Informed by a global database spanning 16 countries our model captured geographic variation in indoor PM2.5 exposure that was instrumental in estimating the global burden of household air pollution for GBD 2013. Future estimates would benefit from more direct exposure measurements by enriching the sparse PM2.5 data landscape.

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CHALLENGES FOR WOMEN IN ACCESSING WATER AND SANITATION FACILITIES IN PERI-URBAN SLUMS OF KENYA

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The vulnerability surrounding insecurity and threat experienced by women when they seek to meet their and their family's daily water and sanitation needs, are not much researched and addressed. A high proportion of women in the developing world are subjected to violence, assault and threat on their daily commutes to fulfill water and sanitary requirements, most of which remain undocumented. This analysis uses quantitative and qualitative data from a larger cross-sectional study on water, sanitation and hygiene (WASH) disparities in peri-urban slums of Kisumu, Kenya during 2014-15. Of the 734 interviewed women, more than 94% had access to toilets. However, more than one-third reported that they felt insecure to access their toilets at night and approximately 11% said they had faced some form of attack, threat or assault on their way to access toilets at night. Despite having toilets in their compounds, accessing them after dark presents severe threat to their personal safety. As a result, women are forced to rely on various unhygienic means of relieving themselves. Although fewer women stated they have been attacked while collecting water, more than 23% reported they felt insecure to gather water for their households at night. Data from semi-structured interviews with 20 women also revealed a fear of violence or threat in accessing WASH facilities within the community. Preliminary results demonstrate installing WASH infrastructure does not always ensure regular usage in communities, when there is an underlying threat of gender-based violence. The lack of safe toilet access at night may give rise to the use of other unsanitary methods of waste disposal, may force a woman to wait until daylight hours, and/or create psychosocial stress all of which can result in other public health risks for women and their families. A systematic and holistic approach is needed to address gender-based violence to ensure safer and higher access to WASH facilities by the women.

1793

EXPLORING THE IMPACT OF HARMFUL ALGAL BLOOMS (HABS) ON THE HEALTH OF COASTAL COMMUNITIES IN THE GULF OF GUAYAQUIL, ECUADOR

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Here we present a methodological framework and initial findings from a transdisciplinary study of harmful algal blooms (HABs) and public health effects in coastal Ecuador. There is a growing scientific consensus that the public health and economic impacts of HABs are indicative of a global epidemic. We have developed a methodological framework to explore the natural and anthropogenic drivers of HABs (red tides) in an estuarine-coastal gradient, and the potential linkages to the public health of three coastal communities in the Gulf of Guayaquil, Ecuador. Previous research identified more than 100 "red tide" events in the gulf since 1968; about 77% of these were associated with the death of fish, birds, or shrimp; however, there is a lack of specific epidemiological information linking HABs to local public health impacts. To address this gap, we are conducting a comprehensive eco-epidemiological study through the following: a) A historical reconstruction (2000-2014) of climate-ocean and anthropogenic conditions associated with HABs events to identify potential triggers, b) an assessment of health practitioners' knowledge and risk perception to explore local health effects of HABs, and c) an ecological study of the ocean-biological conditions that favor the presence and/or dominance of algae associated with toxins through an analysis of remotely sensed imagery and prospective field data collections. This study is being conducted in partnership with Ministry of Health, as establishing a collaborative partnership with public health staff and key local stakeholders from the outset will ensure a participatory process to identify potential interventions and public health priorities surrounding this largely unexplored public health issue.

1794

POLLUTION STUDIES ON THE LOWER MISSISSIPPI MISSISSIPPI RIVER IN PORT GIBSON AREA, MISSISSIPPI DURING THE FALL OF 2013

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Water is critical for the survival of living things including man. It is the driver of life. Its quality is closely associated with the surrounding

environment and the use made of it. It may also depend on whether the water body is lentic or lotic. The purpose of this study was to determine if the Mississippi River in the area of Port Gibson,MS was polluted and compare the result with the Mississippi Water Quality Criteria (MSWQC) and/or the Environmental Protection Agency(EPA) Standard. Water samples were collected from the Mississippi River in Port Gibson Area in the Fall of 2013 for three consecutive times and tested with the LaMotte water pollution test kits. The physical characteristics of the river were taken including the temperature and recorded. The following chemical parameters were tested and analyzed: total alkalinity, ammonia-nitrogen, calcium, carbon dioxide, chlorine, cooper, dissolved oxygen, magnesium, nitrate, hardness, pH, phosphate, and salinity. The parameters tested met the Mississippi Water Quality Criteria (MSWQC) with the exception of total alkalinity, hardness, and phosphate which exceeded the criteria. Coliform bacteria were present in the river water showing evidence of bacteria contamination and the fact that the water was not potable or was unsanitary for human domestic use.

1795

ANTIBIOTIC RESISTANCE OF ENTEROBACTERIA IN TWO WASTEWATER TREATMENT PLANTS OF TWO PERI URBAN COMMUNITIES OF LIMA, PERU

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The increased use of antibiotics in human medicine and agriculture in recent decades has introduced significant selective pressures on bacterial populations in virtually all human-associated environments and lead to the evolution of multidrug-resistant bacteria that compromise our ability to treat infectious disease. Build environments, such as wastewater treatment plants (WWTP) have already been identified as reservoirs of antibiotic resistance genes with high potential for introduction into natural environments. Previous studies regarding the presence of antibiotic resistance bacteria have not been performed in sewage and WWTP in the peri urban shantytowns of Lima, Peru. This is the first report of the isolation and antibiotic resistance of specific Enterobacteria from two WWTP of the districts of San Juan de Miraflores (SJM) and Villa El Salvador (VES), Lima, Peru. A longitudinal sampling was performed from September 2014 until February 2015 in the WWTP of SJM and VES, Lima, Peru. Influent and effluent water samples were taken during each sampling date from each WWTP. Total coliforms, strain isolation and antibiotic resistance to 12 different antibiotics was determined. Antibiotic resistance ranged as follows: 10 to 80% for affluent VES samples, 80 to 100% for VES effluent samples, 30 to 100% for affluent SJM samples and 70 to 80% for effluent samples. Regarding individual antibiotic resistance, SJM affluent and effluent samples were 100% resistant to CF and SXT while effluent samples where 66.6% resistant to CTR. Affluent and effluent samples from VES where 100% resistant to CIP and effluent samples had high resistance percentages to CXM (85.7%), NIF (85.7%) and SXT (87.5%). Isolated bacterial strains included ***E. coli, Enterobacter*** spp., ***Citrobacter*** spp., ***Serratia*** spp., ***Klebsiella pneumonie,*** and ***Salmonella*** spp. In conclusion, results show that effluent waters do carry rasistant bacterial strains into the environment, including pathogenic bacteria. Further studies are needed to evaluate bacterial resistance genes and their behavior in WWTP and their re-entrance into the community's environment.

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UNDERNUTRITION, DIARRHEA AND BREASTFEEDING IN UNDER-FIVE - BENGO PROVINCE, ANGOLA

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New interventions to reduce undernutrition should be design taking into account their basic, underlying and immediate causes, since nutrition is capable of maximize health and minimize morbidity and mortality in early childhood. This study aims to identify factors associated with undernutrition (wasting, stunting and underweight) among children aged less than 5 in Bengo province, Angola. A total of 803 children aged 0 to 59 months were surveyed. Logistic regression analysis was used to examine undernutrition against a set of variables associated with health and water. Children nutritional status was classified as underweight, stunted and wasted if their Z-scores for weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) were less than -2.0 SD of WHO (2006) standards. Children in this study aged mainly 0-23 months (43%), 50.8% were males, 36% were currently breastfeeding, 34% had diarrhea in the last two weeks and 53.4% of the mothers does not treat water. The prevalence of wasting was 5,6%, stunting 30.7 % and underweight 29.0%. The most significant factors for wasting were age (OR 4.5, 2.1-9.3 risk for 0-23 age), being breastfeed (OR 4.0, 2.0-7.7) and having diarrhea episodes (OR 2.0, 1.0-3.7). The most significant factors for stunting were mother´s education (OR 0.5, 0.2-0.9 for mother with secondary or higher education), age (OR 0.5, 0.4-0.8 risk for 0-23 age) and being breastfeed (OR 0.4, 0.3-0.6). The most significant factors for underweight were age (OR 1.7, 1.1-2.6 risk for 0-23 age class), being breastfeed (OR 1.7, 1.1-2.7) and having diarrhea episodes (OR 1.9, 1.3-3.1).The high frequency of reported untreated drinking water in this study may be related to the occurrence of enteric pathogens, and consequently to the occurrence of the diarrhea episodes reported here. On the other hand, our results suggest that breastfeeding may have a protective effect on the occurrence of stunting. Despite the need further investigation, our results suggest the existence of preventable morbidity triggered by undernutrition. Community-based education intervention could represent good strategies to reduce undernutrition.

1797

LABORATORY SURVEY FOR THE PREVALENCE OF ENTERIC BACTERIA IN RESTAURANTS IN THE PERUVIAN AMAZON (IQUITOS, PERU)

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Food and water-borne pathogens are a common and preventable source of diarrheal illness and death worldwide, especially in countries with limited public health infrastructure. A contributing factor to diarrheal illness may be the hygiene and sanitation practices of restaurants. This study investigates the prevalence of pathogenic and commensal enteric bacteria in restaurants in Iquitos, Peru. A total of 17 restaurants were asked to consider participating in the study. Sixteen gave permission to team members to collect an average of 9 food and food preparation contact surface sites for microbiological analysis. Samples were collected by swab in transport media and delivered that day to the microbiology laboratory. A total of 1,280 restaurant samples were cultured for enteric bacterial pathogens (***Salmonella, Shigella, Campylobacter*** and ***Aeromonas***) and also for the presence of normal enteric flora using standard culture techniques. Culture results were then stratified by average cost per meal at the restaurants. Water was also collected

from each restaurant for fecal coliform analysis. ***Salmonella, Shigella, Campylobacter***, and ***Aeromonas*** spp. were isolated from 16 samples (0, 3, 0, and 13, respectively) at 10 of 16 (63%) restaurants. Other fecal flora, most commonly E. coli, Proteus, Enterobacter, or Klebsiella, were isolated from 30 foods/juices and 35 surface sites at 15 of 16 (94%) restaurants. Five of 16 (31%) restaurants yielded positive coliform counts from their water sources including at least one restaurant in each cost stratified group (1 low, 1 medium, 1 high, and 2 very high cost). Our study demonstrates the presence of enteric pathogens and normal human fecal flora in the food, water, and preparation surfaces in restaurants in Iquitos, Peru. While this study does not clarify the relationship between enteric contamination in restaurants and community diarrheal disease, there was no significant correlation between average cost of meal and the presence of enteric bacteria. Future studies include observational and laboratory assessments longitudinally and development of public health based intervention and education strategies.

1798

FLIPPING THE SWITCH TO ACCURATELY DIAGNOSE MALARIA: A COMPARISON OF CLINICAL DIAGNOSIS, RAPID DIAGNOSTIC TEST (RDT), MICROSCOPY AND PCR IN THE DIAGNOSIS OF MALARIA IN CAMEROON

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Accurate diagnosis of malaria is vital for effective disease management and control. In Cameroon, presumptive diagnosis, RDT and microscopy are commonly used in malaria diagnosis. However, these methods lack sensitivity to detect low grade asymptomatic infections. PCR on the other hand permits the detection of sub-microscopic parasitemia that could be a potential roadblock as malaria endemic countries progress towards malaria elimination. In this study we compared the diagnostic test performance of clinical diagnosis, microscopy and RDT against PCR in the diagnosis of malaria. Blood samples were collected from 380 febrile children from Yaoundé and Maroua, Cameroon between February 2014 and October 2014 and their files were reviewed at the end of their hospital visit for clinical diagnosis of malaria. Microscopy, RDT and PCR based prevalence of malaria was 40% (151/380), 56% (213/380) and 63% (239/380), respectively. However, 95% (361/380) participants were presumed to have malaria based on fever, of which 34% (122/361) were negative by PCR and 85% were prescribed quinine. PCR detected 88 and 26 more malaria infections than microscopy and RDT, respectively. As compared to PCR, the sensitivity of microscopy, RDT and clinical diagnosis was 51%, 72% and 99%, respectively; however the specificity was 93%, 62%, and 3.3%, respectively; positive predictive value was 94%, 88% and 63%, respectively; negative predictive value was 55%, 38% and 70%, respectively. Thus, 34% of cases diagnosed as malaria were actually fever cases caused by other pathogens. Eighty-eight (88) sub-microscopic infections were identified by PCR in the study population, suggesting that PCR may be the best tool for accurate diagnosis and control of malaria since the presence of sub-microscopic malaria infections may be a potential hindrance towards malaria elimination. The development of a rapid PCR based test to diagnose malaria could flip the switch to accurate diagnosis, control and elimination agenda of malaria.

1799

IN FIELD ASSESSMENT OF MALARIA RAPID DIAGNOSTIC TEST PERFORMANCE BY COMMUNITY HEALTHCARE WORKERS

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Under ideal conditions, the accuracy of malaria rapid diagnostic tests (RDTs) is considered equivalent to routine microscopy. However, in the field inaccurate test results may occur because of defective RDTs, human processing errors and/or errors reading test results. Data ascertained from 9 implementation studies of Fionet system was used to evaluate the frequency of these errors during routine use of malaria RDTs by healthcare workers. In each study, healthcare workers received training on processing RDTs and integrated an automated RDT reader into their point-of-care case management. The reader features in-process quality control and automated test interpretation for malaria RDTs. In 4 of the 9 studies the workers were blinded to device results allowing an evaluation of in field reading of test results. In these four studies, a total of 19,212 malaria RDTs were processed and had overall agreement of 91.76% (kappa=84.45%). A stronger agreement (96.39%; kappa=92.45%) was observed when the device had a positive or negative test result, consistent across all studies. The discordant results predominantly occurred when the device indicated a quality control warning and the user interpreted that RDT. All nine studies were used to evaluate the proportion of RDTs with quality control warning. The quality control issues were categorized into two main groups: faulty RDTs (e.g. no control line, smearing) and human processing errors (e.g. incorrect volume or placement of solutions, delayed reading, interpreting the wrong RDT). A total of 31,705 tests conducted by 151 healthcare workers were analyzed. There were approximately twice as many (~2200) human processing errors compared to any other error type. A random-effects meta-analysis, using Freeman-Tukey transformation, was used to estimate the average proportion of errors across users and studies. The meta-analyses estimated 7.61% (95% CI: 6.57%, 8.76%) errors due to human processing across. RDT brand was associated with the proportion of defective RDTs. These errors can jeopardize the accuracy of the results. Quality assurance devices would help prevent the use of compromised test results.

1800

EFFECTS OF INTRODUCING MALARIA RAPID DIAGNOSTIC TESTS IN DRUG SHOPS: FINDINGS FROM THE EVALUATION OF A CLUSTER RANDOMIZED TRIAL IN UGANDA

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WHO recommends universal access to malaria diagnosis, encompassing all treatment providers, including the private sector. Rapid diagnostic tests (mRDTs) provide a feasible means of confirming malaria diagnosis in drug shops. As yet, there is limited evidence of the effect of diagnostic testing on antimalarial sales and referral practices by drug shops in Africa. A cluster-randomised trial to evaluate the impact and cost-effectiveness of using mRDTs, compared with presumptive treatment, undertaken in 65 registered drug shops in Mukono District, Uganda in 2010-12, was one of the first investigations of the impact of introducing mRDTs in the private retail sector. Analysis of data routinely recorded by drug shop vendors (DSVs) during the trial found use of mRDTs in drug shops was highly acceptable to both vendors and clients; a finding confirmed by household interviews in a random sample of patients. Adherence to mRDT test results

by DSVs was high with over 95% of treatment decisions consistent with mRDT result, reducing sales of ACTs by 40% compared to drug shops using presumptive diagnosis. Validation of DSV treatment decisions by expert microscopy demonstrated that mRDT testing substantially improved the targeting of ACTs to patients with malaria parasites (72.9% of ACT treatments in shops using mRDTs were correctly targeted vs 33.7% in shops using presumptive diagnosis, P<0.001). Qualitative and economic evaluations amongst drug shop vendors, patients and local health staff, conducted alongside the trial, revealed how pre-existing relationships between DSVs and their clients, and between DSVs and the wider health system shaped the response of drug vendors to the intervention and may have contributed to the high adherence to mRDT results. Potential for less desirous unintended consequences was also revealed. A synthesis of the insights generated by this early ground breaking trial in the retail sector will be presented, drawing evidence from across the epidemiological, ethnographic and economic investigations conducted as part of the study, to illustrate the potential benefits and pitfalls of introducing mRDTs into drug shops.

1801

PERFORMANCE OF MALARIA RAPID DIAGNOSTIC TESTS FOR SCREENING OF PATIENTS TO BE ENROLLED IN CLINICAL TRIALS AND RELATED STUDIES

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Malaria rapid diagnostic tests (mRDTs) are widely used for malaria diagnosis, but their applicability in clinical trials for patients screening and management has not been well assessed. This study assessed the performance of mRDTs when used for screening of patients to be enrolled in clinical trials and other studies, particularly in areas with progressively declining malaria burden. The data was obtained from studies conducted at five health facilities (HFs) of Mkuzi and Muheza, Nachingwea, Rubya and Ujiji in four districts of Muheza, Nachingwea, Muleba and Kigoma, respectively. Patients aged ≥6 months were screened with mRDTs followed by microscopy for possible inclusion in clinical trials and other studies. The performance of mRDTs was compared with microscopy as a gold standard, and factors affecting their accuracy were explored using multivariate logistic regression models. Of the 1,914 participants screened; 1,188 (62.1%) and 1,019 (53.2%) were positive by mRDTs and microscopy, respectively. Parasite positivity rates (by microscopy) were higher at all sites (>50.0%) except Nachingwea (with 35.8%). mRDTs had high sensitivity (>97% at all sites) while the specificity was relatively lower (64.9% - 88.7%). After adjusting for age of patients, fever status , site and the study type, the sensitivity of mRDT was significantly higher in patients with parasite density ≥4000 asexual parasites/ul (OR=6.30, p=0.003). The specificity of mRDT was lower (64.9 - 87.7%) and similar at all sites even after adjusting for the effects of fever status and age of participants (p≥0.525), except at Muleba and Ujiji where the specificity was significantly lower (p≤0.007) due to high rates of false positive mRDT results. In conclusion, the high sensitivity of mRDTs indicate that they can be useful for screening to exclude majority of the patients without malaria and save time and other resources which would be used for microscopy. Due to low specificity which could lead to enrolment of patients without malaria parasites, mRDTs should only be used for initial screening and all positive cases must be confirmed with microscopy.

1802

A NON-AMPLIFICATION, OLIGONUCLEOTIDE-BASED SANDWICH HYBRIDIZATION ASSAY FOR THE DETECTION OF PATHOGENS IN BLOOD

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Novel technologies for the sensitive and reliable detection of infectious agents in blood are still needed. While the standard method of nucleic acid-based pathogen detection generally relies on PCR amplification of target DNA or RNA, complex genome sequences can be resistant to amplification, due to factors such as secondary or tertiary structure, and the potential for nonspecific amplification or sample interference could result in false positive or false negative results. Here, we describe a novel nanoparticle-based sandwich hybridization assay (SHA) for the detection of ***Plasmodium falciparum*** and ***Babesia microti*** parasites without the need for amplification of target sequences in genomic DNA. A uniquely identifiable "barcoded" magnetic microbead and biotinylated silica nanoparticle are conjugated to either ***P. falciparum***- or ***B. microti***-specific 30-mer oligonucleotides corresponding to sequences of the 18S ribosomal gene. For each parasite, the magnetic microbead and silica nanoparticle bead sets hybridize to a unique but adjacent region in the genome. Parasite burden can then be quantified and analyzed upon the binding of an Avidin-PE fluorophore to the target capture complexes via a Bio-Plex 200 instrument. Determination of the analytical sensitivity of the SHA for short complementary oligonucleotide sequences revealed a limit of detection of 10^{-10} M for both ***P. falciparum*** and ***B. microti*** probe sets. Analytical sensitivity studies conducted by spiking human blood with known counts of parasites revealed that SHA can reliably detect up to 10 ***P. falciparum***- or ***B. microti***-infected red cells per mL of blood. For comparison, in our hands PCR can detect 100 ***P. falciparum***- or 1000 ***B. microti***-infected red cells per mL of spiked blood. Thus, SHA offers a 10-100 fold enhanced sensitivity for the detection of these two intraerythrocytic parasites of global public health significance. Studies to determine the clinical sensitivity of SHA for these pathogens are in progress. Details of the method development and sensitivity and specificity data will be presented.

1803

AUTOMATED DETECTION OF MALARIAL RETINOPATHY FOR HIGHLY SPECIFIC DIAGNOSIS OF CEREBRAL MALARIA

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Cerebral malaria (CM) is a lethal clinical syndrome that claims the lives of about 584,000 people annually, 75% of whom are African children. As many as 23% of these cases are misclassified when the standard clinical case definition for CM is used. When malarial retinopathy (MR) detection is included in the case definition, the specificity and positive predictive value (PPV) of the CM diagnosis is greatly increased, and other causes of coma can be sought in patients who are retinopathy-negative. Detection of MR currently requires expensive equipment and well-trained personnel and is thus limited only to research settings in malaria-endemic areas in Africa. We used retinal images from 175 Malawian pediatric patients with MR, captured using a Topcon 50-EX camera to develop and test software algorithms for detecting the presence of MR, and of its associated retinal lesions. Performance assessment was made against manual image grading

as the reference standard. The software achieved specificity of 100% and sensitivity of 95% for MR detection. The individual specificities for detecting retinal abnormalities associated with MR were 91% for retinal whitening, 100% for vessel discoloration, and 96% for white-centered retinal hemorrhages. The system also detects papilledema, a condition associated with death due to raised intracranial pressure, with 100% sensitivity and 81% specificity. The proposed MR detection system can improve the PPV for diagnosis of CM from 68% (using current clinical standard) to 98%. Our MR detection system integrates a low-cost and portable retinal imaging camera with software developed to detect MR. This system is designed to be used by non-ophthalmic personnel with minimal training in low-resource clinical environments.

1804

LOT TESTING OF MALARIA RAPID DIAGNOSTIC TESTS: ACHIEVEMENTS AND LESSONS LEARNED FROM A SEVEN YEARS-LONG EXPERIENCE

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Malaria Rapid Diagnostic Tests (RDTs) have played a key role in fever case management since the 2010 WHO recommendation that every suspected malaria case should be confirmed by parasitological diagnosis before treatment. Sales of RDTs have increased from 46 million in 2008 to 319 million in 2013. The wide range of commercially available products, with variable quality reported in field studies, led to the creation of the WHO-FIND Malaria RDT Evaluation Programme that aims at providing independent comparative performance data to guide procurement (Product Testing) and comprehensive lot verification prior to deployment in countries (Lot Testing). The Lot Testing Programme specifically, with its two reference laboratories in Cambodia and the Philippines, has provided quality data for more than 3600 RDT lots since 2007. RDTs are tested against frozen blood samples collected from malaria patients, standardized at 200 parasites per microliter of blood. The observed mean failure rate of 1.85% is notably low, reflecting the fact that Lot Testing is mainly requested by large funding/procurement agencies selecting only products meeting the WHO recommended minimum performance criteria for procurement. The number of lots tested annually has increased from 59 to 927 in the last 7 years, with the 2014 testing volume covering an estimated 210 million RDTs, equivalent to 66% of the RDT market. Lot-tested RDTs were distributed in 48 countries, with 88% of RDTs destined for sub-Saharan Africa. Lot Testing informs not only parasite detection but also may reveal RDT anomalies, such as red background, incomplete clearing, or problems with kit accessories. The reporting of issues observed on single-use buffer vials in particular has triggered the release of an Information Note to Users by the WHO and field corrective actions by RDT manufacturers. The Lot Testing Programme plays a key role in encouraging manufacturers to maintain the required level of quality from lot to lot. Work is now underway to transition to a decentralized self-funding system, and build local capacity in 12 pilot countries for lot testing in national reference laboratories.

1805

CONDITIONS THAT LEAD TO POLYANDROUS BEHAVIOR IN THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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Upon insemination, ***Aedes aegypti*** females receive seminal fluid proteins from males that render them refractory to subsequent mating. This response is often assumed to be immediate, complete, and lifelong. Nonetheless, several studies have found that polyandry can occur in ***Ae. aegypti***, but little attention has been paid to the circumstances that result in polyandry. This promiscuity could have far-reaching implications. For example, polyandrous behavior could hinder inundative release strategies, which often rely on the assumption of monandry. To our knowledge, no study has determined the timing of the onset of refractoriness, and the degree to which monandry is maintained over time remains unresolved. By using males with fluorescently labeled sperm to identify polyandrous females, we determined how soon females become refractory to additional inseminations and how this refractoriness wanes as females age. We also determined the reproductive success of first and second males under these circumstances. This study clarifies the role of polyandry in the mating system of this important vector and will ultimately guide vector control strategies, predictive models, and experimental design.

1806

HUMAN ADAPTATION TO CLIMATE CONTRIBUTES TO *AEDES AEGYPTI* ABUNDANCE IN THE SOUTHERN MARGINS OF ITS AUSTRALIAN DISTRIBUTION

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The dengue vector ***Aedes aegypti*** has been identified in new areas south of the species' core Australian distribution. It has been hypothesized that an expansion by ***Ae. aegypti*** into these regions would require large water bodies that buffer extremes in temperature and humidity. As part of the human response to drought in the early 2000s, government rebates were offered on large water storage containers such as rainwater tanks (RWT). The installation of over 200,000 RWT has resulted in new habitats for container-breeding mosquitoes throughout the region. Surveys have demonstrated that RWT are key containers for mosquito breeding, however little is known about the role RWT play in vector abundance in cool and dry range margins. The current study was designed to investigate how RWT can contribute to a localized population of ***Ae. aegypti***. To do this we divided the urban landscape into three categories: premises with an exposed RWT, those with a sealed RWT and those with no RWT. Ovitraps and Gravid Aedes Traps (GAT) were used to measure adult population abundance. Traps were retrieved and reset fortnightly for ten weeks, and eggs were counted and reared to fourth instar for counting and identification after each collection. Inspections of study site and surrounding premises documented characteristics of house construction and surrounding urban environment. A multivariate time-series analysis will be performed to examine the effect of the predictor variables on each of the response variables at the scale of the study premises and the study premises plus their neighbours. Results will be presented. Our results show the effect that exposed RWT have on the abundance of ***Ae. aegypti*** when compared with a sealed RWT or no RWT present. The current trend of water storage in Australia is similar to a century ago, when ***Ae. aegypti*** was present and dengue epidemics occurred. Likewise, similar rebate schemes and drought conditions in the United States are increasing the number of large water containers in urban environments, and could provide a potential new habitat for the establishment of ***Ae. aegypti*** and other disease vectors.

1807

EXPLORING HOST ORIENTED FLIGHT AND SPATIAL ASPECTS OF INDOOR MOVEMENT OF *ANOPHELES GAMBIAE* USING INFRA-RED VIDEO TRACKING

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Fundamental to security and shelter, the human home is exploited by many parasitic arthropods, including many vectors of malaria that spend the majority of their adult life bloodfeeding or resting in this environment. Successful vector control methods like indoor residual spraying (IRS) and long-lasting insecticide-treated bednets (LLINs) exploit this behavior. Though central to malaria reduction and elimination plans worldwide, they have limitations with a further serious threat presented by rapidly emerging insecticide resistance. Identifying possible routes to improve control of nocturnal indoor transmitted malaria is a recognized priority. We investigated spatial activity of African ***Anopheles sp.*** during nocturnal host orientation to human subjects, within or without LLINs, using a video system to track flight activity of multiple mosquitoes at high resolution over periods of 60 minutes or more. In tests exploring responses to human-occupied LLINs carried out in a hut in rural Tanzania, behavior of local ***An. arabiensis*** populations was remarkably similar to ***An gambiae s.s.*** Kisumu strain mosquitoes tested in the same environment and as previously reported in our laboratory tests. In the absence of a bed net, approach, landing and biting activity of ***An. gambiae s.s.*** at unprotected human hosts revealed consistent spatial behaviors during approach and departure from the host, most likely in response to thermal and odor cues emanating from the supine human host. We used a different video-tracking system incorporating retro-reflective screens to obtain 3D flight tracks of ***An. gambiae*** as they approached and entered through a 'window' in response to a human host, in the laboratory. Analysis of flight trajectories provided evidence that trajectories passing through the window showed consistent patterns in flight elevation change during room entry and exit. The value of these and related studies on the spatial aspects of mosquito behavior in the domestic environment is considered in terms of its contribution to basic knowledge of vector biology and to the search for new vector control tools and strategies.

1808

POSITIVE AND NEGATIVE EFFECTS OF *WOLBACHIA* INFECTION ON ARBOPATHOGEN TRANSMISSION

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In field trials across the globe, a revolutionary experiment in vector-borne disease control is underway. Artificial Wolbachia infections have been shown to render mosquitoes resistant to transmission of many human pathogens. Wolbachia-infected mosquitoes are currently being released in multiple countries in an attempt to control dengue virus. However, it has become clear that Wolbachia infection does not always lead to pathogen suppression in insects. Multiple studies in a wide variety of vector and non-vector insect species suggest that Wolbachia can enhance certain parasites and viruses in arthropods; a sobering reminder that the pathogen inhibitory effects resulting from Wolbachia infection in some insects cannot and should not be generalized across vector-pathogen systems. I will present mechanistic data demonstrating how Wolbachia can act directly or indirectly to cause enhancement or suppression of vector-borne pathogens. Understanding the specific mechanisms leading to pathogen enhancement and suppression is critical for identifying systems where Wolbachia-based control is likely to succeed, for identifying potential points where Wolbachia-based control is likely to break down and fail in the field, and for planning risk mitigation strategies in the case of unforeseen harmful outcomes.

1809

A CROSS-OVER STUDY TO EVALUATE THE DIVERSION OF MALARIA VECTORS IN A COMMUNITY WITH INCOMPLETE COVERAGE OF SPATIAL REPELLENTS IN THE KILOMBERO VALLEY, TANZANIA

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Malaria elimination is unlikely to occur if vector control campaigns rely entirely on treated bed-nets and indoor residual spraying. There is a need for vector control tools that address vectors that bite outside sleeping hours. Spatial repellents may be able to fill this gap. However it is unclear if malaria vectors will be diverted from households that use spatial repellents to those that do not. The present study was performed for a period of 24 weeks in rural Tanzania. A total of 90 households were recruited and a cross-over design was used to measure the density of resting and blood-engorged mosquitoes in 3 coverage scenarios using 0.03% transfluthrin coils: 1) no coverage; 2) complete coverage and; 3) incomplete coverage. Human blood index of each malaria vector species was calculated for each scenario. Human landing collections were performed and vector biting times were recorded. The main vectors were found to be ***Anopheles arabiensis*** and ***Anopheles funestus*** s.s.. Both species fed outdoors, outside sleeping hours and on humans as well as animals. Data from human landing catches showed that the repellent coils reduced the number of ***An arabiensis*** by 80% but did not reduce the number of host seeking ***An. funestus***, this may be due to potential development of pyrethroid resistance which has been documented in the area. The repellent coils did not reduce indoor and outdoor resting densities of Anophelines nor cause a shift in the human blood index. No diversion of malaria vectors was measured. On the other hand, the spatial repellent coils reduced the household densities of ***Culex*** spp. by 26%, and contributed to 19% diversion of ***Culex*** spp. to non-repellent users. There is strong evidence that large proportion of malaria exposure is not controlled by the current vector control strategy in the Kilombero Valley. The use of 0.03% transfluthrin coils in this area is unlikely to result in malaria reduction since much biting occurred in the morning after coils had gone out. The behavioural responses of pyrethroid resistant mosquitoes and ***Anopheles funestus*** to spatial repellents needs to be further investigated given the increasing importance of this vector in the area.

1810

INSECTICIDES AND POLLUTION EXERT STRONG SELECTION ON NEW CRYPTIC SUBGROUPS OF *ANOPHELES GAMBIAE*

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Ongoing ecological adaptation and lineage splitting within the Afrotropical malaria vector ***Anopheles gambiae s.l***. has the potential to mitigate the effectiveness of both traditional and novel vector control tools. We explored the population structure and identified targets of natural selection in 888 individuals of this species complex collected from contrasting environments. We provide evidence for clear subdivisions within the two sister taxa ***An. coluzzii*** and ***An. gambiae sensu stricto***.

Genome scans of all the new cryptic subgroups reveal pervasive signatures of strong selection around genes involved in metabolic or target-site resistance to insecticides. Notably, a selective sweep containing at least eight detoxification enzymes contributes to local adaptation of urban subgroups that thrive in polluted breeding sites. Our results show that human-induced selection can play a prominent role in driving mosquito population differentiation during the early stages of adaptive evolution with potentially dire consequence for malaria control.

1811

DIRECT, HOUSEHOLD-LEVEL EFFECTS OF SPATIAL REPELLENTS FOR DENGUE CONTROL - A MODELING ASSESSMENT

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In the absence of effective drugs or vaccines, efforts to control and prevent dengue currently rely on interventions acting on the mosquito vector, ***Aedes aegypti.*** Thus, there is need for the development of new, broadly applicable vector control tools to augment the currently available options. Studies to date have shown that spatial repellents (SR) have the potential to reduce malaria transmission, but their impact on other vector-borne diseases, such as dengue, is uncertain. To make accurate quantitative projections of its epidemiological impact, a crucial need is to enhance the understanding of the mechanisms of action underlying its epidemiological impact. SR products could potentially impact multiple components of vectorial capacity (VC), a classical metric that relates different aspects of mosquito life history to transmission potential. VC however, relies on the assumption that each individual has an equal probability of being bitten. The effects of SR on mosquito movement and biting behavior explicitly violate this assumption by potentially causing a reduction as well as a redistribution of bites. This impedes the use of VC in this context. Individual-based models (IBM) are free from key simplifying assumptions of the classical formulation of VC and thus offer an opportunity to explore the epidemiological impact of SR on dengue transmission in a more realistic way. We present a novel adaptation of an IBM for dengue transmission in Iquitos, Peru, to simulate fine-scale heterogeneities in transmission, as informed by extensive entomological and epidemiological data. SR effects on movement, biting behavior, blood feeding and oviposition are incorporated in the model using probabilistic descriptions fit to data from laboratory experiments and experimental hut studies under natural field conditions in disease-endemic settings. We highlight the key factors that underlie the discrepancy between projections from the IBM and VC and demonstrate a positive effect of SR on reducing transmission at the household level. The results from this simulation study warrant further testing and assessment of SR products on a population level.

1812

REVERSE MOLECULAR EPIDEMIOLOGY: INSIGHTS INTO THE INFECTION DYNAMICS OF BLOOD-BORNE HUMAN PARASITES IN A *LOA LOA-, MANSONELLA PERSTANS-* AND *PLASMODIUM FALCIPARUM*-ENDEMIC REGION OF CAMEROON

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Interactions among co-infecting parasites can modify the epidemiology of parasitic infections. We conducted an epidemiologic assessment of prevelances and infection intensities of (co)-infections with ***Loa loa*** (Ll), ***Mansonella perstans*** (Mp), ***Wuchereria bancrofti*** (Wb) and ***Plasmodium falciparum*** (Pf) in 8 villages of eastern Cameroon using highly sensitive and specific quantitative PCR (qPCR) for multiple parasites on archived dried blood spots (20 ul whole blood equivalent per 6mm spot per individual). Resident populations (n=1,144; age range: 2-90 years old) were parasitized with Mp (76%), Ll (38%) and Pf (33%), but not with Wb. Co-infections (49%) were more common than single infections (40%), with 21% having Ll and Mp, 3% with Ll and Pf, 15% with Mp and Pf, and 10 % with Ll, Mp and Pf. Interestingly, those with all three infections (Ll/Mp/Pf) had significantly higher Ll microfilariae (mf) counts than either single Ll (P=0.01) or double Ll/Mp (P=0.03) and Ll/Pf (P=0.05) infected individuals. The estimated counts of Ll mf were positively correlated with the intensities of Mp mf (Regression coefficient =1.43; P<0.0001). Population assessments at the community level showed that despite Mp and Ll both having overdispersed distributions -typical of most filarial infections - the population dynamics of Ll showed much greater overdispersion than did Mp. These data suggest a possible shared host susceptibly to Ll and Mp infection and also provide a method we are terming "reverse molecular epidemiology" that should be broadly applicable to many environmental niches containing infectious organisms for which molecular targets are defined.

1813

THE PREVALENCE OF LYMPHATIC FILARIASIS RELATED HYDROCELE, LYMPHEDEMA AND INFECTION IN MANDALAY REGION, MYANMAR

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Lymphatic filariasis (LF) is highly endemic within Myanmar. Despite the establishment of an elimination programme in 2004, little remains known about the prevalence of LF related morbidity in the country. We therefore conducted a cross-sectional survey to determine the prevalence of LF infection and morbidity and their associated risk factors in 24 randomly selected villages in four endemic townships within the Mandalay region of Myanmar - Amarapura, Patheingyi, Tada-U and Wundwin - between February and March 2015. Within each village, twenty households were randomly chosen for inclusion. Household members one year and older were tested for antigenemia with rapid immunochromatographic card tests (ICT). A night-blood slide was done for those with positive ICT results to quantify microfilaremia. Ultrasound assisted clinical examination was done on household members 15 years and older for signs of LF-related morbidity. Household questionnaires and GPS mapping were completed for risk factor analysis. Of those tested with ICT in 414 households, 45 of 1018 individuals (4.4%, 95% confidence interval (CI) 3.2 to 5.9%) were positive. ICT antigenemia was highest in Amarapura (32/294, 10.9%, 95% CI 7.6 to 15.0%) followed by Tada-U (8/267, 3.0%, 95% CI 1.3 to 5.8%), Wundwin (4/343, 1.2% 95% CI 0.3 to 3.0%) and Patheingyi (1/114, 0.9%, 95% CI 0.02 to 4.8%). Eighteen of the 289 males (99% of those eligible for scrotal examination) had hydroceles (6.2%, 95% CI 3.7 to 9.7%). Thirteen were unilateral and five were bilateral. Of the 23 hydroceles, 14 were stage one, seven were stage two and two were stage three. No cases of limb lymphedema or elephantiasis were found in the 827 individuals examined (0%, 97.5% one-sided CI 0 to 0.4%). The results of this study indicate a high prevalence of LF infection and hydrocele with low levels of limb lymphedema in the Mandalay Region of Myanmar. These results highlight the strong need for further rounds of mass drug administration as well as targeted surgery and morbidity alleviation programmes in the region. Further LF morbidity prevalence studies are needed to elucidate the burden in the remainder of the country.

1814

PROGRESS TOWARD ELIMINATION OF LYMPHATIC FILARIASIS IN HAITI: PRE-TRANSMISSION ASSESSMENT SURVEY ACTIVITIES

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Lymphatic filariasis (LF) is one of the world's most debilitating parasitic diseases; worldwide 120 million people are believed be infected with LF. In Haiti, 11 million people are at risk for LF and the disease remains a serious public health problem. The Ministry of Health aims to eliminate LF by 2020, through annual MDA with albendazole and diethylcarbamazine citrate (DEC). MDA started initially in the areas of highest endemicity called "red zones," where Immunochromatographic (ICT) test positivity among school children was 10-45% when mapped in 2000. Despite a number of critical challenges, including the low level of public health infrastructure, loss of donor funding, and the 2010 earthquake, which disrupted health services throughout the country, the Haiti NTD Program has scaled up progressively. Since 2012, the program has achieved 100% geographic coverage, with reported and surveyed coverage well above the 65% target for disease elimination. In order to assess the program's readiness for transmission assessment surveys (TAS), blood specimens were collected to detect W. bancrofti infection among persons aged ≥2 years. Twenty sentinel and spot check sites were chosen to represent 52 endemic districts; all sampled districts had completed at least 5 consecutive MDA rounds. At each site, approximately 500 samples were taken and tested using ICT. Results showed a significant reduction of filarial infection: 40% of the "red zones" had ICT prevalence of <2% and 48 endemic districts qualified for TAS to determine if transmission has been interrupted. The remaining 4 districts, which had ICT positivity between 31-39% when mapped, now have ICT positivity of 2.35-6.5%. While not yet eligible for TAS, these districts demonstrate a significant reduction in LF infection after 6 rounds of MDA. The Haiti NTD Program is optimistic that these districts will be eligible for TAS after two more MDA rounds. The program has made tremendous progress towards LF elimination in spite of multiple challenges. Haiti expects to reach the 2020 goal to eliminate LF; one of the greatest achievements for the poorest country in the Caribbean.

1815

A COMMUNITY STUDY OF THE IMPACT OF SEMIANNUAL ALBENDAZOLE ON LYMPHATIC FILARIASIS IN CENTRAL AFRICA

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Implementation of mass drug administration (MDA) with ivermectin plus albendazole (Alb) for lymphatic filariasis (LF) has been delayed in Central Africa, because ivermectin can induce serious adverse events in people with very high ***Loa loa*** microfilaremia . Albendazole has activity against ***Wuchereria bancrofti***, and it is safe for use in patients with loiasis. In 2012, the WHO recommended use of Alb MDA together with vector control to combat LF in areas with co-endemic loiasis. In September 2012, we started a 3-year community trial of semi-annual Alb alone on LF in a village with a population of 1055 in Madingou District in the Republic of Congo. Infection with W. bancrofti was diagnosed using the Binax Now Filariasis card test (ICT) for antigenaemia; persons with positive card tests were tested for microfilaraemia (Mf) by night blood smears. We are now presenting results from 24 months, and the final 36 month results will be presented at the meeting. Therapeutic coverage for the population aged >2 years was >80% in all four treatment rounds. Baseline results from 773 subjects aged ≥5 years old revealed a filarial antigenaemia rate of 17.3% and a Mf rate of 5.3%. Evaluation at 24 months (697 tested) showed dramatic reductions in ICT and Mf rates (6.3% and 1.4%, respectively). Mf counts were reduced by 86.3% from baseline values (geometric mean decrease from 202.2 to 27.7 mf/ml, P = 0.01), and total clearance of microfilaraemia was achieved in 71% of those individuals who were microfilaraemic in 2012. We are currently conducting a parallel trial in an area with higher baseline infection rates (31.6% for antigenemia and 11.8% for microfilaremia) in the Democratic Republic of Congo, and 12 month results from that study will be presented at the meeting. These studies will provide strong evidence regarding the use of semiannual MDA with Alb for elimination of LF in central Africa.

1816

HIGHLIGHTING HIGH LYMPHATIC FILARIASIS TRANSMISSION AREAS USING AN SMS REPORTING TOOL: A MORBIDITY MAPPING SURVEY IN DAR ES SALAAM

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The Tanzania Lymphatic Filariasis (LF) Elimination program was launched in 2000 with the aim of distributing Ivermectin and Albendazole to LF endemic populations. The program, which is now integrated with other neglected tropical diseases (NTDs), covers 107 districts, and LF prevalence is now showing signs of decreasing. There is however limited information on the number of people with clinical symptoms of LF i.e. lymphedema and hydrocele cases in Tanzania. This information is required for better planning of morbidity management in the country. The national NTD control program, in collaboration with Liverpool School of Tropical Medicine, conducted a morbidity survey in March 2015, in Temeke municipality, Dar es Salaam (population 1.5 million). The survey was conducted using a bespoke SMS reporting tool, MeasureSMS, which enabled survey data summaries to be viewed instantaneously through a web browser, and downloaded for further analysis. During the survey, community drug distributors visited every house in their designated catchment area, and recorded information on each lymphedema and hydrocele case identified using a paper form. These forms were collated by supervising front line health workers, who then reported the information by SMS to the local phone number allocated to the MeasureSMS tool. Progress was monitored by the national NTD control program throughout the survey using a webpage, and by directly downloading the reported SMS messages to check for missing reports. For data quality assurance, a random subset of reported cases were visited, and their conditions verified. A total of 2547 patients were identified; 987 of patients had lymphedema cases, 1743 had hydrocele, of these 183 had both conditions. Verification is ongoing and will be completed in May 2015. To date, 28 reported hydrocele cases have been verified, of which 88% (25/28) were confirmed to have the condition. Given the high reported morbidity burden in this area, it is now vital that services such as hydrocele surgery and lymphedema management are made accessible to the affected population.

1817

MICRO-MAPPING DISTRIBUTION POINTS, RISK POPULATIONS TO TREATMENT NUMBERS TO MAXIMIZE COVERAGE TO IMPACT OF MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN URBAN DAR ES SALAAM, TANZANIA

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The large rapidly growing urban centre of Dar-es-Salaam in Tanzania has a significant risk of lymphatic filariasis (LF), which is transmitted by Culex spp. mosquitoes that thrive in human domestic environments. Prior to the recent scale up of mass drug administration (MDA) to interrupt transmission, the overall LF infection rate was estimated to be 10%, with young adult males, and informal and peri-urban areas at a significantly higher risk of infection than other sub-groups and areas. In order to improve MDA coverage rates to those at greatest risk, this study aimed to map and examine the spatial patterns of the MDA distribution points, risk populations, and treatments numbers across the three districts of the city with an estimated 4.4 million population at risk. Environmental assessments were also conducted to identify characteristics of low coverage areas, and the specific factors that may place sub-groups at higher risk of infection, including potential breeding sites of Culex spp.. In Temeke district 73 distribution points reported an average coverage of 149%, in Kinondoni district 105 distribution points reported an average coverage of 105% and in Ilala district 63 distribution points reported an average coverage of 101%. Coverage throughout the three districts ranged from 9% (~10 times under) to over 1000% (~10 times over) at specific locations. These excessive coverage values are indicative of population movement both within and into the city. The great variation and distinct spatial patterns found at a micro level suggest that urban populations move dramatically within small geographical areas, as well as at different rates across the different areas of the city, thus making MDA implementation and reaching high coverage challenging. This study takes one important step forward to better understanding and being able to predict the problem areas in a highly dynamic and densely populated city. This is critical and will help the LF Programme to specifically target and increase human resources, training, social mobilisation to where they are needed most.

1818

MULTIPLE IVERMECTIN DOSES ARE MACROFILARICIDAL: IMPLICATIONS FOR THE ELIMINATION OF ONCHOCERCIASIS

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The predominant strategy for achieving the World Health Organization's control and elimination goals for human onchocerciasis is based on mass drug administration (MDA) with ivermectin. The feasibility of achieving these goals crucially depends on the long-term effects of multiple doses of ivermectin on the long-lived ***Onchocerca volvulus*** filarial nematode, which causes onchocerciasis. A single dose of ivermectin rapidly kills the microfilariae while also exerting a temporary sterilization (embryostatic) effect on the female adult worms (macrofilariae). Multiple doses of ivermectin are thought to cause a cumulative effect on macrofilariae manifesting either as permanent reductions in fecundity or a shortened life-span. These assumptions have been incorporated into mathematical models to support elimination efforts. Yet, for decades the nature of this presumed cumulative action has avoided rigorous investigation because scarce longitudinal data on macrofilariae have not been interrogated with suitably powerful analytical techniques. Here, we analyse data on the fecundity and vitality of female worms from the most comprehensive clinical trial of multiple doses of ivermectin treatment (comparing 3-monthly with annual treatment rounds administered during three years in Cameroon) using a recently developed state-of-the-art modelling framework. We demonstrate that multiple doses of ivermectin treatment have a substantial macrofilaricidal effect, even at the doses and frequencies used for routine MDA. We find no evidence that the anti-fecundity activity of ivermectin is enhanced by multiple treatments. We discuss our results in the context of the feasibility to eliminate onchocerciasis in the timeframes set by the global health community.

1819

MODELING EFFECTIVENESS OF DRUG ADMINISTRATION ON A POPULATION INFECTED WITH *SCHISTOSOMA MANSONI*

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Schistosomiasis is one of the most important public health problems that affect human populations, especially those living in poorer regions, with low socioeconomic environment, without adequate sanitation and clean water. WHO recommends treatment without prior diagnosis of the most vulnerable individuals such as school children and adults in endemic areas. In Brazil treatment is based on the infection prevalence. In areas of low and medium prevalence treatment is given only for positive individuals and in situations where the prevalence is greater than 50% treatment is directed to the entire population. The objective is to develop a non-linear mathematical model to evaluate the effectiveness of mass treatment of a population infected with ***S. mansoni***. The evolution of infected and non-infected persons with time was studied by building a 2-dimensional system of differential equations. We consider that a) children born without infection, b) the population is growing with a growth of logistical type, without having reached its maximum growth and c) once treated an individual will be free of infection unless it re-infects. The population was divided into two strata: P0 (t), which corresponds to the number of uninfected persons in year (t) and P1 (t) and those corresponding to the number of infected people during the year (t). It was possible to develop a mathematical model, consisting of a system of differential equations that has in its domain, single global attractor. The 2-dimensional system has a unique global attractor where the number of infected persons is non-zero, due to the re-infection effect. It is proved that the model got its equilibrium. The modeling results suggest that if the treatment is the only intervention in the population, that is, without additional Investments in better sanitation and health education programs, even treating the whole population or just those infected, the prevalence always will be around 10%. The model also suggests that in this way overtime prevalence tends to always keep this level with few possibilities of over spreading disease in the area.

1820

MODELLING EGG COUNTS TO COMPARE EGG REDUCTION RATES IN RANDOMIZED COMPARATIVE TRIALS OF TREATMENTS OF INTESTINAL SCHISTOSOMIASIS

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Treatment efficacy for schistosomiasis and soil-transmitted nematodes is customarily assessed as egg reduction rate (ERR) based on the difference in grouped means between pre- and post-treatment egg counts. This however does not allow comparison of treatment effects in controlled trials. Here, we assess whether Poisson-type modelling could be used to compare efficacy between treatments using a database from 24 trial enrolling 4,740 individuals infected with ***Schistosoma mansoni***, ***S. haematobium***, or ***S. japonicum***, and treated with 40-80 mg/kg praziquantel. Sensitivity analysis used a subset of 856 subjects in a trial comparing 40 vs 60 mg/kg praziquantel. Gender, age, treatment, species, follow-up duration and baseline egg counts were entered in all models as factors. The number of Kato-Katz smears (differences across studies) was used as a proportionality constant (offset) in the models to analyse the sum of counts. Alternative models (quasi-Poisson, negative binomial & zero-inflated Poisson) were fitted and compared. Random variation in risk between individuals and random variation between slides were also allowed for in a multi-level model with the same distributions. 1050 patients with 3577 measurements were analysed. 92% of the observed post-treatment data were zero. The Poisson model of the sum of egg counts and the quasi-Poisson model proved unsuited due to over-dispersion. The negative binomial and the zero-inflated model showed a better fit and predicted 92.07% and 91.98% of zeros. With the multilevel modelling strategy, the Poisson model performed best (95.05% of zero counts predicted). Praziquantel at 40mg/kg, 60mg/kg or 80mg/kg reduced the egg counts with no significant difference between treatments. Baseline counts were significant predictors of post-treatments counts. The sensitivity analysis showed similar results. This study shows that adequate modelling of the sum of post-treatment egg counts or raw egg counts could be useful for comparing treatment effects of anthelmintic treatment.

1821

COST-EFFECTIVENESS OF CHANGING PREVALENCE THRESHOLDS FOR MASS DRUG ADMINISTRATION AGAINST SCHISTOSOMIASIS TO SOIL-TRANSMITTED HELMINTHIASIS

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Current WHO guidelines on mass drug administration (MDA) against helminth infections do not recommend MDA for schistosomiasis and soil-transmitted helminthiasis (STH) below a prevalence of 10% and 20%, respectively, and use separate treatment guidelines for these two helminthiases. We evaluated the cost-effectiveness of changing prevalence thresholds for integrated, annual MDA to school-age children (SAC) and the entire community. We developed a dynamic, age-structured transmission and cost-effectiveness model that simulates integrated MDA programs for schistosomiasis and STH. We simulated a 5-year treatment program with praziquantel plus albendazole at 75% coverage among: (i) SAC alone or (ii) entire community (pre-SAC, SAC, and adults). We tested prevalence values of 1%, 5%, 10%, and 15%. We simulated settings of 10,000 pre-SAC, SAC, and adults with a range of helminth-specific intensities of infection and corresponding transmission parameters. Treatment costs for SAC and pre-SAC/adults were estimated at US$ 0.74 and US$ 1.74, respectively. The incremental cost-effectiveness ratio (ICER) was calculated in 2015 US$ per disability-adjusted life year (DALY) averted, comparing treatment strategies against current WHO recommendations of no treatment. We defined strategies as highly cost-effective if the ICER was less than the World Bank classification for a low-income country (GDP per capita: US$ 1,035). An integrated MDA program against schistosomiasis and STH was highly cost-effective in treating SAC alone at a prevalence of 5% (ICER: US$ 396/DALY averted), 10% (ICER: US$ 297/DALY averted), and 15% (ICER: US$ 285/DALY averted) compared to no treatment. Expanded community-wide coverage was highly cost-effective at a prevalence of 10% (ICER: US$ 902/DALY averted) and 15% (ICER: US$ 796/DALY averted) compared to treatment of SAC alone. Integrated annual MDA programs against schistosomiasis and STH may be highly cost-effective at prevalence thresholds lower than WHO guidelines. These results support re-evaluating global guidelines to consider lowered prevalence thresholds for integrated treatment.

1822

IMPROVING TREATMENT COVERAGE - MASS DRUG ADMINISTRATION AS SEEN FROM THE COMMUNITY HEALTH WORKERS' PERSPECTIVE

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National-scale schistosomiasis control programmes have now been implemented in many endemic countries using mass drug administration with praziquantel. Results from a validated treatment coverage survey after the first mass drug administration in Cote d'Ivoire (CDI) in 2013, highlighted that some districts were under-performing and below the World Health Organization target of treating at least 75% of school aged children. To understand the reasons behind this low coverage, structured interviews were conducted with community health workers (CHWs) in 12 villages in CDI (1 per village). Accounts given by the CHW were captured both on paper and on a digital audio recorder. Transcripts were then translated into English and the data analysed using NVIVO software. To ensure that the information portrayed by the CHW was a true reflection of their thoughts, the interviews were done on a one-to-one basis following free and informed consent. The interviews were led by an independent social scientist and allowed for privacy. Across all interviewees, four themes were repeatedly reported: negative perception by the communities of free medication; limited time for drug distribution due to insufficient human resources; loss of income during campaigns, and inadequate social mobilisation. Other issues were raised but are considered to be country-specific. Results from this survey highlight potential issues which, if addressed, can improve preventive chemotherapy (PCT) coverage. The presenter will discuss the sustainability, the cost and efficiency of proposed solutions. With the emphasis now being on eliminating schistosomiasis, findings from this study are applicable to many PCT control programmes, which will inform national guidelines to fine tune their strategy to ensure that the 2020 targets are met.

1823

THE USE OF A MARKOV TRANSITION PROBABILITY MODEL AS A PROGRAMMATIC TOOL FOR THE CONTROL OF SCHISTOSOMIASIS

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The World Health Organization (WHO), in partnership with the global community, have set ambitious targets for the control and/or elimination of schistosomiasis by 2020. To be able to achieve this it is essential that control programme managers can monitor the impact of treatment and identify areas that are not responding as expected. This will allow suitable adjustments to be made to maximise the impact of the intervention. With this objective, in 2014 a programme-friendly Markov transmission model was developed at the Schistosomiasis Control Initiative (SCI) in collaboration with the WHO to model the changes in the levels of schistosomiasis infection following successive rounds of treatment. The model was parameterized using data obtained from the monitoring and evaluation components of the large-scale deworming programmes in Uganda and Mali. This model is an extension of an earlier Markov model developed for soil-transmitted helminth infection by WHO. Results showed that the transition probabilities derived from baseline and year 1 data can be used to predict the prevalence of each infection intensity group in the following year. The capacity of the model to predict changes in infection prevalence following successive rounds of treatment was then tested on various data sets from 2 countries in sub-Saharan Africa. These data were not used to develop and validate the model, so that only new scenarios could be tested. The model was tested to observe whether it could provide an early warning of the treatment campaigns that failed to meet their targets. The performance of the model was also tested against different parasite species (***S. mansoni*** and ***S. haematobium***), location and underlying endemicity as well as host age. The outputs of the model post-validation will be discussed as well as its suitability as a user-friendly programmatic tool to facilitate the monitoring of schistosomiasis control programmes.

1824

HOTSPOTS OF *SCHISTOSOMA MANSONI* TRANSMISSION TEN YEARS INTO A MASS DRUG ADMINISTRATION PROGRAM

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The Schistosomiasis Control Initiative began mass drug administration (MDA) with praziquantel in Uganda in 2003 with great reductions in infection prevalence, intensities and associated morbidity. However, possible treatment failures have been recorded. In addition, theoretical models have indicated that cessation of MDA may result in higher egg counts than pre-intervention levels in certain individuals. Prevalence and intensity of infection by Kato-Katz were recorded for ***Schistosoma mansoni*** in children from three primary schools in Mayuge District, Uganda. Data were collected pre-, one-week-post- and four-weeks-post-praziquantel in 2004, 2005 and 2006, and pre-, one-week-post- and three-weeks-post-praziquantel in 2013 and pre-praziquantel in 2014. In 2004 and 2013 point-of-care circulating-cathodic-antigen tests (POC-CCA) were also performed. Mean egg reduction rates by three/four-weeks-post-praziquantel from 2004, 2005, 2006, and 2013 were 94.5%, 97.8%, 97.1%, and 95.0% respectively and cure rates 72.3%, 75.7%, 80.7% and 87.2%. Cure rates by POC-CCA in 2004 and 2013 were however significantly lower at 47.8% and 9.4% respectively. Infection prevalence and intensities in 2013 and 2014 were higher than at baseline. We indicate that drug efficacy measured by Kato-Katz has not reduced with MDA, but that cure rates measured by POC-CCA are lower. Although cure rates are often considered to be a less important criteria for morbidity than a reduction in egg output, it is imperative that the causes for the significant differences between Kato-Katz and POC-CCA results, and the higher infection intensities after ten years MDA, are elucidated so we can understand any risks of MDA strategies as well as measure their benefits. Results presented will include model outputs to predict if higher infection intensities observed ten years into the MDA programme are due to high transmission, reduced drug efficacy, or reductions in the development of protective immunity, as control programmes progress.

1825

CONQUERING SCHISTOSOMIASIS IN CHINA: THE FINAL CHAPTER

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Major control efforts over many decades have resulted in a substantial reduction in the prevalence of schistosomiasis japonica in the People's Republic of China, although pockets of new infection continue to arise, particularly in the mountainous areas of the south. As well, the completion of the Three Gorges Dam, which crosses the Yangtze River and other large irrigation projects underway, may have significant environmental and ecological impacts likely resulting in expansion of the habitats for the intermediate snail host ***Oncomelania hupensis*** in some areas, thereby increasing the risk of human and bovine infection, and resulting in potentially new challenging consequences for control. The epidemiological picture for China will be briefly summarised and the current effective control strategies highlighted. The situation in the Philippines will also be briefly outlined but the picture is far less encouraging as there is limited national funding for schistosomiasis control; since the termination of the World Bank Loan program for schistosomiasis control in the late 1990's, both schistosome prevalence and the associated morbidity have rebounded to former levels. Some results of recent surveillance studies we have undertaken in the Philippines will be described which indicate that schistosomiasis japonica is now far more prevalent, both in humans and bovines, than has been appreciated. Results of a large intervention trial we have completed in China - in the highly endemic Dongting Lake area downstream of the Three Gorges Dam, aimed at field-testing integrated strategies, including the use of a bovine transmission blocking vaccine, for schistosomiasis control, will be presented. The results of the trial in China will provide parameters for mathematical modelling of future control methods so as to define the long-term impact and cost-effectiveness of integrated control measures for both China and the Philippines. We believe that such an integrated approach, incorporating bovine vaccination, can lead to the future elimination of schistosomiasis from China.

1826

PLASMODIUM FALCIPARUM GENETIC CROSSES WITHOUT CHIMPANZEES

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Experimental genetic crosses in ***Plasmodium falciparum*** have played a pivotal role in the discovery of genes underlying several important phenotypic traits including drug resistance and host specificity. Previously, ***P. falciparum*** genetic crosses were carried out in splenectomized chimpanzees and in spite of the fact that three successful experimental crosses were generated, ethical and logistical concerns have now rendered this technology obsolete. Here we demonstrate a new model for ***P. falciparum*** experimental genetic crosses: a human hepatocyte-liver chimeric mouse (FRG huHep mouse) injected with human red blood cells (huRBCs) that allows for complete ***P. falciparum*** liver stage development and the transition of exo-erythrocytic merozoites to asexual blood stage development. Using this novel and versatile model, we have rapidly generated and analyzed three experimental crosses, including the identification of unique recombinant progeny from each cross. A chloroquine (CQ) sensitive transgenic strain, NF54HT-GFP–luc, was used as a parent in all three of our new experimental crosses and was crossed with three different strains: GB4 and 7G8, two strains used in a previous chimpanzee experimental cross, and NHP*, a recent field isolate from Southeast Asia. We characterized all crosses using microsatellite markers and further characterized progeny from the NF54HT-GFP–luc × NHP* cross using thousands of single nucleotide polymorphisms (SNPs) from next-generation sequence data and custom genotyping microarrays. These data were used to generate genetic maps and compute recombination rates across the genome. The high-density SNP-based linkage map will be used in conjunction with quantitative trait loci (QTL) mapping to assay a wide variety of quantifiable phenotypes such as drug responses, thus facilitating the study of complex genetic traits in ***P. falciparum***.

1827

DETERMINING THE MECHANISM OF ACTION OF THE IMIDAZOLOPIPERAZINES, A NOVEL CLASS OF ANTIMALARIALS

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Malaria, despite ongoing public health intervention worldwide, remains a tremendous burden globally. While progress has been made recently against malaria, the emergence of artemisinin resistance in ***Plasmodium falciparum*** has accelerated the search for both new and more effective antimalarial drug candidates. One of the most promising new compound classes in clinical development are the Imidazolopiperazines (IZPs), which are not only effective against all stages of ***Plasmodium*** infection, but also prevent transmission and dramatically reduce the rate of future infection. The lead compound of the IZPs, GNF156, is currently in phase IIb clinical trials. Unfortunately, the development of this class of compounds is hampered by a limited understanding of their mechanism of action. Previous ***in vitro*** evolution studies identified a previously uncharacterized gene, designated the ***P. falciparum*** cyclic amine resistance locus, as a potential target for the IZPs. More recent studies, however, have implicated several additional genes/pathways as potential targets of the IZPs. Given this ambiguity about the target and activity of the IZPs, this study will therefore first validate putative drug targets, then go on to examine both the localization and function of those validated targets. We have used the CRISPR/CAS9 system to validate several genes, including PfCARL, in which mutations convey resistance. In addition, we showed that these same mutations protected ***P. falciparum*** gametocytes from IZP activity and that PfCARL protein is localized to the endoplasmic reticulum/Golgi apparatus. Finally, we utilized Saccharomyces cerevisiae as a model to identify several pathways affected by IZP exposure, which were then functionally validated in ***P. falciparum***. By determining the mechanism of action of the IZPs, this study will significantly advance efforts against malaria, both by improving the utility of a promising new class of antimalarials and by identifying new targets for antimalarial intervention.

1828

DEEP SEQUENCING OF *ANOPHELES GAMBIAE* FROM NATURAL POPULATIONS SPANNING SUB-SAHARAN AFRICA - A RESOURCE FOR VECTOR CONTROL RESEARCH

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The ***Anopheles gambiae*** 1000 genomes project (Ag1000G) is using whole-genome sequencing to study genome variation in wild-caught mosquitoes from populations across Africa. In phase 1 of the project high throughput sequence data from 765 specimens from 8 countries have been generated. The sequence data have been used to discover over 52 million single nucleotide polymorphisms - on average 1 SNP every ~2 accessible bases - providing the first genome-wide view of the spectacular natural diversity within and between natural populations. These data have been publicly released and comprise the largest open access genomic resource available for any vector species. Here we provide an overview of the Ag1000G phase 1 data resource and initial results of population genetic analyses, focusing on applications to malaria epidemiology and vector control. First, analyses of population structure in the Ag1000G cohort reveal a complex mosaic, with incomplete speciation, geography, ecology and demography all influencing gene flow across the species' range. These data could be integrated into models informing vector control strategy, and we discuss steps towards this goal. Second, these data enable high-resolution analyses of loci under strong recent selection, including discovery of novel insecticide resistance mutations. We illustrate this with data from the voltage-gated sodium channel gene, and show that multiple independent haplotypes within both West and East African populations have been involved in selective sweeps, cutting across species barriers and major geographic features. We also present preliminary evidence for novel resistance mutations, and discuss how haplotype data from Ag1000G could be used to diagnose the origin and track the spread of insecticide resistance. Finally, we present data from the Ag1000G coastal Kenyan population, where all individuals exhibit long runs of homozygosity consistent with a severe recent population bottleneck. These data demonstrate that demographic events leave a strong genetic signal, and we discuss how genomic data could be used to provide feedback about the impact of vector control interventions.

1829

LARGE-SCALE SCANS FOR GENOMIC REGIONS UNDER POSITIVE SELECTION IN SOUTHEAST ASIAN *PLASMODIUM FALCIPARUM* REVEAL GENES OF PUBLIC HEALTH IMPORTANCE

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As artemisinin resistance in ***Plasmodium falciparum*** continues to simultaneously spread and emerge in new areas throughout Southeast Asia, plans are underway for targeted malaria elimination. Achieving regional elimination will require effective antimalarial drugs, an efficacious vaccine, or both; however, few drugs remain effective in Southeast Asia and currently there is no vaccine. The discovery of new drugs and vaccines is necessary if elimination is to be successful. To identify candidate drug and immune targets, we used three methods - two long-haplotype tests and population differentiation using FST - to locate regions of the ***P. falciparum*** genome that are potentially under positive directional selection among Southeast Asian parasites. Literature review of the previous use of these methods in malaria parasite genomic studies showed little overlap among genes, and differing sampling and genotyping methods prohibit meta-analyses. We genotyped approximately 30,000 loci in over 2,000 samples from 19 geographic sites across the Greater Mekong Subregion using a DNA microarray and whole-genome sequencing. Regions under selection within each geographic site were identified, and meta-analyses identified the most highly-selected genomic regions shared across all sites. Across all analyses there are 245 genes within the most highly-selected regions, including genes associated with drug resistance and encoding current vaccine antigens. Many genes with potential impact in public health are present including genes involved in drug export, vesicle transport, and red blood cell binding. The genes identified in our scans could be under selection for many reasons including association with drug resistance as well as human, vector, or environmental factors. Evidence of positive directional selection may be a useful criterion for selecting new vaccines and drugs for further development and testing. (Additional authors will be listed in the presentation).

1830

EXPLORING UNKNOWN GENES IN MALARIA PARASITES BY A ROBUST GENE REGULATORY SYSTEM

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Malaria is a major health problem in tropical and subtropical countries. The most severe form of malaria is caused by the parasite, ***Plasmodium falciparum***. A limited set of antimalarial drugs is used to treat the disease, but drug resistance is spreading at alarming rate. Hence, there is an urgent need for identification of novel anti-malarial drugs. A major challenge in new antimalarial drug development is identification and prioritization of potential targets for drug discovery. This is mainly due to lack of reliable functional genetics tools for investigating parasite genes. To address this need, we have developed a RNA-protein interaction system that facilitates robust and inducible regulation of target gene translation in eukaryotic organisms, including ***Plasmodium***. Here, we present the application of protein engineering approaches to integrate our synthetic control system with native ***Plasmodium*** translational regulatory mechanisms. In so doing, we have achieved substantially increased regulatory dynamic ranges (up to 200-fold) compared to a 5-10 fold range of the original system. As a proof-of-concept, we have successfully used this system to generate parasite lines in which various proteins of interest can be knocked down to reveal clear growth phenotypes. In addition, we have successfully combined this approach with CRISPR/Cas9 genome editing technology to rapidly validate essential genes. We are currently applying these genetic tools to broadly study parasite genes of unknown function. Since ~60% of the encoded ***P. falciparum*** genes have no known homologs in other eukaryotes, we believe that understanding their functions will aid in identifying potential targets for novel antimalarial drug development.

1831

PARTICIPATION OF INNATE IMMUNE CELLS IN MODULATING BLOOD FLUKE DEVELOPMENT

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Schistosomes develop in the blood stream of their hosts, where they feed on blood cells and produce eggs that cause severe pathology. Previous work has demonstrated that host immune function is required for normal schistosome development, as parasite development is dramatically impaired in some immunodeficient settings. Recently, we presented evidence that regulation of innate inflammatory responses is a requirement for schistosome development to proceed as administration of innate immune stimuli such as LPS, MSU and IL-4 restored parasite development in RAG-1-/ animals. - Here we review our recent progress toward identifying the innate immune cells that are implicated in these host-schistosome interactions. Following administration of various innate immune stimuli to RAG-1-/- mice, splenic neutrophils, monocytes, macrophages and non-classical macrophages were assessed for changes in expression of activation markers and Relm-α by flow cytometry. Administration of pro-inflammatory stimuli LPS and MSU resulted in significant decreases in surface expression of CD204 and CD206 in all cell populations, while treatment with IL-4 cytokine complex resulted in significant increase in CD204 and CD206. Poly I:C, a TLR 3 agonist that did not rescue parasite development, resulted in no significant changes in CD204 and CD206 expression. Taken together, these data suggest that while innate immune cells are likely important environmental factors for parasite development, we are unable to identify changes in innate cell phenotype that correlate with successful completion of the parasite's

developmental cycle. Ongoing studies are assessing changes in innate immune cell metabolism as a possible mechanism facilitating schistosome infection.

1832

THE ADIPOSE TISSUE DERIVED STEM CELLS (ASC) SHOWS IMMUNOREGULATION FUNCTIONS IN SCHISTOSOMIASIS INFLAMMATION ENVIRONMENT

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Numerous reports have shown that mesenchymal stem cells (MSC) appears to be important in therapeutics to regulate immune response invoked in settings such as tissue injury, transplantation and autoimmunity. In this study we investigated the effect of these cells on the immunoregulation activities of adipose tissue derived stem cells (ASC) in a ***Schistosoma mansoni*** experimental model. The ASC were isolated from C57BL/6 mice, expanded ***in vitro*** and phenotypic and functionally characterized. These cells were injected via the tail vein into C57BL/6 mice (n=6) two weeks after ***S. mansoni*** infection. The splenocytes were obtained and the ***in vitro*** proliferative response was determined after six days of culture of splenocytes in the presence or not of ASC, and stimulated with Schistosoma egg crude antigens (SEA) or adult worms (SWAP) or concanavalin A. After thirty days of ASC injection, ***in vitro*** stimulation of splenocytes in the presence of ASC led to a significant increase on proliferation. On the other hand, the addition of ASC to spleen cell cultures from infected mice that did not receive ASC ***in vivo*** showed a decreased proliferative response. Interestingly, a decrease in ***in vitro*** splenocyte proliferation was also observed after sixty days post-ASC injection, even in the presence of fresh ASC. CD4+ T cell activation was analyzed (CD25, CD69, CD28 and CTLA-4) after fifteen, thirty and sixty days post-ASC injection. We observed a decrease in activation of T CD4+ subpopulation, mainly after fifteen and thirty days post-ASC injection. In conclusion, in mice infected with ***S. mansoni***, the ASC were able to modulate the immune response during the early inflammatory process, in a time-dependent manner. These data emphasize the putative role of these cells as candidates for cellular therapy, including the control of inflammation caused by parasitic diseases.

1833

LOW TO MODERATE INTENSITY *SCHISTOSOMA MANSONI* INFECTIONS DO NOT ALTER PROTECTIVE ANTIBODY RESPONSES TO TETANUS TOXOID BOOSTER IMMUNIZATIONS

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Helminths such as schistosomes are remarkable in their ability to modulate host immune responses, which promotes their survival. Immunoregulation begins early in schistosome infection and has been characterized by hyporesponsiveness to parasite antigens and bystander antigens, suggesting schistosome infection at the time of immunizations could lower the protective response to vaccines. To investigate the impact that concurrent helminth infection might have on an individual's responses to vaccine antigens, we recruited participants from Kisumu Polytechnic College in western Kenya. Participants were enrolled, consented and screened for schistosomiasis and soil transmitted helminths (STHs) and assigned to groups based on helminth status. Tetanus toxoid (TT; single dose), hepatitis B (doses at 0, 1 and 6 months), and meningococcus A+C (doses at 0 and 2 months) vaccines were administered. Helminth infections were treated a week after the second hepatitis B immunization. Participants were bled at baseline, 2 months after the start of immunizations and 2 months after the final hepatitis B immunization to evaluate humoral and cellular immune responses to the vaccine antigens using both antibody and cytokine ELISAs. CD3+/CD4+/CD25high T regulatory (Treg) cell levels were also determined at each time point to assess their relationship to vaccine responses. As we have previously reported participants with schistosomiasis had significantly higher proportions of circulating Treg cells than uninfected controls at baseline. These levels increased 1 week after praziquantel treatment, but decreased to uninfected control levels by 7 months after treatment. Anti-TT antibody levels in both infected and uninfected groups were comparable at all measured time-points indicating that schistosome infection did not alter IgG recall responses to this immunization. Preliminary analyses of antibody responses to hepatitis B surface antigen indicate a somewhat lower responsiveness to the primary and booster immunizations in those with ***S. mansoni*** infection. Further analyses are ongoing.

1834

TARGETING THE BURDEN OF SCHISTOSOMIASIS IN MADAGASCAR: GYNAECOLOGICAL MANIFESTATIONS OF SCHISTOSOMIASIS IN AN AREA SCALING UP MASS DRUG ADMINISTRATION OF PRAZIQUANTEL

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Schistosoma haematobium infection frequently causes morbidity in the genital tract, and the disease may increase the risk of HIV transmission. Genital schistosomiasis in women of reproductive age living in endemic areas is under-diagnosed, and efforts are needed to better understand the pathophysiology and to provide control measures for the disease. A study was undertaken in order to determine the female genital morbidity caused by schistosomiasis, and to describe its histopathological correlates in an area of Madagascar prior to mass drug administration (MDA) of praziquantel. Women aged 15-35 years living in an ***S. haematobium***-endemic area in Madagascar underwent pelvic and colposcopic examinations. Women were grouped according to intensity of urinary ***S. haematobium*** infection, and small biopsies were taken from genital lesions and examined microscopically using standard haematoxylin and eosin stain. Updated mapping data of schistosomiasis was collected for the strategic planning, implementation and review of MDA of praziquantel in the same region of Madagascar. Genital lesions named sandy patches and rubbery papules were found in 41 of 118 women (35%). Rubbery papules only reported and described in this study contained an intense cellular immune reaction dominated by eosinophils, epithelial erosion, and viable ova. There was a significant decrease in the prevalence of rubbery papules with age, even after adjustment for urinary ova excretion. The sandy patches with grains showed moderate cellular immune reaction and ova (viable and/or calcified), and were most prevalent in women with low-intensity urinary ***S. haematobium*** infection. These findings in women living in an endemic area of Madagascar indicate a dynamic evolution of inflammatory genital lesions caused by ***S. haematobium*** and a clear need for preventive chemotherapy in school-age children as recommended by the World Health Organization. The authors present the results in light of

the current scale-up of MDA of praziquantel in Madagascar put in place by the Ministry of Health and the Ministry of Education, and supported by the Schistosomiasis Control Initiative (SCI).

1835

LENTIVIRUS HIV-1 INTEGRATES WIDELY THROUGHOUT THE GENOME OF THE HUMAN BLOOD FLUKE *SCHISTOSOMA MANSONI*

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Lentivirus-mediated gene expression manipulation offers advantages for functional genomics of the schistosome, allowing to establish informative lines of transgenic schistosomes and to elucidate gene functions of these pathogens. To investigate the ability of lentiviral vectors to integrate into the schistosomal genome, blood stream forms of the human schistosome, ***Schistosoma mansoni***, including schistosomules and adult female and male parasites were exposed to vesicular stomatitis virus glycoprotein pseudotyped virions of HIV-1. Reverse transcription of the lentiviral RNA genome proceeded, as confirmed by the presence of strong-stop and positive strand cDNAs, in turn confirming the internalization of the lentiviral nucleocapsid into the cytoplasm of schistosome cells. Integration of HIV provirus into chromosomes of the schistosomes followed, as established by anchored PCR targeting integrated provirus in the vicinity of endogenous mobile elements, by high throughput sequencing of lentivirus-anchored PCR products, and by whole genome sequences of the schistosomes. On a population scale, integrations of lentiviral provirus were widely distributed throughout the eight pairs of chromosomes of the schistosomes. Density of integrations was frequently > 10 events per 100 kilobase pair windows. Integration site preference was biased to non-coding regions of the schistosome genome, a preference dissimilar to that of HIV in human T cells. Integrations into exons and introns of protein-encoding loci were also seen. The ability of HIV-1 to complete biochemical processes essential for lentivirus replication was notable and unexpected given that schistosomes are phylogenetically far distant from primates and other mammals naturally infected by the genus Lentivirus.

1836

DEVELOPMENT OF A BIOSENSOR-BASED RAPID URINE TEST FOR DETECTION OF UROGENITAL SCHISTOSOMIASIS

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Schistosomiasis affects up to 300 million people with serious and diverse sequelae arising from infection. Diagnosis of urogenital schistosomiasis (***Schistosoma haematobium*** infection) relies on microscopic identification and enumeration of parasite eggs in urine, however this is time consuming and requires technical skill. A point-of care device for detection of ***S. haematobium*** would significantly reduce the time-to-result with the potential to improve patient care. In this work, we demonstrate the use of our established biosensor platform for bacterial identification for the detection of ***S. haematobium*** in urine. We developed capture and detector probes targeting ***S. haematobium*** rRNA, a robust egg lysis protocol and integration into our established platform. Using the biosensor assay, we demonstrated direct detection of ***S. haematobium*** eggs spiked in human urine at clinically relevant ranges with detection of as few as 30 eggs/ ml urine.

1837

AN ULTRA-SENSITIVE URINE-BASED ASSAY TARGETING THE CIRCULATING ANODIC ANTIGEN (CAA) FOR DIAGNOSIS OF UROGENITAL TO INTESTINAL SCHISTOSOMIASIS IN LOW-ENDEMIC AREAS

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The recent renewed interest in mapping, intensified control and elimination of schistosomiasis has put the need for highly accurate diagnostic assays high on the agenda. The well-studied schistosome antigen detection assays CCA- and CAA-ELISA have now been transformed into a Point-of-Care rapid test (POC-CCA) and an ultra-sensitive UCP lateral flow based strip assay (UCP-CAA), resp. The simple field applicable POC-CCA test may replace the Kato-Katz testing for prevalence mapping of community-level ***Schistosoma mansoni*** infections using a single drop of urine as well as evaluate quickly (within days) the efficacy of treatment. However this test shows variable sensitivity in the diagnosis of ***S. haematobium***. The recently developed UCP-CAA assay detects antigen in serum or urine of all schistosome species at sub-pg levels, a sensitivity allowing detection of single worm infections. The assay has been transformed into a robust, dry-reagent based test, and is currently used in several low-resource settings in Africa. In combination with optimized sampling schedules involving pooled urines this would allow rapid identification of ***foci*** of low prevalence/intensity ***S. haematobium*** and ***S. mansoni*** infections. Recent studies using the 2 ml urine dry reagent UCP-CAA format performed in low prevalence (<2%), ***S. haematobium*** settings (near elimination) show an over 10-fold increase in the prevalence of active schistosome infections. Similar results have been shown for ***S. japonicum*** settings in China and ***S. mansoni*** settings in Brasil and Africa. The UCP-CAA strip assay therefore is a valuable highly sensitive diagnostic tool, applicable for screening and case finding in very low prevalence areas, including pre-elimination settings.

1838

EVALUATING THE IMPACT OF PULSE OXIMETRY ON PNEUMONIA MORTALITY IN CHILDREN UNDER FIVE IN RESOURCE-POOR SETTINGS

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Despite available interventions, pneumonia is still responsible for an estimated 15% of childhood deaths worldwide. Recent research has shown that hypoxia and malnutrition are strong predictors of mortality in children hospitalized for pneumonia. This has led to increasing support for the use of oxygen therapy and monitoring oxygen saturation in the management of severe cases. It is estimated that 15% of children under five hospitalised for pneumonia have hypoxemia and that approximately 1.5 million children with severe pneumonia require oxygen treatment each year. We present a deterministic compartmental model to assess the impact of introducing pulse oximetry as a prognostic to distinguish severe from non-severe pneumonia in under-fives across 15 of the highest-burden countries. Incidence of pneumonia in each country was fitted to data on the mortality rates in each country. The impact of this improved prognostic was compared to the current mortality rates under Integrated Management of Childhood Illness (IMCI) guidelines. We found that, assuming access to supplemental oxygen, pulse oximetry has the potential to avert an estimated 200,000 deaths if implemented across the 15 countries, whereas IMCI was found to have a relatively small impact on mortality. Pulse oximetry can significantly increase the incidence of correctly-treated cases as well as reduce the incidence of incorrect

treatment with antibiotics. We also found pulse oximetry to be highly cost-effective, with median estimates ranging from $2.46 to $9.43 per DALY averted in 14 of the 15 countries analysed (US$). This combination of significant burden reduction and high cost-effectiveness makes pulse oximetry a promising candidate for an intervention against pneumonia in resource-poor settings.

1839

HOUSEHOLD-LEVEL RISK FACTORS FOR SECONDARY INFLUENZA-LIKE ILLNESS IN A RURAL AREA OF BANGLADESH

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Influenza-like illness (ILI) is an important public health concern in Bangladesh. Individuals with ILI are likely to transmit their illness to close contacts, including household members. Household-level risk factors for secondary ILI in a low-income, vulnerable population have not been characterized. We conducted secondary data analysis from participants in the control arm of a randomized controlled trial of handwashing and secondary ILI. We recruited index case-patients with ILI--fever (<5 years), fever, cough or sore throat (≥5years)--from health facilities, collected information on household factors, and conducted syndromic surveillance on all household contacts for ten days after resolution of index case-patients' symptoms. We conducted multivariable negative binomial regression to evaluate the effects of household factors on risk of secondary ILI among household contacts and accounting for clustering by household. A wealth index was created using principal components analysis of household assets. We analyzed data from 1491 household contacts of 184 index case-patients. Mean age was 26 years. Most (71%) reported that smoking occurred in their home, 27% shared a latrine with 1 other household, and 36% shared a latrine with >1 other household. A total of 114 participants had symptoms of ILI during follow-up. Smoking in the home (RRadj 1.91, 95% CI 1.23-2.96), and sharing a latrine with 1 other household (RRadj 2.07, 95% CI 1.18, 3.64) or >1 household (RRadj 3.08, 95% CI 1.81-5.23) compared to not sharing, were independently associated with increased risk of secondary ILI, even after adjustment for wealth. These results suggest that efforts to reduce tobacco use in homes could reduce respiratory illness in Bangladesh. The association between use of shared latrines and household ILI transmission had not previously been demonstrated. It is possible that certain respiratory pathogens could be transmitted effectively through contact with feces or contaminated fomites present in shared latrines; future research could investigate these mechanisms in more depth.

1840

RESPIRATORY VIRAL DETECTIONS DURING SYMPTOMATIC TO ASYMPTOMATIC PERIODS IN YOUNG ANDEAN CHILDREN

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Viruses are commonly detected in children with acute respiratory illnesses (ARI) and in asymptomatic children. Longitudinal studies of viral detections during asymptomatic periods surrounding ARI could facilitate interpretation of viral detections but are currently scant. Methods. We used reverse transcription-polymerase chain reaction (RT-PCR) to analyze respiratory samples from young Andean children for viruses during asymptomatic periods within 8-120 days of index ARI (cough or fever). We compared viral detections over time within children and explored RT-PCR cycle thresholds (CT) as surrogates for viral loads. Results. At least one respiratory virus was detected in 367 (43%) of 859 samples collected during asymptomatic periods, with more frequent detections in periods with rhinorrhea (49%) than those without (34%, p<0.001). Relative to index ARI with human rhinovirus (HRV), adenovirus (AdV), respiratory syncytial virus (RSV), and parainfluenza virus (PIV) detected, the same virus was also detected during 32%, 22%, 10%, and 3% of asymptomatic periods, respectively. RSV was only detected 8-30 days after index RSV ARI, whereas HRV and AdV were detected throughout asymptomatic periods. Human metapneumovirus (MPV) and influenza were rarely detected during asymptomatic periods (<3%). No significant differences were observed in the CT for HRV or AdV during asymptomatic periods relative to ARI. For RSV, CT were significantly lower during ARI relative to the asymptomatic period (p=0.03). Conclusions. These findings indicate that influenza, MPV, PIV, and RSV detections in children with ARI usually indicate a causal relationship. When HRV or AdV is detected during ARI, the causal relationship is less certain.

1841

DESCRIPTION OF THE POPULATION STRUCTURE TO GENETIC DIVERSITY OF *MYCOBACTERIUM TUBERCULOSIS* AMONG PATIENTS OF HAITIAN ORIGIN LIVING IN FLORIDA

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Individuals of Haitian origin have one of the highest tuberculosis (TB) case rates in Florida. However, literature on the propensity of certain M. tuberculosis strains to transmit faster than background rate in this population is limited. We investigated the genetic diversity, occurrence of strain emergence, and determinants of emergent strains among Haitians living in Florida. All culture confirmed TB cases reported to the Florida Department of Health (FDOH) are genotyped using spoligotyping and MIRU. We analyzed data on 482 TB cases of Haitian origin reported to FDOH from 2002 to 2014. We used the web application MIRU-VNTRplus for strain family and shared international types (SIT) assignment. We used SpolTools and the program DESTUS to asses strain emergence, recent transmission index and genetic diversity. In multivariate regression, we measured the socio-demographic and clinical determinants of strain emergence. Of the 482 strains, 136 (28.2%) belonged to the Haarlem lineage, 130 (27.0%) to the LAM lineage and 91 (18.8%) to the T lineage. SIT50, H3 sub-lineage, was the most common spolygotype with 59

strains (12.2%). Sixty two spoligotypes were identified as orphans. The different isolates were characterized into 114 genotypes with average cluster size of 4.2, recent transmission index and clustering rate of 0.76 and 0.24 respectively, and a virtual heterozygosity of 0.040. Adjusting for false discovery rate, The H3 sub-lineage, was identified as emergent (θ=46.84, p=2.44×10-04, q=0.02785), i.e. spreading faster than background transmission rate. A history of incarceration (AOR=7.63, CI: 1.17, 49.79; p=0.0337) was the strongest predictor of strain emergence after controlling for age, gender, years in the US, HIV status, initial drug resistance, site of disease, illicit drug and alcohol use, and homelessness. The H3 sub-lineage seems to be emerging in Haitian communities in Florida, largely driven by exogenous infection among individuals with a history of incarceration. Renewed efforts to track and treat TB patients of Haitian origin are warranted to curtail the spread of this Mtb clone.

1842

EXPANSION OF TYPE 1 CYTOKINE PRODUCING CD4+T CELLS OCCURING IN DISTINCT MEMORY COMPARTMENTS DELINEATES LATENT VS. ACTIVE TUBERCULOUS DISEASE

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Optimal CD4+ responses in the control of tuberculosis (TB) caused by Mycobacterium tuberculosis (Mtb) depends on antigen-specific, protective type 1 cytokine producing stable precursor memory pool. The quality of these responses in active pulmonary (PTB) versus latent TB (LTBI) is, however, poorly understood. We used multiparameter flow cytometry on whole blood from 9 LTBI and 10 PTB subjects to assess the nature of Mtb antigen (Ag) (ESAT6 and CFP10, E6/C10)-specific memory T cell responses. At baseline, PTB subjects compared to LTBI had increased frequencies of total CD4+IFN-γ+ cells (median frequency 0.4 % vs. 0.11, p= 0.01) as well as CD4+IFN-γ+TNF-α- (median frequency 0.35 % 0.1%, p= 0.004) and CD4+IFN-γ+IL-2- cells (median frequency 0.05 % 0.23%, p= 0.04). No differences were seen in the absolute frequencies of total CD4+TNF-α+ or CD4+IL-2+ cells. Interestingly, within CD4+IFN-γ+ cells, PTB subjects showed increased frequencies of cells with a naïve-like phenotype (CD45RA+CCR7+, NV, median frequency 9.2% vs. 2.7%, p=0.05) as well as stem cell like memory cells (CD45RA+CCR7+CD27+CD95+, TSCM) (median frequency 1.5% vs. 0.001%, p=0.02). The PTB group showed increased CD4+IFN-γ+TNF-α- cells frequencies in the TSCM compartment (median frequency 0.6% vs.% 0.001%,p=0.03) while increased CD4+ IFN-γ+IL-2- cells were seen in the TEMRAs (CD54RA+CCR7-) (median frequency 8.3% vs. 2.2 %, p= 0.01). On E6/C10 stimulation, however, LTBI subjects showed an increased net frequency of CD4+IFN-γ+ cells in Central memory (CD45RA-CCR7+, TCM) (median net frequency 8.5 % vs. -3.3%, p=0.05) and TSCM cells (median net frequency 0.01 % vs. -0.4%, p=0.03) compared to PTB. Finally, the LTBI group showed E6/C10-specific increased net frequencies of CD4+IL-2+ cells in the NV compartment (median net frequency 3.09% vs. 0.8% PTB, p=0.05). These data suggest that although increased baseline CD4+ IFN-γ+ responses in are seen in subjects with PTB, Mtb- antigen specific increased IFN-γ and IL-2 within precursor CD4+ memory cells (TCM and TSCM) as seen in LTBI subjects might be associated with infection containment.

1843

INCIDENCE TO RISK FACTORS FOR RESPIRATORY SYNCYTIAL VIRUS TO HUMAN METAPNEUMOVIRUS INFECTIONS AMONG CHILDREN IN THE REMOTE HIGHLANDS OF PERU

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The disease burden and risk factors for respiratory syncytial virus (RSV) and human metapneumovirus (MPV) infections among children living in remote, rural areas remain unclear. Methods: We conducted a prospective, household-based cohort study of children aged <3 years living in remote rural highland communities in San Marcos, Cajamarca, Peru. Acute respiratory illnesses (ARI), including lower respiratory tract infection (LRTI), were monitored through weekly household visits from March 2009 through September 2011. Nasal swabs collected during ARI/LRTI were tested for RSV, MPV, and other respiratory viruses using real-time RT-PCR. Incidence rates and rate ratios were calculated using mixed effects Poisson regression. Results: Among 892 enrolled children, incidence rates of RSV and MPV ARI were 30 and 17 episodes per 100 child-years, respectively. The proportions of RSV and MPV ARI that presented as LRTI were 12.5% and 8.9%, respectively. Clinic visits for ARI and hospitalizations were significantly more frequent (all p values <0.05) among children with RSV (clinic 41% and hospital 5.3%) and MPV ARI (38% and 3.5%) when compared with other viral infections (23% and 0.7%) and infections without virus detected (24% and 0.6%). In multivariable analysis, risk factors for RSV detection included younger age (RR 1.02, 95% CI: 1.00-1.03), the presence of a smoker in the house (RR 1.63, 95% CI: 1.12-2.38), residing at higher altitudes (RR 1.93, 95% CI: 1.25-3.00 for 2nd compared to 1st quartile residents; RR 1.98, 95% CI: 1.26-3.13 for 3rd compared to 1st quartile residents). Having an unemployed household head was significantly associated with MPV risk (RR 2.11, 95% CI: 1.12-4.01). Conclusion: In rural high altitude communities in Peru, childhood ARI due to RSV or MPV were common and associated with higher morbidity than ARI due to other viruses or with no viral detections. The risk factors identified in this study may be considered for interventional studies to control infections by these viruses among young children from developing countries.

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PNEUMONIA FORGOTTEN NO MORE: PNEUMONIA.ORG.AU

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In 1992, pneumonia was described as the "forgotten pandemic" and remains the most common cause of childhood mortality in the developing world. In 2006 at ISPPD5 in Alice Springs, Australia, a declaration was issued for the development of a global action plan against childhood pneumonia. In 2009, at a Tri-Nation (Indonesia, Papua New Guinea and Australia) meeting held in Sydney, Australia, it was resolved to establish a journal dedicated to pneumonia. The pneumonia journal (www.pneumonia.org.au) was launched in 2012. Since that time, pneumonia has strived to be internationally recognised as the premier, open access,

peer-reviewed Journal for publishing on the topic of pneumonia. In December 2014, a Gates Foundation Grant was awarded to the Journal to assist with the development of a sustainable business platform. The esteemed Editorial Board currently has over 40 international members and regional Deputy Editor-in-Chiefs appointed in Australiasia, North and South America, Asia, Africa and Europe. pneumonia is indexed by Google Scholar and the Directory of Open Access Journals. An application has been made for Scopus listing and systems are in place for an application to MEDLINE and PubMed. Recognising the importance of rapid dissemination of research, a continuous publication model is used. To date over 20 manuscripts have been published with as many in the pipeline. The Journal site has been accessed from over 150 countries across all continents and has had over 80,000 page views with greater than 65% of these visits being from new visitors. pneumonia is currently partnered with the Lung Foundation Australia, the Influenza Specialist Group (Australia) and the World Pneumonia Day Coalition to advocate for and increase the awareness of pneumonia appropriate to a broad range of communities globally. pneumonia is published by Griffith University ePress and is supported by the University and philanthropic sources. pneumonia has a major commitment to becoming the international forum for the distribution of quality, peer-reviewed content on pneumonia and raising the global profile of the disease - to be forgotten no more!!

1845

THE ROLE OF VASCULAR REMODELING IN THE IMMUNOPATHOLOGY OF *LEISHMANIA* INFECTION

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Cutaneous leishmaniasis is caused by intracellular protozoan parasites, and has a wide spectrum of clinical presentations mediated in large part by an exaggerated inflammatory response. Since vascular remodeling contributes to the magnitude of the inflammatory response, we hypothesized that manipulation of the cellular and molecular mediators promoting vascular remodeling might provide a novel approach to limit pathology in leishmaniasis. To address this, we first characterized the vascular remodeling that occurs in mice infected with ***Leishmania major***. We found dramatic changes in vessel morphology, structure, and number within leishmanial lesions, and using intravital imaging we visualized a significant increase in vascular permeability that is a consequence of vascular remodeling in leishmaniasis. At the peak of infection, VEGF-A and VEGFR-2 expression were upregulated and correspondingly endothelial cells were proliferating at the site of infection. To determine if these VEGF-mediated responses contributed to the magnitude of the pathology in the disease, we treated ***L. major*** infected mice with neutralizing antibodies directed against VEGFR-2. This treatment led to a decrease in the pathology seen in ***L. major*** infected mice compared with control animals. Taken together these data suggest that VEGF-A-mediated vascular remodeling occurs in leishmaniasis and that blockade of this remodeling can lessen the magnitude of the immunopathology associated with this disease.

1846

SKIN RESIDENT MEMORY CD4+ T CELLS ENHANCE PROTECTION AGAINST *LEISHMANIA MAJOR* INFECTION

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Leishmania-infected patients become refractory to reinfection following disease resolution, and following a primary infection with ***Leishmania major*** C57BL/6 mice are highly resistant to reinfection, yet effective immune protection has not been achieved by vaccines. While circulating Leishmania-specific T cells are known to play a critical role in immunity, the role of memory T cells present in peripheral tissues has not been explored. We have identified a population of Leishmania-specific memory CD4+ T cells present in the skin of mice that have resolved a primary infection with ***L. major***. These cells are present in skin distant from the primary infection, and produce IFNγ in response to ***L. major*** stimulation. Using skin grafts we show that they are resident in the skin, rather than recirculating. Following ***Leishmania*** challenge, these cells enhance the early recruitment of circulating T cells to the skin in a CXCR3 dependent manner, resulting in better control of the parasites. Our findings indicate that protective immunity to Leishmania, and thus the success of a vaccine, may depend on generating both circulating and skin-resident memory T cells.

1847

LEISHMANIA MAJOR INFECTION INDUCES TRANSMISSIBLE ALTERATIONS IN THE SKIN MICROBIOME

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Cutaneous leishmaniasis is a disease characterized by ulcerating skin lesions, the resolution of which requires an effective, but regulated, immune response that limits parasite growth without causing permanent tissue damage. Studies have shown that skin commensals enhance the immune response to ***L. major***, but how the microbiome changes during infection has not been characterized. Using analysis of the 16S ribosomal RNA gene, we found that infection with ***L. major*** causes a loss in bacterial diversity during the peak of infection, resulting in a dominance of the genus Staphylococcus. However once the lesions resolved, bacterial diversity returned to pre-infection levels. Alternatively, when mice developed more severely ulcerated lesions, the proportion of Streptococcus significantly increased on the lesions, demonstrating that ***L. major*** induced alterations in the skin microbiome can vary depending on the severity of disease. Strikingly, we observed similar changes in the microbiome on non-inflamed skin distant from the infection site, as well as in skin from cohoused naïve mice. These results indicate that the altered skin microbiome present at the site of infection is transmissible to uninfected skin despite having different immune responses at these sites. Current studies are directed at defining what factors mediate the changes in the skin microbiome during ***L. major*** infection and how this transmissible microbiome influences disease.

1848

FACTORS ASSOCIATED WITH PROTECTION OR SUSCEPTIBILITY TO DEVELOPMENTS OF DISEASE IN HOUSEHOLD CONTACTS OF CUTANEOUS LEISHMANIASIS PATIENTS

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The majority of infected subjects in an area of L.braziliensis transmission have an asymptomatic or subclinical (SC) infection. While the Th1 response is important for ***Leishmania*** killing, in patients with cutaneous leishmaniasis (CL) T cell activation and production of pro-inflammatory cytokines are associated with pathology. To better determine factors related to susceptibility and resistance to disease we have established a cohort of 308 household contacts (HC) of CL patients without previous or current evidence of CL in the endemic area of Corte de Pedra, Bahia, Brazil. Chemokine and cytokine levels and ***Leishmania*** skin test (LST) were performed at entry and in the years 2 and 4, and the association of the immune response with clinical outcome was analyzed. The immunologic response of HC who develop disease with those who remained disease free was also compared. Based on LST and, or ***in vitro*** IFN-γ production in cultures stimulated with SLA 114 (37%) subjects had evidence of exposure

to L.braziliensis. In 55 (17,8%) of them the LST was positive but in these, IFN-γ was only detected in 36 (65,4%). Moreover in 59 HC only IFN-γ production was observed. After 4 years of follow up CL was documented in 44 (14,2%) of 308 HC. The incidence of CL per 100 persons-years was 0,92 in those who only produce IFN-γ and 4.2 in those with positive LST. The major source of IFN-γ were NK and CD4+ T cells. Monocytes from subjects with SC infection produce less oxidative burst than cells of CL patients upon L. braziliensis infection, but they had great ability to kill ***Leishmania*** independent of ROS and NO production. Our data indicate that a positive LST do not protect against CL due to L. braziliensis. Control of the infection was associated with IFN-γ production and negative LST and greater ability of monocytes to kill leishmaniasis.

1849

DYNAMICS OF ANTIGEN SPECIFIC CD4+ MEMORY T CELL RESPONSE DURING ACTIVE CUTANEOUS LEISHMANIASIS TO SIX MONTHS AFTER TREATMENT

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Human cutaneous leishmaniasis is a devastating tropical disease that affects millions globally and for which no effective vaccine exists. While there are effective treatments, problems with toxicity, compliance and resistance are serious issues that point to the need for the development of new treatments and vaccines. The immunoregulatory environment in individuals actively infected with L. braziliensis is a key factor associated with development or resolution of disease. Our understanding of the balance between memory cell compartments in active disease and following cure is not well understood. Thus, we undertook studies designed to investigate the balance between antigen specific CD4+ T cell memory subpopulations during active disease and six months after treatment. We report findings of CD4+ T cells from three compartments: central memory (CM (CD45RA-,CCR7+)), effector memory (EM (CD45RA-,CCR7-)) and naïve (CD45RA+,CCR7+) as defined using multiparameter flow cytometry in a group of 13 CL patients following overnight cultures in media, with Soluble ***Leishmania*** Antigen (SLA) or polyclonal stimuli (anti-CD3/CD28). Our results indicate that in active disease and 6 months following treatment, the distribution of CD4+ subpopulations did not change and was on average; 30% CM, 45% EM and 20% naïve. We demonstrated significant increases in the frequency of cells expressing CD69, and the inflammatory cytokines; IFN-gamma and TNF-alpha, within the EM cell population following SLA stimulation. Interestingly, IL-10 producing cells where were not biased to the EM population and distributed between EM and CM. Six months post treatment, cultures stimulated with SLA showed a decrease in EM cells expressing CD69, IL-17 and IL-10, while IFN-gamma, TNF-alpha and granzyme A producing EM T cells were maintained at similar levels or increased to that seen in active disease. There was a striking maintenance of the overall subpopulation balance during active disease and six months following treatment. These findings could help understand the balance between CD4+ T cell subpopulations and their maintenance over time after disease resolution.

1850

DEVELOPMENT OF A CANDIDATE LEISHMANIASIS VACCINE LEISH-F3 + GLA-SE

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Key antigens of ***Leishmania*** species identified in the context of host responses in ***Leishmania***-exposed individuals were prioritized for the development of a subunit vaccine against visceral leishmaniasis (VL). Two ***Leishmania*** proteins – nucleoside hydrolase (NH) and a sterol 24-c-methyltransferase (SMT), each of which are protective in animal models of VL when properly adjuvanted – were produced as a single recombinant fusion protein NS (LEISH-F3) for ease of antigen production and broad coverage of a heterogeneous MHC population. When formulated with GLA-SE, a TLR4 TH1-promoting adjuvant, the LEISH-F3 polyprotein induced potent protection against both L. donovani and L. infantum in mice. A robust immune response to each component of the vaccine with polyfunctional CD4 TH1 cell responses, characterized by production of antigen-specific IFN, TNF, and IL-2, and low levels of IL-5 and IL-10, was induced in immunized mice. Based on the sum of pre-clinical data, we prepared GMP materials and performed a Phase 1 clinical study with LEISH-F3 + GLA-SE in healthy, uninfected adults in the United States. The vaccine candidate was shown to be safe and induced a strong antigen-specific immune response, as evidenced by cytokine and immunoglobulin subclass data. These data provide a strong rationale for additional trials in ***Leishmania***-endemic countries.

1851

EVALUATION OF *TRYPANOSOMA CRUZI*-SPECIFIC HUMORAL TO T CELL RESPONSES AFTER THERAPY WITH BENZNIDAZOLE IN CHILDREN IN THE EARLY CHRONIC PHASE OF CHAGAS DISEASE

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We have previously shown that children in the indeterminate phase of Chagas disease have polifunctional T cells specific for ***T. cruzi***, while the overall T cell compartment already shows signs of persistent antigen stimulation. In this study, the effect of treatment with benznidazole on humoral and cellular T cell responses specific for ***T. cruzi***, as well as on the phenotype of the overall T cell compartment was evaluated in 21 ***T. cruzi***-infected children. Treatment with benznidazole induced an early decline in the total frequencies of activated (HLA-DR+) and highly differentiated memory (CD45RA-CD27-CD28-) and effector (CD45RA+CCR7-CD62L-) T cells along with a decrease in ***T. cruzi***-specific IFN-γ- and IL-2-producing T cells. A significant fall in antibody levels specific for ***T. cruzi***, as measured by the conventional serological tests, ELISA, immunofluorescence and hemagglutination, after 36 months following treatment with benznidazole was found. The non-conventional multiplex assay also detected a significant impact on humoral responses by 6 months post-treatment. The early decline in antibody levels found in children is in contrast with that observed in adults in which the fall in antibody levels is very slow. Our results show a significant impact on humoral and T cell responses after treatment with benznidazole which might be indicative of a reduction in parasite load.

1852

SMALL RNA SIGNATURES OF A MIDGUT ESCAPE BARRIER IN *CULEX QUINQUEFASCIATUS* MOSQUITOES INFECTED BY WEST NILE VIRUS

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Small RNA regulatory pathways (SRRPs) are important regulators of endogenous pre- and post-transcriptional control in metazoans. The role of SRRPs such as the exogenous small-interfering RNA (exo-siRNA), PIWI-interacting RNA (piRNA) and microRNA (miRNA) pathways in controlling arbovirus infection in insects has been the subject of intense study in recent years. The role of any of these pathways in vector competence, however, is poorly understood. To evaluate how SRRPs may contribute to vector competence, we identified ***Cx. quinquefasciatus*** mosquitoes that were susceptible to WNV but did not permit virus escape from the midgut into peripheral compartments after 14 days of extrinsic incubation. We then compared sRNA expression from these "elite controllers" of WNV to those that permitted virus dissemination. In particular we compared the intensity of sRNA targeting of the WNV genome, differential production of virus-derived small interfering RNAs (viRNAs) reads, and miRNA expression among these two groups of mosquitoes. We found that viRNAs differentially target 1,519 positions of the WNV genome, and that 177 reads are differentially produced between groups. The most significantly differentially targeted positions and expressed viRNA reads targeted conserved structures in the 3'utr of the WNV genome. Similar results were obtained from analysis of sRNA reads corresponding to a larger size class (24-30nt in length). 1076 WNV genome positions were differentially targeted, but only 3 sRNA reads were differentially produced. These reads also tended to target conserved structures in the WNV 3'utr. A miRNA signature associated with the elite controller phenotype was also detected. These results indicate that SRRPs contribute to the formation of a midgut escape barrier in ***Cx. quinquefasciatus*** mosquitoes and provide novel insights into the molecular mechanisms that underpin vector competence in the Culex-WNV system.

1853

AVIAN INNATE IMMUNE RESPONSES TO WEST NILE VIRUS INFECTION

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Since the first reports of West Nile virus (WNV) in the U.S. in 1999, WNV has been maintained in North America in an enzootic cycle between highly susceptible passerine birds and mosquitoes. However, the mortality of passerine birds in response to WNV infection and the capacity for viral replication vary dramatically between viral strains and avian species. For example, American crows (AMCRs) inoculated with the North American strain of WNV (NY99) manifest high viremias with concomitantly high mortality rates. However, AMCRs inoculated with an African strain of WNV (KEN98) exhibit significantly lower viremias and mortality rates. In contrast, the viremias and mortality rates of NY99 and KEN98 are not significantly different in inoculated house sparrows (HOSPs). In order to understand these viral- and host-specific differences in WNV replication and mortality, the expression levels of avian interferon genes and other innate immune factors potentially involved in regulating WNV replication were measured by qRT-PCR in samples taken from AMCRs inoculated with NY99 or KEN98. To further probe the avian interferon response to WNV infection, AMCRs and HOSPs were inoculated with a WNV mutant lacking 2'-methyltransferase activity (NS5-E218A) that has been shown to be susceptible to restriction by the interferon-stimulated gene family IFIT in mammals. Replication of the NS5-E218A mutant virus was severely retarded in both avian species, suggesting that AMCR and HOSP antiviral responses are robust. However, ***in vitro*** WNV infectivity assays in cells overexpressing avian IFIT genes show that the avian IFIT homolog does not restrict the NS5-E218A mutant virus. Further studies of infected birds, including sequencing of the avian transcriptome, will help to define the passerine innate immune response to WNV infection.

1854

DECONSTRUCTING THE NEUTRALIZING ANTIBODY RESPONSE ELICITED BY A WEST NILE VIRUS VACCINE CANDIDATE

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There is no licensed vaccine or therapy to protect humans against West Nile virus (WNV), a mosquito-borne encephalitic flavivirus. Although neutralizing antibodies (NAbs) are a correlate of protection for existing flavivirus vaccines, the epitopes recognized by anti-WNV NAbs are unknown. The main target of flavivirus NAbs is the E protein, which contains three structural domains (DI, DII, DIII). Two recent studies showed that, despite low sequence conservation, a flexible hinge between DI and DII is a primary target of NAbs against multiple DENV serotypes, raising the possibility that this region is a functionally important target of flavivirus NAbs. To explore the role of the hinge as a target of WNV NAbs, we created a library of E variants containing 31 mutations in surface-accessible hinge residues. Reporter virus particles incorporating each variant were tested for infectivity and characterized using a panel of well-characterized monoclonal antibodies (mAbs) to identify confounding changes in virion conformational integrity. An unexpectedly large fraction (18/31) of the hinge region variants displayed altered sensitivity to mAbs targeting poorly accessible distal epitopes. Preliminary characterization of one of these variants suggests that the overall changes in antigenicity were due to altered virion conformational dynamics, which allows the transient exposure of otherwise inaccessible epitopes. The remaining hinge mutations that did not alter overall antigenicity were tested for neutralization sensitivity to sera from recipients of a candidate WNV vaccine. We identified a combination of two mutations at proximal residues on the E protein that reduced neutralization potency of sera from multiple donors, suggesting a significant contribution of NAbs against the hinge to the overall neutralizing activity of polyclonal sera. Overall, these results will provide insight into the importance of DI-DII hinge in eliciting protective NAb responses against flaviviruses, and in regulating virion conformational changes.

1855

INFECTION, DISSEMINATION TO TRANSMISSION OF JAPANESE ENCEPHALITIS VIRUS IN NORTH AMERICAN *CULEX* MOSQUITOES

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Japanese encephalitis virus (JEV) is a flavivirus transmitted by ***Culex*** species mosquitoes in Asia and sporadically Australia and Western Pacific. Because of the variation in surveillance and diagnostic methods, the true global incidence of Japanese encephalitis virus remains unknown with an estimated annual case number greater than 55,000. The geographic distribution of JEV has changed significantly in the last 30 years as JEV has expanded its geographic range. More recently, the detection of JEV viral nucleic acids was reported in ***Cx. pipiens***in Italy and subsequently raised the concern of the emergence of JEV in Europe. With the presence of several species of ***Culex*** mosquitoes that are vectors in JEV endemic areas, susceptible vertebrate hosts present and no vaccination program implemented, the continuous threat of introduction of JEV into

North America remains a human and veterinary public health concern. Studies to determine the vector competence of North American ***Culex*** populations for the transmission of JEV are currently lacking. In this study, selected ***Culex*** species mosquitoes were orally challenged with JEV and characterized for viral infection and dissemination by the detection of infectious virions of JEV. Transmission of JEV was also evaluated by RT-PCR of viral nucleic acids in mosquito saliva.

1856

POTENTIAL FOR CO-INFECTION OF A NOVEL MOSQUITO-SPECIFIC FLAVIVIRUS TO BLOCK HUMAN FLAVIVIRAL DISEASE AGENT INFECTION AND/OR TRANSMISSION IN MOSQUITOES

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Nhumirim virus (NHUV) represents an example of a unique subset of apparently insect-specific viruses that phylogenetically affiliate with dual-host mosquito-borne flaviviruses. Previous ***in vitro*** co-infection experiments indicated that prior or concurrent infection of mosquito cells with NHUV resulted in a 10,000-fold reduction in viral production of West Nile virus (WNV). In order to assess the potential for ***in vivo*** blockage of medically important flaviviruses in mosquitoes by NHUV, ***Cx. quinquefasciatus*** mosquitoes were intrathoracically inoculated with approximately 1,000 PFU of NHUV and 100 PFU of WNV or solely with WNV as a control. Mosquitoes were allowed to extrinsically incubate the viruses for 3, 5, 7 or 9 days prior to saliva collection by capillary tubes and subsequent trituration of the bodies for assessment of transmissibility and relative replication of the viruses, respectively. Results demonstrated 100% WNV infection of mosquitoes at all four time points in the control groups. Similarly, NHUV and WNV experimental co-infection groups exhibited 100% WNV infection at dpi 5,7 and 9 with 90% infection rates in the co-inoculated group at dpi 3. No significant differences in the mean viral titer from triturated bodies were observed at any time point between the dual and control WNV inoculation groups. Although there was an observed trend for the WNV titers in saliva to be higher in the control group compared to the dual infection group, this difference was not significant. Nevertheless, the proportion of mosquitoes (≥ 21 mosquitoes/ sampling) that were capable of transmitting WNV was significantly lower for the WNV/NHUV group than the WNV control at dpi 3, 7 and 9. By dpi 9, a 40% reduction in transmissibility in mosquitoes from the dual inoculation group was observed compared to the WNV control. These data indicate the potential that infection of ***Culex*** vectors with NHUV could serve as a barrier for efficient transmissibility of flaviviruses associated with human morbidity/mortality.

1857

A THREE-DIMENSIONAL, MULTICELLULAR LIVER MODEL TO STUDY TRANSCRIPTIONAL ACTIVATION OF INNATE IMMUNE RESPONSE TO HEPATIC METABOLISM FOLLOWING INFECTION WITH WILD-TYPE OR ATTENUATED YELLOW FEVER VIRUS

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Most commercial Yellow Fever (YF) vaccines are based on the attenuated YF17D-204 strain derived by serial cultivation of wild-type YF Asibi virus. YF virus is highly hepatotropic ***in vivo*** and can replicate ***in vitro*** in human hepatocytes. No restriction of YF17D-204 replication, compared to YF Asibi, was observed in these cells. However, other liver cells populations like endothelial and macrophage cells were previously shown to be affected by YF virus infection, ***in vivo*** and ***in vitro***, and could play a role in infection control. We have used a cell-spheroid liver model (InSphero™) including major human liver primary cells (hepatocytes, Kupffer and liver endothelial cells) to study transcriptional regulations following infection with YF viruses. Spheroids composed of primary hepatocytes only were used as controls. About 400 genes related to innate immunity and hepatic metabolism were analyzed by PCR array at different times post-infection. No difference in transcriptional regulation of apoptosis-related genes (TNF-α, FASL, TRADD) was seen between the 2 viruses. YF17D-204 infection was shown to induce earlier and stronger activation than YFAsibi infection (p-value<0.05) of type III (IFNλ1 and IFNλ2) and type I β (but not α) interferons, as well as a larger panel of ISGs. YF17D-204 infection also generated distinct JAK/STAT pathway regulations in liver spheroids and in hepatospheroids, suggesting that liver spheroids may respond hierarchically to infection, the non-parenchymal cells response shaping the hepatocytes one. Whole transcriptome analysis was also carried out by RNA-Seq at 11h post-infection. Analysis of upstream transcription regulators (Ingenuity® Pathway Analysis) confirmed the establishment of a strong, early antiviral profile after YF17D-204 infection (z-scores: 2.7-5.0, p-values<10-10) and predicted transcription regulation of liver metabolism-related genes after YFAsibi infection(|z-score|≥2.3, p-value<10-3). Ongoing RNA-Seq studies at later infection times will provide more information on the ability of this liver model to mimic metabolic changes following wt Asibi infection ***in vivo***.

1858

COMPREHENSIVE MUTAGENESIS OF HCV E1/E2 ENVELOPE TO EPITOPE MAP ANTI-ENV ANTIBODIES TO FUNCTIONAL RESIDUES CRITICAL FOR HCV INFECTIVITY

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To obtain epitope maps for anti-HCV Envelope (E1/E2) monoclonal antibodies (MAbs), we individually mutated 552 residues of HCV (H77 strain) E1/E2 to alanine. Each mutant was expressed in human cells and analyzed for its effects on MAb reactivity. This 'Shotgun Mutagenesis' approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as the oligomeric and glycosylated HCV Envelope protein. This approach identified critical amino acids required for the binding of dozens of MAbs, and has also been used to propose E2 disulfide bond cysteine pairs that are not resolved by the available E2 crystal structures. This approach has helped define the range of immunodominant structures on HCV E1/E2 and identify novel neutralizing antibody epitopes that can be used for the development of improved therapeutics, diagnostics, and vaccine candidates. In addition, to identify residues important for HCV infectivity we produced infectious HCV pseudoviruses from each mutant Env clone in the library. These pseudoviruses were used to evaluate each Env clone for infectivity on target cells. This allowed us to identify critical E1/E2 residues whose mutation eliminated HCV infectivity, identifying crucial HCV E1/E2 structural components that enable HCV infectivity.

1859

UNDERSTANDING THE DISTRIBUTION OF CHOLERA BURDEN TO RISK IN AFRICA: SPATIAL MODELING TO GUIDE PREVENTION TO CONTROL EFFORTS

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There are an estimated 2.8 million cholera cases per year globally, but the majority of these cases are not detected or reported. Because of inadequate surveillance and reporting the global distribution of cholera risk and its public health burden are poorly known. Previous attempts to determine the burden of cholera globally, and specifically in Africa, have

relied on a limited number of case studies that do not capture the broad range of settings and environmental conditions where cholera occurs. Here we assemble a large database of cholera surveillance and incidence reports from a variety of government, scientific, and non-governmental agency sources, with a particular focus on sub-Saharan Africa where a majority of cholera cases have been reported in the past several decades but where the distribution of risk and burden is still poorly understood. We develop a hierarchical Bayesian modelling framework to estimate the cholera incidence and risk at a 5km scale across the entire African Continent. Our method allows us to synthesize environmental and socioeconomic variables with cholera incidence reports at different spatial and temporal scales and estimate cholera incidence at a high spatial resolution. Preliminary results for 10 countries in West Africa indicate a cumulative cholera incidence rate of 3.3 per 100,000 since 2009 with local incidence rates as high as nearly 8,000 per 100,000. Over four million people in these countries live in area with an annual incidence rate >100 per 100,000. The resulting maps can be used to identify areas of high or low incidence (and risk) at finer spatial scales than the country-level, such as the province, district, or city-level. Work is ongoing to expand the model globally and incorporate additional explanatory variables. Improving our understanding of the spatial distribution of cholera and associating incidence with climate, environmental and socioeconomic factors will provide a basis for planning public health preventions to reduce cholera transmission.

1860

LABORATORY EVALUATION OF IMMUNOCHROMATOGRAPHIC RAPID DIAGNOSTIC TESTS FOR CHOLERA IN HAITI

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Rapid diagnostic tests (RDTs) for cholera play a key role in responding to cholera epidemics. Because microbiological culture capacity is often non-existent in resource-poor settings where most cholera epidemics occurs, RDTs can allow early identification of ***Vibrio cholerae*** as the causative agent in outbreaks, facilitating the deployment of rapid public health measures. Despite their importance, evidence on the performance characteristics of the many available RDTs for cholera is scarce. This study evaluated the performance characteristics of two cholera RDTs: Span Diagnostic's Crystal VC O1/O139 RDT, and Standard Diagnostic's SD Bioline Cholera O1/O139 RDT, in a regional laboratory in Haiti, where a protracted cholera epidemic is now in its fifth year. We retrospectively reviewed de-identified records from May 2014 to January 2015 of a laboratory-based surveillance program for ***Vibrio cholerae*** at Hôpital Saint-Nicolas in Saint-Marc, Haiti. We analyzed 189 Crystal VC RDT results and 233 SD Bioline RDT results and compared these to culture results as the gold standard. Of 236 cultures, 125 were positive for O1, 15 were positive for non-O1, and 96 were negative for ***V. cholerae***. Crystal VC demonstrated a sensitivity of 98.8% (95% CI: 96.5%-100%) and specificity of 72.1% (95% CI: 63.5%-80.7%). Notably, we documented a high percentage of ***V. cholerae*** O139 positive results, with 39% of all tests showing positive results for O139. Given the absence of ***V. cholerae*** O139 in Haiti, these are likely a result of cross-reactivity. SD Bioline demonstrated a sensitivity of 77.7% (95% CI: 70.3%-85.1%) and specificity of 93.7% (95% CI: 89.2%-98.2%). SD Bioline did not demonstrate the O139 cross-reactivity seen with Crystal VC, as no O139 positive results were documented. This study highlights the suboptimal specificity of Crystal VC, and brings attention to a high rate of O139 cross reactivity, a characteristic that may adversely impact its interpretation in the field. Additionally, it is the first study to document the performance characteristics of the SD Bioline RDT that unlike other available cholera RDTs, shows high specificity but low sensitivity.

1861

'O ANTIGENIC' POLYSACCHARIDE SPECIFIC MEMORY B CELL RESPONSES IN YOUNG CHILDREN, OLDER CHILDREN TO ADULTS INFECTED WITH *VIBRIO CHOLERAE* O1 OGAWA IN BANGLADESH

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Infection with ***Vibrio cholerae*** O1 causes the cholera, which can be life threatening if quick and proper treatment is not given especially in severe dehydrating cases. It has been considered that long term protection against cholera may be mediated by anamnestic memory B cell responses after natural infection with ***V. cholerae*** O1. Protection against cholera is serogroup specific, and serogrouping is defined by the O-specific polysaccharide (OSP) of lipopolysaccharide (LPS). We determined Ogawa O-specific polysaccharide (OSP) and Lipopolysaccharide (LPS) specific memory B cell responses as well as other immune responses in Bangladesh in patients with cholera. They included young children (2 to 5 years of age; n= 11), older children (6 to 17 years of age; n=21) and adults (18 to 55 years of age; n=28). Patients were studied after hospitalization (day 2) as well as at convalescence at day 7, day 30, day 90 and day 180. We observed vibriocidal antibody responses that lasted up to the follow up periods ($P<0.05$) in both older children and adults while responses decreased to the baseline levels by 3 months in younger children. Patients of all age groups developed OSP and LPS specific plasma IgA, IgG and IgM antibody responses within 7 days ($P<0.01$) of onset of disease which decreased to baseline values at convalescence at 6 months; adults showed higher OSP and LPS specific antibody responses than children. We also found that in all age groups OSP specific IgA and IgG memory B cell responses developed within 30 days of illness, which persisted throughout the follow-up period. In addition, they also developed LPS specific IgA memory B cell by day 30 ($P <0.02$). All age groups had comparable memory B cell responses to OSP and LPS while children had higher OSP IgA memory B cell responses than adults ($P <0.05$) on days 30 and 180. We describe for the first time OSP-specific memory B cell responses in all age groups of cholera patients and show that the responses persist over the study period, which suggest that OSP-specific memory B cell may play a role in mediating long term immunity against cholera.

1862

CHARACTERIZATION OF DUODENAL MUCOSAL ASSOCIATED INVARIANT T (MAIT) CELLS IN *VIBRIO CHOLERAE* INFECTION

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Cholera is an acute dehydrating diarrheal disease caused by ***Vibrio cholerae*** O1 infection. The mechanisms of protection against cholera are not well known. MAIT cells are recently described innate-like T cells with adaptive capacity. We have previously shown that circulating MAIT cells are activated during cholera and associated with ***V. cholerae*** LPS-specific antibody responses. The objective of this study was to characterize MAIT

cells in the intestinal mucosa during cholera. We collected peripheral blood and duodenal biopsy specimens by endoscopy from adults with confirmed ***V. cholerae*** O1 infection at days 2 and 30 after onset of disease. Preliminary data using multispectral fluorescence microscopy suggest that MAIT cells are mostly present in the crypt of the duodenal lamina propria and are more abundant at day 2 post illness compared with day 30. Using flow cytometry, we found that a greater percentage of duodenal MAIT cells are activated (CD38+) than those in the periphery at both time points. Stool alpha-1-antitrypsin, a marker of intestinal permeability, was correlated with a decrease in % of activated MAITs between days 2 and 30. More complete results, including analysis of corresponding LPS-specific antibody responses, will be available at time of presentation.

1863

SMALL INTESTINE BACTERIAL OVERGROWTH IN BANGLADESHI TWO YEAR OLDS

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Small intestine bacterial overgrowth (SIBO) has been associated with adverse nutritional outcomes, intestinal inflammation, and increased intestinal permeability when overgrowth is secondary to preexisting intestinal pathology. Children living in unsanitary conditions have high rates of SIBO despite having no predisposing condition. Limited information is known about the detrimental effects of SIBO when overgrowth develops in the setting of poor sanitation. We tested for SIBO via glucose hydrogen breath testing at 2 years of age in a cohort of 90 Bangladeshi infants. Children had C-reactive protein (CRP), endotoxin core- antibody (endocab), fecal Reg 1β, Lactulose:Mannitol ratio (L:M), and anthropometric indices measured. All children were previously enrolled in the PROVIDE study, a longitudinal birth cohort designed to investigate oral vaccination failure and environmental enteropathy. As part of PROVIDE, an extensive collection of biomarkers for enteropathy as was available. This data was analyzed via multivariate logistic regression and smoothly clipped absolute deviation (SCAD) to identify possible predictors of SIBO from a set of 50 biomarkers collected during the first 24 weeks of life. The incidence of SIBO was 16.7% (15/90). Children with SIBO had a greater mean loss in height-for-age Z score from birth to 2 years than those without (-.86 versus -.35, p value 0.03). Mean Reg 1β was higher in the children with overgrowth (116.75 µg/ml versus 65.62 µg/ml, p value .022). CRP, L:M, and endocab, were not statistically different in children with SIBO. Serum zinc level and fecal calprotectin were identified via both logistic regression and SCAD as possible predictors of SIBO. This data shows that SIBO due to unsanitary living conditions is associated with intestinal epithelial cell damage but not increased systemic inflammation. An increased rate of stunting in the first 2 years is associated with the development of SIBO which, along with increased fecal calprotectin and serum zinc levels in infancy, may represent a phenotypic predisposition to development of SIBO in the developing world setting.

1864

A COMPARISON OF DIARRHEAL SEVERITY SCORES IN A MULTISITE COMMUNITY BASED STUDY

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There is a lack of consensus on how best to measure diarrheal severity. Several severity scores based on caregiver-reported symptoms have been published, but the performance of these in relation to child health outcomes is unclear. The MAL-ED study is a multi-site, prospective birth cohort. In this report, we describe a modified Vesikari score (MAL-ED score), as well as two previously published severity scores, one by Clark and colleagues, and one derived from a Peruvian cohort study (Community Diarrheal Severity (CODA) score). The association between these scores and the risk of hospitalization was tested by means of receiver operating characteristic analysis, and each score was also related to short-term weight gain, illness etiology, and moderate-to-severe diarrhea (MSD) as defined by dysentery, health-care worker diagnosed dehydration, or hospitalization. A total of 9,803 episodes of non-persistent diarrhea from 1681 unique children were considered. There were 135 cases of hospitalization and 1,147 episodes (11.7%) met the definition of MSD. The area under the curve of each score as a predictor of hospitalization was 0.82 (Clark), 0.84 (MAL-ED), and 0.87 (CODA). Agreement with MSD diarrhea was lower (AUC= 0.65, 0.68, 0.70 for Clark, MAL-ED, and CODA respectively). Relative to enteropathogen-negative episodes, mean scores were higher during rotavirus- and adenovirus-positive diarrhea and lower during campylobacter- and giardia-positive diarrhea; and the mean MAL-ED or CODA scores were also significantly higher during astrovirus-, ETEC-, norovirus- and cryptosporidium-positive illness. Although families were more likely to seek care for severe diarrhea across all sites, our results also suggest that a large percentage of these cases never reached the health care system. Diarrhea severity scores based on maternal report are able to predict relevant child health related outcomes. They may therefore be useful in estimating the burden of disease, and the impact of disease control interventions, in community-based studies where not all episodes will necessarily reach the health care system.

1865

ASSOCIATION BETWEEN ENTEROPATHOGENS TO MODERATE-TO-SEVERE MALNUTRITION IN CHILDREN AGED 6-24 MONTHS IN DHAKA, BANGLADESH (MAL-ED): A CASE-CONTROL STUDY

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The association between individual enteropathogens or cumulative pathogen burden and malnutrition in the absence of diarrheal disease remains poorly understood. As part of the MAL-ED study, we sought to identify pathogens associated with malnutrition by performing a case-control study of children presenting to a nutrition clinic in Dhaka, Bangladesh. Moderate to severely underweight children [weight for age Z score (WAZ) <-2] aged 6-24 months were enrolled as cases, and better nourished children aged 6-24 months (WAZ > -1) were enrolled as controls. All stools (500 cases and 480 controls) were tested using conventional bacterial culture as well as PCR for diarrheagenic E. coli

subtypes and by enzyme immunoassay (EIA) for rotavirus, adenovirus, astrovirus, ***Entamoeba histolytica, Giardia***, and ***Cryptosporidium***. A random subset of 202 cases and controls were also tested for a broader range of pathogens with arrayed, singleplex quantitative PCR using TaqMan Array Cards (TAC). The association between pathogens and malnutrition was estimated using age- and sex-adjusted logistic regression. By conventional culture, only detection in stool of ***Campylobacter*** was associated with malnutrition. In the subset tested by TAC, ***Campylobacter***, LT-ETEC, Aar-negative EAEC, Shigella, Norovirus GII and Giardia detection were associated with malnutrition. This set of pathogens was used to define a pathogen burden score. In a multivariate model, age, drinking water outside of the home and pathogen burden were associated with malnutrition, and higher income was associated with better nourished children. In this exploratory analysis, a subset of pathogens were identified for incorporation into a metric of pathogen burden, which could be validated in other studies of early childhood growth. Pathogen burden was an independent risk factor for malnutrition.

1866

URBAN MALARIA RISK CHARACTERISTICS FROM MULTIPLE METRICS OF THE URBAN-TO-RURAL CONTINUUM: RELATIONSHIPS WITH *ANOPHELES* VECTOR ABUNDANCES IN BLANTYRE, MALAWI

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Malaria risk in urban, peri-urban, and urban slum settings is becoming a major concern as urbanization swells in malaria endemic countries. Analysis of such malaria risk is complicated by multiple definitions of what is "urban," but also by uncertainty in where transmission occurs. We analyzed household-level Anopheles mosquito abundance patterns across an urban-to-rural continuum in and around the city of Blantyre, Malawi. As the country's "commercial capital," Blantyre (~1.1M pop.) has highly diverse land use/land cover (LU/LC) inside its ~225 sq km city limits. Mosquito abundance was sampled during the rainy and dry seasons of 2015 with light-traps and aspiration at 320 houses in 64 locations situated ~2.5km equidistant along 8 transects radiating outward from the city center. Using household geolocations, GIS-based data on geographical features, and satellite-image derived LU/LC, the urban-rural status of each household was classified by various metrics, including census population, house density, peri-domestic vegetation/agriculture, proximity to roads/infrastructure and a PCA-derived "urbanicity score." Multi-level statistics (e.g. households, locations, census areas) and spatial statistics (e.g. clustering, autocorrelation, interaction) were used to evaluate mosquito abundance in relation to traditional and derived urban-rural classifications. Results indicated that Anopheles indoor abundance generally increased with distance from the city center, but this explained less than one-third of the variation, making this designation of urban-rural was not strongly predictive of Anopheles abundance. LU/LC classifications that accounted for urban agriculture and topography improved predictability, regardless of geographic location. This association remained after controlling for clustering. Our findings have important implications for understanding household- and community-level "urban" malaria risk, and for identifying locations where malaria prevention and control efforts might be most effective.

1867

CHARACTERIZING THE SPATIAL DISTRIBUTION OF MALARIA VECTORS IN AN AREA OF HIGH TRANSMISSION IN NORTHERN ZAMBIA: EVIDENCE OF SPATIAL-TEMPORAL VARIATION IN THE DISTRIBUTION OF *ANOPHELES FUNESTUS* TO *ANOPHELES GAMBIAE* TO IMPLICATIONS FOR TARGETED INTERVENTIONS

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Characterizing the spatial distribution of malaria vectors is critical to understanding transmission dynamics, with implications for the effectiveness of targeted control interventions. Active entomological surveillance was conducted at randomly selected households in Nchelenge District in Zambia from April 2012 to December 2014. At each visit, a questionnaire was administered and a CDC light trap left overnight for indoor mosquito collections. A total of 4,591 ***Anopheles funestus*** s.s. and 548 ***An. gambiae*** s.s. were captured at 461 households. Counts of ***An. funestus*** and ***An. gambiae*** were modeled using zero-inflated Poisson regression models as a function of environmental variables. Both species were significantly associated with closer proximity to category 1, 2, and 3 streams and with increasing degree of topographical slope. Both species had higher abundances inland compared to along the banks of Lake Mweru. Overall, ***An. funestus*** did not display seasonal variation, but the abundance of ***An. gambiae*** was four times higher in the rainy season as compared with the dry season (RR=4.1; 95% confidence intervals: 2.9, 6.0). In the rainy season, ***An. funestus*** abundance increased 10% in proximity to category 2 streams (RR=1.1; 95% CI: 1.0, 1.1) and 10% per increased degree of topographical slope (RR=1.1; 95% CI: 1.0, 1.3). While ***An. funestus*** abundance was higher in proximity to roads in the dry season, this association reversed in the rainy season when ***An. funestus*** abundance decreased 50% with proximity to roads (RR=0.5; 95% CI: 0.4, 0.6). ***An. gambiae*** abundance was not associated with proximity to roads but decreased in proximity to roads in the rainy season (RR=0.3; 95% CI: 0.2, 0.5). These findings suggest differences in breeding site preferences between these species. Regression models expressing the significant spatial and seasonal variation in the abundance of ***An. funestus*** and ***An. gambiae*** mosquitoes, modeled as a function of identified environmental features, were used to generate maps of species-specific predictive abundance. These maps can be used to maximize the impact of targeted vector control interventions such as IRS or larviciding.

1868

SPATIO-TEMPORAL VARIATION IN MALARIA TRANSMISSION INTENSITY IN RWANDA

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Knowledge of vector distribution and bionomics is critical for vector borne diseases control program strategy. Rwanda made steady progress in the control of malaria over the past decade by scaling up malaria control prevention, treatment, community case management, and behavior change communication. The use of long-lasting insecticidal nets (LLINs) and insecticide indoor residual spraying (IRS) has altered vector bionomics in Africa. This is the first report from Rwanda that determined the abundance, species composition, and infectivity of mosquitoes collected from entomological monitoring in seven sentinel sites by pyrethrum spray collection (PSC) and human landing catches (HLC). The monthly collections

between 2010 and 2013 identified 340,684 mosquitoes to species level using morphological characteristics and grouped into anophelines (26.2%) and culicines (73.8%). The species composition comprised of ***An. gambiae*** s.l, and ***An. funestus*** as the major malaria vectors and six other anophelines. The culicines comprised of Culex, Mansonia, Coquellittidia and Aedes mosquitoes. For ***Anopheles gambiae*** s.l. (n = 84,189), the proportion of mosquitoes host-seeking indoors and outdoors was assessed as well as infection the rates. The mosquitoes collected indoors dropped from an average of 51.3% (95% CI = 44.7, 51.0) in 2010 to 44.9% (95% CI = 43.1, 49.8) in 2013 ($p < 0.005$). We also calculated mean densities and entomological inoculation rates (EIR). The mean resting density for ***An. gambiae*** s.l was 0.3 mosquitoes/house/night with the highest resting density reported in Mashesha (1.0 ***An. gambiae*** /h/n). The average annual EIR 99.5 (range 1.0 - 329.8) with Mashesha showing the highest EIR of 329.8 (a rice growing area) followed by Busoro and Kicukiro with 107.5 and 103.6 infective bites per person per year respectively. We plan to collect and analyze mosquitoes from additional sentinel sites to provide a comprehensive database on vector bionomics which will be the critical data informing vector borne diseases control policies in Rwanda.

1869

IMPROVED MONITORING OF IRS COVERAGE ON BIOKO ISLAND THROUGH THE USE OF A GIS-BASED CAMPAIGN INFORMATION MANAGEMENT SYSTEM

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Starting in 2014, the Bioko Island Malaria Control Project (BIMCP) has used a GIS-based Campaign Information Management System (CIMS) to plan, implement and monitor Indoor Residual Spraying (IRS). The CIMS uniquely identifies each household based on geographical location. The maps can be easily updated to reflect changes due to new houses, or destroyed or burnt down houses, thus enhancing the accuracy of the house count that is used as the denominator for IRS coverage rates, and eliminating the ability of Spray Operators to report fictitious houses. High resolution maps that clearly identify households, enable Supervisors to track and verify that spraying has occurred in houses reported by Spray Operators. This has virtually eliminated the ability of Spray Operators to falsify spray records, thus inflating the numerator in IRS coverage rates. The CIMS also permits identification of non-compliant households, thus enabling focalized intervention where Spray Operators and community leaders return to low-compliance areas for mobilization in an effort to increase spray coverage. Prior to 2014, Spray Operator data obtained from SP1 cards suggested that spray coverage was consistently at least 80%, while retrospective 6-month recall data from annual Malaria Indicator Surveys (MIS) suggested that IRS coverage was no higher than 65% (95% CI = 61-69%) in 2010 and as low as 47% (95% CI = 41%-54%) in 2013. Without the CIMS it was impossible to discern whether the discrepancy was due to errors in the housing count and/or falsification of IRS spray data or to poor recall by MIS respondents. In 2014, using the CIMS, the spray data revealed 57% coverage during the spray round, while the MIS reported that 60% (95% CI= 51- 68%) of surveyed households were sprayed in the previous 6 months. By linking the CIMS and MIS data it was determined that MIS data were 85% accurate compared to CIMS spray data, confirming a high level of sprayer falsification and under reporting of the number of houses to be sprayed in previous years. The CIMS has improved coverage monitoring for IRS and confirmed that a high refusal rate exists in Bioko Island,undermining coverage and program performance.

1870

EVALUATION OF A NOVEL LONG LASTING INSECTICIDAL NET TO INDOOR RESIDUAL SPRAY PRODUCT, SEPARATELY TO TOGETHER, AGAINST MALARIA TRANSMITTED BY PYRETHROID RESISTANT MOSQUITOES IN NORTHWEST TANZANIA: A CLUSTER RANDOMIZED CONTROLLED TRIAL

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The scaling up of the primary malaria vector control interventions - long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) - are responsible for the major reduction in malaria burden in Africa. We need to develop a more rational approach to deployment of chemical control, one which reduces selection pressure for resistance but continues the present gains in malaria control. To help define future strategy of LLIN and IRS deployment in East Africa we are conducting a 4 arm cluster randomised controlled trial (CRT) in North-West Tanzania where the initial prevalence of malaria infection was 65% and resistance to pyrethroids in the predominant vector ***Anopheles gambiae*** exceeded 90% and consisted of at least two resistance mechanisms. The base arm of the trial is universal coverage of Olyset LN, the pyrethroid LLIN which constitutes the current standard of care. The second arm is universal coverage with Olyset Plus, a LLIN which combines pyrethroid and the synergist PBO that in bioassay can overcome pyrethroid resistance mediated by mixed function oxidases; this intervention is a potential future standard of care. The third arm combines Olyset LLIN and IRS with Actellic CS, a long lasting organophosphate insecticide for IRS. The fourth arm combines the LLIN with the synergist PBO and the IRS with the long lasting OP - and is the combination intervention most likely to control malaria transmitted by highly pyrethroid resistant vector populations. The study area comprises 48 clusters and population 150,000 inhabitants, allocated into 12 clusters per arm using restricted randomisation to ensure balance between study arms. The interventions were rolled out in January 2015 and achieved the required coverage to achieve community impact ahead of the long transmission season. Analysis of mosquito population surveillance and malaria infection prevalence in children in the four arms will be presented. The findings will help to define context-specific interventions and their potential to maintain or accelerate present progress and prevent further selection of insecticide resistance.

1871

IMPACT OF SEASONAL PATTERNS TO PARASITE ASEXUAL STAGE ON *ANOPHELES GAMBIAE* SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* INFECTION IN BURKINA FASO

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Transmission reduction is a key component of global efforts to control and eliminate malaria. A wide range of novel transmission-reducing drugs and vaccines are currently under development. Currently, it is unclear how the densities of the parasite stages or the season influence the infection rate and its intensity. Here, we highlighted the importance of the ***Plasmodium falciparum*** stages seasonal pattern in ***Anopheles gambiae*** infections success. Parasitological data were obtained by blood slide processing from

child volunteers with parental consent. Larvae were sampled from natural pools, reared to adulthood before experiment. Gametocytes carriers' infectiousness to mosquitoes was determined at the peak and end of wet season and dry season via membrane feeding assay. Infection prevalence and intensity were determined one week after feeding by midgut dissection. About 28062 mosquitoes offered blood meal and 29.6% fed and survived until dissection, seven days later. The average number of dissected mosquitoes 75 (range 18 - 207) was quite the same according to the assays period. In 71.8% (79/110) of feeding experiments, at least one mosquito was infected. The median percentage of infected mosquitoes per infectious experiment was 15.7% (IQR: 07.3- 89.2 %) with a median oocyst number of 2 (range 1 - 101). The prevalence of infected blood meal was similar across season (70.0%, 72.7% to 70.1% at the dry, the peak, and the end of the wet season. Mosquitoes' infection rate also did not show any significant variation within season. The infection success was higher for asexual parasites carriers (91%) than non carriers (9%). However, mosquitoes' infection rate and oocyst load did not significantly vary according to the asexual forms carriage. This highlights the need to carefully interpret evaluations, regarding asexual parasites and transmission season for malaria control program.

1872

QUANTIFYING BIAS TO OVERESTIMATION IN SELF-REPORTED ITN USE: A SYSTEMATIC REVIEW TO META-ANALYSIS

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Insecticide-treated bednets (ITNs) are one of the most cost effective malaria prevention measures. In 2013, the WHO recommended universal coverage with ITNs for all 3 billion individuals at risk of malaria world-wide. Evidence for this recommendation is based on robust evidence of an 18-23% reduction in child mortality among households using ITNs. However, these studies used self-reported ITN use as a proxy for actual ITN use. We hypothesized that self-reports would overestimate actual ITN use, because self-reported ITN use may be subject to recall and social desirability bias. We performed a systematic review of the literature to identify studies which included both self-reports and another more objective measure of ITN use (surprise sleeping visits, visual confirmation of mounted ITNs, etc) in the same population in order to determine the magnitude of overestimation. We searched for relevant terms in Pubmed and Embase, reviewing 464 titles and abstracts. We performed full article reviews of 193 studies and eventually included 19 studies for analysis. The studies examined were published from 1989 through 2013, including countries from Southeast Asia and sub-Saharan Africa. Six of 19 studies used surprise night visits to confirm self-reported use, while the rest used visual confirmation of a mounted ITN. Self-reported ITN use over-estimated actual ITN use ranging from -2.9% to 32.1%, with a mean of 9.1%. This 9.1% mean difference between self-reported and observed ITN use represents a 23.5% overestimation of actual ITN use. The significant and consistent overestimation of ITN use reported by self-reports suggests that 1) ITNs may be more effective than currently thought, i.e. the accepted relationship between ITN use and malaria outcomes may be based on an overestimate of their actual use, and 2) self-reported ITN use may not be an appropriate measure of actual ITN use, indicating the need to shift from self-reports towards more valid measures of ITN use in malaria prevention studies, most especially related to adherence behaviors and cost-effectiveness modeling.

1873

TARGETING THE CELL STRESS RESPONSE OF *PLASMODIUM FALCIPARUM* TO OVERCOME ARTEMISININ RESISTANCE

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Artemisinin derivatives (ARTs) are recommended, in combination regimens, as first line antimalarials in most countries where malaria is endemic. However their mechanism of action is poorly understood and their usefulness is threatened by the emergence of drug resistance. We undertook a detailed kinetic analysis of the drug responses of K13 wildtype (sensitive) and mutant (resistant) isolates of ***Plasmodium falciparum*** sourced from a region in Cambodia (Pailin). We demonstrate that ART treatment induces growth retardation and an accumulation of ubiquitinated proteins, indicative of a cellular stress response that engages the ubiquitin/ proteasome system. We show that resistant parasites exhibit lower levels of ubiquitinated proteins and delayed on-set of cell death, indicating an enhanced cell stress response. We found that the stress response can be targeted by inhibiting the proteasome. Accordingly, clinically-used proteasome inhibitors strongly synergize ART activity against both sensitive and resistant parasites, including isogenic lines expressing mutant or wildtype K13. Synergy is also observed against ***P. berghei in vivo***. Our work provides a rationale for improving the detection of ART resistance in the field and for treatment strategies that can be employed in areas with ART resistance.

1874

ROLE OF CELL CYCLE REGULATORS IN ARTEMISININ INDUCED DORMANCY IN *PLASMODIUM FALCIPARUM IN VITRO*

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Artemisinin-induced dormancy provides a plausible explanation for the variable rates of recrudescence reported in the field following artemisinin monotherapy. This phenomenon is reminiscent of cell cycle arrest in mammalian cells where cell cycle regulators, such as cyclin dependent kinases (CDKs) and cyclins play an important role. We investigated the transcription dynamics of 6 ***P. falciparum*** CDKs and 4 cyclins as well as protein expression and kinase activity of selected CDKs before and after dihydroartemisinin (DHA) treatment. The role of CDKs in dormancy was further investigated by evaluating the effect of 3 CDK inhibitors on the induction of and parasite recovery from dormancy. Transcription analysis revealed an up-regulation of two plasmodial CDKs throughout the dormancy period, and a down-regulation of genes encoding the remaining 4 CDKs and cyclins. The 3 inhibitors demonstrated different effect on parasite recovery from dormancy, one of these inhibitors completely blocked the parasite recovery from dormancy when it was added at the early stage of dormancy. The results suggest that plasmodial CDKs play an essential role in artemisinin induced dormancy, most likely control of parasite entry into S phase and mitosis. These findings provide new insights of cell cycle regulation in ***P. falciparum*** and in artemisinin induced dormancy.

1875

ARTESUNATE/AMODIAQUINE VERSUS ARTEMETHER/LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN UGANDA: CHANGING EFFICACY PATTERNS CONSISTENT WITH CHANGES IN TREATMENT PRACTICES TO DRUG SENSITIVITIES

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With older therapies limited by widespread drug resistance, standard treatment for uncomplicated falciparum malaria is now artemisinin-based combination therapy, with nearly all endemic countries in sub-Saharan Africa recommending either artemether/lumefantrine (AL) or artesunate/amodiaquine (AS/AQ). In Uganda, AL has shown superior efficacy compared to AS/AQ and is the first-line regimen. However, recent changes in treatment practices and evidence of shifting drug sensitivities prompted a reassessment of the relative efficacies of these regimens. We enrolled 602 patients aged 6-59 months from health centers in the Apac, Mubende, and Kanungu Districts of Uganda in 2013-14. Children with uncomplicated falciparum malaria were randomly assigned treatment with AL or AS/AQ, and 594 (98.7%) of those enrolled were followed for 28 days. Recurrent infections were genotyped to distinguish recrudescence from new infection, and ***Plasmodium falciparum*** resistance-mediating polymorphisms were characterized for all infections. At each site the risk of recurrent parasitemia was lower in children treated with AS/AQ compared to those treated with AL (overall 28.6% vs. 44.6%; p <0.001). Recrudescences were uncommon, but all occurred in the AL treatment arm (0% vs. 2.5%; p = 0.006). Recovery in hemoglobin was greater in the AS/AQ arm (1.73 vs. 1.39 g/dl, p = 0.04). Both regimens were well tolerated; serious adverse events were uncommon (1.7% for AS/AQ and 1.0% for AL). In new infections after therapy the two regimens selected for opposite polymorphisms in pfcrt and pfmdr1, with each selecting for polymorphisms associated with decreased sensitivity to the partner drug. Polymorphisms in the K13 propeller domain, which have been associated with artemisinin resistance in Asia, were uncommon (1/76) and not associated with recurrent parasitemia. Although both regimens were highly efficacious, AS/AQ showed superior efficacy, contrasting with older data, and consistent with recent changes in parasite drug sensitivity. Malaria treatment guidelines should consider multiple or rotating regimens to maintain the efficacies of leading treatments.

1876

PARASITE CLEARANCE IN CHILDREN UNDER FIVE YEARS OF AGE WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AFTER TREATMENT WITH ARTEMETHER-LUMEFANTRINE IN NCHELENGE DISTRICT, ZAMBIA

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Artemether-lumefantrine (AL) has been used in Zambia as first line treatment since 2002. The World Health Organization (WHO) reported a decline in malaria prevalence of over 60% in Zambia and this has been partly attributed to prompt and effective treatment with AL. While efficacy of AL remains high, there have anecdotal reports of delayed clearance of parasites after treatment with the drug combination. Countries in South-east Asia, including Thailand, Cambodia and Myanmar, have reported artemisinin drug resistance, manifested by delayed parasite clearance and rare cases of treatment failure. Therefore, there is need to examine the efficacy of AL in Zambia and document whether or not some patients experience delayed parasite clearance despite adequate treatment with the drug. This study aimed to examine the efficacy of AL and delayed parasite clearance in children in Nchelenge District, Zambia. Febrile children aged 6 to 59 months, with uncomplicated ***Plasmodium falciparum*** infection, were enrolled into the study beginning in December 2014. The children were treated for three days with directly observed therapy of AL based on weight according to the WHO protocol and followed-up 35 days to evaluate drug efficacy by monitoring clinical and parasitological parameters with Giemsa-stained blood smears and PCR. The study results showing parasite clearance times, parasite slope half-life (using the WWARN Parasite Clearance Estimator) and associated clinical parameters, as well as the presence of K13 propeller mutations, will be presented. Preliminary analysis of the first 20 participants who have complete data sets (of a planned total of 100), revealed that all study participants cleared parasites within 48 hours, with 60% of the children having a lag phase before parasite clearance began, and a subsequent median clearance half-life of 2.69 hrs (IQR 1.64, 3.13).

1877

EFFICACY OF CHLOROQUINE TO PRIMAQUINE IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM VIVAX* MALARIA, CRUZEIRO DO SUL, ACRE, BRAZIL, 2014

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The World Health Organization recommends the regular monitoring of antimalarial treatment policies. We evaluated the ***in vivo*** efficacy of chloroquine and primaquine for the treatment of uncomplicated ***P. vivax*** malaria in Brazil. The study was conducted in Cruzeiro do Sul from February to December 2014. We enrolled patients ≥5 years of age with parasitologically confirmed ***P. vivax*** monoinfection (parasitemia between 250 and 100,000 parasites/uL) and no evidence of severe disease. Patients were treated under direct observation with chloroquine for 3 days (daily dose of 25 mg/Kg). After laboratory confirmation of normal glucose-6-phosphate dehydrogenase (G6PD) activity levels, primaquine (daily dose of 0.5mg/Kg) was administered, also under direct observation, for 7 days. We monitored patients clinically and parasitologically on Days 1, 2, 3, 7, 14, 21, 28 and then every 4 weeks until Day 168. Seven neutral microsatellites were used to differentiate genetic profile of parasites detected at the time

of enrollment and recurrent infection. We enrolled 132 patients, 13 were excluded (nine with G6PD deficiency, and four with either low-density parasitemia or ***P. falciparum*** infection). Among the 119 valid patients, 65 (54.6%) were male and the median age was 24 years. The geometric mean of asexual parasitemia at admission was 3,373 per uL. At Day 28, no patient, among 110 patients to reach this time point, presented with recurrent ***P. vivax*** parasitemia; while at Day 168, 28 (30.1%; 95% CI: 21.0-40.5%) presented with recurrent ***P. vivax*** infection. Microsatellite typing demonstrated that 13 of those infections were due to a different strain (i.e., reinfection), while 15 were caused by a genetically identical strain compared to Day 0 and were most likely relapses. This finding brings the corrected rate of relapse to 18.8% (95% CI: 10.2-27.3%). Treatment with chloroquine and primaquine appears to remain efficacious to treat ***P. vivax*** malaria and prevent recrudescence, but it is likely associated with relapses 6 months after acute disease. Revision of the primaquine regimen with possible increase in total dose or use over 14 days should be considered.

1878

DISSECTING THE MUTATIONAL ACQUISITION OF PFCRT-MEDIATED ANTIMALARIAL DRUG RESISTANCE

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Named for its primary role in mediating resistance to chloroquine (CQ), the ***Plasmodium falciparum*** Chloroquine Resistance Transporter (PfCRT) is increasingly recognized as a multi-drug resistance transporter with pleiotropic effects on parasite susceptibility to our present arsenal of antimalarials. Field studies of drug selective forces on the pfcrt locus have historically centered on the single-nucleotide polymorphism (SNP) K76T, a critical determinant of CQ resistance that is always accompanied by at least three mutations in all CQ-resistant isolates. The impact of these mutations on parasite drug resistance, fitness, and the mutational paths accessible to parasites as they acquire resistance remains unclear. To explore this, we used pfcrt-specific Zinc-Finger Nucleases (ZFNs) to dissect the mutational trajectories leading to the evolution of the Ecuadorian pfcrt allele Ecu1110, which harbors the fewest polymorphisms in a CQ-resistant parasite (namely K76T, A220S, N326D, I356L). We generated a collection of otherwise isogenic asexual blood-stage parasites expressing the wild-type (CQ-sensitive) PfCRT haplotype as well as each possible single-, double-, triple-, and quadruple-SNP combination of the four SNPs that comprise Ecu1110 PfCRT. We also generated isogenic parasites expressing the related quintuple-SNP 7G8 PfCRT variant (C72S, K76T, A220S, N326D, I356L) that is highly prevalent throughout South America and the Western Pacific. Parasite susceptibility to a panel of clinically employed antimalarials was determined via standard IC50 assays and was complemented with data from flow cytometry-based ***in vitro*** growth competition assays to model the mutational paths of PfCRT-mediated drug resistance. Our studies indicate that multiple PfCRT SNPs have a direct impact on parasite susceptibility to various antimalarial compounds, define mutational pathways that likely led to CQ resistance, and delineate growth rate constraints imposed on parasites as they evolve resistance.

1879

MUTATION OF THE MU-SUBUNIT OF THE *PLASMODIUM FALCIPARUM* CLATHRIN-ASSOCIATED AP2 ADAPTOR PROTEIN COMPLEX (PFAP2-MU) TO ITS EFFECT ON PARASITE SUSCEPTIBILITY TO ANTIMALARIAL AGENTS

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Reports of ***Plasmodium falciparum*** infections with reduced susceptibility to artemisinin are now widespread throughout the Greater Mekong sub-region and threaten to undermine Artemisinin-based Combination Therapies (ACTs), currently the first-line WHO recommendation for malaria treatment. While the genetic basis for this reduced sensitivity to artemisinin in Asia has been associated with mutations in kelch13 (PF3D7_1343700), other candidate genes are thought to be involved in resistance developing independently elsewhere. Selection for parasites surviving artemisinin exposure in the murine malaria P. chabaudi uncovered a novel locus associated with antimalarial resistance, pcap2-mu. This gene encodes the mu chain of an adaptor protein complex that functions in clathrin-mediated endocytosis. Sequencing of submicroscopic parasites surviving ACT treatment in Kenyan children revealed an increased survival of parasites harbouring the Ser160Asn polymorphisms in the ***P. falciparum*** homologue, pfap2-mu. We have recently reported that the episomal expression of the mutant form of pfap2-mu (160Asn) in Dd2 parasites resulted in a significantly reduced susceptibility to dihydroartemisinin using the standard 48 hour ***in vitro*** growth inhibition assay. Furthermore, expression of the mutant pfap2-mu also resulted in reduced susceptibility to quinine. No differences in susceptibility to dihydroartemisinin were observed when comparing wild type with mutant parasites using the ring stage assay (RSA0-6h). We seek to expand on our previous findings by using gene editing CRISPR-Cas9 and Zinc Finger Nuclease approaches to replace the endogenous wild type Ser160 pfap2-mu gene with the mutant 160Asn gene associated with residual parasitemia after ACT treatment in Kenya. We will report the effects of this mutation on the ***in vitro*** parasite drug sensitivity to a range of antimalarial agents assessed with growth inhibition assays employing both the standard 48 hour exposure and newer 6 hour pulse assays.

1880

IMMUNOMODULATION BY *LITOMOSOIDES SIGMODONTIS* INFECTION TO FILARIAL ANTIGEN ADMINISTRATION IMPROVES GLUCOSE TOLERANCE IN DIET-INDUCED OBESITY MICE

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Adipocyte necrosis in obesity causes pro-inflammatory macrophages to infiltrate into the adipose tissue and results in chronic inflammation and insulin resistance. Since filariae induce a regulatory immune response in their hosts and trigger type 2 immune responses that counter regulate type 1 immune responses, we investigated whether immunomodulation by ***Litomosoides sigmodontis*** (L.s.) improves glucose tolerance in diet-induced obesity (DIO) mice. After 10 and 16 weeks on high fat diet, respectively, L.s.-infected and L.s. antigen (LsAg)-treated mice were tested for glucose tolerance and immunological analysis was performed. Both L.s. infection and LsAg administration in DIO mice increased eosinophil and alternatively activated macrophage frequencies within the epididymal

adipose tissue and improved glucose tolerance, suggesting enhanced insulin sensitivity. This improved glucose tolerance by L.s. infection was dependent on eosinophils, as was shown with eosinophil deficient dblGATA mice. Total epididymal adipose tissue B-cell numbers were further reduced in L.s.-infected DIO mice and consisted substantially of B1-cells, whereas uninfected DIO controls had increased numbers of B2-cells. Consistently, L.s.-infected DIO-animals produced less IgG2a, which was previously shown to contribute to insulin resistance. qPCR arrays of epididymal adipose tissue further suggested a suppressed adipogenesis in L.s.-infected DIO mice. Accordingly, the differentiation of 3T3-L1 preadipose cells to mature adipocytes was suppressed by LsAg treatment ***in vitro***. However, two weeks of LsAg treatment did not suffice to reduce adipose tissue weight and adipocyte size in DIO mice. In conclusion our investigation indicates that L.s. infection and LsAg treatment restores a cellular composition within the adipose tissue of DIO mice that is dominated by eosinophils and alternatively activated macrophages and improves glucose tolerance in an eosinophil dependent manner. Additional protective mechanisms may include a suppressed adipogenesis and the induction of regulatory responses and are currently further investigated.

1881

CHEMOKINES TO THE REGULATION OF EOSINOPHILIA IN LOIASIS

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Although eosinophilia is considered a hallmark of human helminth infection, the degree of blood eosinophilia varies considerably among infected individuals. Whereas some of this variation is likely due to differences in parasite burden, the host immune response to parasite antigens clearly plays an important role. To explore the role of chemokines in the regulation of blood eosinophilia in patients with microfilaremic loiasis, patients with loiasis were divided into low and high eosinophil groups based on the median absolute eosinophil count (AEC). Chemokines were measured by multiparameter immunoassays in antigen (***Brugia malayi*** antigen [BMA])- or mitogen (PMA/ionomycin ([PI])-sitmulated PBMC supernatants from 11 patients with loiasis. Chemokine levels in unstimulated supernatants were comparable between the two groups with the exception of IL-8, that was significantly increased in the low eosinophil group (GM 9.4 vs. 3.8 mcg/ml in the high eosinophil group, $p<0.01$) and showed a negative correlation with AEC overall ($r = -0.75$, $p = 0.001$). No significant differences were detected in BMA-stimulated chemokine levels between the two groups, although eotaxin 1, RANTES and MCP-4 showed significant negative correlations with AEC overall ($r= -0.77$, $p=0.007$ for eotaxin-1 and $r= -0.65$, $p=0.37$ for RANTES and MCP-4). TARC levels were increased in PI stimulated supernatants in the high eosinophil group ($p=0.056$). Microarray analysis was performed using PBMC from a separate group of 24 patients with loiasis divided into high and low eosinophil groups. Preliminary analysis of chemokine expression reveals upregulation of TARC and eotaxin-2 in the high eosinophil group and upregulation of RANTES in the low eosinophil group. Although IL-5 expression is comparable between the two groups, genes associated with Th1 and inflammatory responses appear to be upregulated in the low eosinophil group. Taken together these data suggest that differences in chemokine secretion by PBMC may contribute to the regulation of blood eosinophilia in loiasis.

1882

TISSUE-SPECIFIC TRANSCRIPTOMICS TO PROTEOMICS OF *DIROFILARIA IMMITIS* TO ITS WOLBACHIA ENDOSYMBIONT

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Recent evidence for the development of drug resistance among parasitic nematodes has accentuated the need for development of new treatment modalities. Although transcriptomic and proteomic characterization of filarial nematode life cycles using whole worms have greatly enhanced our knowledge of the biology of these organisms, it is clear that tissue-level approaches are needed to provide spatial resolution of gene expression that can aid drug discovery and vaccine studies. Here, we describe the first concurrent tissue-specific transcriptomic and proteomic profiling of a filarial nematode (***D. immitis***) and its Wolbachia endosymbiont (wDi) in order to better understand tissue functions and identify tissue-specific antigens that may be used for the development of new diagnostic and therapeutic tools. Hierarchical clustering of the ***D. immitis*** tissue transcriptomes, along with the recently published whole-worm adult male and female ***D. immitis*** transcriptomes, revealed that the whole-worm transcriptome is typically dominated by transcripts originating from the gonads (testis and uterus). The uterus appears to have the most variable transcriptome, and may reflect variance in worm age and fecundity. Although many functions are shared between the reproductive tissues, the most significant differences in gene expression were observed between the uterus and testis. Interestingly, wDi gene expression in the male and female body wall is fairly similar, yet slightly different to that of Wolbachia gene expression in the uterus. Proteomic methods verified 32% of the predicted ***D. immitis*** proteome, including over 700 hypothetical proteins of ***D. immitis***. Of note, hypothetical proteins were among some of the most abundant Wolbachia proteins identified, which may fulfill some important yet still uncharacterized biological function. The spatial resolution gained from this parallel transcriptomic and proteomic analysis adds to our understanding of filarial biology and serves as a resource with which to develop future therapeutic strategies against filarial nematodes and their Wolbachia endosymbionts.

1883

GENETIC VARIATION IN *ONCHOCERCA VOLVULUS* TO WOLBACHIA ENDOSYMBIONT REVEALS STRUCTURED PHYLOGEOGRAPHY IN RELATION TO PHENOTYPIC DIVERGENCE OF PATHOGENICITY

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Onchocerca volvulus, the filarial nematode that causes onchocerciasis, is widely endemic in sub-Saharan Africa and in parts of Latin America. The pathology ranges from sub-clinical symptoms to dermatitis and ocular disease, and epidemiological studies have shown that blinding onchocerciasis is much more common in dry savannah regions than in rain forest regions of West Africa. To develop a comprehensive understanding of the population genetics and structure of ***O. volvulus***, we investigated the genomic diversity within and between ***O. volvulus*** populations by analyzing whole-genome (nuclear, mitochondrial and Wolbachia) sequences of >30 clinical parasite samples. Of these, 20 were collected from seven countries in Africa (forest or savanna) and 10 from Ecuador in South America. At a mean depth of 60x coverage per sample, we identified and annotated ~1 million segregating SNPs and small indels

(including 65k missense and 1k nonsense variants, together affecting 9k genes), as well as ~300 genomic regions (with a combined total length of >5Mb) displaying copy number variations. Admixture analysis of the inter- and intra-continental patterns of nuclear, mitochondrial and Wolbachia sequence variations provided genetic insights regarding the phylogeography of the parasite at an unprecedented resolution. Signatures of differential selection, identified through genomic markers of both the nuclear genome of the parasite and the Wolbachia endosymbiont, provided evidence of local adaption underpinning the phenotypic divergence of pathogenicity between the forest and the savanna strains. The study also provides new information on the genetic variability of potential vaccine, drug, and diagnostic targets.

1884

HELMINTH PARASITES TO CANCER-CAUSING TUMORS CONDITION HUMAN MONOCYTES SIMILARLY: INSIGHTS INTO PARALLEL MECHANISMS OF IMMUNE EVASION

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A number of features of the host-parasite interface are reminiscent of those that are also observed at the host-cancer interface. In particular, both cancer cells and parasites establish a tissue microenvironment that leads to immune evasion and likely reflects an alteration of the function of various antigen-presenting cells. The present study investigated how the phenotype and function of circulating human monocytes is altered by exposure to live filarial parasites and if these functional and phenotypic alterations parallel those induced by exposure to tumors. Thus, human monocytes were exposed either to live microfilariae (mf) of ***Brugia malayi*** - a causative agent of lymphatic filariasis - or to three different cancer cell lines MDA-MB-231 (breast cancer), OVCAR (ovarian cancer), or U-87 (glioblastoma). After 2 to 7 days of culture, cells were harvested, flow-sorted and assessed for mRNA expression, phenotype and function. To our great interest, following exposure to either mf or any of the 3 cell lines, the cell surface expression of the inhibitory ligands PDL1 and PDL2 were highly induced. Furthermore, monocytes exposed to tumor cell lines for even 2 days showed markedly upregulated expression of M1-associated (TNF-α, PDL1; > 6-fold), M2-associated (PDL2, MRC1; >5 fold) and Mreg-associated (IL-10, IDO, TGF-β; > 4–10–fold) genes, as well the expression of MMP-9 and VEGF, both known to be pro-angiogenic. The expression of these markers was also elevated in mf-exposed monocytes but most profoundly only after 7 days of exposure. Conditioning the human monocytes with either mf or tumor cell lines clearly altered their function as shown by a diminution of production of IP10, TNF-α, CCL22, and IL-10 in response to TLR3 and TLR7 agonists. Collectively, our data suggest that helminth parasites and tumor cell lines share similar abilities to alter the phenotype and function of human monocytes and may provide common insights into immune evasion.

1885

A MULTI-OMICS APPROACH FOR DEVELOPING IMPROVED DIAGNOSTIC TOOLS FOR ONCHOCERCIASIS

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Onchocerciasis is a neglected tropical disease that is responsible for significant morbidity (blindness and severe skin disease) in sub-Saharan Africa and parts of Latin America. Large-scale public health programs based on mass distribution of ivermectin have reduced ***Onchocerca volvulus*** infection rates in many areas, and plans are being developed to scale up activities to eliminate the disease. Improved diagnostic methods are needed for identifying hypoendemic areas that were excluded from prior control programs and for determining when transmission has been interrupted. The recently introduced Ov-16 antibody test is a major step forward, but it has only moderate sensitivity in subjects with microfiladermia. We have used a multi-omics approach to identify novel diagnostic antigens with high levels of sensitivity and specificity that may be useful for immunoassays to support the onchocerciasis elimination program. The approach included: i) development of a relational database that includes the annotated genome, developmental transcriptome (including MF, L3, adult male and adult female), and adult female proteome of ***O. volvulus***; ii) affinity purification ***O. volvulus*** antigens using IgG from onchocerciasis patients for identification via mass spectrometry. A total of 180 immundominant proteins showed favorable interactions with patient and control sera. These included many of the major diagnostic antigens that have been identified over the past 25 years (e.g., Ov16, Ov3.6, Ov7) plus many new candidates. The immunodominant proteins were characterized based on their conservation with orthologs in relevant species, expression pattern across the life cycle, and expression among eight individual female worms, and 19 candidates were prioritized for recombinant expression and laboratory testing in serodiagnostic assays. These candidate proteins, as well as our extensive genomic, transcriptomic, and proteomic datasets, are offered to the community (http://nematode.net) to further basic and translational onchocerciasis research in diagnostics and other areas.

1886

FURTHER CHARACTERIZATION OF A CARBOHYDRATE-RICH CIRCULATING *WUCHERERIA BANCROFTI* ANTIGEN AS A MEMBER OF THE JUV-P120 GENE FAMILY

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Rapid diagnostic tests (RDTs) used in the Global Programme to Eliminate Lymphatic Filariasis detect a 200 kD circulating filarial antigen (Wb-CFA) in blood and serum samples from humans infected with ***W. bancrofti***. Prior studies have shown that Wb-CFA is an adult worm excretory-secretory (E-S) product that is recognized by monoclonal antibodies AD12.1 and DH6.5. These antibodies bind to a single repeated epitope that is not specific to ***W. bancrofti*** and is likely responsible for false positive RDT results seen in some persons with loiasis. The exact nature of the epitope is unknown, but its immunoreactivity is destroyed by metaperiodate and is not affected by pronase B. In the present study, the Consortium for Functional Glycomics (Emory University) assessed the binding specificity of AD12.1. The antibody bound strongly to only 2 of 609 targets in their mammalian glycan array (GlcAß1-3GlcNAc and GlcAß1-3Gal). These disaccharides have been detected in O-linked carbohydrate side chains in glycoproteins from other nematodes including C. elegans. To identify the protein associated with the glycan moiety, we used tandem mass spectroscopy to analyze a tryptic digest of Wb-CFA that had been immunoaffinity-purified from pooled patient sera using antibody DH6.5. Three peptide sequences were identified in sequences encoded by two predicted ORFs situated adjacent to each other on a single 5,762 base pair contig in the ***W. bancrofti*** draft genome assembly (GenBank accession LM003610.1). These peptide sequences were not observed in human or common contaminant proteins. Although there is some ambiguity in the contig assembly in the vicinity of these ORFs, comparisons with ***Brugia*** sequences suggest that the two predicted ORFs actually combine to form a single ORF encoding protein of ~550 amino acids with high homology (66% identity, E score 1e-164) to a ***B. malayi*** protein (GenBank accession AHB87099.1) that is an orthologue of the ***L. sigmodontis*** E-S protein

Juv-p120 (GenBank accession AAS92593.1). Work in progress may provide insights regarding the function of Wb-CFA and lead to a more specific assay for the antigen.

1887

NEW EVIDENCE THAT *ANOPHELES GAMBIAE SENSU LATO* DISCRIMINATE AQUATIC HABITATS FOR OVIPOSITION: COULD THIS LIFE TRAIT BE EXPLOITED TO CONTROL RESIDUAL MALARIA TRANSMISSION?

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Residual malaria transmission in Africa is sustained by vector populations that resist insecticides and bite outdoors. These could be conceivably controlled by targeting gravid ***Anopheles gambiae*** s.l. searching for oviposition sites. Thus the aim of this study was to investigate if ***An. gambiae*** s.l. make informed choices when selecting egg-laying sites and to identify physical, chemical and biological parameters associated with this choices. Egg-count bioassays were used to evaluate oviposition responses to habitat water and test if bacterial volatiles affect the selection of the aquatic habitat. A cross-sectional case–control study of aquatic habitats was done on Rusinga Island in Lake Victoria, Western Kenya to compare the characteristics of habitats colonised and not colonized by early instar Anopheles larvae. Factors evaluated included biological characteristics of the sites, zooplankton, invertebrate fauna, physical parameters, nutrients, bacteria communities and volatile chemicals released from the water. Experiments showed that wild and caged ***An. gambiae*** s.l. discriminate between potential aquatic habitats using chemical cues. In the field no evidence was found that bacteria from natural habitat water influence habitat selection. Although chemical cues were highly diverse analysis suggests that cases and control habitats differ in the headspace volatile profiles. High turbidity >200 NTU was the only environmental factor strongly associated with cases. Other risk factors were higher grass coverage (positive association), and the abundance of creeping water bugs of the family Naucoridae and fish (negative associations). This study demonstrates that gravid ***An. gambiae*** choose suitable habitats for oviposition using a complex system of chemical and visual cues from water bodies. Habitats preferred by ***An. gambiae*** exhibited distinct and measurable characteristics that can be potentially exploited to attract and kill gravid mosquitoes. Such strategies would additionally target insecticide resistant and outdoor vector improving vector monitoring and control.

1888

QUANTIFYING DISPERSAL BEHAVIOR FOR *CULEX QUINQUEFASCIATUS* TO *AEDES ALBOPICTUS IN* COLLEGE STATION, TEXAS

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To better control populations of mosquitoes and break the transmission cycle of vector-borne diseases, it is crucial to understand the dispersal of adult mosquitoes. We performed a stable isotope mark-capture study, focusing on ***Culex quinquefasciatus*** and Aedes albopictus, to characterize dispersal distance and behavior. We enriched (i.e. marked) naturally occurring larval mosquitoes in container habitats with ^{13}C-glucose or ^{15}N-potassium nitrate at two different locations (~0.5km apart) in College Station, Texas in 2013. We used 32 CDC light trap, 32 gravid trap, and 16 BG Sentinel at different trap locations within a two-kilometer radius of the enriched larval habitats. Each location was trapped once per week and all mosquitoes collected were identified and numerated. ***Cx. quinquefasciatus*** and ***Ae. albopictus*** were pooled and tested for West Nile virus (WNV) by RT-PCR or tested by stable isotope analysis. In total, 720 trap nights were completed from July to August 2013 yielding a total of 32,140 ***Cx. quinquefasciatus*** and 7,722 Ae. albopictus. Overall, 69 marked female mosquitoes (n=2,758) and 24 marked male mosquitoes (n=350) were captured throughout the study period. Mean distance traveled (MDT) was calculated by sex and species. Female ***Cx. quinquefasciatus*** have a MDT of 0.94km, female ***Ae. albopictus*** of 0.38km, male ***Cx. quinquefasciatus*** of 1.16km, and male ***Ae. albopictus*** of 1.04km. This study provides a greater understanding of the dispersal of two important mosquito vectors capable of transmitting diseases in urban environments. We also confirm the ability to use stable isotope enrichment as a means to study the biology of mosquitoes.

1889

MINIMUM INFECTIOUS TIME 50: A METRIC NOVEL FOR STANDARDIZED COMPARISONS OF VECTOR COMPETENCE

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Chikungunya (CHIKV) virus has seen an increase in its transmission intensity and geographic range. Two particular events - the Indian Ocean and the ongoing outbreak in the Caribbean, each resulting in hundreds of thousands of cases - have been caused by different lineages of the virus. It has been reported that phenotypic differences between lineages of CHIKV are critical to understanding the scope of outbreaks. After the emergence of East Central South African sublineage in the Indian Ocean (ECSA-V), laboratory studies indicated increased efficiency of this viral type in a historically secondary vector, Ae. albopictus. Since then, this combination of lineage-vector has been the focus of most of the CHIKV vector competence studies. However, the ongoing outbreak in the Caribbean has been attributed to a strain belonging to the Southeast Asian genotype and ***Ae. aegypti***, suggesting that this focus might be short-sighted. There is a paucity of data directly comparing the vector competence of these two lineages in ***Ae. aegypti***. Further, mathematical models of transmission, used often to predict and forecast outbreak trajectories, rely on vector competence studies to parameterize their models by extrapolating the extrinsic incubation period (EIP) of the virus within the mosquito vector(s). These two measures- vector competence and EIP- are parts of a single process, but are rarely considered together either for the design of experimental investigations or for interpretation of vector competence to parameterize models. To bridge these gaps, we directly compare experimental vector competence results of these two lineages and subsequently propose the MIT50, the mosquito infectious time 50 (the time it takes post-exposure for 50% of exposed mosquitoes to become infectious). We show that the phenotypic differences detected between the two lineages are more directly comparable and more likely to be appropriately interpreted for model parameterization with the MIT50. We demonstrate the effect of mosquito mortality on transmission by calculating the mortality reduced transmission rate as the probability that a mosquito survives to transmit at MIT50.

1890

NATURAL *WOLBACHIA* INFECTIONS IN *ANOPHELES GAMBIAE SENSU LATO*

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Wolbachia is an intracellular bacterial endosymbiont that infects the germlines of many arthropod species. It has been proposed as a tool to control malaria transmission in Anopheles mosquitoes for two key

properties. First, Wolbachia can spread through natural populations due to reproductive phenotypes induced in the host insect, principally cytoplasmic incompatibility (CI). In CI, Wolbachia-uninfected females produce no fertile progeny with Wolbachia-infected males, while Wolbachia-infected females produce fertile progeny with any male, thus driving the infection into the population. Second, Wolbachia infection can block human pathogen development in several insects, recently extended to include the inhibition of *Plasmodium* development in female Anopheles mosquitoes. Central to the future of using Wolbachia in malaria control, our laboratory has identified for the first time strong evidence of natural Wolbachia infections in field populations of ***An. gambiae*** sensu lato in Burkina Faso, Africa. Whole-genome shotgun metagenomic sequencing has placed this strain in a novel phylogenetic group – wAnga – distinct from, but related to, other dipteran Wolbachia strains. Infection persisted at low frequency (20-40%) over four years despite imperfect vertical transmission to progeny. We have successfully colonized mosquitoes harboring this infection and have examined whether these bacteria affect the reproductive success of their mosquito host. Moreover, fluorescent in situ hybridization targeting 16S rRNA sequences localizes wAnga within the female germline, consistent with vertical transmission. Our results suggest a role for Wolbachia in modulating aspects of mosquito biology that are highly relevant to vectorial capacity.

1891

REGULATION OF THE MOSQUITO MIDGUT BACTERIA BY THE STEROID HORMONE 20-HYDROXYECDYSONE

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Anopheles gambiae and ***Aedes aegypti*** are mosquito species that cause high morbidity and mortality by spreading pathogens such as *Plasmodium* parasites and arboviruses. As the midgut of the mosquito is the main barrier to dissemination of pathogens from a bloodmeal into the hemolymph, understanding midgut immune mechanisms and their regulation is critical. Blood feeding triggers signaling pathways required for successful egg development. We hypothesized that these pathways may play additional roles in modulating immunity. The steroid hormone 20-hydroxyecdysone (20E) signaling cascade plays an important role in egg development. 20E is rapidly produced in the ovaries in response to blood feeding and is released into the hemolymph where it can signal to peripheral organs. Previously, studies of the relationship between 20E and mosquito immunity have been focused on 20E's effect on the fat body. To date, no studies have examined the midgut as a potential target of 20E signaling. We found that directly injecting 20E into non-blood fed ***Ae. aegypti*** and ***An. gambiae*** resulted in a significant increase in midgut bacteria measured by colony counting and 16S qPCR. The increase in bacteria parallels what is observed after blood feeding. Metagenomic, transcriptomic, and functional analyses of 20E treated mosquitoes are underway to explore mechanisms leading to expansion of midgut bacteria. Our work provides evidence that 20E signaling influences midgut bacteria levels and that this mechanism is conserved across diverse mosquito species. Understanding regulation of the mosquito commensal population is crucial as their abundance and species composition have been implicated in disease transmission.

1892

GENOMIC DIVERGENCE TO LOCAL ADAPTATION IN CRYPTIC SUBGROUPS OF *ANOPHELES FUNESTUS*

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Anopheles funestus, the second most important vector of human malaria in Sub-Saharan Africa, is splitting into multiple cryptic species and subgroups, which have yet to be fully delineated. We used high-throughput sequencing to explore the genomic patterns of divergence and local adaptation in wild-caught ***An. funestus*** from Cameroon. Genotyping of 6605 SNPs across 101 individuals confirmed the presence of cryptic ecotypes differentially adapted to the savannah and rainforest. Genomic regions of high differentiation between the two ecotypes are clustered on the 3R chromosome, particularly within the 3Ra and 3Rb chromosomal inversions. Comparative mapping of chromosomal arms showed that several outlier regions of genetic differentiation within the 3Rb inversion of ***An. funestus*** map to the 2La inversion of ***An. gambiae***, which is also associated with adaptation to aridity. Despite 50 million years of divergence, it appears that ***An. funestus*** and ***An. gambiae*** utilize the same repertoire of genes to adapt to contrasting environment - a truly remarkable case of convergent evolution. Further functional analyses of genes within the overlapping chromosomal regions of ***An. funestus*** and ***An. gambiae*** will provide a deeper understanding of the direct mechanisms underlying parallel adaptation in these two rapidly evolving species.

1893

EFFICACY OF SISAL STRIPS IMPREGNATED WITH TRANSFLUTHRIN SPATIAL REPELLENT DISPENSED AT ROOM TEMPERATURE TO PROTECT AGAINST OUTDOOR BITING MALARIA VECTORS

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In most African settings, people spend time outside houses in the early evening before they are under the protection of long lasting insecticide treated nets thus are exposed to infectious mosquito bites. An ideal spatial repellent is likely to provide long range repellency outdoors and reduce mosquito bites. The objectives of this study were to determine protective efficacy of sisal strips treated with Transfluthrin and to determine protection to non-users, as well as degree of diversion of mosquitoes from users of Transfluthrin sisal strips to the non-users. Experiments were conducted in a rural village, southeastern Tanzania. The main malaria vectors are Anopheles arabiensis mosquitoes that bite both indoors and outdoors. Efficacy of Transfluthrin sisal strips was determined by measuring the biting rate of mosquitoes of human landing catchers with treated Transfluthrin sisal compared to untreated sisal. Durability of efficacy of treated sisal was determined as well as protection of treated to non-user at different distances (10m, 20m, 30m, 40m and 60m) from the user of treated sisal. Over 8000 persons hours of human catches were conducted. Treated strips prevented more than 80% bites for a period of 30 weeks and at least 50% bites were prevented 90 weeks after the strips had been treated. Treated strips provided 40% relative protection to non-users at 5m and no evidence of increased risk to non-users beyond that distance was obvious. Analysis of all 4032 person hours of human landing catches from the dose-response reveal that; 0.01ml Transfluthrin provided 80% protection against mosquito bites, 1ml Transfluthrin provided 70% protection, 2ml provided 70% protection and 5ml Transfluthrin provided 70% protection. Sisal fibers are excellent substrates for dispensing vapour active spatial repellents, including Transfluthrin without the need of external sources of heat. This study shows that treated sisal strips reduced bites of outdoor biting An. arabiensis hence are likely to protect against malaria occurring outdoors LLINs may not be sufficient.

1894

NORTH AMERICAN PARAGONIMIASIS: IDENTIFICATION OF A NOVEL INTERMEDIATE SNAIL HOST FROM MISSOURI

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Paragonimiasis is a foodborne trematode infection that affects 23 million people mainly in Asia. The infection is locally of great public health

importance and can cause a serious lung disease that is often misdiagnosed as tuberculosis or cancer. In North America, paragonimiasis is caused by ***Paragonimus kellicotti***. Infection is rarely seen in humans but is common is the animal definitive host in some states. After a small outbreak of human paragonimiasis in southern Missouri, the second intermediate host and source of human infection was identified as common crayfish species ***Orconectes punctimanus, O. luteus*** and ***O. virilis***. Although the infection rates in crayfish were locally very high (>60%), the first intermediate host remained unknown. Only the walker snails ***Pomatiopsis lapidaria*** and ***P. cincinnatiensis*** (family Hydrobiidae) have been reported as first intermediate hosts for ***P. kellicotti***. These snail species have not been observed in areas with high infection rates in crayfish. In order to identify the snail host of ***P. kellicotti*** in our study area, we collected more than 2,000 snails of the three most common species and screened them by light exposure/cercariae shedding and dissection for developmental stages of ***P. kellicotti***. We detected cerariae and rediae of ***P. kellicotti*** in the endemic ***species Elimia potosiensis*** (family Pleuroceridae). Trematode identity was confirmed by PCR and DNA sequencing. ***E. potosiensis*** is also the first intermediate host to at least two undescribed trematode species. According to ITS-2 sequence, these appear similar to ***Lecithodendrium spathulatum***, a parasite of bats, and ***Collyriclum faba***, a parasite of birds. Our findings indicate that ***P. kellicotti*** has a wider first intermediate host range than previously assumed, and can use high abundance snail species of different families for development. The flexibility of the host range of all developmental stages of ***P. kellicotti*** may explain why this parasite species can be locally so abundant.

1895

ASSESSING THE EFFECT OF WATER QUALITY MARKERS ON THE VIABILITY OF SCHISTOSOME-BEARING SNAILS

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Schistosomiasis, infection by ***Schistosoma*** worms, is a disease that affects over 200 million people worldwide, mostly in developing areas in Africa and Asia. ***Biomphalaria, Bulinus***, and ***Oncomelania*** snails are the intermediate hosts for ***Schistosoma***, which emerge from these snails as cercaria, the larval stage which infects humans. At the Biomedical Research Institute (BRI), we maintain the life cycle for multiple species of schistosomes and provide these parasites for researchers around the world. Given the importance of optimizing viability of lab-cultivated snails to our mission, we are investigating the effects of water quality markers on the growth and viability ***of Biomphalaria, Bulinus***, and ***Oncomelania*** snails. We have determined the baseline values for various water quality markers such as pH, chlorine content, conductivity, total dissolved solids, salinity, hardness and surface tension. Adjustment of multiple water quality markers affects the viability of schistosome-bearing snails. Our work has implications for not only optimal laboratory cultivation of snails, but may also help identify novel means by which water properties can be manipulated in order to reduce infestation by schistosome-bearing snails.

1896

SPATIAL ANALYSIS TO HUMAN FECAL WATER CONTAMINATION BY MICROBIAL SOURCE TRACKING: ASSOCIATION WITH RISK OF SCHISTOSOMIASIS FOLLOWING TREATMENT IN BRAZIL

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Schistosomiasis is to a degree a disease of contact with fecally contaminated surface waters, rather than ingestion. In a community in rural Bahia, Brazil that straddles a shallow river, we previously developed a model which showed that proximity from a person's home to the nearest water contact site and concentration of human fecal contamination at this point explained ~50% of the risk of infection. This result was for data obtained during a community survey prior to treatment when prevalence of infection was 45%. We also observed that human fecal contamination increased considerably in the downstream portion of the village. Human fecal contamination was assessed by determination of a widely used Lachnospiraceae marker, which we validated by pyrosequencing and qPCR of local stool samples. In this opportunity, we used QGIS to test our model in this same village following treatment. All individuals who tested positive were treated in 2009 and at follow up in 2012, prevalence had declined by 48% and intensity by 44%. Those infected were again treated in 2012 and the village was re examined in 2013 when prevalence declined by 32% and intensity by 30%. Re infection rates were 33.7 and 18.4% in 2012 and 2013, respectively. Incidence was 22.0 and 12.9%. Our model explained 73% and 81% of the risk of infection in 2012 and 2013, respectively. Improvement in identification of risk by the model over time can be explained by the effect of treatment. People who live closer to water with high human fecal contamination are more likely to be re infected and people who live in proximity to water with low contamination are likely to remain infection free. This is further supported by spatial analysis which showed that over time, new infections clustered downstream. Our findings give more insight into the importance of human fecal water contamination for schistosome transmission. Models of schistosome transmission and spatial analysis may aid in identifying areas at risk of re infection in communities with high prevalence of infection undergoing treatment.

1897

DETERMINANTS OF *SCHISTOSOMA JAPONICUM* INTERMEDIATE HOST MIGRATION IN SICHUAN, CHINA

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Schistosomiasis is targeted for elimination in China, and monitoring for re-emergence in previously controlled areas in this country has become a major public health priority. Host and vector dispersal can facilitate the spread and re-emergence of infectious diseases, but little is known about the movement of ***Oncomelania hupensis***, the intermediate host for ***Schistosoma japonicum***. This study characterized intermediate host snail movement between villages in Western China and examined environmental factors that affect host dispersal in this setting. Snail migration rates between 32 sites in Sichuan Province, China, were estimated using Bayesian assignment tests conducted on genotypes of 833 ***O. hupensis*** snails. Bi-directional effective geographic distances (EGD) between each site pair were estimated based on geographic weightings representing nine environmental models: Euclidean, topography, incline, wetness, land use, distance from watershed, stream use, streams and channels, and stream velocity. Median, first quartile and minimum EGD values were analyzed as to their ability to explain estimated paired migration rates using mixed effects models. Among sites, 7.8% to 32.7% of sampled snails were identified as migrants, with 20 sites containing over 20% migrants. Migration rates were generally low between sites; however, for 12 sites, as much as 80% of the migrant population and over 10% of the overall snail population originated from a proximal site. EGD was found to be a significant predictor of migration rate, with the log of the minimum EGD emerging as the best predictor across all environmental models. Odds of migration decreased as EGD increased (OR from Euclidean model: 0.70, 95% CI: 0.63, 0.78). Models accounting for topography and wetness explained a significantly larger proportion of the variance in migration between sites as compared to the Euclidean model. Random intercept models suggest nearly 50% of the variation in migration rate can be attributed to site-specific factors, and roughly 10% can be attributed to EGD. Our findings have important implications for controlling the geographic spread of schistosomiasis in China.

1898

DOES FLOODING DETERMINE THE DISTRIBUTION OF SCHISTOSOME-TRANSMITTING SNAILS IN MIDDLE TO LOWER REACHES OF THE YANGTZE RIVER, CHINA?

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Schistosomiasis, caused by blood fluke genus ***Schistosoma***, is one of the most devastating tropical diseases in the world. ***Oncomelania hupensis*** is the only intermediate host of schistosoma and its growth and development are sensitive to environmental factors. In China, these snails are only found in the 12 Provinces located along the Yangtze River and south of it. Hunan Province, located in the southeastern hinterland of China, along the middle and lower reaches of the Yangtze River, is one of the schistosomiasis endemic areas in China, and ***Oncomelania*** snails are mainly distributed in the vast floodplains of Dongting Lake region, the northeastern part of Hunan Province, while no snail is found in the southwestern part of Hunan Province, where the altitude is much higher. In this study, we focused on the influence of extreme low air temperature, annual precipitation and time of flooding on the distribution of ***Oncomelania*** snails in Hunan Province, China. The distribution areas of snails in Hunan Province were marked and the data of annual extreme low air temperature, annual precipitation and the time of flooding from 1995 to 2002 was collected or calculated. Nonparametric tests suggest that in Hunan Province, the extreme low air temperature and precipitation are not the main factors that impact the distribution of ***Oncomelania*** snails, while the time of flooding is a crucial factor that influences the distribution of ***Oncomelania*** snails in Hunan Province, and the favorable time for the snail's survival ranges from about 2 to 7 months.

1899

THE *SCHISTOSOMA MANSONI* CYTOCHROME P450 (CYP450) IS ESSENTIAL FOR WORM SURVIVAL

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Schistosomiasis affects millions of people in developing countries and is responsible for over 200,000 deaths annually. There is only one drug, praziquantel, available for its treatment. Recent data suggest that drug resistance could soon be a problem. There is therefore the need to identify new drug targets and develop drugs for the treatment of schistosomiasis. Unlike many other organisms, e.g., humans have 57 CYP450 genes and C. elegans has 83 CYP450 genes, ***Schistosoma mansoni*** has only one CYP450 gene encoded in its genome. Analysis shows that the predicted 1452 bp open reading frame encodes a characteristic heme-binding region in its catalytic domain and a hydrophobic sequence that is usually present as the membrane interacting region in other CYP450s. The highest identity to human CYP450s is 22%. Using 5' RACE and analysis of cDNAs from worm populations and different developmental stages, we found no evidence that the schistosome CYP450 mRNA is differentially spliced or processed, suggesting that a single CYP450 protein is present in worms. Analysis of CYP450 mRNA abundance indicates that it is differentially regulated with the egg and schistosomula having the highest levels. Additionally, adult female worms have higher message levels than adult male worms. We used reverse genetic and chemical tools in order to determine if the CYP450 is essential for parasite survival. RNA interference caused large reductions in CYP450 mRNA and resulted in schistosomula death. CYP450s are targets of many antifungal and cancer therapies. Antifungal azoles target 14α-demethylase (CYP51A1), a vital CYP450 enzyme for sterol metabolism and cell membrane stability in fungi. We found that imidazole antifungal agents related to miconazole targeted CYP450 and resulted in larval and adult worm death at low micromolar concentrations. In addition, combined sub-lethal concentrations of both CYP450-specific dsRNA and miconazole showed an additive effect on larval worm death indicating that the effect of miconazole is CYP450 specific. Our results indicate that the schistosome CYP450 is essential for worm survival and could be an ideal drug target.

1900

SCHISTOSOME ABC TRANSPORTERS: ROLES IN DRUG SUSCEPTIBILITY TO PARASITE PHYSIOLOGY

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Praziquantel (PZQ) is essentially the only drug available for the treatment of schistosomiasis, a disease affecting hundreds of millions. Although invaluable, PZQ has significant limitations. It is largely ineffective against juvenile schistosomes, and there are several reports of treatment failures in the field. Clearly, there is an urgent need for new therapeutics. ATP binding cassette (ABC) transporters such as P-glycoprotein (Pgp) mediate efflux of toxins and xenobiotics from cells and are associated with drug resistance in many organisms, including parasitic helminths. They show broad substrate specificity and are inhibited by several drugs currently in clinical use. ABC transporters are also implicated in a variety of normal physiological activities and transport many potent signaling molecules with high affinity, including several with immunomodulatory activity. We hypothesize that schistosome ABC transporters offer attractive candidate targets for new or repurposed drugs that act either as antischistosomals on their own or as adjuncts that enhance parasite susceptibility to PZQ. Schistosomes with reduced PZQ sensitivity show higher basal expression of ABC transporters such as Pgp (SMDR2) and multidrug resistance associated protein (SmMRP1). ABC transporters are also upregulated in response to sub-lethal concentrations of PZQ. PZQ is both an inhibitor and likely substrate of schistosome Pgp. Disruption of transporter function (by inhibition) or expression (by RNAi) increases the activity of PZQ against adult parasites, and results in PZQ-refractory juvenile worms becoming susceptible to the drug. ABC transporters also appear to play a role in parasite egg production. We are currently investigating the role of schistosome ABC transporters in PZQ susceptibility of the parasite within the host. We are also assessing the role of these transporters in the parasite's modulation of host immune responses. These studies should provide important insights into the role of ABC transporters in PZQ susceptibility and parasite physiology, and may offer targets for novel or repurposed therapeutics.

1901

EBOLA EPIDEMIC IMPEDES MALARIA CARE DELIVERY IN GUINEA

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The current Ebola epidemic has had significant impact on the overall functioning of the healthcare system in affected countries. Due to the overlap of symptoms of Ebola virus disease and malaria, and malaria's position as the leading cause of health facility visits, malaria care delivery is

particularly sensitive to healthcare system perturbations due to the Ebola epidemic. A survey of 60 randomly-selected health facilities in prefectures highly affected by Ebola and 60 randomly-selected health facilities in Ebola-unaffected prefectures was performed in Guinea in December 2014. Study teams abstracted malaria case management indicators from registers for January-November for 2013 and 2014 and interviewed healthcare workers and community health workers. Nationwide weekly surveillance data on suspected malaria cases reported for 2011-2014 were also analyzed. The expected number of cases in 2014, estimated from the trends in 2011-2013, was compared to the observed surveillance data for 2014. There were substantial reductions in all-cause patient visits (-42%), fever cases (-46%), and patients treated with oral and injectable antimalarials (-58% and -69%) in adults and children >5 in Ebola-affected prefectures, with smaller but still substantial reductions in other age groups and in Ebola-unaffected prefectures. In Ebola-affected prefectures, only 74% of community health workers were operational, and only 48% of those working reported actively treating malaria cases, compared to 66% before the Ebola epidemic (p-value <0.01). Nationwide, the Ebola epidemic was estimated to have resulted in 74,000 (95% CI: 71,000-77,000) fewer malaria cases seen at health facilities from the start of the Ebola epidemic to the end of 2014. The decrease in malaria care delivery at the health facility and community level due to the Ebola epidemic threatens malaria control in Guinea. Untreated and inappropriately treated malaria cases lead to excess malaria morbidity and mortality, and thus more fever cases in the community. This makes identification of suspect Ebola cases more difficult, potentially hampering an effective Ebola outbreak response.

1902

DEATHS DUE TO *PLASMODIUM KNOWLESI* MALARIA IN SABAH, MALAYSIA, 2012-2014: REDUCED FATALITY RATE WITH IMPROVED USE OF INTRAVENOUS ARTESUNATE

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Plasmodium knowlesi is the most common cause of malaria in Malaysia, and an important cause of fatal malaria in Sabah. In a review of malaria deaths in Sabah during 2010-2011, fatal outcomes were associated with misdiagnosis of knowlesi malaria as ***P. malariae*** and consequent delayed initiation of parenteral therapy. In Malaysia intravenous artesunate is now the recommended firstline treatment of severe malaria of any species. We reviewed malaria notification data and case notes of mandatorily-reported malaria deaths in Sabah 2012-2014, to describe clinical details of ***P. knowlesi*** deaths and to assess for trends in ***P. knowlesi*** case fatality. Sixteen malaria deaths were reported: 7 ***P. knowlesi***, 7 ***P. falciparum***, and 1 ***P. vivax*** (all PCR-confirmed), and one microscopy-diagnosed "***P. malariae***". Median age of fatal ***P. knowlesi*** cases was 61 years, and four were female. Complications of PCR-confirmed ***P. knowlesi*** cases included jaundice (N=5), metabolic acidosis (N=5), acute kidney injury (N=3), shock (N=6), and respiratory distress (N=7). Four ***P. knowlesi*** cases were diagnosed by microscopy as ***P. malariae***, 1 as ***P. falciparum***, 1 as ***P. vivax*** and 1 as ***P. knowlesi***. All recognised to have severe malaria on admission received intravenous artesunate. A total of 3138 cases of microscopy-diagnosed ***P. malariae/P. knowlesi*** were notified in Sabah during 2012-2014, giving a fatality rate of 2.23 per 1000 notifications, compared to a fatality rate of 5.5 per 1000 notifications during 2010-2011 (1087 notifications with 6 ***P. knowlesi*** deaths). Despite ongoing microscopic misdiagnosis of ***P. knowlesi***, management of severe malaria in Sabah appears to have improved. Although the study demonstrates the ability of ***P. knowlesi*** to cause fatal disease despite optimal therapy, increased use of artesunate has been associated with a reduction in ***P. knowlesi*** fatality-rates.

1903

CLUSTERING OF IMPORTED MALARIA CASES IN A SETTING OF VERY-LOW MALARIA INCIDENCE IN NORTHERN SENEGAL

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Richard-Toll district (pop. 157,915) in northern Senegal is home to industrial activities among which are sugar cane harvesting and refining, with about 2,000 seasonal and 3,000 permanent workers. Recognizing very low transmission and numbers of infected residents in the district, a malaria case investigation strategy was initiated in Richard-Toll by the NMCP in 2012 and has continued as a routine district-wide intervention. RDT or microscopy-confirmed cases presenting to health facilities are followed to their homes and all members of the index case household and the 5 nearest households are tested for malaria and given treatment if positive. In 2013, 234 passively detected cases of malaria were investigated and 10,071 contacts were tested, with 42 positive secondary cases. Of the 276 total positive cases, 175 (63%) had recent travel history. In 2014, 134 passively detected cases of malaria were investigated and 5,751 contacts were tested, with 14 positive secondary cases. Of the 148 total positive cases, 104 (70%) had recent travel history. Travel appears to play an important role in malaria transmission and there appears to be clustering of travel-related cases around Richard-Toll city during October-November: in 2013 and 2014, 79% and 80% of cases had recent travel (53% and 62% of total imported cases in district) and 51% and 57% occurred in October and November. In 2014, 82% of cases with recent travel were concentrated in 4 neighborhoods. This period coincides with the beginning of the school year and the sugar cane harvest, as well as a major religious holiday in 2014. These results suggest that opportunities exist to improve targeting of malaria elimination strategies within the district, including focused screening of higher-risk populations such as seasonal workers, pupils and travelers. During 2012-2014, 478 cases were passively identified in health facilities and 72% were associated with travel and likely imported, suggesting that local transmission within the district is extremely low (average of 0.28/1000 pop./year) and with attention to reducing the risk of imported infections, the district could rapidly become malaria-free.

1904

TOLERABILITY TO SAFETY OF WEEKLY PRIMAQUINE AGAINST RELAPSE OF *PLASMODIUM VIVAX* IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT TO NORMAL CAMBODIANS

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Primaquine (PQ) is not implemented in many malaria endemic countries, including Cambodia, to prevent ***Plasmodium vivax*** relapse for fear of precipitating PQ-induced acute haemolytic anaemia (AHA) in patients with glucose-6-phosphate dehydrogenase deficient (G6PDd). Reluctance to

use primaquine is reinforced by a lack of quality safety data. From January 2013-January 2014, Cambodians with acute vivax malaria were treated with dihydroartemisinin-piperaquine on Days (D) 0, 1 and 2 plus weekly primaquine 0.75 mg/kg x 8 doses (D0, 7, 14 to D49) and follow to Day 56. G6PD genotype and measured G6PD activity confirmed G6PD status. The primary outcome was treatment completion without primaquine toxicity defined as any one of: (i) severe anaemia (Hb<7 g/dL), (ii) a fractional fall in Hb >25% vs. D0, (iii) blood transfusion, (iv) haemoglobinuria, (v) acute kidney injury (AKI, an increase in baseline serum creatinine > 50%), or (vi) methaemoglobinaemia (metHb) > 20%. The Trial registration number is ACTRN12613000003774. 75 patients were enrolled with a median (range) age of 24 (5-63) years; 63 (84%) patients were males. D0 G6PD activity ranged from 0.1-1.5 U/g Hb (median 0.85 U/g Hb) in 18 G6PDd patients (17/18 had the Viangchan variant) and 6.9-18.5 U/g Hb (12 U/g Hb) in 57 G6PD normals (G6PDn). Median (range) D0 Hb concentrations were similar (p=0.46) between G6PDd vs. G6PDn: 13 g/dL (9.6-16) vs. 13.5 g/dL (9-16.3) and nadired on D2 in both groups: 10.8 g/dL (8.2-15.3) vs. 12.4 (8.8-15.2) g/dL (p=0.006), respectively. By D7, five G6PDd patients (27.7%) had > 25% fall in Hb vs. 0/57 in G6PDn (p=0.00049), including one G6PDd male who required a blood transfusion (D0-D5 Hb 10.0-7.2 g/dL). No patient developed severe anaemia, haemoglobinuria, metHb concentration > 4.9% or AKI. In conclusion, vivax infected, G6PDd patients suffered significant, mostly transient, falls in Hb and one was transfused. Weekly primaquine in G6PDd patients mandates medical supervision and pretreatment screening for G6PD status. The feasibility of implementing a package of G6PDd testing and supervised primaquine should be explored.

1905

IMPROVING MALARIA DATA QUALITY TO USE IN HEALTH MANAGEMENT INFORMATION SYSTEM, TANZANIA

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Malaria data is an important aspect for monitoring malaria burden and intervention coverage to inform evidence-based programmatic decisions. Anecdotal evidence suggests that the quality of malaria data from routine health management information system (HMIS) is poor which made it difficult to analyze, interpret and ultimately the use of these data is limited. The situation analysis was conducted to assess, identify gaps, and setting up priorities of malaria data quality and use in Tanzania's HMIS. Desk reviews and field-based data collection was conducted to gather information that describes current practice, barriers and needs for malaria data quality improvement. A stakeholder's workshop was convened to triangulate data, identify gaps, propose solutions, prioritize activities and develop a work plan to improve data quality and use. The findings suggest that malaria data quality in Tanzania is poor. The main issues reported at the facility level were data inconsistencies (in the registers, tally sheets and summary forms), data collection tools not being available and not being filled out. The findings also showed that most of health facilities do not complete the tally sheets. At the district and regional levels, the main challenge encountered was unreliable internet connectivity that hindered timely processing of data into the DHIS2 system. The stakeholders proposed priorities for improving data quality and use, including: 1) data quality and use of data to be introduced as permanent agenda item in Health Management teams at district and regional level; 2) develop guidelines on data quality and use; and 3) supporting CHMTs to conduct regular, comprehensive and effective supportive supervision and, including data quality assessments. HMIS that produce complete, timely, reliable, and valid data are needed by national malaria control programs to monitor malaria burden and intervention coverage at national and sub-national levels to inform evidence-based programmatic decisions. The challenges appear to be behavioral and organizational, relating to how staff value and use data in their everyday work.

1906

SEASONAL MALARIA CHEMOPREVENTION, THREE YEARS OF IMPLEMENTATION

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In 2012, Médecins Sans Frontières (MSF) started implementing seasonal malaria chemoprevention (SMC), following WHO recommendation for children in areas with high seasonal transmission using sulphadoxine-pyrimethamine and amodiaquine. MSF works in southern Mali, Chad and Niger where children suffer 2-3 episodes of malaria/year and where the malaria season is accompanied by a malnutrition peak causing an increase in admissions. SMC started in 2012 in MSF projects in Mali and Chad, and in 2013 in Niger. Monthly contacts with families at SMC delivery, give opportunities to provide other health interventions. In 2013 SMC was combined with screening for malnutrition, and in 2014, in Chad, with Pentavalent vaccination for children under 2. In Niger we included SMC in a preventive package (PP) which includes nutrition screening and prevention and EPI. Programs were evaluated through surveys to determine coverage, adherence and acceptability of SMC. We assessed the impact of the programs on incidence of simple and severe malaria, hospital mortality, all-cause hospitalizations and admissions with severe malarial anaemia, using program data. Estimates of SMC efficacy were made from the proportion of malaria cases who had received SMC and SMC coverage, using the screening method. Side-effects where monitored through re-inforced national pharmacovigilance systems. The prevalence of molecular markers of resistance to SMC drugs was assessed through surveys before and after SMC implementation. There has been a high level of acceptability of SMC in the communities. Monthly coverage of SMC exceeded 85% in rural areas but was lower in an urban area. When combined with a PP and delivered at the health center, coverage was similar to SMC alone. Pentavalent vaccine coverage increaased when combined with SMC in Chad. SMC led to substantial reductions of both simple and severe malaria cases in Mali, Chad and Niger. We observed a reduction in cases of severe malarial anaemia, but no reduction in the number of cases of cerebral malaria. Integration with other interventions is feasible and can prevent missed opportunities in the child health package.

1907

MALARIA INDICATORS IN THE DEMOCRATIC REPUBLIC OF THE CONGO, 2014

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Malaria continues to be a major health problem in DRC, accounting for an estimated 40% of outpatient visits by children under five and 19% of the overall mortality in the same age group. The implementation of large scale malaria interventions in the country continues to face serious challenges. The USG funding for malaria activities in the Democratic Republic of Congo (DRC) has increased from $18 million in FY 2010, to 50 million in FY 2014, a substantial increase which has allowed the DRC to obtain the successful results seen in the last Demographic and Health Survey (2013). Results have shown: a) the increase in the use of mosquito nets by children under five, from 38% to 56%; b) the increase in their use by pregnant

women, from 43% to 60%; and c) the malaria prevalence in children under five, with a rate of 30.8% obtained by RTDs, and 22.6% obtained through microscopy tests. The aim of this study is to review malaria cases throughout ten years, and describe the different malaria interventions carried out during those same years and their impact. The NMCP is reviewing this data to define its new country malaria strategy 2015-2020. Additionally, data will be presented from the 5 provinces supported by PMI to show the impact of five years of interventions in those provinces. The US President's Malaria Initiative in the Democratic Republic of Congo has been supporting the majority of interventions in five provinces, and together, with the support of the Global Fund, the World Bank, and the Department for International Development of the British Government (DFID), the coverage of the country has reached all the health zones, even those with very difficult access.

1908

DEVELOPMENT OF EFFECTOR MOLECULES TO BLOCK *LEISHMANIA* TRANSMISSION UTILIZING THE PARATRANSGENIC APPROACH

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Leishmaniasis is a neglected tropical disease that affects 13 million people worldwide. This disease is caused by the protozoan parasite Leishmania, and is transmitted by the bite of an infected female phlebotomine sand fly. Our laboratory utilizes the paratransgenic approach to control parasite transmission by host vectors. In this approach, symbiotic or commensal bacteria residing within the gut of the vector are genetically modified to express molecules that target the parasite. In previous work, we had reported on the toxicity of honey bee melittin against ***Leishmania*** spp. Leishmaniacidal activities of human histones H2A and H2B has been shown by others. In this study, we are examining the combined toxicity of these two antimicrobial peptides (AMP's) on ***Leishmania major*** promastigotes in an effort to develop a broader panel of effector molecules for use in the paratransgenic strategy. In experiments measuring live/dead ratios of cultured promastigotes by flow cytometry and fluorescent plate assays, the IC100 for melittin and H2B are observed at 5 uM and 7 uM, respectively. Synergistic toxicity is observed when the AMP's are used in combination treatments, decreasing the calculated IC100 values by more than half. There results were further verified by con-focal microscopy. Similar experiments are underway to characterize the activity of these two AMP's against other strains of ***Leishmania*** as well as ***Trypanosoma cruzi***, the causative agent of Chagas disease. The addition of a second AMP to paratransgenic systems that result in more efficient parasite killing has multiple advantages. Specifically, it decreases the absolute expression levels required from the transformed commensal bacteria within the gut of the sand fly. Additionally, the use of multiple AMP's drastically reduces the incidence of acquired resistance by the parasite

1909

EVOLUTIONARY COMPARISON OF THE GENOME OF *LEISHMANIA DONOVANI* CLINICAL ISOLATES FROM INDIA WITH ITS ANCESTOR *LEPTOMONAS*

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Flagellates of the family Trypanosomatidae fall into two natural groups. The ancestral genera is ***Leptomonas*** which is confined to invertebrates, the more evolved genera is ***Leishmania*** which uses both vertebrate and invertebrate hosts. Utilizing next generation sequencing technology we explore the genomes of the two clinical isolates, ***Leishmania*** and ***Leptomonas*** to answer a wide range of evolutionary and pathological questions. We find that the genome of ***Leishmania*** donovani was similar to size and coding capacity with other ***Leishmania*** genomes. However, genome of ***Leptomonas*** is much smaller but contains in contrast more protein coding genes. It was seen that this genome expansion is mostly due to larger number of repetitive sequences and analysis of SNPs showed that the core genome was more variable in this isolate. Aneuploidy indicated there is significant opportunity for generation of SNPs that may have many applications in molecular epidemiology. Transposable elements (TEs) provided a source of genetic variation in ***Leptomonas***. Variation in the Ras protein superfamily between ***Leishmania*** donovani and ***Leptomonas*** was observed. Absence of urea cycle in ***Leptomonas*** was found to be an ancestral feature. Our whole-genome sequence data reveals genetic structural differences between ***Leishmania*** and it's ancestral species ***Leptomonas***, which cannot be identified by using multilocus typing alone. Our research finding has immense potential for creating renewed impact of the parasite genome on biomedical research, contributing to a paradigm shift in research activities that will lead to new diagnosis and treatment.

1910

ELECTRONIC VOLUMETRIC ASSESSMENT DURING SODIUM STIBOGLUCONATE THERAPY IN PATIENTS WITH CUTANEOUS LEISHMANIASIS: A PROMISING TECHNIQUE USING DIGITAL 3D MODELS

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Cutaneous Leishmaniasis (CL) is susceptible to bias and subjectivity during assessment of treatment response and follow-up. As a part of standard of care, methods to estimate the extent of lesions are usually performed through manual or visual methods which are non-uniform and difficult to standardize in low-income settings. This work shows a novel, non-invasive method to obtain metrics of depth and volume by creating digital models of CL lesions. Digital models of CL lesions were created by acquisition of point-clouds with a commercial 3D scanner. The data were exported using the .XYZ file format that provided the point coordinates to recreate 3D mesh models. After IRB approval, patients with CL were enrolled, and lesions were digitalized: twenty-six before treatment, and 8 at the end of treatment. Two volumes were considered from each lesion: inflammatory volume (IV, a volume between an imaginary line following normal skin surface and an imaginary surface located at the top of raised edges) and an ulcerative volume (UV, a volume between an imaginary line following normal skin surface and the deepest point of the ulcer). Three metrics (in mm3) and one coefficient were obtained for each lesion: global volume (GV: IV+UV), IV, UV, and the volume coefficient (VC: IV/UV proportion). Mean duration of disease was 2.2 ± 0.89 months. 12 of 26 (46%) were classified as ulcer-predominant (if UV was higher than IV), and 14 (54%) as inflammatory-predominant (if IV was higher than UV). Median metrics at baseline evaluation were: 221.6 for GV, 143.14 for IV, 36.6 for UV, and 1.66 for VC. According treatment outcome, there were no significant differences in these metrics at baseline evaluation. In patients with volumetric evaluations after treatment, GV was higher in those classified as treatment failure compared to those classified as cured after 1-3 months of follow-up (2218.5 vs. 224, p=0.04). As a proof of concept, this study represents initial evidence to support the utility of electronic volumetric assessment of treatment response and follow-up of CL, with future possible extension to other transmissible and non-transmissible dermal diseases.

1911

THE DILEMMAS OF CONGENITAL CHAGAS DISEASE SCREENING IN AN ENDEMIC SETTING

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Concomitant with successful trans-national disease control programs across Latin America, Chagas disease has shifted from a neglected, endemic parasitic infection of the rural poor to an urbanized chronic disease and now a potentially emergent global health problem. Congenital transmission has become proportionately more important, estimated to account for 22% of new Trypanosoma cruzi infections in 2015. Treatment during infancy is significantly more efficacious and better tolerated than later, but current diagnostic methods, even when optimally executed, fail to detect over half of infected neonates and <20% of infants complete 9-months of follow-up. We recruited pregnant women presenting for delivery in two urban hospitals in Santa Cruz department, Bolivia and monitored infants of infected women at birth, 1, 6 and 9 months to evaluate the performance of quantitative PCR (qPCR), IgM Western blots (using trypomastigote excreted-secreted antigen; TESA-blots) and micromethod (microscopic observation of trypomastigotes) for newborn screening for congenital Chagas disease. Of 518 at-risk infants from 507 seropositive women, unequivocal congenital transmission was identified in 32 infants of 29 mothers, including 3 sets of concordantly infected twins (5.7% transmission rate); 6 additional neonates were diagnosed at 6 months or later and vectorial or intrapartum transmission could not be excluded. In cord blood, qPCR, TESA-blot and micromethod displayed sensitivity/specificity of 82.8%/97.3% (median of 7143.6 parasites/ml; interquartile range of 5.0-187788.9 parasites/ml), 71.4%/99.5% and 20.7%/100%, respectively. When birth and 1 month results were combined, cumulative sensitivity reached 96.9%, 89.7% and 38.7% for qPCR, TESA-blot and micromethod, respectively. qPCR has the potential to facilitate earlier diagnosis and circumvent loss to follow-up. We critically discuss the technical, logistical and economic obstacles of implementing routine molecular screening for congenital Chagas disease in resource-limited settings and describe the future prospects for improvement.

1912

GALECTIN 3 IS ASSOCIATED WITH THE MORE SEVERE FORM OF CHAGAS CARDIOMYOPATHY

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Chagas Chronic Cardiomyopathy (CC) is characterized by a diffuse myocarditis with intense myocardial remodeling, fibrosis, cardiomyocyte hypertrophy and damage. Gal-3 is involved into two pathophysiological mechanisms (fibrosis and inflammation), hence GAL-3 levels maybe a good biomarker to stratify Chagas Disease patients and identify those who could benefit from early treatment strategies. The objective of this study was to evaluate if Gal-3 level in plasma is associate with the development of the severe form of CC and prognosis. We have used samples collected in a previous retrospective cohort that enrolled ***T cruzi*** seropositive, and seronegative blood donors (BD) in São Paulo and Montes Claros, Brazil. This cohort was supplemented with CC patients from a tertiary hospital (Heart Institute). All subjects underwent a health clinical evaluation, ECG, and echocardiogram (Echo). ECG and Echo were reviewed blindly by centralized reading centers. The subjects were classified as with or without signs of CC by a blinded panel of 3 cardiologists. Gal-3 (VIDAS® galectin 3 France - bioMerieux Inc.) was available to test 441 samples: 101 negative controls, 190 BD without CC, 60 BD with CC, and 90 patients with CC. The median level of GAL-3 was 13.1ng/mL for negative controls, 12.ng/mL for BD without CC, 13.1ng/mL for BD and 15.4ng/mL for CC patients. The proportion of individuals with Gal-3 level > 17.8 was significantly higher ($p<0.0001$) among the CC patients (37.8%) as compared to negative controls (14.8%), BD without CC(11.1%) and BD with CC (16.7%). Ejection fraction <50 was also associated with higher levels of Gal3 ($p=0.0001$). The Gal3 showed correlation with lCAM-1, MPO, PAI-1, CRP, IL6, TNFα, IL8, VEGF, Troponin and NT-proBNP, with p values <0,01. Follow up date on mortality was available for the CC patients. In this group, we could detect an association of Gal-3 level and subsequent death in the follow up 5 years. ($p=0.0004$). In conclusion, our data suggest that Gal-3 is associated with the more severe form of CC and risk of death.

1913

A FIELD-APPLICABLE MOLECULAR TOOL TO DIAGNOSE AMERICAN CUTANEOUS LEISHMANIASIS

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Dermal and mucosal leishmaniasis is widely distributed in Central and South America affecting an estimated 190,000- 300,000 people annually. Microscopy is the most common diagnostic method used in endemic regions but its sensitivity is low (≤70%) and tends to decrease further with disease chronicity. Serology is variable and does not distinguish between current and past infections. Conventional or quantitative PCR from dermal or mucosal samples have high sensitivity (≈87-98%) and specificity (≥87%) but require expensive equipment, trained personnel and lab facilities beyond the possibilities of resource-limited health infrastructure of endemic areas. We developed a novel point of care molecular test to diagnose dermal and mucosal leishmaniasis produced by ***Leishmania Viannia*** spp., which are responsible for the majority of cases. We designed primers and probes that targeted the kinetoplast DNA minicircles. ***Leishmania*** DNA was extracted using the Qiagen® kit and detected by isothermal Recombinase Polymerase Amplification coupled with a lateral flow immunochromatographic strip (RPA-LF). The test has sensitivity similar to real time PCR (gold standard) detecting as few as 0.1 parasites per reaction. It does not require expensive equipment and the results are read with the naked eye in < 1 hour. The RPA-LF specificity for the L. Viannia subgenus was confirmed by the amplification of ***L. braziliensis, L. panamensis, L. guyanensis, L. peruviana*** and ***L. lainsoni***. There was no cross amplification with ***L. chagasi, L. major, L. mexicana, L. amazonensis*** or ***T. cruzi***. Preliminary data indicated that RPA-LF has an excellent agreement with PCR as determined in patient samples from endemic areas of Peru. We are evaluating additional primer sets capable of amplifying the ***Leishmania*** subgenus with the goal of developing an RPA- multiplex lateral flow test that encompasses all species that produce cutaneous leishmaniasis. This novel method could fill the need for a field applicable diagnostic tool critical to cutaneous and mucosal leishmaniasis management and control.

1914

ACTIVATION OF BICYCLIC NITRO DRUGS BY A NOVEL NITROREDUCTASE (NTR2) IN *LEISHMANIA*

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The novel nitroimidazopyran agent, (***S***)-PA-824, has potent antibacterial activity against ***Mycobacterium tuberculosis in vitro*** and ***in vivo*** and is currently in Phase II clinical trials for TB. In contrast to ***M. tuberculosis***, where the (***R***) enantiomer is inactive, our previous studies demonstrated that (***R***)-PA-824 shows potent cidal acitivity against ***Leishmania donovani***, the causative agent of visceral leishmaniasis (VL). In the murine model of VL, (***R***)-PA-824 led to >99% suppression of parasite burden when administered orally at 100 mg kg^{-1}, twice daily for 5 days. Defining the mechanism of action of this promising anti-leishmanial has now become the focus of our current studies. In general, nitro drugs are believed to function as pro drugs requiring enzyme mediated reduction before becoming cytotoxic. In trypanosomatids, a type I nitroreductase (NTR) has been demonstrated as catalysing the bio-activation of the bicyclic nitro drugs nifurtimox, benznidazole and fexinidazole. However, transgenic ***L. donovani*** promastigotes overexpressing ***L. major*** NTR do not show an increased sensitivity to (***R***)-PA-824 suggesting that this nitroimidazopyran is not activated by NTR. (***R***)-des-nitro-PA-824 is inactive against ***L. donovani in vitro***, confirming that the nitro group is important for the anti-leishmanial activity of PA-824. Thus, if the mechanism of action of PA-824 does involve bio-activation, then this putative reduction is mediated by an as-yet unidentified enzyme(s). Here, we describe our use of genome-wide sequencing, proteomics and genetic approaches to identify NTR2, a novel nitroreductase involved in the bio-activation of (***R***)-PA-824 and several other bicyclic nitro drugs.

1915

METABOLIC REGULATION OF MACROPHAGE FATE: THE ROLE OF MTORC2 SIGNALING IN ALTERNATIVELY ACTIVATED MACROPHAGES

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The mammalian target of rapamycin complexes, mTORC1 and mTORC2 have emerged as important regulators of environmental cues for development of immune cells. mTORC1, the most well studied mTOR complex whose activity is highly responsive to changes in cellular glucose and amino acid levels. Intriguingly, however, it has been shown that the constitutive activation of mTORC1 negatively regulates the alternative (M2) macrophage activation. In contrast, it is still unknown that the role of mTORC2 in macrophage activation. Here we report that myeloid lineage specific deletion of Rictor, a critical adaptor protein of mTORC2 in macrophages, they failed to fully become M2 macrophages during the ***Heligmosomoides polygyrus*** infection and have protective immunity to the parasite. Also, importantly, we observed that level of IRF4 expression and glycolytic/mitochondrial metabolism was markedly decreased from Rictor-deficient M2 cells during the infection. Since IRF4 is required for aerobic glycolysis and development of CD8 T cells, we found that expression of IRF4 was elevated in IL-4 stimulated macrophages, and conditional IRF4 deletion in macrophages affected neither mTORC2 activity nor the phosphorylation of Stat6, but led to a significant reduction of glucose metabolism and polarization of M2 macrophages. Taken together, our finding suggests that mTORC2-IRF4 signaling links to metabolic reprogramming and activation of macrophages.

1916

EXPORTED EPOXIDE HYDROLASES MODULATE ERYTHROCYTE SIGNALLING LIPIDS DURING PLASMODIUM FALCIPARUM INFECTION

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Erythrocytes are storage reservoirs for epoxide-containing lipid signalling molecules, including epoxyeicosatrienoic acids (EETs) and epoxy octadecenoic acids (EpOMEs). EETs/EpOMEs function as vasodilators and anti-inflammatory modulators in the blood stream. These bioactive epoxides are hydrolysed by epoxide hydrolases, converting them into less active diols. We have identified and characterized two epoxide hydrolases (EH) of ***Plasmodium falciparum***, PfEH1 and PfEH2. Both proteins are exported to the periphery of infected erythrocytes. Recombinantly expressed PfEH1 can hydrolyze a non-physiological reporter epoxide, and both enzymes convert several EET regioisomers to the corresponding diols. Overexpression of PfEH1 or PfEH2 in parasites results in a significant alteration in the epoxide fatty acids stored in the infected erythrocyte phospholipids. Although we were able to knock out PfEH1 and 2 ***in vitro***, we propose they may have an increased importance ***in vivo***. ***P. berghei ANKA*** has no predicted exported EHs, but chemical inhibition of the host EH enzyme reduced the percentage of mice that developed severe, cerebral malaria, without reducing parasitemia. We hypothesize that the parasite disruption of EETs/EpOMEs leads to perturbed vascular function, and favorable conditions for binding and sequestration of infected erythrocytes to the microvascular endothelium.

1917

A *PLASMODIUM* SPECIFIC KINASE IS AN ESSENTIAL S PHASE PROMOTING FACTOR DURING BLOOD-STAGE SCHIZOGONY

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Like invasion and host cell remodeling, ***Plasmodium*** schizogony is a parasite specific process, offering an attractive route for intervention. During schizogony a multinucleated cell is formed after which daughter parasites bud off the mother cell. In search of potential new drug targets, we assessed all schizont-stage kinases for their essentiality in ***Plasmodium falciparum***, employing the inducible destabilization domain system in a loss-of-function knockdown screen. We identified the ***P. falciparum*** cdc2-related protein kinase (CRK) 4 as essential for proliferation in the blood-stage and for transmission to anopheline mosquitoes. Knockdown of ***P. falciparum*** CRK4 led to a complete block of DNA replication at the onset of schizogony. Organellar development, however, was not impaired in mutant parasites. This suggests that the regulation of nuclear and organellar development is independent. By quantitative phosphoproteomic profiling, we identified key effects for ***P. falciparum*** CRK4 on pathways that co-ordinate cell cycle events including the initiation of DNA replication, histone modifications, and regulation of gene transcription. ***P. falciparum*** CRK4 was also found to be required for subsequent rounds of DNA replication, which characterize schizogony. Together our data indicate that chemotherapeutic targeting of ***P. falciparum*** CRK4 would be possible throughout schizogony, which in addition to blocking transmission, are attractive features for drug development.

1918

SCHISTOSOMA MANSONI INFECTION INDUCES ANTI-ATHEROGENIC TRANSCRIPTIONAL CHANGES IN HEPATIC MACROPHAGES

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Hepatic macrophages play an essential role in the granulomatous response to infection with the parasitic helminth ***Schistosoma Mansoni***, but the transcriptional changes that underlie this participation are poorly understood. To explore this, we sorted the two previously recognized hepatic macrophage populations (perivascular and Kupffer) from naïve and ***S. mansoni*** infected mice and performed microarray analysis as part of the Immunological Genome Project. Consistent with the pattern of great diversity identified in other organ macrophages, the two hepatic macrophage populations displayed signatures distinct from all other macrophages, with the two populations exhibiting remarkable differences between them. However, this diversity was greatly reduced following infection with ***S. mansoni***, and in fact, many of the transcripts identified as uniquely perivascular or Kupffer cell specific were lost following infection, raising the possibility that both populations may be replenished by monocytes following infection. Our analysis showed a profound alteration in phospholipid and cholesterol metabolic pathways, including prostaglandin signaling, in addition to alterations in M2 markers. These changes suggested a possible mechanism for the previously reported atheroprotective effects of ***S. mansoni*** infection. Indeed we find that ApoE null mice fed a high fat diet in combination with ***S. mansoni*** infection have reduced body mass and increased glucose tolerance in addition to reduced plaque area as compared to control mice.

1919

A GENOME-WIDE CRISPR/CAS9 SCREEN REVEALS GENES THAT ARE CRITICAL FOR *TOXOPLASMA GONDII* GROWTH IN HUMAN FIBROBLASTS

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Despite recent advances in apicomplexan genetics, the available tools have remained low-throughput. Although RNAi can be used to elucidate gene function in ***Trypanosoma brucei***, differences in the underlying machinery have precluded its use in apicomplexans. Recently, we and others used CRISPR/Cas9 to generate double-stranded DNA breaks and efficiently induce precise mutations in ***Toxoplasma.*** We have now used CRISPR/Cas9 to perform pooled genetic screens. By transfecting a ***Toxoplasma*** strain that constitutively expresses Cas9 with a library comprised of ten different sgRNAs against each gene, we mutated every gene in the ***Toxoplasma*** genome, in multiple ways, in seven days. Quantifying the fold-change in abundance of each sgRNA over the course of this experiment using next-generation sequencing allowed us to identify genes that contribute to the fitness of ***Toxoplasma*** when grown in human fibroblasts. The power of having ten independent mutations per gene allowed us to identify a set of genes that we can confidently say are critical under these conditions. In a CRISPR/Cas9 screen performed in the presence of a toxic uracil analog, sgRNAs against a component of the uracil scavenging pathway were enriched by approximately 1000-fold, demonstrating that our platform yields expected results and holds potential for identifying mechanisms of drug resistance. These results give us insight into ***Toxoplasma's*** large set of uncharacterized genes on an unprecedented scale. Our work now focuses on characterizing a set of 58 hypothetical genes that are conserved within Apicomplexa and confer significant fitness defects when mutated.

1920

GENETIC MODIFICATION OF THE DIARRHEAL PATHOGEN *CRYPTOSPORIDIUM PARVUM*

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Cryptosporidium is the second most important pathogen after rotavirus to cause diarrhea in young children. ***Cryptosporidium*** is also an opportunistic pathogen and causes severe disease in HIV/AIDS patients and organ transplant recipients. Currently, there are no fully effective drugs or vaccines to treat or prevent cryptosporidiosis. The main roadblock in the development of drugs and vaccines is the overall poor tractability of ***Cryptosporidium*** due to lack of continuous culture system, poor animal models, and lack of genetic tools. We report here a robust approach to genetically modify this important human pathogen. We demonstrate the transfection of ***Cryptosporidium parvum*** sporozoites in tissue culture and optimization of regulatory sequences and electroporation conditions to drive expression of a luciferase reporter gene. We developed a mouse model for ***C. parvum*** infection to inject electroporated sporozoites directly into the small intestine of IFN-γknockout mice. We used the CRISPR/Cas9 system to knockout the parasite's thymidine kinase (***tk***) gene and replaced it with a cassette expressing the luciferase reporter fused to a neomycin resistance gene that conferred paromomycin resistance ***in vivo***. Quantitative PCR and luminescence measurements were used to monitor infection in mice, and development of paromomycin-resistant transgenic parasites. We confirmed loss of ***tk*** gene in the stable transgenics, and evaluated the use of transgenic oocysts for performing drug assays. Using this powerful approach, we have generated stable transgenic parasite lines that express other reporters such as fluorescent protein and red-shifted luciferase. We used the red-shifted luciferase transgenic to monitor and quantify the real-time dynamics of ***C. parvum*** infection in mice using ***in vivo*** bioluminescence imaging. Ongoing efforts are directed towards testing the efficacy of potential anti-cryptosporidial compounds in mice using this transgenic parasite. Our ability to genetically engineer ***C. parvum*** will help to answer key questions related to parasite biology and accelerate the development of novel therapeutics for disease intervention.

1921

TRYPANOSOMA BRUCEI INFECTION ACCELERATES THE MOUSE CIRCADIAN CLOCK

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By living in a 24-hour world, organisms are subjected to daily environmental changes. Many organisms have evolved molecular mechanisms to anticipate such changes. In humans, the internal circadian clock regulates many physiological functions, including sleep/wake cycle and metabolism. Although the internal clock is controlled by the brain, all cells have an intrinsic clock. Patients with sleeping sickness show alterations of sleep/wake cycle, body temperature and endocrine secretion, which have led to the hypothesis that sleeping sickness may be a circadian rhythm disorder. We first infected mice with ***T. brucei*** and we recorded the circadian behavior using a running-wheel assay. We observed that infected animals run 2-fold less during the active phase and are 7-fold more active in the rest phase than healthy mice, confirming the changes in circadian behavior observed in patients. When we infected circadian reporter mice and measured the circadian parameters of several organs ***ex vivo,*** we observed that, although all organs have a robust circadian rhythm, those with higher parasite load have an internal clock that runs two hours faster than non-infected organs. These alterations were reproduced ***in vitro,*** when parasites were directly co-cultured with isolated

fibroblasts, suggesting that parasites may have a direct effect on the host cell circadian clock. Finally we observed that expression of clock genes ***in vivo*** is significantly affected in peripheral tissues, especially in those with the highest parasite load. These results show that (i) ***T. brucei*** mouse infection reproduces circadian behavior changes observed in humans; (ii) ***T. brucei*** infection accelerates the mouse circadian rhythm by two hours; (iii) this effect may be partly caused by a direct interaction with the parasite.

1922

THE INOSITOL PHOSPHATE PATHWAY CONTROLS TRANSCRIPTION OF TELOMERIC EXPRESSION SITES IN TRYPANOSOMES

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African trypanosomes evade host antibody clearance by periodically changing their variant surface glycoprotein (VSG) coat. They transcribe only one VSG gene at a time from one of about 20 telomeric expression sites (ESs), and they undergo antigenic variation either by switching transcription between telomeric ESs or by recombination of the VSG gene expressed. We found that the inositol phosphate/phosphatidylinositol (IP) pathway controls both transcription of telomeric ESs and VSG antigenic switching in ***Trypanosoma brucei***. Conditional knockdown of phosphatidylinositol 5-kinase (PIP5K), phosphatidylinositol 5-phosphatase (PIP5Pase) or overexpression of phospholipase C (PLC) derepresses numerous silent telomeric ESs in ***T. brucei*** bloodstream forms. This derepression is specific to telomeric ESs and coincides with an increase in the number of telomeric and RNA polymerase I foci that colocalize outside of the nucleolus. Monoallelic VSG transcription resumes after re-expression of PIP5K; however, most of the resultant cells switch the VSG gene expressed. PIP5K, PLC, their metabolic substrates and products localize to the plasma membrane, whereas PIP5Pase localizes in the nucleus proximal to telomeres. PIP5Pase associates with repressor/activator protein 1 (RAP1), and their telomeric silencing function is altered by PIP5K knockdown. The results show that the IP pathway controls ES transcription and antigenic switching in ***T. brucei*** by epigenetic regulation of telomere silencing, which likely involve a signal transduction process.

1923

EXPLORING UNKNOWN GENES IN MALARIA PARASITES BY A ROBUST GENE REGULATORY SYSTEM

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Malaria is a major health problem in tropical and subtropical countries. The most severe form of malaria is caused by the parasite, Plasmodium falciparum. A limited set of antimalarial drugs is used to treat the disease, but drug resistance is spreading at alarming rate. Hence, there is an urgent need for identification of novel anti-malarial drugs. A major challenge in new antimalarial drug development is identification and prioritization of potential targets for drug discovery. This is mainly due to lack of reliable functional genetics tools for investigating parasite genes. To address this need, we have developed a RNA-protein interaction system that facilitates robust and inducible regulation of target gene translation in eukaryotic organisms, including Plasmodium. Here, we present the application of protein engineering approaches to integrate our synthetic control system with native Plasmodium translational regulatory mechanisms. In so doing, we have achieved substantially increased regulatory dynamic ranges (up to 200-fold) compared to a 5-10 fold range of the original system. As a proof-of-concept, we have successfully used this system to generate parasite lines in which various proteins of interest can be knocked down to reveal clear growth phenotypes. In addition, we have successfully combined this approach with CRISPR/Cas9 genome editing technology to rapidly validate essential genes. We are currently applying these genetic tools to broadly study parasite genes of unknown function. Since ~60% of the encoded P. falciparum genes have no known homologs in other eukaryotes, we believe that understanding their functions will aid in identifying potential targets for novel antimalarial drug development.

1924

A NETWORK OF PROTEIN INTERACTIONS REQUIRED FOR TRAFFICKING OF *PF*EMP1 IN *P. FALCIPARUM*-INFECTED ERYTHROCYTES

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Plasmodium falciparum is the most virulent human malaria parasite. Parasites invade red blood cells (RBCs) and extensively modify the structure and morphology of their host cell - including the generation of virulence complexes on the surface of the erythrocyte. These complexes comprise of a collection of exported parasite proteins that assemble at knob-like structures under the surface of the erythrocyte membrane and allow infected RBCs to cytoadhere and sequester within the microvasculature of the host. One key parasite protein, ***P. falciparum*** Erythrocyte Membrane Protein-1 (PfEMP1), is largely responsible for this adhesion. It is becoming clear that PfEMP1 is trafficked with the aid of a complement of host and parasite-structures and compartments that are generated ***de novo*** during the approximate 48-hour lifecycle of the parasite inside its host RBC. In this work, we have made use of mini-PfEMP1 constructs to examine this complex export process. We have delineated discrete trafficking compartments that these virulence proteins associate with during export and used immunoprecipitation followed by mass spectrometry to identify the 'interactome' of virulence proteins in these compartments. Known and novel interacting-proteins were identified across multiple parasite and host compartments, consistent with our current knowledge of the ***Pf***EMP1 trafficking pathway. This includes parasite proteins localized to the parasite secretory pathway, parasitophorous vacuole (such as PTEX) and exported proteins found in the host RBC compartment. We also present the identification of a number of human chaperone-type proteins that may represent a mechanism for parasite recruitment of host factors in the export of parasite proteins such as ***Pf***EMP1. Using inducible knockdown systems we show that depletion of these identified proteins results in the reduction of ***Pf***EMP1 export to the surface of the RBC. Ultimately, understanding and targeting the export of parasite virulence proteins may ultimately allow us to ablate parasite virulence ***in vivo***.

1925

PROGRAMMATIC USE OF MOLECULAR XENOMONITORING AT THE LEVEL OF EVALUATION UNITS TO ASSESS PERSISTENCE OF LYMPHATIC FILARIASIS IN SRI LANKA

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Sri Lanka's Anti Filariasis Campaign (AFC) distributed 5 annual rounds of MDA with DEC plus Alb. to all LF endemic regions in the country from 2002-2006. Post-MDA surveillance has consistently documented microfilaremia rates <1% in all sentinel and spot check sites, and all implementation units easily satisfied WHO TAS targets in 2013. However, recent studies have shown that Sri Lanka has low level persistence of LF in some areas based on several criteria, especially molecular xenomonitoring (MX, detection of filarial DNA in mosquitoes). Some of the highest signals for persistence of LF were observed in sentinel sites in Galle district. The purpose of this study was to demonstrate the use of MX at the program

level and to field test different sampling methods. Galle district (population 1.1 million) was divided into two evaluation units (EUs). These included a coastal EU with persistent LF and a low risk inland EU. Mosquitoes were systematically sampled from ~300 trap sites in 30 randomly selected clusters (lower health administrative units) per EU. Approximately 28,000 blood fed, gravid or semigravid Culex quinquefasciatus mosquitoes were collected with CDC gravid traps, sorted into pools, and tested for filarial DNA by qPCR. 92/620 pools (14.8%) from the coastal EU and 8/583 pools (1.4%) from the inland EU were positive for filarial DNA. 16/30 clusters in the coastal EU had one or more positive pools compared to 3/30 in the inland EU. Maximum likelihood estimates (MLE) for filarial DNA rates calculated by Poolscreen2 were essentially the same when the same number of pools were collected and tested from 75, 150, or 300 trap sites. The range of filarial DNA rates calculated in the coastal EU with the different samples was 0.61-0.72%, and the range in the inland EU was 0.04-0.06%. The ability to use a smaller number of trap sites reduces the cost and time required for mosquito sampling. The MX results suggest there is widespread, low-level persistence of LF in coastal Galle district 8 years after the last round of MDA. This study has shown MX can be used by national programs to assess and map the persistence of LF at the level of large EUs in regions with Culex transmission.

1926

EPIDEMIOLOGICAL, CLINICAL, AND LABORATORY EVALUATION OF ONCHOCERCIASIS IN AN AREA OF HIGH PREVALENCE -TSHOPO PROJECT AREA, DEMOCRATIC REPUBLIC OF THE CONGO, 2014

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Onchocerciasis, a neglected parasitic disease caused by Onchocerca volvulus, affects at least 37 million people globally. Efforts to eliminate this disease are based on mass drug administration of ivermectin (IVM MDA). As part of a study to evaluate tools for measuring the impact of elimination efforts, we evaluated different diagnostic tests for O. volvulus. We performed convenience sampling in Tshopo Project area in the Democratic Republic of Congo, where there was a baseline nodule prevalence of 50-70%, and IVM MDA had been ongoing for 1-5 years before the study. Risk factor and clinical data were collected for 500 people. Blood smears for Loa loa and Mansonella perstans, immunochromatographic card tests for lymphatic filariasis (LF), and skin snips for onchocerciasis were evaluated on-site. Plasma, serum, blood smears, dried blood spots, and preserved skin snips were sent to CDC-Atlanta for further testing. Median participant age was 50 years (range: 6-88 years); 193 (39%) were female. Past-year ivermectin use was reported by 60 (12%), though 117 (34%) reported having taken it at least once before. At least one nodule was present in 253 (51%) people, with a median of three (range 1-11) nodules. Onchocercal eye disease was present in five (1%) people; one (0.2%) had microfilaria (MF) in the anterior chamber of the eye. The skin snip was positive for O. volvulus in 205 (41%) people. Among those with at least one positive snip, the mean MF load per snip, determined by averaging the number in both snips, was 27.5 (range: 0.5-341.5). On-site tests for other filariae showed 273 (55%) people were infected with M. perstans, 108 (22%) with L. loa, and 25 (5%) with LF. Six (1%) people were infected with all four filarial parasites, 51 (10%) with three, and 135 (27%) with two. The OV-16 antibody test for O. volvulus was positive in 333 (67%) people. Additional laboratory testing is pending to define OV-16 test sensitivity and specificity in this setting. A better understanding of the performance of the OV-16 antibody test in the African context, particularly in the setting of co-endemic filarial infections, is needed to ensure proper usage by elimination programs.

1927

TOWARDS THE ELIMINATION OF LYMPHATIC FILARIASIS IN MALAWI: CESSATION OF MASS DRUG ADMINISTRATION NATIONWIDE AFTER TRANSMISSION ASSESSMENT SURVEYS

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Malawi, a small land-locked country in south-eastern Africa was shown to be highly endemic for lymphatic filariasis (LF) when disease mapping at national scale was completed in 2008. LF is a debilitating mosquito-borne parasitic infection that is endemic in 34 countries in Africa. Mapping delineated 26 LF endemic districts out of 28 and mass drug administration (MDA) to eliminate the disease started in 9 districts in 2008. In 2009, MDA was scaled up to 100% geographic coverage for the target population of 13 million people at risk. Between 2008 and 2014, treatment coverage surpassed 80% for each MDA round. In 2014, after 6 consecutive rounds of MDA, Malawi met all the criteria to conduct transmission assessment surveys (TAS) to determine if transmission of LF has been interrupted. Pre-TAS sentinel site surveys involving >300 parsons, five years or older, per site were conducted at 48 sites. None of the sentinel sites was shown to have a microfilaria (MF) prevalence rate of 1% or greater. In 2014, TAS was conducted in 11 evaluation units (EUs) following WHO guidelines and infection status determined using a point of care ICT method. The survey was conducted in 30 randomly selected schools per EU and altogether 18,337 children, about 6 and 7 years old were enrolled from 330 schools. A total of 34 (0.19%) children from all 11 EUs were found to be ICT positive. The LF infection rate for each EU was below the critical cut-off level suggesting that all EUs met the criteria to stop MDA. The NTD Regional Programme Review Group (RPRG) for the WHO African Region reviewed and approved the TAS results in 2015 indicating that MDA could be stopped in Malawi. However Malawi is endemic for two other debilitating neglected tropical diseases targeted with the same drugs as LF: onchocerciasis and soil transmitted helminthiasis. The endemicity status of these two diseases should be taken in to consideration before any decision to stop distribution of ivermectin and albendazole can be made. The RPRG report singled out Malawi for a special commendation as a success story. Stopping MDA in Malawi represents a big step forward for the global LF elimination programme as we approach the 2020 target.

1928

SUCCESSFUL FINAL TRANSMISSION ASSESSMENT SURVEY FOR LYMPHATIC FILARIASIS USING ICT AND STRIP TEST SIX YEARS AFTER STOPPING MDA IN TOGO

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Lymphatic filariasis (LF) was once endemic in 8 of the 40 districts in Togo. From 2000 to 2009, the National Lymphatic Filariasis Control Program of Togo conducted between seven and nine rounds of mass drug administration (MDA) with albendazole and ivermectin in these 8 districts. In 2010, based on consistently high MDA coverage and low prevalence of nocturnal microfilaremia (<1%) at sentinel and spot-check sites, MDA for LF was stopped following a successful Transmission Assessment Survey (TAS). According to World Health Organization (WHO) recommendations, two TAS should be conducted 2 to 3 and 5 to 6 years after stopping MDA. Togo conducted and passed its first post-MDA TAS in 2012, and its second, final post-MDA TAS in January 2015, to reconfirm that there is no transmission of Wuchereria bancrofti. We report on this latter survey here. The survey was conducted according to WHO recommendations. The 8 endemic districts were grouped into 4 evaluation units (EU); in the north, Tone, Cinkassé and Kpendjal districts comprise EU1; in the northeast, Binah and Doufelgou districts make EU2; Kozah district in central eastern Togo is EU3; and Amou and Haho districts in the south are EU4. School-going children aged 6 to 7 years old were selected for testing using cluster sampling. Children were tested for LF filarial antigen using both an immunochromatographic test (ICT) and an LF test strip in EU1 and EU2, and ICT alone in EU3 and EU4. Test results were scored 0 for negative tests and 1 to 4 according to the intensity of a positive result. An EU was considered to have successfully passed the TAS when the number of positive cases found was inferior to a critical cut-off number, which varied from 18 to 20 among the EUs. Of the children tested – 1701 in EU1, 1547 in EU2, 1542 in EU3, and 1564 in EU4 – there were, respectively, 6 (0.35%), 0 (0%), 0 (0%) and 0 (0%) positive cases found, well below the critical threshold in every EU. All six cases were positive by both test methods. The risk of recrudescence of LF remains very low in these 8 previously endemic districts more than six years after stopping MDA for LF in Togo. Togo is preparing a dossier to submit to WHO for validation of elimination of LF.

1929

SHRINKING THE LYMPHATIC FILARIASIS MAP: DOES GABON NEED TREATMENT TO ELIMINATE LYMPHATIC FILARIASIS?

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Historical records suggest that lymphatic filariasis (LF), was found in Gabon, but there is little information on its presence. MDA has never been conducted. Establishing the endemicity of LF is challenging using traditional parasitological and serological methods because of the limited skills for microscopy and coendemicity with Loa loa (loa). Loa and Wb microfilaria are difficult to distinguish microscopically and Loa antigens have been shown to cross react with Wb antigens in individuals with high Loa microfilaria counts who are negative for Wb test positive by ICT. We conducted a nationwide integrated survey of LF, onchocerciasis and loiasis. For LF, 114 villages were surveyed across the 9 provinces. Individuals aged 15 years or above were tested using ICT cards. A total of 28 ICT positive individuals were found in 22 communities, from 18 different districts. To further investigate if the ICT positives found during the survey were truly due to Wb and not a cross reaction due to Loa infection, we collected dried blood spots (DBS) to perform multiplex assays for Wb123 and Bm14, two antigens for filarial parasites. Antibody based assays to detect Wb123 has been shown to be sensitive and specific for LF infection while Bm14 has lower specificity and is recognized by a proportion of individual with other filarial infections. DNA was extracted from each DBS and amplified by PCR and qPCR using pan filarial primers. Only one sample had borderline positive responses to Wb123, but 13 had positive serology to Bm14, suggesting that ICT positivity was related to Loa and not Wb. All 28 samples had positive results for Loa by both PCR approaches. Our inability to confirm that ICT positivity was related to Wb suggests that LF is no longer present and therefore no intervention is required against LF. More robust epidemiological methods may be required to confirm that transmission of LF has been interrupted. These results would represent a tremendous step forward for the Global Program to Eliminate Lymphatic filariasis, shrinking the LF map by one more country and helping to cross the bridge towards the achievement of the ultimate goal of worldwide elimination of LF by 2020.

1930

PROGRESS TOWARDS LYMPHATIC FILARIASIS ELIMINATION IN SIERRA LEONE

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Lymphatic filariasis (LF) is endemic across Sierra Leone, and mass drug administration (MDA) with ivermectin and albendazole started in 2007, reaching 100% geographical coverage in 2010 with effective epidemiological coverage. Baseline microfilaraemia (mf) level was assessed before the first MDA. Mid-term assessment (after the 3rd MDA) and pre-TAS (after the 5th MDA) was conducted in 12 rural health districts (HDs) at sentinel sites (SS) and spot check sites (SC), using midnight blood samples from at least 300 people at each site. Two HDs were paired into each group (6 groups) according to population size and epidemiological characteristics, sharing SS and SC. At baseline (14 sites), the mean mf prevalence in 12 HDs was 2.6% (95% CI: 2.3-3.0%), ranging 0-6.9%. The mean mf density was 1.3mf/ml (95% CI: 1.0-1.7). Ten HDs had mf prevalence ≥1% at SS. At mid-term (6 SS and 6 SC), the mean mf prevalence was 0.3% (95% CI: 0.2-0.5%), ranging 0-1.6%. The mean mf density was 0.1mf/ml (95% CI: 0.0-0.1). Eleven HDs showed mf prevalence <1% at SS and SC. In 2013 a pre-TAS was conducted in 6 SS and 7 SC. Overall mf prevalence was 0.5% (95% CI: 0.4%-0.8%), ranging 0-2.7%. The mean mf density was 1.0mf/ml (95% CI: 0.30-1.8). Nine HDs showed mf prevalence <1%, persistent from the mid-term. Compared to baseline there was significant decrease in mf prevalence and mf density ($p<0.001$). Eight districts qualified for and will be subject to transmission assessment survey (TAS). Three HDs (Bombali, Kailahun, and Koinadugu) that showed mf prevalence ≥1% had baseline mf prevalence 6.9%, 5.7% and 2.6% respectively, and, together with Kenema (though mf prevalence <1% at pre-TAS), share borders with Guinea and/or Liberia where 100% geographical coverage of MDA has not been reached. These 4 HDs will continue MDA for additional 2 years, while the cross border issues are addressed. Significant increase in mf density from the mid-term was mainly due to a SC in hard to reach areas in Bombali. This highlights the importance of SC in identifying hot spot of LF prior to TAS.

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