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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.