ASTMH
AMERICAN SOCIETY OF TROPICAL MEDICINE & HYGIENE
ADVANCING GLOBAL HEALTH SINCE 1903

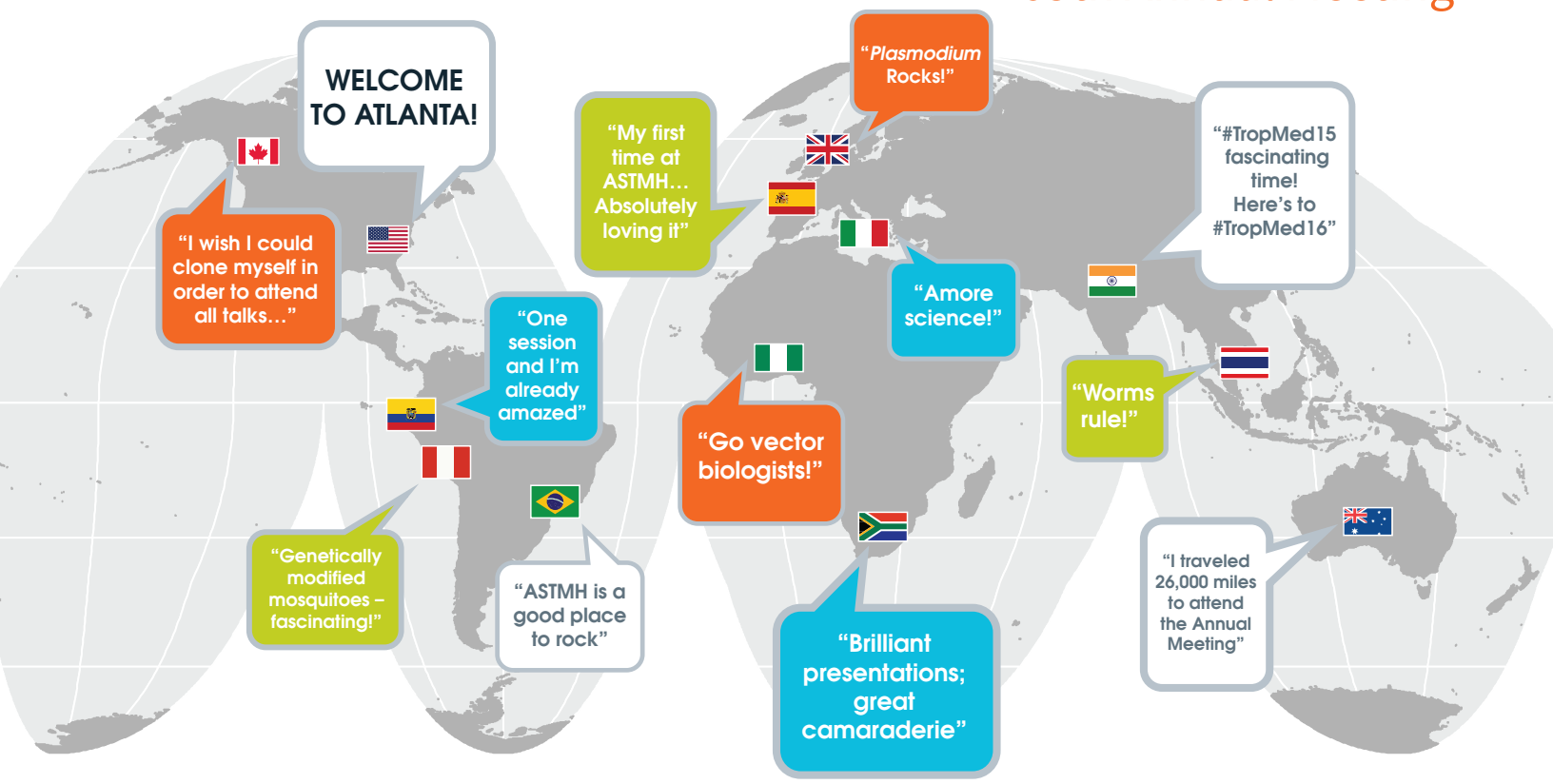
VOLUME 95 NOVEMBER 2016 NUMBER 5 SUPPLEMENT

Abstract Book

November 13–17, 2016

Atlanta Marriott Marquis
Atlanta, Georgia USA

65th Annual Meeting



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Supplement to
The American Journal of Tropical Medicine and Hygiene

1

LYMPHATIC FILARIASIS RESIDUAL TRANSMISSION HOTSPOTS IN AMERICAN SAMOA

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To achieve elimination of lymphatic filariasis, American Samoa carried out 7 rounds of mass drug administration from 2000 to 2006 and passed Transmission Assessment Surveys (TAS) in 6-7 yr-old children in 2011 and 2015, although 1 or 2 ICT-positive children were found on each occasion in the same school. Serology studies using Og4C3, Wb123 and Bm14 in 807 adults in 2010 identified higher antigen prevalence in two spatial clusters, one of which included the school attended by ICT-positive children in both TAS. Prevalence was higher in men and those residing in American Samoa for <5 yrs. To follow up these findings, a targeted study in 2014 tested three groups of individuals: 124 residents aged 3-70 yrs in the two putative 'hotspots'; 337 children aged 7-13 yrs in the school where ICT-positives had been identified in TAS; and 650 adult workers (residing across the island) attending a pre-employment clinic or working in the tuna cannery. Overall prevalence (N=898 to 1,111 depending on the test) was 2.1% (95% CI 1.3-3.1%) for ICT and Og4C3, 5.7% (4.2-7.3%) for Wb123, and 10.2% (8.5-12.2%) for Bm14. The study confirmed elevated prevalence of ICT and Og4C3 (both 8.1%) antigen as well as Wb123 (9.8%) and Bm14 (23.6%) antibody in all ages in the two suspected hotspots. Bm14 antibody prevalence was higher in males than females in all groups (32.1% vs 17.1% in hotspot villages (p=0.05); 19.9% vs 7.0% in adult workers (p<0.001); and 3.3% vs 0.6% in children aged 7-13 yrs (p=0.06)). All ICT positive persons were treated and had slides taken. Microfilariae (Mf) with density from 8 to 3267/ml were observed on 4 of 20 slides examined, with all Mf positive persons residing in hotspots. Thus age, gender and residence in a hotspot village were the predominant risk factors for being positive for diagnostic markers of LF. This study has confirmed the suspected hotspots previously identified in 2010 from a spatially representative adult sample as sites of continuing transmission and potential sources of resurgence. The results further support the potential use of spatial epidemiological methods for identifying residual foci of infection in the endgame phase of LF elimination programs.

2

EXCESS MORTALITY ASSOCIATED WITH HIGH *LOA LOA* MICROFILAREMIA IN THE EAST REGION OF CAMEROON: A RETROSPECTIVE COHORT STUDY

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Loiasis affects more than 10 million people, most in Central Africa. Besides its classical signs ("eye worm" and "Calabar swelling"), it has also been reported as a cause of renal and cardiac complications. However, the burden of loiasis has never been assessed and it is still considered a benign condition. To assess whether loiasis bears any excess mortality, we conducted a retrospective cohort study in the East Region of Cameroon. In 2001, 3,627 individuals living in 28 villages were included in a survey during which thick blood smears (50 µL) were analyzed for *Loa loa* microfilarial (mf) density. In 2016, these villages (where no mass ivermectin treatment has ever been organized) were visited again to assess whether the subjects examined 15 years before were still alive. The vital status could be determined for 3,301 individuals (91%). Data analyses included (a) an analysis at the community level between the age- and sex-standardized prevalence of (hyper)-microfilaremia in 2001 and the standardized mortality rates (SMR); (b) an assessment, using multivariate accelerated failure model, of the excess mortality relative to the initial mf density (4 classes: 0, 1-8,000, 8,000-30,000 and >30,000 mf/mL); (c) the calculation of the population-attributable fraction of mortality due to presence (vs. absence) of a Loa microfilaremia. At community level, the SMRs increased by 5.5% when the proportion of subjects with >30,000 mf/ml increased by 1% (P=0.040). A similar trend was observed when the threshold was 8,000 mf/ml (2.9%/1%; P=0.068). People aged >25 years with more than 30,000 mf/mL in 2001 died significantly earlier than those with lower mf densities (Time Ratio=0.67, 95% CI: 0.48-0.95, P=0.024). Lastly, 14.5% (95% CI: 6.5-21.8) of all-cause mortality was attributable to the presence of *Loa* mf. In conclusion, high *Loa* microfilaremia was associated with increased mortality in the study site. There is a need to validate our observations in other Loa areas, as they are likely to have implications on the status of loiasis in terms of public health, and the implementation of onchocerciasis and lymphatic filariasis elimination programs in Central Africa.

3

PROGRESS TOWARDS ONCHOCERCIASIS ELIMINATION IN THE PARTICIPATING COUNTRIES OF THE AFRICAN PROGRAM FOR ONCHOCERCIASIS CONTROL: EPIDEMIOLOGICAL EVALUATION RESULTS

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The African Programme for Onchocerciasis Control (APOC) was created in 1995 in order to control onchocerciasis as a public health problem by implementing sustainable Community Directed Treatment with Ivermectin (CDTI). When research showed that mass treatment can lead to complete elimination of the infection in Africa, APOC shifted its target to elimination of infection and interruption of transmission. Epidemiological evaluations have been undertaken from 2008 to 2014 in evaluation areas with ≥6 years of effective treatment. We analyzed these unique data, to assess progress towards elimination. Epidemiological evaluations were done per area, in two phases. First, parasitological surveys were done in about 10 selected high risk communities per area with high pre-control endemicity level. By comparing observed prevalence levels to expected trends (as predicted by the established ONCHOSIM model, developed at Erasmus MC Rotterdam, and extensively used for policy support in onchocerciasis control in Africa), using Bayesian methods and Monte Carlo simulation, we classified the progress towards elimination as "faster than predicted", "on track", or "delayed". Second, in areas close to elimination, additional parasitological surveys were done in more communities to assess whether mass ivermectin treatment can safely be stopped. Initial parasitological surveys covered 54 areas, 639 villages and 127,665 people out of 53 million total population. The decline in prevalence was faster than predicted in 23 areas, on track in another 23 and delayed in 8 areas. Additional surveys were done in 22 areas and 13 of these met the epidemiological criteria for stopping treatment. Overall, 32 areas (25.4 million people) had reached or were close to elimination, 18 areas (17.4 million) were on track but required more years treatment, and in 8 areas (10.4 million) progress was unsatisfactory. Great progress has been made by APOC in realizing elimination beyond its prime goal. Elimination is reached or close for millions of people. Extra effort is needed in areas with unsatisfactory progress.

4

DETECTING INFECTION HOTSPOTS: MODELING THE SURVEILLANCE CHALLENGE FOR ELIMINATION OF LYMPHATIC FILARIASIS AND OTHER DISEASES

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Confirming elimination of lymphatic filariasis (LF) is a challenge due to its existence in small endemic foci (microfoci; µf). The capacity of post-treatment surveillance (PTS) to detect µf is unclear. We used microsimulation modeling to assess the ability of different types of PTS to detect LF µf in a background of low prevalence. Five µf of radius 1, 2, or 3 km with infection marker prevalence (intensity) of 3, 6, or 10 times background were placed in each simulation, run in R Version 3.2. Tests included microfilaremia, immunochromatographic test (ICT), and Wb123 ELISA. We set population size (360,000) and area (60 x 60 km) and based demographics on literature; background ICT prevalence in 6-7 year olds was 1.0%. Adults≥18 years, women 15-40 ('WCBA') years, 6-7 year olds, or children≤5 years were sampled. Cluster (CS) or simple random sampling (SRS) was conducted, with follow-up testing of nearest 20, 100, or 500 persons to each positive. A threshold count of positive persons in follow-up testing indicated a suspected µf. Suspected and true µf were compared for predictive value positive (PVP). Each parameter set was referred to as a protocol. Protocols were scored by efficiency, defined as the most µf identified and fewest persons tested. Of 3402 protocols, 384 (11.3%) identified all 5 µf (PVP 3.4-100.0%) testing 0.73-35.6% of the population. All used SRS; 378 (98.4%) only identified all 5 µf if they were 2-3 km diameter or intense infection (6x or 10x). 374 (97.4%) required ICT or Wb123 and 281 (73.0%) required sampling adults or WCBA. The most efficient CS protocol identified 2/5 µf. After limiting to 1-km radius µf with 3x intensity (n=378), 8 protocols identified all 5 µf; all used SRS and ICT but required testing 31.2-33.3% of the population. Nine protocols identified 4 µf, all using SRS and ICT with adults or WCBA and testing 3.5-9.7% of the population. Of those using CS (42.2%), only 14 (8.6%) identified any µf (1 of 5). Of the protocols using microfilaremia tests, only 6 (3.7%) identified any µf (1 of 5). In this model, SRS, ICT and sampling of adults maximized µf detection efficiency. The model provides many PTS protocols that can be selected for optimal outcomes.

5

USE OF AN ADULT TRANSMISSION ASSESSMENT SURVEY TO ASSESS PERSISTENCE OF LYMPHATIC FILARIASIS AT THE EVALUATION UNIT LEVEL IN GALLE, SRI LANKA

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Sri Lanka's Anti Filariasis Campaign (AFC) conducted 5 annual rounds of mass drug administration (MDA) with DEC plus albendazole in all endemic regions in the country between 2002-2006. Post-MDA surveillance has consistently documented microfilaremia (Mf) rates <1% in all sentinel and spot check sites, and all implementation units easily satisfied WHO transmission assessment survey (school-based TAS) targets in 2013. However, alternate surveillance methods documented persistence of LF in some areas. For example, molecular xenomonitoring (MX) surveys showed alarmingly high rates of filarial DNA in mosquitoes in the coastal Galle evaluation unit (EU) in southern Sri Lanka. The purpose of this study was to explore the utility and feasibility of AdultTAS for detecting low level persistence of *W. bancrofti* infection at the EU level using coastal Galle (population 0.7 million) as a positive control study site. We used Survey Sample Builder to randomly select two samples of 30 evaluation areas (EAs, mean population per EA 3,000), and approximately 1,800 adults were tested for filarial antigenemia with the Alere Filariasis Test Strip (FTS) in each of EA samples. Thirty of these EAs had previously been assessed for persistent LF by MX. The FTS rate for the entire study sample (N=3,612) was 1.8% (CI 1.4-2.2), and rates for the two sets of 30 EAs were 1.5% (CI 1.0-2.2) and 2.0% (CI 1.4-2.7), respectively. Thus the two EA samples provided similar results for the EU with upper CI values that exceeded the 2% target. FTS rates by EA were highly variable (range 0-11%) and exceeded 5% in 6 EAs. FTS rates in adults and filarial DNA rate in mosquitoes for 30 EAs tested by both methods were significantly correlated (r = 0.43; P=0.02). This study has shown that AdultTAS is more sensitive than school-based TAS and more convenient than MX for detecting persistent LF following MDA. Results from AdultTAS may be useful for identifying hot spot areas that require mop-up activities. AdultTAS is feasible for use by national LF elimination programs at the EU level, and it should also be more useful than school-based TAS for remapping "non-endemic" areas that were not included in MDA programs.

6

IMPACT OF LONG LASTING INSECTICIDE TREATED BEDNETS ON LYMPHATIC FILARIASIS PREVALENCE IN PAPUA NEW GUINEA

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Vector control efforts against malaria may accelerate lymphatic filariasis (LF) elimination in coendemic areas with anopheline LF transmission such as Papua New Guinea. Long lasting insecticide treated bednets (LLIN) were distributed nationwide in Papua New Guinea in 2009. Mathematical models have predicted that LLIN could be sufficient to end transmission in some of these study communities based on the previously described decrease in anopheline mosquito density and LF vectorial transmission potential, as reported previously. Here, we evaluate the impact on human markers of LF infection 5 years after LLIN distribution in the absence of mass drug administration (MDA) in communities previously shown to have different LF transmission levels. 1,262 Night-time finger prick blood samples were collected January-May, 2015. These samples were assessed for microfilaria (MF), ICT antigen card test, and BM14 antibody test. Current bednet usage was self-reported to be >80%. The moderate transmission communities had zero MF positive individuals and only one ICT-positive individual among children under 10 years of age (living most of their lives post-LLIN). Although age adjusted prevalence had decreased from 5.0% to 2.4% (p<0.001) during this time, older age groups were observed with mf prevalence up to 14.6%. Nearby higher transmission study communities (between 2 and 15km away) maintained similar infection profiles to pre-LLIN with mf prevalence ranging from 3.8% in the children <10 years to 21.9% in the older age groups and ICT card positivity of 53.7%. Furthermore, 29% of MF-negative individuals in the study were antibody positive, including 46 of 529 (8.7%) MF-negative children under 10 years of age (considered the sentinel population for exposure). These results indicate that adults remain a reservoir for MF in these communities and antibody assays indicate continued LF transmission, even after the distribution of LLIN. These results indicate that LLIN are insufficient to interrupt transmission in this area and MDA will be necessary to achieve elimination endpoints.

7

IMPACT OF ANNUAL AND SEMIANNUAL MASS DRUG ADMINISTRATION IN AREAS CO-ENDEMIC FOR *BRUGIA TIMORI* AND *WUCHERERIA BANCROFTI* IN EAST NUSA TENGGARA, INDONESIA

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Lymphatic filariasis is targeted for global elimination by the year 2020. Key strategy is the annual mass drug administration (MDA) with single dose of DEC combined with albendazole for at least five years. In order to shorten the duration of MDA, more rounds may be needed to achieve similar outcome. Here we compared the impact of annual and semiannual MDA on the prevalence of *Brugia timori* and *Wuchereria bancrofti* in Sikka District in Flores Island. Two villages (Paga, *B. timori* only and Lewomada, co-endemic) were assigned annual MDA with single dose of DEC/albendazole, while the third village (Pruda, co-endemic) was assigned semiannual MDA. Blood samples were collected from individuals aged 5 years and older before and 1, 2, and 3 years after the first treatment. The overall compliance with MDA was ranging from 67-90%. Microfilaremia (mf) was determined by microscopic examination of thick night blood smears for all years. Detection of filarial-specific IgG4 (Brugia Rapid, BR, for B. timori) or filarial antigen (ICT cart test for W. bancrofti) were performed at baseline and after three years post initial treatment. The mf prevalence in Pruda village decreased after 5 semiannual treatments from 14.2% to 1.3%, while in other two villages the mf prevalence decreased after 3 annual treatments from 3.9 and 5% to 0 and 0.3%, respectively. The ICT positivity rate in Pruda and Lewomada decreased from 22.9 and 6.5% to 7 and 0.8%, while the BR positivity rate in Pruda, Lewomada and Paga decreased even stronger from 28.9, 31.7 and 12.5% to 3.6, 4.1 and 1.8%. Mf prevalence and BR as well as ICT positivity rates show similar levels of reduction in both MDA regimens, but Pruda started out at much higher levels. We conclude that in our setting 3 annual rounds of MDA are sufficient to reduce mf rates to less than 1% in the population, but semiannual MDA is helpful for higher prevalence settings to reach the same degree of reduction.

8

EL NIÑO AND THE SHIFTING GEOGRAPHY OF CHOLERA IN AFRICA

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In the wake of the 2015-2016 El Niño event, there has been an explosion of cholera cases in East Africa, including the largest outbreak since the last strong El Niño (1997-1998) in Tanzania. El Niño conditions are associated with increased rainfall in East Africa and decreased rainfall in Southern Africa, West Africa, and parts of the Sahel. Because of the key role of water supplies in cholera transmission, a relationship between El Niño events and cholera incidence is highly plausible, and previous research has shown a link between El Niño patterns and cholera in Bangladesh. However, there is little systematic evidence for this link in Africa. Using high-resolution mapping techniques, we find that El Niño profoundly changed the annual geographic distribution of cholera in Africa from 2000-2014, shifting the burden to continental East Africa, where almost 50,000 additional cases occur during El Niño years. Cholera incidence during El Niño years was higher in regions of East Africa with increased rainfall, but incidence was also higher in some areas with decreased rainfall suggesting a complex relationship between rainfall and cholera incidence. Here we show clear evidence for a shift in the distribution of cholera incidence throughout Africa in El Niño and non-El Niño years, likely mediated by El Niño's impact on local climatic factors. Knowledge of this relationship between cholera and climate patterns coupled with El Niño forecasting could be used to notify countries in Africa when they are likely to see a major shift in their cholera risk. Effective case management enormously decreases mortality in cholera outbreaks and new control tools (e.g., oral cholera vaccines) may be able to prevent outbreaks altogether. Therefore the ability to step up preparedness and surveillance when local risk is high can have a major impact on saving lives and lowering the disease burden.

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SURVEILLANCE FOR CHOLERA MORTALITY DURING AN URBAN EPIDEMIC—DAR ES SALAAM, TANZANIA, 2016

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Cholera can cause profuse watery diarrhea, severe dehydration, and death within hours. Effective treatment with oral rehydration salts (ORS) and intravenous fluids (IVF) can lower the case fatality rate from >30% to <1%. In August 2015, a cholera epidemic occurred in Tanzania, and by 27 October 3,773 cases and 36 deaths were reported in Dar es Salaam. Because of rumors of additional deaths in the community, we conducted a mortality investigation. We defined a suspected cholera death as death from acute watery diarrhea in a person ≥2 years old in Dar es Salaam after 15 August 2015. We obtained information about health facility deaths from cholera treatment centers and referral hospitals, and community deaths from municipal burial permits. We interviewed family members about decedents' demographic characteristics, timing and location of death, care-seeking behavior, and treatment. We identified 96 cholera deaths in 2015; 56 (58%) were not captured by surveillance. We were unable to interview family members of 40 (42%) of 96 decedents; 35 (85%) could not be found, 3 (8%) had moved, and one entire family of 2 (5%) died. Family interviews revealed that 56 decedents ranged in age from 2 - 80 years (median 23 years); 32 (57%) were male; 35 (63%) had primary school education or less; and 45 (80%) died within 24 hours of symptom onset. Of 56 decedents, 33 (59%) died in the community or en route to care, 22 (39%) in a health facility, and 1 (2%) in an unknown location. Ten percent of decedents reportedly took ORS at home after becoming ill, and 31% waited >6 hours to seek care. Of 43 decedents who sought care at a health facility, 15 (35%) received neither ORS nor IVF. Of 33 community decedents, 24 (72%) had sought care at a health facility and were discharged before death. Most cholera deaths were not captured by surveillance. ORS use at home was inadequate, care-seeking was often delayed, and most community decedents were discharged from a health facility before death. For many decedents treated in health facilities, rehydration therapy was inadequate. To address these problems, case management trainings were held and cholera messages disseminated to the population.

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THE PLASMA AND MUCOSAL ANTIBODY RESPONSE TO THE COMPLETE *VIBRIO CHOLERAE* O1 PROTEIN IMMUNOME AND O-SPECIFIC POLYSACCHARIDE IN ADULTS WITH INABA OR OGAWA CHOLERA IN BANGLADESH

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Cholera is a severe dehydrating illness of humans caused by *Vibrio cholerae* O1 serotype Inaba or Ogawa. Characterization of immunogenic *V. cholerae* antigens could lead to a better understanding of protective immunity in human cholera infection. Using microarrays, we immuno-probed > 3500 *V. cholerae* antigens with plasma and antibody-in-lymphocyte supernatant (ALS) collected from 7 adult Bangladeshi cholera patients infected with *V. cholerae* O1 (4 Ogawa; 3 Inaba). Antibodies in ALS peak within a week of infection, and reflect transiently circulating activated lymphocytes migrating to lamina propria; as such, antibodies in day 7 ALS are surrogate markers for humoral responses in intestinal tissue. Overall, we identified 151 *V. cholerae* O1 immunoreactive antigens (61 in plasma and 103 in ALS). We rank ordered immunoreactivity, and identified 16 antigens with significantly higher immunoreactivity at convalescence (day 7 and day 30) compared to acute phase plasma (day 2), and 18 antigens with higher immunoreactivity in ALS of cholera patients collected at day 7 compared to age, gender, and ABO-matched healthy Bangladeshi controls. A number of the identified antigens have previously been demonstrated to be immunogenic and associated with virulence (e.g. *V. cholerae* O-specific polysaccharide (OSP); cholera toxin A and B subunits (CtxA, CtxB); toxin co-regulated pilus A (TcpA); and *V. cholerae* cytolysin, VCC/HlyA). Additional identified antigens included sialidase (NanH), a virulence factor required for mucosal colonization; and flagellin proteins FlaC and FlaD, involved in motility. Thus far we have confirmed plasma immunoreactivity to sialidase and FlaC via standard ELISA, and are in the process of confirming immunoreactivity of these and other identified antigens using ALS. This study is the first profiling of the mucosal and systemic antibody responses to the complete *V. cholerae* O1 protein immunome and O-specific polysaccharide. Our analysis has identified novel antigens that may be involved in host-pathogen interactions, and the results may aid in the development of an improved cholera vaccine.

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THE EARLY B CELL RESPONSE TO THE *VIBRIO CHOLERAE* O1 ANTIGEN IS CHARACTERIZED BY A HIGH DEGREE OF CLONALITY, SOMATIC HYPERMUTATION AND RECALL OF PRIOR ANTIGEN EXPOSURE

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Vibrio cholerae is non-invasive, induces long lasting protective immunity, and is an example of a highly effective human mucosal immune response. Plasmablasts, or early activated antibody secreting cells, which are found briefly in the circulation after cholera are strongly predictive of subsequent duodenal responses, suggesting that these cells provide a transient window into immunologic memory at the mucosal surface. In this study, we investigated early B cell responses to cholera using a single-cell, single-antibody analysis of the immunoglobulin repertoire, specificity, and functional characteristics of cholera induced plasmablasts. Analyzing an array B-cell receptor sequences and a panel of 140 monoclonal antibodies generated from these sequences, we found that cholera induces a plasmablast response marked by high levels of somatic hypermutation and clonality, and that the majority of B cell expansions following cholera produce antibodies that target the immunodominant antigens of *V. cholerae*: CT and LPS. In addition, *V. cholerae* sialidase was a novel major target of the early B cell response following cholera. We found that effective cholera toxin neutralizing responses targeted both the A and B subunits, and were likely impacted by prior exposure to Enterotoxigenic *E. coli* in the study population. Most notably, *V. cholerae* O1 LPS responses uniformly target the O-specific polysaccharide, but varied widely in serotype specificity and functional characteristics. Unexpectedly, we found that antibodies which bind preferentially to the previously circulating *V. cholerae* O1 Inaba serotype were characterized by high levels of somatic hypermutation and but additional mutations provided the ability to adapt to the Ogawa serotype. These findings suggest the existence of bona fide immunologic memory against a canonical T-cell independent antigen and provide an underlying mechanism for the long term immunity seen following cholera.

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IMMUNE RESPONSE TO ORAL CHOLERA VACCINE (SHANCHOL) IN INTERNALLY DISPLACED PERSONS IN SOUTH SUDAN

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Large scale outbreaks of cholera outside of historically endemic regions have renewed interest in new cholera control toolkits, including oral cholera vaccines (OCV). Extensive evidence supports the use of OCV in South Asia where the disease is endemic, but there is a dearth of efficacy studies outside of historically endemic regions where differential demographics, health status and co-circulation of other pathogens could influence vaccine response. We conducted an immunogenicity study during a 2015 pre-emptive OCV campaign in internally displaced persons (IDPs) in South Sudan. We report the immunological responses to Shanchol in a subset of volunteers (n=205), the impact of age, one versus two OCV doses and baseline titers on vaccine response. Consistent with recent circulation of cholera, high baseline titers (>80) were observed in 21% of the study participants. Amongst those without evidence of recent exposure to cholera (baseline vibriocidal titers ≤80), 90% of young children, 73% of older children and 72% of adults seroconverted (≥4 fold changes in vibriocidal titers) after the 1st OCV dose against serotype Inaba; with similar percentages of individuals seroconverting after 2nd dose; responses against serotype Ogawa were similar. Immunological endpoints (vibriocidal titers, isotype antibody levels) did not differ between the 3 age

groups post vaccination suggesting the beneficial effects of Shanchol in all. Adults and older children had baseline titers >80 more frequently than younger children (consistent with higher probability of previous cholera exposure), which was inversely associated with seroconversion. For all age groups, responses did not differ significantly between one or two doses of vaccine. Immunological priming (high baseline titer) is reflective of the increasing endemicity of *Vibrio cholerae* in South Sudan. Our results indicate Shanchol is immunogenic in a cohort of internally displaced individuals in South Sudan, and that a single dose alone may be sufficient to achieve a similar immunological response as the currently licensed two-dose regimen.

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THE EFFECTIVENESS OF ONE DOSE OF ORAL CHOLERA VACCINE IN RESPONSE TO AN OUTBREAK

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Oral cholera vaccines (OCV) represent a new effective tool to fight cholera and are licensed as two-dose regimens with 2-4 weeks between doses. Evidence from immunologic studies and secondary analyses from epidemiologic studies suggests that a single-dose of OCV may provide significant protection against cholera. A one-dose regimen, if effective, would be cheaper and easier to use in outbreaks to rapidly protect large at-risk populations. During a cholera outbreak starting in May 2015 in Juba, South Sudan, the Ministry of Health, Médecins Sans Frontières and partners engaged in the first field deployment of a single-dose of OCV (Shanchol®) to enhance their outbreak response. We conducted a case-cohort study to estimate the short-term effectiveness of one-dose of Shanchol, enrolling suspected cholera cases from 9-August-2015 through 29-September-2015 and an 898-person cohort. Suspected cholera was confirmed through multiple diagnostic tests including PCR and culture. Unadjusted and adjusted vaccine effectiveness were estimated with proportional-hazards regression models. We enrolled 87 suspected cases into the study from cholera treatment centers throughout Juba with 34 classified as cholera positive, 52 as cholera negative and 1 with indeterminate results. None of the 858 cohort members who completed a follow-up visit developed clinical cholera during follow-up. The unadjusted single-dose effectiveness was 80·2% (95% CI 61·5,100·0) and after adjusting for potential confounders, 87·3% (95% CI 70·2,100·0). One dose of Shanchol was effective in preventing medically-attended cholera in this study. These results support the use of single-dose strategy in response to outbreaks.

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ORAL CHOLERA VACCINE STUDIES IN HIGH CHOLERA ENDEMIC SETTINGS IN BANGLADESH

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Bangladesh bears the highest burden of endemic cholera with an estimated 450,000 cholera cases and over 4,500 deaths annually. From 2011 to 2016 large scale vaccination studies have being conducted with the oral cholera vaccine (OCV) ShancholTM in high risk urban areas in Bangladesh. The objective these studies is to assess the feasibility of delivery and vaccination strategies utilizing the existing national immunization facilities. To study effectiveness, thermal stability, optimal dosage, host factors including immune responses and interval of vaccination needed for making the OCV program successful in Bangladesh. Vaccination with a 2 dose of Shanchol through the routine public health care system in urban settings of Bangladesh is feasible, acceptable and impactful with over 50% protection evident in all age groups and sustained for 2 years. In the rural settings the program was successful and 92% of the 1st dose recipients received 2nd dose of OCV. A Phase III protective efficacy study carried out in urban slums of Dhaka city among 205,600 participants and efficacy measured one year and above. Overall results from these studies have shown that OCV is safe and satisfactory immune response is elicited after intake of vaccine stored in the cold or at elevated temperatures of storage. This latter observation makes the cold chain not obligatory for vaccine delivery in large campaigns such as in epidemics and outbreaks. The vaccine could be delivered to people in high risk densely populated settings in over 500,000 people in children and adults with support of the EPI. However, to better understand the optimal strategy for vaccination the population to immunize against cholera; surveillance for cholera is ongoing in 22 sites in Bangladesh to gauge the prevalence of the disease. In summary to implement OCV uptake in national immunization programs factors such as the target age group to vaccinate, feasibility of program, cost-effectiveness as well as the availability of enough cholera vaccine doses as funding to meet the large demand for a successful and sustainable OCV program in the country.

15

EVIDENCE TO OPTIMIZE THE DESIGN OF SCHOOL-BASED INTERVENTIONS AGAINST MALARIA

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In Malawi, the prevalence of *Plasmodium* infection is highest in school-age children, making this age group an attractive target for interventions to decrease the burden of malaria. However, prior trials of school-based malaria control interventions have had mixed results. Two potential reasons for intervention failures are insensitive diagnostics used in screen-and-treat efforts, and/or frequent reinfection following the intervention. We conducted school-based cohort studies to further evaluate the dynamics of *Plasmodium* infection in this age group and to help improve intervention efficacy. Students were randomly selected and enrolled in observational, school-based, cohort studies in the rainy (N=405) and dry (N=381) seasons of 2015 in Malawi. All students were followed at 1, 2, and 6 weeks after baseline, using surveys of malaria symptoms and bed net use, microscopic examination of blood smears, and PCR testing of dried blood spots. At baseline, students with positive rapid diagnostic tests (RDTs) were treated with artemether-lumefantrine. Thirty-eight percent of participants were RDT-positive and treated at baseline and an additional 11% had PCR-

based infection that was below the limit of detection (LOD) of RDTs (Negative predictive value 81%, CI: 77-85%). Among students who were RDT positive and treated at baseline during the rainy season, 35% were RDT-positive, and 44% were PCR-positive at week 6. Among RDT-negative, PCR-positive students who were not treated at baseline, 75% had infection detected at least one more time during the 6-week follow up. Nearly one quarter were PCR-positive at each of the four visits. More detailed longitudinal analysis and dry season results will also be presented. RDTs frequently do not detect low-density *Plasmodium* infections. However, the high rate of reinfection following treatment in school-age children highlights the need to tailor the timing of treatment intervals to the prophylactic period of the drug and the epidemiologic context.

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EVALUATION OF A PRIMARY SCHOOL-BASED MALARIA CASE MANAGEMENT PROGRAM ON SCHOOL ATTENDANCE IN SOUTHERN MALAWI

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Despite school-age children harbouring a large proportion of malaria parasites in the community, evidence suggests they are the age-group least likely to be taken for treatment at a health facility, or to sleep under a bed net. Evidence also indicates malaria is an important cause of morbidity in school children and a potentially significant contributor to school absenteeism. Primary schools present a pragmatic opportunity to address this disease burden and improve access to malaria diagnosis and treatment in this age group. A cluster randomized controlled trial was conducted in 58 primary schools in Zomba District. In 29 randomly selected schools, 2-4 teachers were selected to diagnose and treat uncomplicated malaria using rapid diagnostic tests (RDTs) and artemisinin-based combination therapy (ACT) as part of a basic first aid kit (Learner Treatment Kit or LTK). School attendance of children in Standards 2, 4 and 6 in was monitored using daily teacher-recorded registers and unannounced attendance 'spotchecks' by the study team. Prevalence of malaria parasitaemia, anaemia and educational performance was assessed at the end of the study. Between December 2013 to March 2015, 92 trained teachers in 29 primary schools provided 32,193 unique consultations to school children seeking care. During the peak transmission season significantly more children sought care; fulfilled diagnostic criteria for testing by RDT; and were found malaria positive. Despite a significantly greater proportion of consultations provided to female children between 6 and 14 years old, no difference was observed in the type of symptom reported. No significant difference was observed in the proportion of child-days recorded as absent in teacher registers (unadjusted OR=0.90 (0.59-1.36), p=0.614) or of children absent during 'spotchecks' throughout the intervention. There was no significant difference in prevalence of malaria parasitaemia, anaemia or education scores between the groups at the end of the intervention. In spite of this apparent lack of impact this programme of school-based malaria case management was a highly utilised and acceptable source of care.

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FACILITY-BASED ENHANCED MALARIA SURVEILLANCE TO MEASURE VECTOR-CONTROL INTERVENTION IMPACT IN WESTERN KENYA, 2012-2015

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Reliable monitoring and evaluation platforms to measure the impact of malaria control interventions are important for programs. We implemented enhanced malaria surveillance at 10 health facilities in western Kenya to monitor the impact of discontinuing indoor residual spraying (IRS). One health facility in 10 sub-counties was purposefully selected for enhanced surveillance for 1 week per month from August 2012 to April 2015. We collected clinical data and malaria rapid diagnostic test (RDT) results for all persons in outpatient clinics with a documented temperature of ≥37.5°C or history of fever. We compared the change over time in facility-specific malaria test positivity rates in relation to historical IRS implementation. During 155 enhanced-surveillance days over 33 months, 84,365 persons presented to facilities; 65.1% fit criteria for suspect malaria, of whom 45.1% were RDT positive. Older children ages 5-14 years (66.4%) were more likely to have confirmed malaria than children ages <5 years (49.1%; OR=1.35; 95% CI: 1.25-1.45; p<0.0001). Males of all ages (48.4%) were more likely to have confirmed malaria than females (42.6%; OR=1.14; 95% CI: 1.11-1.17, p-value <0.0001), which was most pronounced in males aged ≥15 years. The test positivity rate was significantly lower (31.5%) in IRS sub-counties in the first 9-months after spraying compared to the following 9 months (42.7%) after discontinuing IRS (OR=0.74, CI: 0.62-0.88, p=0.0008). Over the surveillance period, there was no difference in malaria positivity rate trends between facilities in historical IRS areas (i.e., with 1-5 years of spraying) and those without IRS. Monitoring the effects of a dynamic vector-control strategy was facilitated by enhanced malaria surveillance at a few facilities. Males and older children were most likely to have confirmed malaria perhaps because of prevention efforts focused on pregnant women and young children. Although reductions in malaria positivity rates were not detected as a long-term effect of IRS, the results highlight the importance of complementary vector-control activities as priorities and strategies shift.

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COMPARISON OF THREE VERSUS FOUR ROUNDS OF SEASONAL MALARIA CHEMOPREVENTION ON THE INCIDENCE OF CLINICAL MALARIA IN MALI

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Seasonal malaria chemoprevention (SMC) is a simple and effective strategy recently recommended by WHO for malaria control in children between 3 to 59 months living in Sahelian countries with seasonal transmission

like Mali. SMC entails administration of curative doses of sulfadoxine -pyrimathamine and Amodiquine at monthly intervals during the high transmission season. SMC efficacy in Mali, Senegal and Burkina Faso was demonstrated with three treatment courses, and there are no data to support additional benefit provided by a 4th treatment course ass suggested in the WHO recommendation. Considering the logistics of an additional treatment course, we sought to determine the benefit of 4 versus 3 courses of treatment. Children aged 3-59 months in 17 villages in two health sub-districts in Oulelessebougou were randomized to receive either 3 or 4 SMC rounds during the transmission season, starting in August 2015 using the door-to-door delivery method. Incidence rate of clinical malaria over the transmission season (August to December) measured by passive surveillance was compared between the arms. Overall, 3578 children were enrolled and followed during the 2015 transmission season. The incidence rate of clinical malaria was 0.26 episodes/child/season in children who received 3 courses of SMC and 0.20 episodes/child/season in those who received the 4th SMC treatment course, corresponding to a reduction of 25% in incidence of clinial malaria in chidren who received the 4th SMC treatment course (incidence rate ratio (IRR) = 0.75 , 95% Confidence Intervals (CI) 0.63-0.90, p = 0.002). After adjustment for age and gender, using negative binomial regression, the reduction remained unchanged (IRR = 0.75, 95% CI 0.63, p = 0.001). A fourth treatment course of SMC during the malaria transmission season provided additional protection against malaria clinical episodes.

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SUBPATENT MALARIA INFECTION IS NOT ASSOCIATED WITH POOR BIRTH OUTCOMES IN KENYAN WOMEN RECEIVING INTERMITTENT SCREENING AND TREATMENT OR INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY

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Malaria in pregnancy has significant adverse consequences. However, the impact of subpatent infections on pregnancy outcomes remains unclear. We assessed the impact of subpatent infections in a longitudinal cohort of women enrolled in a study evaluating the efficacy of intermittent screening and treatment in pregnancy with dihydroartemisinin-piperaquine (ISTp-DP), and intermittent preventive treatment in pregnancy (IPTp) with DP compared to IPTp-sulfadoxine-pyrimethamine (SP). HIV-negative women were enrolled at 12-32 weeks gestation. At each visit, samples were collected for malaria detection by microscopy and polymerase chain reaction (PCR), and by RDT for women in the ISTp-DP arm. Logistic and linear regression were used to assess the cumulative effect of patent and subpatent infections (compared to no infection) on birthweight (BW), low birth weight (LBW, <2500 gm), maternal haemoglobin (Hb) at delivery, and maternal anemia (Hb<11 g/dL), adjusted for relevant covariates. Among 1523 singleton pregnancies, 54% were paucigravid women (G1/2) and 46% multigravid women (G3+); 33% had malaria by PCR at enrolment. Neither patent nor subpatent parasitemia was associated with LBW (adjusted odds ratio (aOR) and 95% CI for patent: 1.2, 0.45-3.33; subpatent: 0.3, 0.03-1.87, or mean BW (adjusted mean difference (MD) and 95% CI for patent: -101g, -213 to 11; subpatent: 86g, -21- 192). Both patent and subpatent parasitemia were associated with reduced mean maternal Hb, though this was significant only for patent (MD, 95% CI for patent: -0.50g/dL, -0.87- -0.13; subpatent: -0.15, -0.50, 0.20). Subpatent parasitemia was associated with an increased risk of maternal anemia among G1/2 (aOR 2.9, 1.5-5.8) but not G3+ (aOR 0.75, 0.34-1.66); patent parasitemia was not significantly associated with anemia in either G1/2 or G3+ (aOR 1.61, 0.83-3.12 and 1.48, 0.62-3.57, respectively). In the context of routine screening (ISTp-DP) or IPTp in a high malaria transmission setting in western Kenya, we found no evidence that patent or subpatent parasitemia were associated with LBW or mean birthweight, despite associations with maternal anemia and Hb.

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IGNORING PEOPLE 'WHO ARE NOT THERE' MAY MITIGATE SUCCESS OF MASS DRUG ADMINISTRATION FOR MALARIA: FINDINGS FROM A MIXED-METHOD STUDY IN THE GAMBIA

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The Gambia has achieved great reductions in malaria morbidity but complete elimination still presents a challenge. Mass drug administration (MDA) has been proposed for eradication of malaria in low- and moderate-transmission settings but no MDA programme has been successful in sub-Saharan Africa. This social science study aimed to determine the consequences of the assumption that rural villages are static entities and exclusion of mobile people from MDA on malaria transmission in villages. The study was nested into a cohort study in twelve villages in The Gambia, which implemented two MDA campaigns. In sequential mixed-methods study design a quantitative survey in four villages complemented findings from qualitative research. Active case-finding identified individuals not enrolled in the cohort but present in the village at the time of study. 1384 people from four villages were enrolled in the cohort at baseline. In December 2015, 112 individuals who stayed in the villages but were not included in the MDA cohort were interviewed and screened for parasiteamia. The main reasons for not being included in the cohort study were mobility (travelled or moved); not meeting the study's eligibility criteria; withdrawal or lack of awareness. Among surveyed individuals 9.8% were parasitemic and 74.1% were not adequately protected by bed net. Males (OR=1.14, P<0.05) had increased odds of malaria parasitemia and sleeping under long-lasting insecticide-treated net (LLIN) was protective from malaria (OR=0.7, P=0.007). The findings confirm the importance of human mobility for malaria elimination in two ways. Mobile people might constitute a reservoir of malaria infection that is missed but in addition the people not involved in the cohort study used little protective measures and were thus at increased risk of infection. The use of census lists to identify the beneficiaries could mitigate success of MDA interventions. To achieve sustainable reduction of malaria, MDA interventions have to take into consideration the fluidity of villages and expand their eligibility criteria to include targeting people who are mobile and may not be documented as residents.

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COST-EFFECTIVENESS OF FOCAL MASS DRUG ADMINISTRATION AND MASS DRUG ADMINISTRATION WITH DIHYDROARTEMISININ-PIPERAQUINE FOR MALARIA PREVENTION IN SOUTHERN PROVINCE, ZAMBIA: RESULTS OF A COMMUNITY RANDOMIZED CONTROL TRIAL

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The Zambian National Malaria Elimination Program has embarked on a national strategy of malaria elimination because of recent successes and challenges in sustaining malaria control efforts. As part of this strategy, community-based treatment approaches, combined with vector control, are being tested in a large scale trial with the intent of possibly interrupting malaria transmission and reducing the burden of malaria in southern Zambia. The trial compared three arms: 1) Standard of Care including high vector control coverage with LLIN and IRS using Actellic®, roll-out of community case management for malaria and efforts to improve the quality of diagnostics and treatment, 2) Mass Drug Administration (MDA) using dihydroartemisinin-piperaqunie (DHAp) and 3) Focal Mass Drug Administration (fMDA) with DHAp. Cost was measured at the health facility catchment level to estimate the costs and cost-effectiveness of the MDA and fMDA strategies. Results differed by outcome (infections averted vs. clinical cases averted) and transmission strata (high vs. low), but in all cases MDA showed superior cost-effectiveness to fMDA. Cost-effectiveness acceptability curves produced in probabilistic sensitivity analysis indicated that both MDA and fMDA would be highly likely (>80%) to be considered cost-effective interventions (≤3x GDP per capita per disability adjusted life year (DALY) averted by WHO standards when infections averted were used as a basis for modelling DALY outcomes, but that neither intervention was considered highly cost-effective in these settings (<1x GDP per capita per DALY averted).

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DISCOVERY AND DEVELOPMENT OF A MULTISTAGE ANTIMALARIAL WITH NEW MECHANISM OF ACTION USING NEXT GENERATION SYNTHESIS

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Despite increased efforts in the last decade, the antimalarial drug discovery and development pipeline still lacks compounds with non-erythrocyte stage activity or with novel mechanism of action (nMOA). Antimalarial leads have thus far been derived mainly from two sources - natural products and synthetic 'drug-like' compounds. At the Broad Institute, we created a diverse collection of synthetic compounds having three-dimensional features reminiscent of natural products and underrepresented in typical screening collections (Diversity Oriented Synthesis compound collection). A novel series, the bicyclic azetidine, was found with robust *in vivo* efficacy in erythrocytic, hepatic and sexual stages. The compound series inhibits phenylalanine t-RNA synthetase activity, a novel molecular target, with a low propensity to induce resistance and shows efficacy with a single dose efficacy in both *Plasmodium berghei* and NSG *P. falciparum* models. Extensive pharmacokinetic and preclinical safety data supports the progression of this novel antimalarial agent towards pre-IND development.

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TARGETING RESISTANCE: EXPLOITING EVOLUTION IN DRUG DISCOVERY

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Significant strides have been made in the past decade to control malaria, however these fragile gains are in danger of being lost due to the rise and spread of ACT resistance. New strategies are needed to combat resistance and extend the lifespan of antimalarial chemotherapies. As evidenced by the emergence of ACT failure, traditional approaches of ad hoc drug combinations are not sufficient. Here we explore a new paradigm for protecting the efficacy of antimalarial therapies by identifying partner drugs that decrease the selective advantage of drug resistant cells from emerging. We propose suppressing resistance with a population biology trap: by identifying situations where resistance to one compound confers hypersensitivity to another, we can design combination therapies that not only kill the parasite, but also guide its evolution away from resistance. We applied this concept, termed "targeting resistance," to the malaria enzyme dihydroorotate dehydrogenase (PfDHODH). We have demonstrated that resistance mutations quickly arise from *in vitro* parasite selection with single PfDHODH inhibitors. Characterization of these resistant parasites showed that resistance to one PfDHODH inhibitor rendered them hypersensitive to other structural classes. To further develop this concept, we performed a high-throughput screen to identify inhibitors selective for mutant PfDHODH. As part of a Tres Cantos Open Lab Foundation project, we screened select GSK libraries and identified 25 mutant-selective and 74 wild-type-selective compounds. Of particular interest are an additional 28 compounds that were equally potent against both the wild-type and mutant enzymes. These molecules were then validated in cellular assays and prioritized candidates identified, representing promising starting points for further development. Furthermore, pairing wild-type and mutant-selective PfDHODH inhibitors largely suppressed the emergence of resistant parasites in *in vitro* experiments. We believe that this approach is widely applicable to other antimalarial targets and represents a new drug development strategy for a variety of diseases.

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A NEW BENZOXABOROLE WITH AN APPARENT NOVEL MECHANISM OF ACTION AGAINST *PLASMODIUM FALCIPARUM*

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New antimalarial drugs are needed. We have synthesized several oxaboroles with nanomolar activity against cultured malaria parasites and excellent efficacy in murine malaria models. AN13762 is a current lead candidate. It was active against multiple laboratory strains (IC50 for Dd2: 50 nM, W2: 30 nM, 3D7: 41nM, 7G8: 70 nM, and VS1: 73 nM) and fresh field isolates of *Plasmodium falciparum* (mean IC50 91nM). Pharmacokinetic assessment showed that AN13762 was ~100% orally bioavailable with t1/2 5-10 h in mouse, rat, and dog. AN13762 had

potent activity in murine models, with ED90 6.3 mg/kg against *P. berghei* and 0.85 mg/kg against *P. falciparum*-infected mice, with *in vivo* rates of clearance similar to those for artesunate. No genetic toxicology safety concerns for AN13762 were identified in Ames assays or *in vivo* rat micronucleus studies. Cytotoxicity was not seen at concentrations up to 100 μM in human cell lines. Treatment of cultured *P. falciparum* with AN13762 for 8 h intervals across the erythrocytic life cycle demonstrated maximal activity against rings and trophozoites, and parasites did not develop beyond this stage. To gain further insight into mechanisms of action we selected *P. falciparum* with decreased sensitivity to AN13762 by culturing Dd2-strain parasites in step-wise increasing concentrations or a single high concentration of AN13762. Parasites selected rapidly for resistance, with IC50 values of 400-2500 nM after selection. AN13762-resistant parasites were analyzed by whole genome sequencing. Compared with sensitive parental parasites, resistant parasites consistently had SNPs in genes predicted to encode a microtubule and actin binding protein (PFC0960c), lysophospholipase (PF07_0040), SUMO-activating enzyme subunit 2 (PFL1790w), and a protein of unknown function (PF14_0594). In summary, the benzoxaborole AN13762 represents a promising new class of antimalarial compounds, for which the mechanism of resistance appears to be complex.

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NOVEL CLINICAL STUDY AND PHARMACOMETRIC MODELLING TO FIND THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF A NEW ANTIMALARIAL

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Determination of the MIC of antimalarial drugs in the clinical setting may provide a better alternative to empirical approaches to effective dose finding. This was an open-label, dose-ranging (de-escalation), phase IIa study in Vietnamese adults (n=25) with uncomplicated *Plasmodium falciparum* malaria to estimate prospectively the in-vivo MIC of cipargamin. Patients were treated sequentially with single cipargamin doses (30, 20, 10, or 15 mg). Population PK/PD modelling of plasma cipargamin concentrations, serial parasite densities assessed by microscopy and real-time quantitative PCR were used for estimating the in-vivo MIC. PK properties of cipargamin were described by a flexible transit-absorption model followed by a one-compartment disposition model, resulting in a high predictive performance. Individual PK estimates were then imputed into the PK/PD model to assess the cipargamin-dependent parasite killing effect. As no parasite growth data were available before drug administration, the parasite multiplication rates were fixed to 10-fold multiplication/parasite life-cycle (48 hours) based on malariatherapy and volunteer data. Initial implementation of the PK/PD model assumed a homogenous parasite population and drug-dependent killing of parasites (EMAX model). Population and individual parasite clearance curves showed a biphasic pattern of parasitaemia decline, suggesting the presence of dormant (non-sensitive) parasite population. The fraction of sensitive/dormant parasites and the activation of dormant parasites were estimated. Higher doses and plasma cipargamin concentrations were associated with significantly faster maximum parasite killing. A total of 23 patients were characterized accurately as either cured, having had early treatment failure or recrudescent infection using the final model. Median (range) MIC was estimated at 0.126 (0.0375-0.803) ng/mL occurring at median (range) time of 7.45 (5.29-11.4) days. The developed PK/PD model demonstrated an informative approach for determining the in-vivo MIC, providing a rational framework for dose finding in antimalarial drug development.

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ASSESSING THE SPEED OF CLEARANCE OF *PLASMODIUM VIVAX* FROM THE BLOOD FOLLOWING TREATMENT WITH A LICENSED AND EXPERIMENTAL ANTIMALARIALS

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The rate of clearance of malaria parasites from the blood of patients is a key determinant of treatment success. For example, in falciparum malaria the rate of clearance in early infection is inversely associated with mortality. Although it is considered that drug response in *Plasmodium vivax* infection is generally faster, there is a paucity of information, both because *in vitro* culture is not possible and because clinical trials have been fewer. To investigate the relative activity of four antimalarials against *P. vivax* we undertook clinical trials entailing induced blood stage infection with *P. vivax*. Artemether/lumefantrine (A/L) and chloroquine (CQ) were administered at the approved doses, while artefenomel (OZ439) and DSM265 are scheduled to be administered as a single dose of 200mg and 400mg, respectively. Subjects were treated at symptom onset, and rate of clearance of asexual parasitemia measured by qPCR. Parasite clearance half life (PCt1/2) and parasite reduction ratio (PRR) were derived from the slope of the parasite clearance curve. Data from 10 subjects treated with A/L indicate a PCt1/2 of 1.6 hrs (95%CI: 1.5-1.7) and a Log10PRR of 9.1 (95%CI: 8.4-9.7). Data from 8 subjects treated with CQ indicate a PCt1/2 of 5.0 hrs (95%CI: 4.7-5.4) and a Log10PRR of 2.9 (95%CI: 2.7-3.1). Similar data from the OZ439 and DSM265 cohorts will be available for presentation. Deriving these key pharmacodynamics variables of antimalarials against *P. vivax* will be of major assistance in selecting optimal drugs, combinations, doses and regimens so that optimal systems are developed for treatment of this widely prevalent and important pathogen.

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FOSMIDOMYCIN-PIPERAQUINE AS NON-ARTEMISININ-BASED COMBINATION THERAPY FOR ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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The combination of fosmidomycin and piperaquine, with the attributes of rapid blood schizonticidal activity and prolonged post-treatment prophylaxis, is being developed to meet the challenge of emerging artemisinin resistance. As a potent inhibitor of 1-Deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, fosmidomycin possesses a unique mode of action through blockade of the non-mevalonate pathway of isoprenoid biosynthesis. In contrast, piperaquine is thought to bind to heme, inhibiting its detoxification within the malaria parasite. It is further postulated that the toxic build-up of heme increases the membrane permeability of red cells favouring the influx of fosmidomycin. The efficacy, tolerance and safety of the combination for the treatment of acute uncomplicated *Plasmodium falciparum* mono-infection are being evaluated in a proof of concept study in Gabon. A total of 100 symptomatic patients including 10 adults aged >14 years, 40 older children aged 5 to 14 years, and 50 younger children aged one to five years with initial parasite counts between 1,000 and 150,000/μL have been treated with fosmidomycin, in twice daily doses of 30mg/kg, and piperaquine, in a once daily dose of 16mg/kg, orally for three days and followed-up for 63 days. The primary efficacy endpoint is the per protocol PCR-corrected Day 28 cure rate.

Preliminary results at all ages show the combination is highly effective with an excellent tolerance and there are no safety concerns. The full results will be presented at the ASTMH meeting in November 2016. Meanwhile, proposals are being drawn up for dose optimisation studies aimed at achieving a therapeutic regimen of once daily dosing administered over three days.

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MALARIA, MALNUTRITION, AND ADVERSE BIRTH OUTCOMES AMONG PREGNANT WOMEN: A POOLED ANALYSIS

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Malnutrition and malaria infection commonly co-exist, afflicting pregnant women in resource-poor settings. Prior small studies have indicated that the effect of malaria on low birthweight (LBW; <2500g) may depend upon maternal nutritional status. We investigated the interaction between malaria infection during pregnancy and maternal nutrition with regards to the risk of LBW using data from 14,635 singleton, live birth pregnancies from women who participated in 13 pregnancy studies conducted in malaria endemic countries across Africa and Asia from 1996-2015. Study-specific effect estimates and measures of interaction were calculated using linear and log-binomial regression models, adjusted for confounders (maternal age, gravidity, area of residence, HIV infection, anemia) using inverse probability of treatment weights, and pooled across studies using a restricted maximum likelihood random effect model. Nine of the thirteen studies assessed malaria (microscopy or RDT) and mid-upper-arm circumference (MUAC) at enrollment. Across these 9 studies, 75% of women were well-nourished (MUAC≥23 cm) and malaria-uninfected at enrollment, 10% were well-nourished but malaria infected, 12% were malnourished and not malaria infected, and 2% were both malnourished and malaria infected. Compared to women who were well-nourished and uninfected, the pooled risk ratios for LBW were: malaria alone, 1.18 (95% confidence interval [CI]: 0.93, 1.48); malnutrition alone, 1.55 (95% CI: 1.29, 1.85); and malaria and malnutrition together, 1.75 (95% CI: 0.90, 3.37). The pooled interaction contrast was -0.03 (95% CI: -0.11, 0.06; p=0.57), with minimal statistical heterogeneity across studies (=0.0014, Cochran Q=11.63 [p=0.11]). While MUAC<23cm was associated with an increased risk of LBW, malaria infection at enrollment was not as strongly associated and there does not appear to be synergism between these two factors. Additional analyses to be presented will consider: mean birth weight; preterm birth; malaria infection at delivery; malnutrition defined by BMI; meta-regression for subgroup effects; selection bias by excluding pregnancy loss.

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ASSESSING ULTRASONOGRAPHY AS A DIAGNOSTIC TOOL FOR PORCINE CYSTICERCOSIS

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Neurocysticercosis caused by the pork tapeworm, *Taenia solium*, causes 30% of epilepsy in poor rural communities of the developing world. Use of the ring strategy is a promising control intervention targeting treatment of humans and pigs living near heavily infected pigs. Tongue examination for *T. solium* cysts provides a crude means of identifying heavily infected pigs. However, as prevalence decreases over time in treatment communities, higher sensitivity methods are needed to achieve full treatment coverage. This study evaluates ultrasonography as an alternative method to detect pigs infected with varying burdens of *T. solium* cysts. We collected blood samples and purchased 158 seropositive pigs living in eight villages of Piura, a province of northern Peru where *T. solium* is endemic. Tongue examination and ultrasonography of the limbs were performed in these animals, followed by fine dissection necropsy to determine cyst burden. We used necropsy as a gold standard and compared the sensitivity and specificity of ultrasonography with tongue examination for their ability to detect heavy infection (≥ 100 viable cysts) in pigs. Compared to tongue examination, ultrasonography was more sensitive (92% vs. 83%) but less specific (90% vs. 97%) detecting pigs with heavy cyst burdens, although these differences were not statistically significant. The improved sensitivity of ultrasound resulted in the detection of one additional heavily infected pig compared to tongue examination, but also resulted in more false positives (14 vs. 3) due to poor specificity. Ultrasonography was highly sensitive in detecting pigs with heavy cyst burdens and may allow for better treatment coverage in endemic areas compared to tongue examination. In its current form, however, the high rate of false positives results in a substantial number of unnecessary treatments, and must be improved before ultrasound can replace tongue examination as the preferred screening tool for pigs in ring strategy interventions. With improvements in training and technology, the use of ultrasound could potentially benefit local elimination strategies where previous efforts have stalled.

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VASCULAR LEAKAGE IN THE BRAIN IN PORCINE NEUROCYSTICERCOSIS IS ASSOCIATED WITH ANGIOGENESIS

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Anthelmintic therapy for neurocysticercosis, brain infection by *Taenia solium* cysts (larvae), frequently exacerbates neurological symptoms. We have used naturally infected pigs and Evans Blue (EB) to show that the acute post treatment immune response is associated with increased vascular permeability of the blood-brain barrier (BBB). Here we studied potential mechanisms for this increase. We used 11 infected pigs, 3 controls (T0) and 8 treated with a single dose of Praziquantel, euthanized 2 and 5 days later (T2 and T5). EB was injected 2h before sacrifice; biopsies of brain tissue around cysts (capsules) were analyzed using immunohistochemistry (IHC) and real time quantitative PCR for selected markers.EB-stained (blue), "leaky" capsules from all groups had more newly formed vessels (strong IHC reaction for cadherin, vascular endothelial growth factor (VEGF) and von Willebrand factor (vW)) than clear ("non-leaky") capsules and also had higher expression of VEGF, vWf, transforming growth factor beta and ephrine genes, as evidence of angiogenesis. Immature new vessels, with incomplete basal lamina and weak cellular junctions, demonstrated perivascular lymphocytic cuffing, with clear recruitment of immune cells, consistent with high permeability. These effects were significantly more intense in T5 compared to T2 and T0. The "leaky", highly vascularized capsules also showed increased astroglyosis (extreme IHC reaction for glial fibrillar acidic protein, GFAP) and the deposition of beta-amyloid peptide compared to clear capsules. A decrease in transcripts for GFAP and the amyloid precursor protein (APP) on T5 versus T2 and T0 suggested downregulation of these markers and a possible restabilization of the BBB. This is consistent with early remodeling by matrix metalloproteinases (MMP)-2 and MMP-9, suggested by their high gene expression on blue capsules at T5.We conclude that the increase of vascular permeability as a result of treatment-induced inflammation happens mainly around newly formed vessels; we also suggest the existence of a restabilization process of the BBB that follows the rapid post treatment disruption.

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AMYLOID-β PEPTIDE AND EXPRESSION OF AMYLOID PRECURSOR PROTEIN GENE (APP) ARE INDUCED BY ANTHELMINTIC TREATMENT IN PORCINE NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), hyperendemic in Peru, is the brain infection with the larval stage (cyst) of *Taenia solium*; a main frequent clinical manifestation are seizures. This disease has been well documented clinically, but the complex host-parasite interaction and the resulting inflammation, a main factor of the severity of the symptoms, are not completely understood. Among many inflammatory markers, amyloid-beta peptides (Aβ) have been associated with neuropathological processes including glial activation, synaptic dysfunction and neuronal apoptosis. However, the role of Aβ in the neuropathology of NCC has not been explored yet the effect of anthelmintics on this molecule is unknown. We used brain sections of eight pigs naturally infected with *Taenia solium* cysts. Five animals received a single dose of 100 mg/kg praziquantel (PZQ), which is demonstrated to trigger inflammation, and three untreated pigs served as controls (T0). Three pigs were sacrificed after two days of treatment (T2) and two after five days (T5). Two hours before sacrifice, all animals were sedated and injected with Evans Blue to delineate increased permeability of the blood-brain barrier (BBB), shown to correlate with inflammation. Eighty-two cyst capsules (23 from T0, 36 from T2 and 23 from T5) were examined to evaluate the presence of Aβ in host tissue by immunohistochemistry (IHC). The relative expression of the amyloid precursor protein gene (app) was analyzed by quantitative real time PCR in 20 capsules (7 from T0, 7 from T2 and 6 from T5). We found that PZQ induced both the presence of Aβ and app expression, this was more evident when the BBB had been affected. IHC results showed similar values for T2 and T5, both being significantly higher that T0, but app expression was clearly higher for T2 than T0 and T5, indicating post-transcriptional regulation. Besides, we observed less Aβ in degenerated cysts, which suggests the possibility of a regeneration process that follows the acute inflammatory phase. Together, these findings suggest that Aβ is a component of the acute inflammatory response in NCC and that its expression is regulated at some point of the cyst degeneration.

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TAENIA SOLIUM CYSTICERCOSIS SEROCONVERSION AND SEROREVERSION CUMULATIVE INCIDENCE IN A LARGE-SCALE COMMUNITY TRIAL IN BURKINA FASO

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Cysticercosis is a debilitating yet neglected disease caused by *Taenia solium*, a zoonosis transmitted between humans and pigs. Knowledge on the disease transmission patterns is crucial to develop effective control strategies. Our objective was to estimate the seroconversion (SC) and seroreversion (SR) cumulative incidence as well as related risk factors in a large scale community randomized controlled trial in Burkina Faso. The study was conducted in all pig-raising departments of the provinces of Nayala, Boulkiemdé and Sanguié. Two villages per department meeting eligibility criteria were randomly selected and clustered sampling based on pig raising was used to select 80 concessions per village. In each concession, one household was randomly selected from which one eligible individual was randomly chosen. Sixty people were asked to consent to provide three blood samples over the course of three years in each village. Socio-demographic factors, pork cooking and eating practices and pig management practices were assessed through questionnaires. The presence of excretory secretory circulating antigens (Ag) of *T. solium* metacestodes was measured in sera using the B158/B60 enzyme-linked immunosorbent assay (ELISA). Sera were collected at baseline and 18 months follow-up from 2211 consenting individuals. The SC and SR cumulative incidences were 3.3% (95% confidence interval (CI) 2.6-4.2) and 35.8% (95% CI 24.7-48.5), respectively. Univariable analyses indicated higher SC in Boulkiemdé vs. Sanguié (4.0 vs. 2.2%, cumulative incidence ratio (CIR) = 1.8, 95% CI 1.0-3.3). While SC tended to be higher among individuals older than 40 years versus those of 6-17 years (4.3

vs. 2.5%, CIR = 1.7, 95% CI 1.0-3.1), SR was much lower in the older group (18.9 vs. 80.0%, RR = 0.2, 95% CI 0.1-0.5). Lastly, SC was higher in individuals eating pork at the village market compared to those never eating pork (4.7 vs. 2.0%, RR = 2.4, 95% CI 1.1-5.2). This is the largest cohort study of cysticercosis SC and SR ever conducted in West Africa. Multivariable and hierarchical analyses will be conducted to explore the impact of village-level and other individual level factors.

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USE OF DIFFERENTIALLY EXPRESSED MONOCYTE GENES TO DISTINGUISH BETWEEN NEUROCYSTICERCOSIS-ASSOCIATED EPILEPSY AND IDIOPATHIC EPILEPSY

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Neurocysticercosis (NCC) is a parasitic infection of the brain that accounts for 34% of active epilepsy cases in Vellore district, India. New diagnostic methods are needed due to the high cost of brain imaging and because antibody detection misses 34% of NCC patients.. We conducted a cross-sectional study of patients aged 18 to 51 years seeking care at the Dept. of Neurological Sciences, Christian Medical College and Hospital, Vellore between Jan. 2013 and Oct. 2014. Subjects were categorized as patients diagnosed with NCC-associated epilepsy with at least one seizure in the past 7 months (Group 1, n=76); recovered NCC (RNCC) patients without seizures and brain lesions for at least two years (Group 2, n=10); patients with epilepsy with at least one seizure in the past 7 months without any structural brain lesion on imaging (Group 3, n=29); patients who had a normal brain CT scan or MRI, no epilepsy or head trauma (Group 4, n=17). Groups 3 and 4 were negative for antigens or antibodies to T solium larvae in serum. Group 1 was sub-divided into those with Solitary Cysticercus Granuloma (SCG, n=29), single calcified cysts (SCC, n=20) and multiple cysts (MNCC, n=27). We used mRNA arrays of CD14+ blood monocytes from 6, 6 and 4 patients in Groups 1, 3 and 4, respectively, to identify differentially expressed genes linked to inflammation, host defenses or central nervous system processes. We identified 15 genes including GTPase's (4), and genes linked to immune regulation (4) enzymes with role in signaling (3), neurogenesis (1), and other immune function (3). Expression of these genes was measured by qPCR in all participants and fold-change in NCC cases (Groups 1-3)/Control (Group 4) was calculated. Highest expression levels were observed in patients with NCC-associated epilepsy, followed by RNCC and finally by those with idiopathic epilepsy. Expression levels of 7 of 15 genes differed among NCC patients with different types of brain lesions with expression decreasing as lesions became calcified. In contrast, one gene (RAP1A), increased as lesions calcified. This study suggests measuring expression of key blood monocytes genes may be useful to diagnose and stage NCC.

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VENTRICULAR NEUROCYSTICERCOSIS IN THE UNITED STATES: TREATMENT, COMPLICATIONS AND OUTCOME IN A TERTIARY REFERRAL CENTER

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Ventricular involvement in neurocysticercosis (NCC) has distinct clinical presentations, complications and treatments. Here we review the clinical course of 21 patients with one or more ventricular cysts referred to the National Institutes of Health (NIH) over 31 years. 21 patients with one or more ventricular cysts had a median age of 29.6 yr. (range 22.4-52.6), 52.4% male and the median follow up was 3.1 yr. (range 0.12-27.7). Most of the patients presented with a single cyst (19/21, 90.5%); two patients had 2 and 3 cysts, respectively. The 4th ventricle was involved alone in 12/21(57.1%) persons but a single cyst occupied more than one ventricle, migrated or there were multiple cysts in 16/24 (66.7%). Most of the patients presented with one or more additional manifestations of NCC, 5/21 (23.8%) with viable/degenerating parenchymal cysts, 9/21 (42.9%) with parenchymal calcification(s), and 8/21 (38.1%) with subarachnoid cysts. Only 4 had a single 4th ventricular cyst without any other accompanying types of NCC. Most of the symptoms were due to acute or chronic hydrocephalus (76.1%) including headache, vomiting, nausea, syncope, confusion, dizziness, coma, and vision disturbance, in decreasing order. A majority of patients were initially treated with cyst removal and/or shunt placement before referral to NIH. Patients with non-resectable cysts were treated medically. A number of complications were noted, mostly related to surgery prior to referral and to residual long-lasting symptoms.Of the 6 persons with only ventricular cysts and/or calcifications, 4 had sufficient follow-up and serial evaluations to assess if resolution occurred. Negative or decreasing cestode antigen levels in the CSF predicted resolution after cyst removal without the need for cysticidal treatment. Low WBCs counts in the CSF mostly corroborated the cestode antigen findings but, in one case, interpretation was complicated by the use of corticosteroids and presence of an infected shunt. Careful follow up of patients with surgically removed ventricular cysts, allows determination of the need for treatment.

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RISK FACTORS FOR SEIZURE RECURRENCE AFTER SUCCESSFUL ANTIPARASITIC TREATMENT IN PARENCHYMAL NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), caused by the larval stage of the pork tapeworm *Taenia solium*, is a major risk factor for seizures worldwide. In this retrospective cohort analysis based on two randomized clinical trials in which patients had a systematic post treatment follow up of 18 months, patients in whom all viable cysts resolved after antiparasitic treatment were identified to assess risk factors for seizure recurrence from 6 to 18 months after treatment onset. Out of 188 patients, 85 (45.2%) had complete resolution of viable cysts demonstrated on follow up MRI at 6 months. Eight (9.4%) of these patients had at least one seizure between 6 and 18 months after treatment. A seizure in the initial month after the onset of antiparasitic treatment (present in 30 cases) was associated with seizure relapse between months 6 and 18 (6/30, 20% versus 2/55, 4%, relative risk 5.6, 95% confidence interval 1.2-25.6, p=0.02). Similarly, a seizure in the initial two months or in the initial 6 months after treatment onset were associated with further seizure episodes in the same period (2-months: 7/33, 21.2% versus 1/52, 1.9%, relative risk 11.0, 95% confidence interval 1.4-85.6, p=0.003; 6 months: 8/36, 22.2% versus 0/49, 0%, risk reduction 0.22, 95% confidence interval 0.09-0.36, p<0.001). The risk of seizure relapse was also significantly higher in patients with a history of previous courses of antiparasitic treatment (RR 6.7, 95% confidence interval 1.6-29.3, p=0.03), but no significant differences in risk of seizure relapse were found in regards to their length of seizure history, number of seizure episodes in the 12 months before antiparasitic treatment, number of cysticercotic lesions, presence of calcifications, proportion of lesions with inflammation at baseline, or length of antiepileptic drug treatment. Our results show that early post-treatment seizures will be associated with further seizure relapse in a subgroup of NCC patients. Enhanced seizure control in the first months should be considered to reduce this risk.

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FUNCTIONAL ANALYSIS OF DIVERGENT GPCR-LIKE PROTEINS DURING *PLASMODIUM CHABAUDI* BLOOD-STAGE DEVELOPMENT

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Whilst malaria infection results in a complex range of responses and disease in the host, the parasite must respond in turn to changing host conditions to optimise its infectivity and transmission capacity. The components of parasite signaling pathways, therefore, have great potential as anti-malarial therapeutic targets. Signaling pathways operating through G-Protein Coupled Receptors (GPCR) are the best-established class of therapeutic targets and, although almost nothing is known about the identities of *Plasmodium*-encoded components of GPCR signalling pathways, chemical library screens and experimental approaches suggest that they operate during *Plasmodium* blood-stage development. We have therefore investigated the function of two *Plasmodium* proteins that are members of ancient and divergent GPCR families using the rodent *in vivo* malaria model, *P. chabaudi.* Gene deletion and mutation has revealed that *Plasmodium* GPCR-like genes play a role in parasite egress and invasion and in host-parasite interactions necessary for the establishment of both acute and chronic infection. The function of GPCR-like family members in regulating the signalling pathways involved in these processes will be discussed.

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A NOVEL POPULATION OF TCRαβ-EXPRESSING CD11BHIGHCD14+F4/80+ MACROPHAGES IS INDUCED BY *PLASMODIUM BERGHEI* ANKA MURINE MALARIA

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Macrophages are equipped with a wide array of invariant receptors that facilitate their phagocytic function and the regulation of inflammation. In malaria, macrophages mediate both immune protective (parasite clearance) and pathogenic (cerebral malaria and severe malaria anemia) processes. Using the virulent asexual stage of the *Plasmodium berghei* ANKA (*Pb-A*) parasite which causes experimental cerebral malaria in C57BL/6 mice and severe anemia in Balb/c mice, we have identified a novel population of $CD11b^{high}CD14^{+}F4/80^{+}$ macrophages that express TCRαβ during malaria infection. This population expands rapidly during a *Pb-A* infection and preferentially sequesters in the brain during ECM. Proliferation of malaria specific TCRαβ-expressing macrophages requires a threshold level of parasite burden and optimal expression of TCRαβ on $CD11b^{high}$ cells requires coexpression of CD14 and F4/80. Furthermore, in depth flow cytometric analysis demonstrates that these unique TCRαβ-expressing macrophages are $CD3^{-}CD4^{-}CD8^{-}$ and the Vβ TCR repertoire of macrophages is distinctly different from conventional T cells during *Pb-A* infection. Identification of this unusual macrophage population that uses combinatorial TCRαβ expands our knowledge of macrophage biology during malaria and adds another layer of complexity to malaria immunology while providing new considerations in vaccine and drug design.

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PLASMODIUM FALCIPARUM PHISTC PROTEINS ARE REQUIRED FOR ANTIGEN DELIVERY TO THE INFECTED ERYTHROCYTE SURFACE

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Plasmodium falciparum extensively remodels its host cell to mediate nutrient exchange and avoid immune and splenic pressure during blood stage infection. This remodeling includes changes to the adhesive and biophysical characteristics of infected cells and is mediated by exported parasite proteins. Here, we investigate the role of *Plasmodium* PHISTc proteins in erythrocyte remodeling. PHISTc proteins are exported and contain a helical core domain shared by all PHIST paralogs. They are conserved across primate malarias, and several are expressed in both asexual and sexual blood stages, suggesting conserved function in host pathogen interactions. While several proteins from the PHISTb sub-class have a role in cellular rigidity or cytoskeletal architecture, little is known about the function of PHISTc proteins. To investigate the role of PHISTc proteins in asexual and sexual stage remodeling, we knocked out 9 of the 16 *P. falciparum* paralogs in the reference line 3D7 and in a second line, CS2. Flow cytometry showed that 6 of the 9 PHISTc knock outs have decreased asexual surface reactivity to serum from malaria patients, suggesting that they are required for efficient surface antigen trafficking. Of these 6 PHISTc knockouts with decreased surface antigen display, 2 showed a complete absence of the VAR2CSA PfEMP1 variant at the erythrocyte surface by flow cytometry and immunofluorescence microscopy while 2 others showed normal VAR2CSA display. These data suggest differential specificity for surface antigen delivery between PHISTc proteins. Finally, microsphere filtration showed that none of the 9 PHISTc knock outs affects cellular rigidity of asexual stages, and immunofluorescence microscopy showed that PHISTc disruption also does not affect trafficking of knob or Maurer's clefts markers. Altogether our observations suggest that PHISTc proteins, in contrast to PHISTb proteins, play a specialized role in antigen delivery to the host cell surface without drastically altering cellular architecture. We hypothesize that each PHISTc protein may differentially contribute to the trafficking of specific antigen families or PfEMP1 variants.

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EXPLORING THE ROLE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN 1 IN INVASION OF DUFFY-NULL AFRICANS

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The ability of the malaria parasite *Plasmodium vivax* to invade red blood cells (RBCs) is dependent on the expression of the Duffy blood group antigen on RBCs. Consequently, Africans who are null for the Duffy antigen are not susceptible to *P. vivax* infections. Recently, *P. vivax* infections in Duffy-null Africans have been documented, raising the possibility that *P. vivax*, a virulent infection in other parts of the world, may expand malarial disease in Africa. In our study, we have identified two Duffy-null Ethiopians infected with *P. vivax*. For invasion, *P. vivax* binds the Duffy blood group antigen through its Duffy binding ligand 1 (PvDBP1). Previously several mutations were observed in PvDBP1 from Madagascar, India, and Brazil and we identified unique mutations in PvDBP1 in Duffy-

null Ethiopians by sequencing. We aimed at understanding whether the mutations in PvDBP1 results in binding to another receptor on Duffy-null RBCs. We determined PvDBP1 from these parasites failed to bind Duffy-null RBCs, but bound strongly to Duffy-positive RBCs, indicating that mutations in DBP1 did not account for the ability of *P. vivax* to infect Duffy-null Africans. Interestingly, by real-time quantitative PCR we identified three and eight copies of PvDBP1 in the two Duffy-null Ethiopians suggesting that it may be selected to bind low copy number of Duffy blood group antigen if expressed on Duffy null RBCs or another new receptor on the RBCs by increasing its gene expression. Moreover, Sal I *P. vivax* invades Squirrel monkeys despite the failure of PvDBP1 binding to squirrel RBCs. It is surprising to know that despite the high similarity between the Duffy blood group antigens from Squirrel, Aotus monkeys and humans, Sal I PvDBP1 does not bind Squirrel monkey erythrocytes. Furthermore, we determined *P. vivax* DBP1 from India and Brazil bound squirrel monkey RBCs as strongly as Aotus RBCs. Therefore, Sal I *P. vivax* infects Squirrel monkeys in the absence of PvDBP1 binding to Squirrel monkey RBCs. We conclude that *P. vivax* Sal I and perhaps *P. vivax* in Duffy null patients may have adapted to use new ligand-receptor pairs for invasion.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY PREVALENCE: GENETIC VARIANTS AND THEIR INFLUENCE ON HEMOLYTIC EFFECT IN MALARIA ENDEMIC AREAS OF COLOMBIA

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Glucose 6-phosphate dehydrogenase (G6PD) is an enzyme involved in prevention of cellular oxidative damage, particularly protecting erythrocytes from hemolysis. An estimated 400 million people present variable degrees of inherited G6PD deficiency (G6PDd) which puts them at risk for developing hemolysis triggered by several risk factors including certain foods and multiple drugs including Primaquine (PQ). Intensification of malaria control programs worldwide are recommending a more extensive use of PQ and related drugs in populations with different levels of G6PDd prevalence. The aim of this study is to assess the prevalence of G6PDd in representative malaria endemic areas of Colombia and the influence of PQ administration on the induction of hemolysis. A total of 426 volunteers from Buenaventura, Tumaco, Tierralta and Quibdó were evaluated for G6PD enzymatic activity by using a quantitative G6PD test and a subset of samples were analyzed by PCR-RFLP to determine the frequency of the three most common G6PD genotypic variants: A-, A+ and Mediterranean. Preliminary results indicate a high frequency of G6PD A- genotype, followed by A+ genotype. A total of 28 individuals (6.56%) displayed either severe or intermediate G6PDd. The highest prevalence (3.51%) was found in Buenaventura, whereas G6PDd prevalence was lower (<1%) in Tierralta and Quibdó. G6PD A alleles were the most frequent (15.23%) particularly in Buenaventura and Tumaco. In order to determine the hemolytic effect of PQ administration for treatment of *P.vivax* infections, in a second phase, individuals attending the malaria control program are being studied. Blood samples from *P.vivax* malaria patients are being collected over a period of three weeks after initiation of PQ treatment and are being analyzed to assess hematologic parameters such as hemoglobin, hematocrit, and bilirubin as well as G6PD phenotype and genotype. Final results of these studies will be presented. Assessment of G6PDd prevalence in malaria endemic areas is crucial in view of possible mass drug administration (MDA) for malaria elimination in these regions.

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ACUTE KIDNEY INJURY IS COMMON IN UGANDAN CHILDREN WITH SEVERE MALARIA, AND STRONGLY ASSOCIATED WITH SEQUESTERED PARASITE BIOMASS AND MORTALITY

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Acute kidney injury (AKI) is a common complication in severe malaria that portends adverse clinical outcomes. A major challenge in assessing AKI relates to defining baseline kidney function. A number of approaches have been evaluated in pediatric cohorts, but none have been validated in African children. This study was performed at Mulago Hospital, Kampala, Uganda. Children with cerebral malaria (CM), severe malaria anemia (SMA), or community children (CC) were enrolled if they were between 18 months and 12 years of age. In this study we validate an approach to estimate baseline renal function in children assuming a normal glomerular filtration rate (GFR) of 120mL/min/1.73m2 to back-calculate creatinine (Cr) using the Schwartz equation. We compared the estimated baseline Cr (eCr) values to a population-derived normal curve of Cr for height using CC (n=169). The eCr calculated a GFR of 120mL/min/1.73m2 correlated very well with values derived from the reference population (R2=0.997, p<0.0001). AKI was defined using the Kidney Disease: Improving Global Outcomes (KDIGO) classification where a 1.5x increase in baseline Cr constituted AKI. Rates of AKI classification using the two approaches were identical with 39.5% of children with CM (n=99/257), and 21.0% of children with SMA (n=46/219) meeting a definition of AKI. AKI was associated with the sequestered parasite biomass in children with CM and SMA, p<0.0001 and p=0.005 respectively. In children with CM, AKI was associated with increased odds of in-hospital and all-cause 24-month mortality (odds ratio, 95% confidence interval: in-hospital mortality, 1.59, 1.04-2.45, p=0.009; 24 month mortality, 1.69, 1.09-2.61, p=0.003). This study validates a GFR of 120mL/min/1.73m2 as an appropriate estimate of baseline renal function in Ugandan children. AKI was strongly associated with sequestered, but not circulating, parasite biomass in children with both SMA and CM. In children with CM, AKI was also strongly associated with increased short- and long-term mortality. Interventions that decrease sequestration should be studied for their potential to reduce AKI and mortality in children with CM.

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PFHRP2 PERSISTENCE IN "ONCE INFECTED RBC" ENABLES A RAPID PREDICTION OF POST-ARTESUNATE DELAYED HEMOLYSIS

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Artesunate rapidly cures patients with severe malaria but frequently induces anemic episodes called Post-Artesunate Delayed Hemolysis (PADH) for which a simple predictive method is urgently needed. The concentration of "once-infected" red blood cells - appearing when

artesunate-exposed parasites are expelled from their host RBC by pitting - is predictive of subsequent PADH. In 103 French travellers patients, we observed that *Plasmodium falciparum* Histidine-Rich Protein 2 (HRP2) persists in the whole blood (not plasma) of artesunate-treated patients at significantly higher levels than quinine-treated patients (p=0.035). This HRP2 persistence was also observed in 70 Bengladesh patients. Using an optimized membrane permeation method, HRP2 was observed by immunofluorescence, Western blot and electron microscopy to persist in once-infected red cells from artesunate-treated patients. HRP2 deposition followed a membrane-bound pattern similar to that of Ring-Erythrocyte Surface Antigen (RESA), the conventional marker of these cells. Based on these observations, we developed a semi-quantitative titration method using a widely available HRP2-based RDT. Positivity of this RDT using a 1:500 dilution of whole blood collected after parasite clearance (2-4 days after start of treatment) predicted subsequent PADH with 93% sensitivity and 74% specificity. These results immediately open the way to the adaptation and adoption of existing HRP2-based malaria RDTs for cheap, bed-side prediction of PADH several days before it occurs.

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EVALUATE MOSQUITO NETS FASTER AND CHEAPER: RESULTS FOR PUBLIC HEALTH INTEREST

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The World Health Organization (WHO) promotes the use of long-lasting insecticidal nets (LLINs) to prevent malaria and also advises national programs to evaluate insecticidal activity on nets. To measure insecticidal activity, the WHO guidance mentions the use of a cone test, which needs 100 female mosquitoes per tested net. However, it requires a major and steady effort to produce thousands of insecticide-susceptible female mosquitoes, involving also expensive materials. The aim of the present study is to improve LLIN bio-efficacy testing by reducing cost and efforts while guaranteeing the accuracy of results. We evaluated two alternative methods to fulfill this objective: reducing the number of mosquitoes and evaluating a mosquito-free method. First, we compared the use of one, two, three or four cones (i.e. 25, 50, 75 or 100 mosquitoes) on each piece of LLIN with its Bayesian probability to be a valid LLIN. The result showed that using two cones has a limited impact on accuracy (93%) as compared with actual standard of four cones (94%). In a series of several LLINs, the average error in the measured proportion of valid LLINs was <1%. This result shows that it is possible to halve the time of lab processes without loss of accuracy for Public Health recommendations. Second, we evaluated a Colorimetric Field Test (CFT), a chemical method to measure surface levels of insecticide on LLINs. From three brand of LLINs collected in Madagascar, cut off values corresponding to the threshold value of 80% mortality with bio-assay test were determined at 0.35 µg, 0.60 µg and 0.07 µg per sample to consider a LLIN as 'good net'. The results showed 92% sensitivity and 100% specificity, demonstrating that CFT is an excellent tool for assessing the residual activity of the insecticide. By adopting one of these methods, one could save much time and money to make a faster and cheaper decision for malaria control programs. A decision that could save human lives!

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INTERROGATING MOSQUITO-PATHOGEN COMMUNITIES USING HIGH-THROUGHPUT MICROFLUIDICS

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Eliminating mosquito-borne diseases requires intimate knowledge of the ecology of vectors. Such knowledge can for instance be used to design effective vector control strategies, or to understand the eco-evolutionary processes that drive range expansions of vectors and pathogens. Current techniques in vector ecology, such as human landing catches or chemically baited traps, are very labor intensive and therefore severely limited in throughput. These limitations prevent the detailed interrogation of mosquito-pathogen communities in the field. We demonstrate a low-cost automated screening tool that enables dissection-free, high-throughput molecular analysis of individual vectors and their pathogens. We exploit the fact that mosquitoes transmit pathogens by expectorating saliva to autonomously collect saliva droplets resulting from single mosquito bites. Multiple cues (e.g. temperature, odorants, texture) are integrated on a microfabricated substrate mimicking human skin, the substrate is designed to maximize its attractiveness to mosquitoes and induce them to bite, thereby depositing saliva. We present behavioral data extracted from laboratory experiments that allow us to quantitatively assess the interaction of mosquitoes with the device. The use of high-throughput microfluidics enables us to perform small volume biochemical analyses on a huge number of pico-to-nanoliter saliva samples in parallel, greatly reducing reagent cost and processing time. We implement multiplexed microfluidic assays that enable the simultaneous characterization of the biting mosquito's genetic make-up and its pathogens. This platform provides us with a means of high-throughput, high-resolution sampling of individual insects in field and laboratory settings. Large scale application of this tool in public health surveillance may provide early warnings for epidemics, detect the emergence of drug resistance, and track the spread of emerging infectious diseases.

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A PROMISING NEW MODE OF ACTION CHEMISTRY INDOOR RESIDUAL SPRAY PRODUCT TO CONTROL RESISTANT MOSQUITOES

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Widespread insecticide resistance of Anopheline mosquitoes to pyrethroids has dramatically reduced their effectiveness and as a consequence their use in indoor residual spray (IRS) programs has declined dramatically. Currently there are only three other mode of action insecticides recommended by the World Health Organization that are currently in use as IRS adulticides: carbamates, organophosphates and organochlorines, all of which also have cases of resistance reported. A new mode of action IRS chemistry is therefore urgently needed to control resistant populations and also to help prolong the life of existing IRS chemistry by rotating applications over time between different classes. SumiShield™ 50 WG is an indoor residual spray product based on the neonicitinoid insecticide clothianidin that has previously not been used in vector control. Initial laboratory studies have shown excellent residual activity of this water dispersible granule (WG) formulation on typical indoor surfaces such as mud, wood and cement. Experimental hut and semi field studies have also been conducted that confirm these initial findings and have shown residual activity of over 6 months against both susceptible and resistant strains. These findings are discussed in detail. This clothianidin based IRS product is currently undergoing evaluation by the World Health Pesticide Evaluation Scheme (WHOPES) in both experimental huts and also in village scale trials. Introducing an alternative mode of action chemistry to the marketplace will allow use of IRS products to be rotated and thus help facilitate the implementation of the WHO Global Plan for Insecticide Resistance Management (GPRIM). When used alongside other interventions such as bed nets and other modes of action IRS products

SumiShield 50 WG will be a valuable tool to help control both susceptible and resistant mosquitoes and bring us closer to the goal of eliminating and ultimately eradicating malaria.

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SHAZAM FOR MOSQUITOES : CROWDSOURCING VECTOR SURVEILLANCE BY USING MOBILE PHONES AS ACOUSTIC SENSORS

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One of the most ubiquitous, powerful and easily accessible data collection tools that we possess today is the mobile phone. Here we show that mobile phones can be harnessed to identify insects around us through acoustic measurements. We demonstrate that a variety of mobile phones, from recent smart phones to the feature-phone models of the 1990s, can be used to record sufficiently sensitive signals for the acoustic identification of individual insects. Through signal processing analyses, we create an "acoustic fingerprint" for disease vectors such as mosquitoes, which can be used to infer its species and physiological characteristics like sex and blood-feeding status. We outline a citizen science initiative to create an open database of acoustic signatures, with insect recordings from all over the world being processed for crowd-sourced, real time surveillance of vector ecology. This technique makes it possible to collect vector surveillance data at extremely high spatio-temporal resolutions, which can help plan disease control strategies, monitor invasive species and gauge infection risks for vector-borne diseases.

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AUTOCIDAL MOSQUITO CONTROL: ALLOWING MOSQUITOES TO HELP US WITH OUR WORK

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The ongoing problem of mosquito borne disease provides an impetus to develop additional methods for the control of invasive mosquito species and against the globalization of mosquito-vectored pathogens. In addition to the development of new active ingredients, there is need also to develop additional methods for delivering pesticides. Autocidal methods rely on the use of mosquitoes to 'self-deliver' pesticides and may provide a useful compliment to traditional application methods. Here, the results of recent field trials will be presented. The trials are based on the release of male mosquitoes that have been either 1) infected with a naturally-occurring bacterium "Wolbachia" to cause sterility in a targeted population or 2) dusted with pyriproxyfen, which is a powerful inhibitor of immature mosquito development. The Wolbachia method is non-GMO and categorized by the EPA as a microbial biopesticide. The Wolbachia method is species specific and has been developed for multiple mosquito species, including *Aedes albopictus*, *Ae. aegypti* and *Anopheles stephensi*. The pyriproxyfen dusting approach can be used alone, or combined with classical Sterile Insect Technique, Wolbachia and GMO approaches, to increase the overall impact of the introduced male mosquitoes. The different approaches will be discussed and contrasted, and their relevance to different mosquito control contexts, including areal delivery, will be discussed. The results of recent field trials will be summarized.

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TRANSGENIC INSECT KILLING FUNGUS BETTER KILLS INSECTICIDE-RESISTANT, MALARIA-VECTOR MOSQUITOES

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The continued success of malaria control efforts requires the development, study and implementation of new technologies. Here we present a significant advancement in the development of insect killing fungi as a means of mosquito biocontrol. We have combined the natural abilities of *Metarhizium* spp. fungi and the field of arthropod-derived toxins to engineer a highly specific and potent pathogen of mosquitoes. Our studies show significant improvements in the rate of mosquito mortality due to the transgene in both insecticide-susceptible and wild-caught, insecticide-resistant populations. We further discovered the enhanced ability of the transgenic fungus to decrease a critical aspect of mosquito behavior for the spread of malaria (blood feeding behavior). In only 5 days, the transgenic fungus not only decreases disease transmission in mosquitoes faster than the wild-type fungus, but it decreases blood feeding to a greater degree. This research characterizes our transgenic entomopathogen as an effective and rapid mosquito control technology, which can be readily applied to mitigate risks involved with existing insecticide-based control methods.

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COMPARING THE EFFICACY OF INSECTICIDE MIXTURE AND COMBINATION STRATEGIES FOR IMPROVED CONTROL AND MANAGEMENT OF PYRETHROID RESISTANT MALARIA VECTORS IN SOUTHERN BENIN, WEST AFRICA

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The Global Plan for Insecticide Resistance Management (GPIRM) recommends the use of a combination/mixture of unrelated insecticides against insecticide resistant malaria vectors for improving control and managing resistance. This concept can be applied by using insecticide mixtures on mosquito bed nets, co-formulated insecticide mixtures for indoor residual spraying (IRS) or deploying non-pyrethroid IRS together with pyrethroid bed nets. The uptake of these strategies at national levels has been slow due to lack of effective new mixture/combination tools and insufficient evidence on efficacy to guide decision making. Mixtures on bed nets or IRS being single interventions could be cost effective and more desirable than the combined intervention approach if comparable levels of impact can be demonstrated in terms of their ability to improve vector control and manage resistance. The current study compared the efficacy of a newly developed chlorfenapyr (a pyrolle) and alphacypermethrin (a pyrethroid) mixture long-lasting bed net (Interceptor G2) to an IRS mixture of chlorfenapyr and alphacypermethrin and a combined chlorfenapyr IRS and alphacyperthrin long-lasting net (Interceptor 1) intervention in experimental huts against wild pyrethroid resistant malaria vectors in Cove, Benin. Mortality rates were very low with alphacypermethrin IRS alone (5%) and Interceptor 1 alone (24%) owing to high levels of pyrethroid resistance in the vector population. Mortality with the mixture IRS (42%) was unexpectedly lower than IRS with chlorfenapyr alone (51%) ($P<0.005$). The mixture net (Interceptor G2) and the combined IRS and bed net approach provided the highest mortality rates and these were similar between both treatments (75% vs. 69% respectively, $P>0.05$). Blood feeding rates were also significantly reduced with these treatments compared to the control and the mixture IRS ($P>0.05$). The results demonstrate that Interceptor G2 could be a more cost-effective and

reliable strategy for improving the control of pyrethroid resistant malaria vectors and managing resistance compared to the combined intervention approach and the mixture IRS.

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LYMPHATIC FILARIASIS ELIMINATION IN AMERICAN SAMOA: EVALUATION OF MOLECULAR XENOMONITORING AS A SURVEILLANCE TOOL IN THE ENDGAME

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has made significant progress toward interrupting transmission of lymphatic filariasis (LF) through mass drug administration (MDA). Operational challenges in defining endpoints of elimination programs have been identified, including the need to determine appropriate post-MDA surveillance strategies. As humans are the only reservoirs of LF parasites, one such strategy is molecular xenomonitoring (MX), the detection of filarial DNA in mosquitoes using molecular methods (PCR), to provide an indirect indicator of infected persons nearby. MX could potentially be used to evaluate program success, provide support for decisions to stop MDA, and conduct post-MDA surveillance. American Samoa has successfully completed MDA and passed WHO recommended Transmission Assessment Surveys in 2011 and 2015, but recent studies using spatial analysis of antigen and antibody prevalence in adults (aged ≥18 years) and entomological surveys showed evidence of possible ongoing transmission. This study evaluated MX as a surveillance tool in American Samoa by linking village-level results of the recent human and mosquito studies. Of 32 villages, seropositive persons for Og4C3 antigen were identified in 11 (34.4%), Wb123 antibody in 18 (56.3%) and Bm14 antibody in 27 (84.4%) of villages. Village-level seroprevalence ranged from 0%-33%, 0%-67% and 0%-100% for Og4C3, Wb123 and Bm14 respectively. PCR-positive *Aedes polynesiensis* mosquitoes were found in 15 (47%) villages, and their presence was associated with a significantly higher probability of seropositive persons for Og4C3 (67% vs 6%) and Wb123 (87% vs 29%), but not Bm14. In villages with seropositive persons for Og4C3 and Wb123, PCR-positive *A. polynesiensis* were found in 91% and 72% respectively. In villages without Og4C3-positive persons, PCR-positive *A. polynesiensis* were absent in 94%. Our study provides promising evidence to support the potential usefulness of MX in post-MDA surveillance in an *Aedes* transmission area in the Pacific Island setting, and to predict sub-national areas where LF transmission may be continuing.

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FILARIASES IN GABON: EMPIRIC ASSESSMENTS REDEFINE DISTRIBUTION AND TREATMENT STRATEGIES FOR ONCHOCERCIASIS AND LOIASIS

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Previous surveys for Rapid Epidemiological Mapping of Onchocerciasis (REMO) in Gabon predicted very low prevalence of onchocerciasis (0 – 5%) across the country, whereas Rapid Assessment for Loiasis (RAPLOA) predicted more than 40% prevalence in the entire country. Ivermectin was never given in Gabon due to the low onchocerciasis prevalence and the considerable risk of serious adverse events (SAE) to ivermectin in highly-prevalent *Loa loa* endemic areas where individuals with *Loa loa* infections with intensities of >30,000 mf/ml may reside. Due to the new global target to eliminate onchocerciasis, there is an urgent need to determine which treatment intervention is required to eliminate the disease from Gabon. Therefore, in 2014-2015, 7,108 individuals in 93 communities in 34 districts were tested for onchocerciasis by skin snip and/or Ov16, and 10,214 individuals in 176 communities in 43 districts were tested by blood smear to detect *L. loa* microfilaremia. Prevalence of onchocerciasis was found to be much higher in some communities than predicted by REMO; 9% of districts were hyper-endemic (at least one community > 60% infected) and 12% of districts were meso-endemic (at least one community 40 - 60% infected). In contrast, *L. loa* prevalence was lower than predicted by RAPLOA; 82% of individuals tested were negative, and in one district the maximum prevalence found in any community was 0% despite a RAPLOA prediction of >40%. Of 93 communities tested for onchocerciasis, 67 communities (72%) were endemic (any positive results from skin snip or Ov16). All 67 communities had some *L. loa* prevalence (ranging from 2% to 51%), but, importantly, 31 communities (46%) had no individuals with high intensity (30,000 mf/mL+) infection. Our results have shown that it is possible to find areas with very little *L. loa* infection and high onchocerciasis burden where treatment with ivermectin could be appropriate. They also emphasize the importance of empiric assessments to refine our understanding of the distribution of these two diseases in Central Africa and guide treatment interventions to achieve onchocerciasis elimination.

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HIGH PREVALENCE OF EPILEPSY IN ONCHOCERCIASIS ENDEMIC REGIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC)

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Between 2014 and 2016, we conducted epilepsy prevalence surveys in 61 villages in onchocerciasis endemic areas in the DRC. A case control study was performed in Titule (Bas-Uélé), Salambongo (Tshopo) and Draju (Ituri). Cases were patients with active convulsive epilepsy and controls were age matched persons without epilepsy randomly selected from the same village. A high prevalence of epilepsy was observed in villages located closely to Simulidae (blackfly) infested rivers: 2.3-6.4% in Bas-Uélé, 1.5-6.0% in Tshopo, and 3.0-5.6% in Ituri. Epilepsy cases showed a marked spatial pattern with clustering of cases occurring within and between adjacent households. Individual risk for epilepsy was found to be associated with living close to the river. Peak onset of epilepsy was around the age of 14-15. Nodding syndrome was not observed but adolescents with epilepsy and with severe stunting and without secondary external signs of sexual development were observed in several villages. Phenobarbital was the anti-epileptic drug most frequently used but rarely continuously. In villages where Ivermectin was distributed for at least 10 years, no difference in the presence of *Onchocerca volvulus* (OV) DNA in the skin was observed between cases and controls. On the other hand in Draju, where Ivermectin was never distributed, OV microfilaria were observed in skin snips of 55.9% (33/59) of epilepsy cases compared to 29% (20/69) of controls ($p = 0.002$); mean density of microfilaria (Mf) in skin snips of cases was 33.6 parasites/mg skin compared to 3.8 parasites/mg skin in controls ($p = 0.002$); and 45.8 % (27/59) of cases had OV16 antibodies compared to 26.1% (18/69) of controls ($p = 0.002$). A quantitative real-time PCR assay showed that the amount of Wolbachia ftsZ gene (bacterial OV endosymbiont) in skin snips was significantly higher in cases than in controls ($p < 0.01$). In conclusion, the prevalence of

epilepsy in villages in onchocerciasis endemic areas in the DRC was 2-10 times higher than in non-onchocerciasis endemic regions in Africa. Our study confirms that OV infestation is associated with epilepsy.

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SIMULATING THE EFFECT OF EVALUATION UNIT SIZE IN DETERMINING ELIGIBILITY TO STOP MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN HAITI

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The Transmission Assessment Survey (TAS) was designed as a decision-making tool for program managers to determine when transmission of lymphatic filariasis (LF) is presumed to have reached a level low enough that it cannot be sustained even in the absence of mass drug administration (MDA). The geographic area over which a TAS is applied is referred to as an evaluation unit (EU). EUs may comprise one or multiple program implementation units (IUs) and should have no more than 2 million people. In 2015, TAS was conducted in 14 EUs in Haiti, many comprising a single IU. Of these 14 TAS, two failed and one had a borderline result (i.e., the number of positive results was equal to the critical threshold). Simulations were used to understand what the programmatic conclusions would have been had Haiti chosen to form larger EUs. Eight "combination-EUs" were formed through various groupings of existing adjacent EUs. Several simulation approaches to replicate TAS were carried out in these combination-EUs, using bootstrapping to simulate the expected data. Each approach was replicated 1000 times, with the number of "passing" and "failing" TAS results recorded. The simulations showed that when the combination-EU was comprised of discordant EUs - at least one "passing" and one "failing" - the combination-EU would pass the TAS 71% - 100% of the time, with exception of one combination-EU, where the TAS failure rate of the combination-EU was never more than 83%. Combining IUs to form large EUs is common practice, as it can result in considerable cost savings and detailed prevalence data to better inform such decisions is often lacking. Our results demonstrate the high potential for misclassification when the prevalence of LF in the combined IUs differs. Of particular concern is the risk of "passing" larger EUs that include focal areas where prevalence is high enough to be potentially self-sustaining. Our results reaffirm the approach that Haiti took in forming smaller EUs, based on historical knowledge, and suggests that in areas where such information is lacking greater detail, perhaps gathered through additional spot check sites, may be useful to inform EU creation.

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PREVALENCE OF OV16 ANTIBODIES AMONG SCHOOL-AGE CHILDREN AFTER TWENTY YEARS OF MASS TREATMENT WITH IVERMECTIN IN TOGO

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The Onchocerciasis Control Program of Togo (OCP) is in the process of shifting its focus from control to elimination of onchocerciasis. Thirty-two of Togo's 40 districts are endemic for onchocerciasis and have been receiving annual treatment with ivermectin in villages with population ≤2000 for more than 20 years, and the 15 northern most districts receive two rounds of treatment per year. Yearly epidemiological surveillance focuses on approximately 300 villages where onchocerciasis remains prevalent according to skin snip surveys. In preparation for the move to elimination, Togo's Ministry of Health conducted a survey to obtain preliminary data on the distribution of antibodies to the Ov16 protein of *Onchocerca volvulus* in school-age children outside of the areas of ongoing surveillance. The survey was integrated with an impact assessment for other neglected tropical diseases (NTD). In 2015, in each of 1126 schools serving as NTD sentinel sites, a convenience sample of 8 school-going children aged 6 to 9 years had finger-stick blood drawn for the Ov16 rapid test. Altogether, 9007 children were tested by Ov16 rapid test and 60 (0.7%) children tested positive. A map of the locations of the 60 children with positive results shows that most were from areas where onchocerciasis is believed to be low, and one child was from a district that was categorized as non-endemic according to baseline mapping and has never received mass treatment with ivermectin. The OCP is conducting follow-up visits for all 60 children to document residency and travel history, to repeat the Ov16 rapid test, and to conduct skin snip testing with treatment if indicated. While there are challenges and limitations associated with use of the Ov16 rapid test for onchocerciasis, this survey provides important information for Togo's OCP in moving toward onchocerciasis elimination in Togo.

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TOWARDS ELIMINATION OF LYMPHATIC FILARIASIS IN MALI BY 2020

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In Mali all 63 health districts (HD) were endemic for lymphatic filariasis (LF) and an initial mapping with ICT cards in 2004 revealed a national prevalence of 7.1% (from 1% in the north to 18.6% in the south). The country committed to the goal of eliminating LF as a public health problem by 2020. The implementation of mass drug administration (MDA) with albendazole and ivermectin began in areas co endemic with onchocerciasis in southern Mali in 2005, and scaled up to other endemic areas in northern Mali, reaching 100% geographical coverage in 2009. Since 2008, Mali has benefited from the support from Helen Keller International with funding from the USAID's NTD Control Program and ENVISION Project, managed by RTI International. According to World Health Organization guidelines, LF transmission assessment surveys (TAS) were conducted in districts which had had at least 5 effective rounds of MDA. The Survey Sample Builder (SSB) was used to determine sample sizes and the selection of clusters. Evaluation units (EU) were approved by the RPRG before the implementation of the surveys. By 2016, transmission assessment surveys have been conducted in 31 health districts (11 EUs) across Mali. The 31 districts surveyed included all 10 districts in the Koulikoro region, all 6 communes in the district of Bamako, 9 out of 10 districts in Sikasso region, and 6 out of 8 districts in Segou region. A community based cluster sampling (in 9 EUs) and school-based cluster sampling (2 EUs) strategy was used depending on school enrollment rates. All 11 EU surveyed passed the TAS with the number of positive cases being below the critical cut-off values determined by the SSB (18 or 20 positive cases). Currently, 49% (31/63) of the originally LF endemic districts have reached the criteria to stop MDA and evaluations are in preparation for the 13 other districts in the center and south Mali and should be completed by June 2016. Insecurity in the north has caused some MDA rounds

to be cancelled and made it impossible to carry out evaluations. If the security situation improves, Mali will be on course to achieve national LF elimination objectives by 2020.

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EMPLOYING THE NEW OV16 RAPID DIAGNOSTIC TEST (RDT) TO EVALUATE ONCHOCERCIASIS IN AFRICA

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Elimination of onchocerciasis in Africa is targeted to be achieved by 2025. Over the next decade, thousands of people will need to be tested to identify where treatment interventions are needed, when transmission interruption has been achieved, and for surveillance to verify that elimination has been attained. Nodule palpation and skin-snip assessment of microfiladermia have been the traditional diagnostic tools, but a principal limitation of both is their lack of sensitivity in low prevalence settings. Because the Ov16 ELISA test has already been proven a useful tool in the Americas and a few settings in Africa to assess the interruption of transmission of onchocerciasis, World Health Organization (WHO) guidelines now recommend Ov16 serology as a diagnostic tool for onchocerciasis elimination programs. A new, point-of-care rapid diagnostic test (RDT) which detects antibodies to the parasite antigen Ov16 has recently been developed by PATH and its partners. While benefits of the RDT include requiring no cold chain, its ease of use, and being relatively low-cost, this test still requires evaluation at scale before it can be recommended for routine programmatic use. Therefore, in a multi-country study to compare skin snip tests with the new Ov16 RDT, a total of 31,633 people were tested in 3 post-treatment settings (in Mali, Malawi, Guinea Bissau) and in 3 settings where treatment for onchocerciasis has never been given (in Gabon, Nigeria, the Democratic Republic of the Congo). In the post-treatment settings, the prevalence of onchocerciasis as diagnosed by a positive Ov16 RDT was 3.18% (95% CI: 2.74-3.62) but 0.00% when diagnosed by skin snip. In the treatment-naïve settings, the prevalence via Ov16 RDT was 3.30% (95% CI: 3.11-3.55) and by skin snip was 1.00% (95% CI: 0.90-1.10). The Ov16 RDT was found to have a higher sensitivity compared with the skin snip diagnostic for all age groups in both the pre- and post-treatment settings. It is likely that as similar experience accumulates in other studies, the Ov16 RDT will become the standard diagnostic tool in the future for monitoring all programs aiming to achieve elimination of onchocerciasis in Africa.

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A SINGLE DOSE OF TV005 ELICITS COMPLETE PROTECTION AGAINST CHALLENGE WITH THE HETEROTYPIC DENV-2, RDEN2Δ30

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Over the past several decades dengue has become hyper-endemic in all tropical and sub-tropical regions of the world. Current estimates report nearly 100 million symptomatic and 300 million asymptomatic dengue infections annually. Dengue can range from an asymptomatic or mildly symptomatic illness to one that results in bleeding diatheses, plasma leakage, and vascular collapse. The recently licensed dengue vaccine Dengvaxia™, was found to be significantly less efficacious in persons who were sero-naive to dengue at the time of vaccination and regulatory authorities, including the WHO SAGE committee, have recommended that it be administered only to children 9 years or older living in highly dengue endemic regions. Previously, we demonstrated that the live attenuated tetravalent dengue vaccine (LATV) TV003 elicited complete protection against DENV-2 challenge using our controlled dengue human infection model (DHIM). As a first assessment of the protective efficacy of our second LATV dengue vaccine candidate TV005 in sero-naïve subjects, we conducted a randomized, placebo-controlled, double-blind trial utilizing this same DHIM. Forty-eight flavivirus-naïve subjects were enrolled. On Study Day 0, 24 subjects received TV005 and 24 subjects received a placebo. Six months later, 43 subjects returned and received 1,000 PFU of the DEN2Δ30 challenge virus (22 TV005 recipients and 21 controls). The DEN2 challenge virus is a different genotype than that included in the vaccine. All 21 controls had detectable viremia following challenge and all developed rash. None of the TV005 recipients had detectable DENV-2 in the blood following challenge and none developed rash. The LATV TV005 induced complete protection against the both viremia rash. In addition, 70% of TV005 recipients demonstrated sterilizing immunity to challenge as evidenced by lack of detectable virus in the blood and a < 4-fold rise in serum neutralizing antibody to DEN2. These data demonstrate that TV005 induces strong protection in subjects who are flavivirus-naïve at the time of vaccination.

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HUMAN CD4+ T CELL RESPONSES INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE PARALLEL THOSE INDUCED BY NATURAL INFECTION, IN MAGNITUDE, HLA RESTRICTION AND FINE ANTIGEN SPECIFICITY

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Four dengue virus (DENV) serotypes are responsible for a growing number of infections, making DENV the most frequent mosquito-transmitted viral disease in humans, and creating a unique challenge for vaccine development. While considerable debate exists regarding which immune mechanisms may confer protection, a hallmark of live attenuated vaccines (LAV) is their ability to induce both humoral and cellular immune memory. We previously demonstrated that DENV-specific CD8+ T cell responses elicited by live attenuated DENV vaccines resemble those elicited by natural infection. CD4+ T cells are also a key component of cellular immunity, and contribute to host protection directly through cytokine production, and indirectly by providing help for CD8+ and antibody responses. Here, we characterize for the first time CD4+ T cell responses after live attenuated dengue vaccination and compare them to responses observed in natural infection with dengue virus. PBMCs from study participants receiving the tetravalent live attenuated DENV vaccine (TV-003), developed by the U.S. National Institutes of Health were screened in IFNγ ELISPOT assays with pools of HLA matched, predicted class II binding peptides covering the entire DENV proteome. CD4+ T cell responses were detected with magnitude and breadth similar to natural dengue infection. In natural

infection and vaccines alike, DENV specific CD4+ T cells are focused dominantly focused on the capsid, NS3 and NS5 antigens, while the envelope protein is a minor target for CD4+ DENV specific T cells.

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A PHASE I CLINICAL TRIAL EVALUATING THE IMPACT OF TETRAVALENT RECOMBINANT SUBUNIT DENGUE VACCINE BOOST ADMINISTERED TO SUBJECTS WHO HAVE PREVIOUSLY BEEN VACCINATED WITH A LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE

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With the increasing global burden of dengue, there remains an urgent need for dengue vaccines. The V180 vaccine candidate consists of four truncated, soluble, dengue envelope glycoproteins (DEN-80E). The vaccine has been shown to induce robust virus-neutralizing antibody responses when formulated with ISCOMATRIX™ adjuvant and administered to flavivirus-naïve volunteers in a Phase I clinical trial (NCT01477580). V180 was also tested in a Phase I clinical trial (NCT02450838) where subjects who had previously received the live-attenuated tetravalent vaccine (LATV) developed by the National Institute of Allergy and Infectious Diseases (NIAID) were administered a V180 booster dose. The study was designed to assess whether a recombinant subunit vaccine is able to boost the trivalent or tetravalent responses induced by the LATV vaccine, which have proven difficult to boost with an additional dose of the LATV itself. The study was a randomized, placebo-controlled, double-blind study of safety and immunogenicity of the V180 vaccine. Twenty subjects who had previously received one or two doses of LATV were randomized and received a single dose of V180 non-adjuvanted (N=8), V180 adjuvanted with Alhydrogel™ (N=8), or placebo (N=4). Vaccine safety (solicited and unsolicited adverse events) was assessed using a Vaccination Report Card for 28 days following vaccination. Serious adverse events were captured from the time of informed consent through the final study visit at 6 months postvaccination. Immunogenicity was assessed using a plaque reduction neutralization test at Day 0, Day 14, Day 28, and Month 6 relative to vaccination. The results of the study demonstrate that the vaccine is generally well tolerated and immunogenic in these dengue-experienced volunteers.

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TETRAVALENT DENGUE HETEROLOGOUS PRIME-BOOST VACCINATION - SAFETY, HUMORAL, AND CELL-MEDIATED IMMUNITY AT 1 AND 6 MONTHS

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Heterologous prime boost approaches have been explored as vaccination strategies in a number of infectious diseases. We studied whether sequential administration of live and inactivated tetravalent dengue vaccine candidates could improve safety and immunogenicity performance metrics. Tetravalent dengue virus live-attenuated vaccine (LAV) and tetravalent dengue virus purified inactivated vaccine (PIV) have been evaluated previously using homologous two-dose strategies. This phase 1, randomized, open-label, primary vaccination study conducted in non-dengue endemic region (Maryland, USA). Eighty subjects were enrolled into 4 heterologous prime-boost vaccination treatment groups (N=20): Group 1 = LAV (day 0), PIV (1 month); Group 2 = PIV (day 0), LAV (1 month); Group 3 = LAV (day 0), PIV (6 month); Group 4 = PIV (day 0), LAV (6 month). Subjects were followed for 6 months after vaccination. Safety (primary end point), microneutralization (MN50) antibody titers, RNAemia, and cell-medicated immunity (CMI) were assessed. At one month after the second vaccination, there were no related severe adverse events (SAEs), no related medically attended adverse events (AEs), and no grade 3 related local AEs. Grade 3 related systemic AEs occurred in small numbers (N=11), with the majority in Group 4. LAV associated rash was observed in 8 subjects. Post LAV dengue RNAemia was detected in 18%, 26%, 20%, and 61% of the subjects in groups 1, 2, 3, and 4 respectively. At one month, PIV priming followed by LAV boost generated superior MN50 antibody titers and broader seroconversion rates with no clear difference between Groups 2 and 4. One month CMI was assessed by an IFN-γ ELISpot assay and overlapping peptide pools from all four serotypes. PIV priming appeared to result in the highest magnitude of response and the most balanced median IFN-γ spot counts. These results suggest a PIV prime, LAV boost strategy against dengue has further developmental potential despite manufacturing challenges. All follow up study visits were completed in March 2016. Safety, MN50, RNAemia, and CMI at 6 months post boost dose will also be presented.

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TAKEDA'S TETRAVALENT DENGUE VACCINE (TDV) CANDIDATE PROGRESSES TO PHASE III: SAFETY AND IMMUNOGENICITY OF TDV

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Takeda's live attenuated tetravalent dengue vaccine candidate (TDV) contains a molecularly characterized dengue serotype 2 virus (TDV-2) and three recombinant viruses expressing the pre-membrane (prM) and envelope (E) structural genes for serotypes 1, 3, and 4 in the attenuated TDV-2 backbone. On the path to phase III, Takeda has investigated different formulations, routes of administration, dosage schedules and vaccine presentations, through four phase I and four phase II studies involving more than 3800 participants (adults and children in endemic and non-endemic countries). The TDV clinical program has followed the WHO guideline for dengue vaccine development. The safety and immunogenicity profile of TDV supports continued clinical development. Key data from the development program will be presented and their implications for decisions affecting the schedule and formulation will be discussed.

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CONTRIBUTIONS OF SILENT INFECTIONS TO DENGUE VIRUS TRANSMISSION

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A considerable fraction of dengue virus (DENV) infections is thought to result in either no detectable symptoms (asymptomatic) or symptoms that are sufficiently mild that they go undetected by surveillance systems (clinically inapparent). The unknown contribution of undetected infections to the transmission of DENV and other emerging mosquito-borne viruses, like Zika virus, raises questions about the effectiveness of reactive practices for detecting and responding to outbreaks. Despite estimates that 293 million people experience either asymptomatic or clinically inapparent infection each year, it has been assumed that these individuals contribute little to onward transmission. Recently, however, blood-feeding experiments with *Aedes aegypti* showed that people with asymptomatic DENV infections are capable of infecting mosquitoes. We combined those findings with models of within-host DENV dynamics and human demographic projections to: (1) quantify the net infectiousness of individuals that experience either asymptomatic or symptomatic (either clinically inapparent or clinically apparent) infections, and (2) quantify the contributions of asymptomatic and symptomatic infections to DENV force of infection, which depends not just on their infectiousness but also on their numerical prominence in a population. Our calculations indicate that individuals with asymptomatic infections have a lower net infectiousness than symptomatic infections, yet they are still capable of making appreciable contributions to DENV transmission. We estimate that approximately two-thirds of infections could result from individuals with undetected infections. Among infections that result from clinically apparent infected individuals, more than half could result from mosquitoes biting during the pre-symptomatic phase of the infection. Our findings emphasize the need to reorient current practices for responding to outbreaks of dengue and Zika viruses, to pre-emptive interventions that take account of the role of undetected infections in DENV transmission dynamics.

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PREDICTORS FOR SEVERE DENGUE: RESULTS FROM A PROSPECTIVE MULTI-CENTRE STUDY IN EIGHT COUNTRIES ACROSS ASIA AND LATIN AMERICA

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The clinical spectrum of symptomatic dengue is broad, ranging from mild febrile illness to severe disease with potentially life-threatening complications such as bleeding, organ impairment, and plasma leakage that may result in hypovolemic shock. Without an effective therapy patient outcomes depend primarily on appropriate triage and judicious use of intravenous fluids. However although the revised 2009 WHO guidelines indicate a number of warning signs to identify potentially severe cases early, the evidence base to support these recommendations is presently limited. A prospective multi-centre observational study recruiting out-patients aged ≥ 5 yrs with symptoms consistent with possible dengue within 72 hrs of fever onset is in progress in 8 countries across Asia and Latin America, aiming to enrol around 3000 participants with confirmed dengue by June 2016. A broad range of clinical and laboratory features are assessed daily during the acute illness, and at follow-up 1 week later. We aim to identify clinical and/or simple laboratory predictors of severe dengue, in particular to explore the value of repeated measurements during the febrile phase. Severe outcomes are defined according to the need for hospitalisation or intravenous fluid administration, as well as by the WHO 2009 guidelines. After assessing heterogeneity of outcomes across countries, we will investigate the predictive value of a range of candidate predictors in classical prognostic models depending on baseline covariates only, and also explore dynamic prognostic models which take into account longitudinal data. Despite heterogeneity in clinical management between sites, preliminary findings in 2254 patients with laboratory-confirmed dengue from 5 countries suggest certain parameters, eg haematological indices such as the platelet count, to be of interest. A detailed description of the disease spectrum and risk factors identified among ~3000 dengue cases will be presented, representing the most comprehensive dataset available to date. We anticipate these results will have substantial impact on future triage and management policies in dengue endemic countries.

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ACT PARTNER DRUG EROSION—EVIDENCE OF PPQ RESISTANT PARASITES FROM CAMBODIA

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With the emergence and spread of artemisinin (ART) resistance, more pressure is put on the partner drugs (e.g., piperaquine) used in ART combination therapies (ACTs). Recent data from Western Cambodia indicate that ART and piperaquine (PPQ) resistance has emerged in this region, leading to declining frontline ACT efficacy. To assess whether ART-resistant parasites also show resistance to PPQ, we obtained 157 cryopreserved isolates from Pursat and Pailin, Western Cambodia that were collected by the Tracking Resistance to Artemisinin Collaboration (TRAC). We culture-adapted 68 parasites and focused on parasites with slow clearance rates in patients and high % survival values in the ring-stage survival assay (RSA) *in vitro* – two ART-resistance phenotypes. We first exposed parasites to a single high PPQ dose (2 μM) for 72 hours and found that some parasites were able to recover. We then profiled 23 isolates by standard EC50 assays. A PPQ-resistant subset of these isolates showed a bimodal response to PPQ with increased survival under higher drug pressure, while their primary EC50 values were comparable to sensitive isolates (5 nM). This bimodal response was not due to the presence of mixed parasite populations within the isolate, as daughter clones retained the phenotype. Exposure of parasites to PPQ at consecutive 12-hour intervals throughout the lifecycle showed that later stages (schizonts) are less susceptible than earlier stage parasites. Use of available whole-genome sequencing data and independent interrogation of these PPQ-resistant isolates did not identify any of the previously-reported markers of PPQ resistance. These results confirm the existence of PPQ-resistant parasites in Cambodia. Ongoing studies aim to identify the underlying mechanism of PPQ resistance.

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ARTESUNATE-MEFLOQUINE EFFECTIVELY TREATS DIHYDROARTEMISININ-PIPERAQUINE-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA IN CAMBODIA

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Artemisinin (ART) combination therapies (ACTs) are recommended worldwide for the treatment of *Plasmodium falciparum* malaria. Dihydroartemisinin-piperaquine (DHA-PPQ) was adopted as the frontline ACT in Cambodia in 2008, and was safe, tolerable, and 96-98% efficacious. However, the rapid spread of ART resistance, defined as a parasite clearance half-life >5 h following treatment, exposes a larger biomass of parasites to the unprotected partner drug – due to the short (~1 h) elimination half-life of the ART derivative, and long elimination half-life of the partner drug. We postulated that parasites in areas with ART resistance would readily develop partner drug resistance, resulting in decreased ACT efficacy. Among patients treated with DHA-PPQ in western, northern, and eastern Cambodia (in Pursat, Preah Vihear, and Ratanakiri provinces) in 2012-2013, 46%, 16% and 2% of patients experienced recrudescent infections, respectively, over 63 days of follow-up. Recrudescent parasites had higher prevalence of *K13* mutations (molecular markers of ART resistance), higher PPQ 50% inhibitory concentrations (IC50s), and higher prevalence of Exo-E415G (a molecular marker of PPQ resistance). They also showed lower mefloquine (MQ) IC50s and did not have >1 *pfmdr1* gene (a molecular marker of MQ resistance). We thus hypothesized that these parasites could be effectively treated with artesunate-MQ (AS-MQ) – the former frontline ACT in Cambodia. In 2014-2015, while DHA-PPQ was still the frontline ACT in Cambodia, we measured the efficacy of AS-MQ in 144, 60, and 92 patients in Pursat, Preah Vihear, and Ratanakiri provinces, respectively. In Pursat, Preah Vihear, and Ratanakiri the prevalence of parasites with *K13* mutations increased (77, 34, and 11% to 94, 93, and 18%) and similarly the prevalence of Exo-E415G increased (74, 20, and 3% to 76, 88, and 17%) from 2012-2013 to 2014-2015. Meanwhile, the prevalence of >1 *pfmdr1* gene decreased (8, 14, and 2% to 0, 0, and 0%). Only 1/296 patients presented with a recrudescent infection over 42 days of follow-up, indicating that AS-MQ effectively treats DHA-PPQ-resistant malaria.

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SIGNIFICANT DIFFERENT LEVELS OF ARTEMISININ MONOTHERAPY EFFICACY ON *PLASMODIUM FALCIPARUM* IN MALI

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Resistance to artemisinin derivatives is associated with delayed parasite clearance. In Mali, five years after ACTs were recommended as the first-line treatment for uncomplicated malaria, a prospective artesunate monotherapy efficacy study observed no delayed parasite clearance time. In the context of regular monitoring of artemisinin resistance we repeated the above studies in two sites of Mali. From October 2015 to March 2016, we conducted a prospective study to evaluate the efficacy of artesunate monotherapy in subjects aged 6 months and longer in Bougoula-Hameau and Faladje. Patients with uncomplicated malaria were treated with artesunate for 7 days and followed for 28 days. Parasitaemia was evaluated every 8 hours until three consecutive slides were negatives. MSP2, Ca1 and TA99 polymorphisms was assessed by PCR to distinguish new infections from recrudescent infections. PCT and parasite clearance half-life were calculated using the online WWARN parasite clearance estimator software (PCE).Results were compared with the studies conducted in Bougoula-Hameau in 2011.We included 100 patients in Bougoula-Hameau and 120 others in Faladje. Adequate Clinical and parasitological response (ACPR) was 92.0% and 79.2% in Bougoula-Hameau and Faladje, respectively. After molecular correction cACPRs was 100% at both sites. By 24 hours after treatment initiation, 28% of participants had cleared parasitemia in Bougoula-Hameau, compared with 2.5% in Faladje (P<0.0001). The median parasite clearance time was 32 hours in Bougoula-Hameau (similar to the Bougoula-Hameau 2011 results) but 40 hours in Faladje (P<0.001). The parasite clearance half-life was 2.0 hours (1.66 to 2.23) in Bougoula-Hameau and 2.8 hours (2.39 to 2.99) in Faladje ($p < 0.001$). Only two participants still had parasites at 48 hours in Faladje and no participant in Bougoula-Hameau. Artesunate monotherapy remains effective on *P. falciparum* in Mali but there are significant differences in the level of susceptibility of parasites from different settings of the country.

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CLINICAL EFFICACY OF ARTEMETHER-LUMEFANTRINE IN RELATION TO DRUG EXPOSURE IN CHILDREN WITH UNCOMPLICATED SEVERE ACUTE MALNUTRITION

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In the Sahel, malaria and malnutrition frequently overlap. Severe acute malnutrition (SAM) affects almost all organs and has been associated with a loss of intestinal villosities reducing intestinal absorption of medicines. However, very limited information is available on the pharmacokinetic properties of antimalarial drugs in children with SAM. We assessed artemether-lumefantrine (AL) clinical efficacy in children with SAM compared to those without SAM, with respect to lumefantrine concentration in their blood. Children under 5 with uncomplicated *P. falciparum* malaria were enrolled between November 2013 and January 2015 in Mali and Niger, one third with uncomplicated SAM and two thirds without SAM. The three-day twice daily AL treatment was directly observed and children were followed for 42 days, with PCR-corrected adequate clinical and parasitological response (ACPR) at day 28 as the primary outcome. Lumefantrine capillary blood concentrations were assessed in a subset of participants at different time points including systematic measurement on day 7. A total of 399 children were enrolled. Children with SAM were younger than their non-SAM counterparts (mean 17 versus 28 months, P<0.0001). PCR-corrected ACPR at day 28 was 100% (95% CI: 96.8%-100%) in SAM versus 98.8% (96.4%-99.7%) in non-SAM, P=0.236. In the age stratified analyses, SAM was associated with a greater risk of reinfection by day 28 in children older than the median of 21 months (hazard ratio=2.25 [1.12-4.48], P=0.022) and day 7 lumefantrine concentrations were significantly lower in SAM (median 251 versus 365 ng/ml in non-SAM, P=0.049). This study shows comparable clinical efficacy of standard doses of AL in children with and without SAM, but a higher risk of reinfection in older children suffering of SAM probably associated with poorer exposure to ACTs, as documented by a lower lumefantrine concentration on day 7. Sub-therapeutic concentration of a drug does not necessarily translate into lower efficacy but could contribute to selecting resistant parasites at population level. Further studies of dose optimization of AL in SAM children are urgently needed.

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ZINC-FINGER NUCLEASE-MEDIATED GENE EDITING ILLUSTRATES THE ROLE OF PFMDR1 N86Y IN MODULATING *PLASMODIUM FALCIPARUM* SUSCEPTIBILITY TO ARTEMISININ-BASED COMBINATION THERAPIES

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Malaria chemotherapy relies heavily on the use of artemisinin-based combination therapies (ACTs), which pair a fast-acting, short half-life artemisinin derivative with a longer half-life partner drug. The current arsenal of partner drugs include lumefantrine, mefloquine, amodiaquine and piperaquine. The recent emergence of resistance to artemisinin has placed increased pressure on these partner drugs, with piperaquine failures increasingly documented in in western Cambodia. Epidemiological studies have suggested that *Plasmodium falciparum* susceptibility to many of these agents can be modulated by mutations at positions 86 or 184 in the digestive vacuole-resident transporter protein PfMDR1. To characterize the role of these PfMDR1 mutations, we employed zinc-finger nuclease-mediated genome editing to modify pfmdr1 in genetic strains harboring Asian/African or South American alleles of the related transporter PfCRT. Our data show a significant role for PfMDR1 N86Y in increasing *P. falciparum* susceptibility to lumefantrine, mefloquine and dihydroartemisinin. This polymorphism also decreased parasite susceptibility to chloroquine and amodiaquine. We also observed a modest, strain-dependent reduction in susceptibility to piperaquine with the PfMDR1 N86/184F haplotype. We also mapped these PfMDR1 variants, as well as copy number changes, globally using 2,500 African and Southeast Asian genomes recently released by the MalariaGEN consortium. These data, combined with findings from our pfmdr1-modifed lines, help inform region-specific drug use policies by predicting changes in parasite susceptibility to current ACTs.

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UNSUPERVISED PRIMAQUINE FOR *PLASMODIUM VIVAX* RADICAL CURE LACKS EFFECTIVENESS IN SOUTHERN PAPUA

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Primaquine is the only licensed drug for eradicating *Plasmodium vivax* hypnozoites and therefore preventing relapses. It is efficacious when prescribed in clinical trials but its effectiveness in real-world settings is unknown. Using individualised hospital surveillance data from southern Papua, Indonesia, we conducted a retrospective cohort study to determine the effectiveness of unsupervised primaquine in combination with dihydroartemisinin-piperaquine for preventing representation to hospital with vivax malaria. Between April 2004 and December 2013 there were 62,492 episodes of vivax malaria available for analysis. The risk of representation with vivax malaria within one year was 34·4% (95% Confidence Interval (95% CI) 33·8-35·0%) after initial vivax monoinfection. Prescription of any dose of primaquine was associated with an Adjusted Hazard Ratio (AHR) for representation with vivax malaria of 0·89 (95% CI 0·85-0·93, p<0.001). Limiting the comparison to high dose (≥5mg/kg total dose) versus no primaquine in the period during and 6 months either side of a large, unplanned primaquine stock outage attenuated substantially this difference (AHR 0·95, 95% CI 0·88-1·02, p=0·15). Unsupervised primaquine for *P. vivax* malaria, prescribed according to international guidelines, had minimal impact on the risk of clinical recurrence within one year. New strategies for effective radical cure are needed urgently.

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ESTIMATING THE RISK OF *PLASMODIUM VIVAX* RELAPSES IN AFGHANISTAN

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Plasmodium vivax is responsible for more than 90% of laboratory-confirmed malaria cases in Afghanistan and continues to cause considerable morbidity in several areas of the country. Control of vivax malaria requires radical cure with primaquine to prevent relapse from

hypnozoite forms, although the proportion of cases with liver forms has not been determined. In Nangarhar and Kunar provinces, Afghanistan, between 2009 and 2014, we undertook an open, randomised controlled trial in patients with vivax malaria, comparing chloroquine plus primaquine (0.25mg/kg/day for 14 days) with chloroquine treatment alone. In patients randomised to the primaquine arm, G6PD deficiency was excluded by the fluorescent spot test prior to treatment. Patients were followed-up for one year; at recurrence, treatment was as at baseline. Preliminary analyses indicate that 588 patients were enrolled (295 chloroquine, 293 chloroquine plus primaquine) with 84.9% completing follow-up or having a *P. vivax* recurrence by 12 months. During the first 6 months, 45 / 263 (17.1%) patients in the chloroquine group had *P. vivax* recurrence, while only 7 / 262 (2.7%) had recurrence in tbe chloroquine plus primaquine group (p<0.0001). In the 6-12 month period, 40 / 205 (19.5%) patients in the chloroquine group had *P. vivax* recurrence, while 30 / 242 (14.0%) had recurrence in the chloroquine plus primaquine group (p = 0.127). This large randomised study shows clearly that there is a signicant store of hypnozoites in patients presenting with *P. vivax* in Afghanistan, and that unsupervised primaquine reduces relapse in the first 6 months by at least 5-fold. G6PD testing will be needed for primaquine deployment at scale. Further analyses will be presented in the meeting.

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IMPACT OF NEW COMBINATION LLINS ON ENTOMOLOGICAL MEASURES OF MALARIA TRANSMISSION IN MALI

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Long-lasting insecticidal nets (LLIN) are a key component to malaria control in sub-Saharan Africa, but widespread pyrethroid resistance may threaten their effectiveness. Combination LLIN containing pyrethroid insecticides plus piperonyl butoxide (PBO) are a possible mitigation strategy, but field evaluations are needed to determine if performance against resistant vectors is superior to standard LLIN. In this study, 16 Malian villages in areas where pyrethroid resistance had previously been documented were randomized to receive one of four types of PBO-containing or traditional LLIN (permethrin+PBO, deltamethrin+PBO, permethrin only, or deltamethrin only). Indoor resting mosquitoes were collected in all villages bimonthly over two years (2014-2015) using pyrethrum spray catch and Prokopack aspirator. The primary outcome measure was the sporozoite rate (proportion of An. gambiae s.l. mosquitoes positive for *P. falciparum* sporozoites); a secondary outcome was indoor resting vector density. Bottle bioassays containing permethrin or delamethrin plus PBO resulted in significantly greater mortality than assays containing only permethrin or deltamethrin ; however, none of the PBO-containing assays restored full insecticide susceptibility (mortality >97%). During high malaria transmission seasons (June, August, October) in both years, sporozoite rates were not significantly different between the permethrin+PBO and permethrin only net arms: 5.1% (95% CI: 4.0, 6.2) versus 5.5% (95% CI: 4.2, 6.7) (p=0.67). Sporozoite rates were significantly lower in the deltamethrin+PBO arm compared to the deltamethrin only arm: 1.9% (95% CI: 1.2, 2.7) versus 3.7% (95% CI: 2.6, 4.8) (p=0.01). Over the 2014-15 rainy season morning indoor resting collections yielded a mean of five and three An. gambiae s.l. per house/night for the deltamethrin+PBO and deltamethrin only net arms, respectively. Overall, there was some evidence that deltamethrin+PBO LLIN were more effective in reducing sporozoite rates in pyrethroid resistant An. gambiae s.l.; however, deltamethrin+PBO nets did not reduce vector density compared to deltamethrin only LLIN.

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IMPACT OF COMBINING INDOOR RESIDUAL SPRAYING AND LONG-LASTING INSECTICIDAL NETS ON *ANOPHELES ARABIENSIS* IN ETHIOPIA: PRELIMINARY FINDINGS OF A RANDOMIZED CONTROLLED TRIAL

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The current malaria vector control interventions, indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) have been used in combination in sub-Saharan Africa with inconclusive evidence that the combined intervention is more effective than either IRS or LLINs alone. In Ethiopia, both interventions target *Anopheles arabiensis*, the sole primary malaria vector. This study compared the impact of combining IRS and LLINs with either intervention alone in south-central Ethiopia. Villages were randomly allocated to four study arms: IRS + LLIN, IRS, LLIN, and control. LLINs (PermaNet 2.0) were provided free of charge. IRS with propoxur was applied before the main malaria transmission season in 2014 and 2015. Adult mosquitoes were collected in randomly selected villages in each arm using CDC light trap catch (LTC) set close to a sleeping person, pyrethrum spray catch (PSC), and artificial pit shelter (PIT), for measuring host-seeking density (HSD), indoor resting density (IRD), and outdoor resting density (ORD). Human landing catch (HLC) was performed in selected villages to monitor An. arabiensis biting behaviors. Mean densities were compared using incidence rate ratio (IRR) calculated by negative binomial regression. A total of 1786 female anophelines of four species was collected of which An. arabiensis (n=574) was highest in the control arm (51.4%) followed by LLIN (31.5%), IRS (9.2%), and IRS+LLIN (7.9%). The mean HSD of An. arabiensis in the IRS+LLIN arm was similar to either the IRS arm (0.03 vs. 0.03/ house/LTC/night) or the LLIN arm (0.03 vs. 0.10/house/LTC/night, p=0.07) and so was the difference in IRD and ORD between the IRS and LLIN compared to the IRS arm. However, both IRD and ORD were higher in LLIN compared to IRS+LLIN (p < 0.001 for indoors). In all study arms, An. arabiensis was actively biting indoors and outdoors throughout the night with an early night biting peak before the local people retire to bed. IRS+LLIN compared to IRS had equal powerful impact on resting density of An. arabiensis, but LLIN had the least impact.

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IMPLEMENTATION OF A NON-PYRETHROID INSECTICIDE-TREATED DURABLE WALL LINING FOR MALARIA CONTROL UNDER OPERATIONAL CONDITIONS IN RURAL TANZANIA

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Despite considerable reductions in malaria achieved by scaling-up long-lasting insecticidal nets (LLINs) and indoor residual spraying, maintaining community protection is challenging. An insecticide treated wall lining (ITWL) has been developed that can be attached to inner house walls and releases a mixture of two non-pyrethroid insecticides over 3-4 years. The operational success of ITWL will be determined not only by its demonstrable efficacy (being evaluated in a 44-cluster randomized trial in Muheza, Tanzania) but also its feasibility, household acceptability and durability under field conditions. To install the ITWL, we recruited 110 teams comprising two local installers and one team leader. Cluster supervisors each supervised 5 teams. Over 6 months in 2015-16, these teams installed ITWL in 5666 rural households (67.7% of all enrolled houses). Completion rate by village varied dramatically (range 42.5%-95.8%). Principal reasons for household refusals included rumors and skepticism, concerns about wall damage, and fear of changing house appearance. Concurrent political elections, higher socio-economic status and skin and eye irritation among 5% of installation workers also lowered initial intervention uptake in some villages. Determining an appropriate scheme for paying installers given differences in house size, providing adequate protective equipment, and reimbursement were a serious challenge. Furthermore, approximately 8% of homeowners consented to installation in only some of eligible rooms, which has potential epidemiological implications. Cross-sectional durability surveys conducted 3 months after installation indicated that ITWLs were no longer installed properly in 33.3% of surveyed rooms, principally due to failed installation fixings (71.8%) and that 91.0% of ITWLs had developed holes from general wear and tear (44.3%) or during actual installation (23.6%). Lessons learned included the need for better communication among supervisors, installers and households, prompt payment of installers, and better protective equipment. Such insights can facilitate future scale up.

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EXPERIMENTAL HUT EVALUATION OF A NEW NON-PYRETHROID INSECTICIDE-TREATED DURABLE WALL LINERS FOR CONTROL OF PYRETHROID RESISTANT *ANOPHELES GAMBIAE* AND *AN. FUNESTUS SENSU STRICTO* IN MUHEZA, TANZANIA

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A new insecticide-treated durable wall liner (ITWL) has been developed which mimics the effect of IRS but is designed to last for 3-4 years. Meant to be used in combination with LLILN, it is made of high-density polypropylene treated with two slow-release non-pyrethroid insecticides. A 9 week experimental hut trial was conducted in Muheza, Tanzania between May-July 2015 in an area with pyrethroid-resistant *Anopheles gambiae* and *An. funestus s.s*. to compare the efficacy of several interventions: including the new ITWL + WHOPES recommended LLIN, ITWL alone, LLIN alone, and pyrethroid wall liner alone. Ceilings were not covered with ITWL. Performance was measured primarily in terms of insecticide-induced mortality. ITWL produced mortality 40-50% of *An. funestus s.s.* and *An. gambiae*. Against *An. funestus s.s*. ITWL alone produced 47% mortality, which was not significantly different to that of LLIN alone (29%, P=0.306) or ITWL + LLIN (35%, P=0.385). Although the numbers of *An. gambiae* were lower, results were similar, with ITWL producing 43% mortality compared with 26% for LLIN. The ceilings provided an untreated refuge for resting mosquitoes. Partial covering of the eaves with DL (1 versus 4 eaves open) had no impact on numbers of either species entering the huts. LLIN provided added personal protection against *An. funestus s.s* over ITWL alone (24% blood-fed for ITWL + LLIN compared with 69% for ITWL only, 1 eave (P=0.001) and 56% (P=0.001) ITWL only, 4 eaves. Cone bioassays of ITWL material after the hut trial produced 98% mortality using pyrethroid-susceptible *An. gambiae* kisumu from the insectary, while F1 offspring of field-collected *An. gambiae* showed lower mortality (80%). Comparison of cone and cylinder bioassays suggested some irritancy from the ITWL. The effect of high community-level coverage of ITWL on epidemiological and entomological parameters of malaria transmission is currently being evaluated in Muheza, Tanzania.

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THE AVECNET TRIAL TO ASSESS WHETHER ADDITION OF PYRIPROXYFEN, AN INSECT JUVENILE HORMONE MIMIC, TO LONG-LASTING INSECTICIDAL MOSQUITO NETS PROVIDES ADDITIONAL PROTECTION AGAINST CLINICAL MALARIA OVER CURRENT BEST PRACTICE IN AN AREA WITH PYRETHROID-RESISTANT VECTORS IN RURAL BURKINA FASO: A CLUSTER-RANDOMIZED CONTROLLED TRIAL USING A STEP-WEDGE DESIGN

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Recent reductions in malaria in sub-Saharan Africa have been associated with increased coverage with long-lasting insecticidal nets (LLINs). Pyrethroids are currently the only insecticide class used for treating nets, and the rapid increase in resistance to pyrethroids in vector mosquitoes may jeopardize future vector control. We assessed whether nets containing a novel combination of permethrin, a pyrethroid, and pyriproxyfen, an insect juvenile hormone mimic, (PPF-LLIN) provide incremental protection against malaria over current best practice of LLINs and prompt treatment in an area with pyrethroid-resistant vectors. In this two-armed step-wedge, cluster-randomized, controlled efficacy trial in Burkina Faso, we used a computerized algorithm to allocated PPF-LLINs randomly to 5 clusters and LLINs to 35 clusters at the start of the trial. One month later, and each subsequent month during the malaria transmission seasons, LLINs were exchanged for PPF-LLIN by groups of 5 clusters, so that 3 months before the end of the 2 year trial all participants had received a PPF-LLIN. A cohort of ~2000 children aged 6 months to 5 years was enrolled by random sampling proportionate to cluster size, and surveyed at the start of the 2014 transmission season and followed in 2014 and 2015 by passive case detection for clinical malaria. Exposure to malaria parasites was assessed by collection of mosquitoes indoors using CDC light traps. The primary endpoints were clinical malaria incidence measured by passive case detection and entomological inoculation rate from *Anopheles gambiae* sensu lato mosquitoes. Generalised Linear Mixed Models will be used to carry out a likelihood ratio test of this primary effectiveness estimate (with alpha=0.05), using Poisson errors, and person-time-at risk as an offset variable. Microscopists reading the slides were blinded. The results from this trial will be presented at the meeting.

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ODOR-BAITED TRAPS AS A NOVEL TOOL FOR MALARIA CONTROL - THE SOLARMAL TRIAL

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Recent progress in malaria control promises that elimination is feasible but to achieve this aim, current tools need to be complemented by novel strategies to combat insecticide and drug resistance. We explored the impact of solar powered, odor-baited mosquito traps on malaria incidence and prevalence on Rusinga Island, Kenya; a location where malaria is endemic and LLINs and drug treatment were already widely available to the population of 25,000. *Anopheles funestus* and An. gambiae s.l. were the main vectors. A system containing a mosquito trap, solar panel, battery, mobile phone charging port and LED lights was termed a SMoTS (solar-powered mosquito trapping system). A novel stepped wedge cluster-randomized control design was used to implement the roll-out of the SMoTS across the island, from zero to complete coverage over 24 months. Three times per year a health and demographic surveillance (HDSS) was conducted covering the entire population while malaria prevalence was measured in a randomly selected 10% of the population. Mosquitoes were monitored over successive six-weekly intervals in 80 randomly selected houses per round. A contemporaneous comparison of clinical malaria incidence was insufficiently powerful to measure an intervention effect due to the unexpectedly low number of clinical cases recorded. Malaria prevalence was significantly lower in intervened compared with non-intervened clusters (29.8% reduction, 95% CI: 20.9 - 38%) and mosquito densities were similarly reduced in intervened clusters, with highest effect on An. funestus (69.2% reduction, 95% CI: 29.1 - 87.4%). It is concluded that the odor-baited traps led to significant reductions in malaria transmission, an effect comparable in size to the impact of LLINs. Sociological investigations showed that the provision of electric light contributed to the high degree of compliance and approval of the SMoTS technology, whereby the population undertook the maintenance of the traps. Mass trapping of mosquitoes should be considered a viable tool for future malaria interventions.

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ANOPHELES DIRUS EFFICIENTLY TRANSMITS MIXED SPECIES AND MULTIPLE CLONE MALARIA INFECTIONS IN CAMBODIA

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Mixed species and multiple clone infections occur commonly in human malaria. It remains unknown whether species and clonal diversity are maintained during transmission in spite of a bottleneck in parasite numbers during Plasmodial transmission to the mosquito. In a study of 12 Cambodians with mixed infections whose blood was membrane fed to reared *Anopheles dirus* mosquitoes, we answered a twofold question. We assessed, first, the relative transmissibility of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) and second, preservation/loss of Pv/Pf genotypes from patient blood to mosquitoes by deep sequencing at targeted loci. DNA from 30 membrane-fed mosquitoes per patient were subject to real-time species-specific PCR. We found that Pv was transmitted more frequently (7/12) than Pf (2/12) from mixed species infections prior to dihydroartemisinin-piperaquine treatment. Pf was the only species transmitted post-treatment. While few mosquitoes caught in the wild have demonstrated mixed infection, we found that 21% (46/222) of mosquitoes fed on mixed patient blood carried both species. All doubly infected mosquitoes had fed upon blood from patients with concomitant microscopic Pf/Pv gametocytemia. We compared diversity of Pf ama1 and Pvmsp1 haplotypes in patient blood to those at the parasite oocyst and sporozoite stages. Clone diversity (multiplicity of infection roughly 2.6 in both mono and mixed patients) was transmitted to mosquitoes intact, often even on an individual-mosquito basis. In 6/7 patients, all pvmsp1 clones present within infected patients were also found in both the oocyst and sporozoite stages within the mosquito. Assessed individually, mosquitoes often carried multiple clones, with multiplicity of infection similar to that in the blood. Pf infections were mostly monoclonal, but findings were similar. In conclusion we see transmissibility of both Pv and Pf simultaneously from mixed infection patients and the absence of a transmission bottleneck in terms of genetic diversity. The observed transmission efficiency of Pv has important implications for malaria control and models of transmission.

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MESOAMERICAN NEPHROPATHY (MEN) IN NICARAGUA: ACUTE INTERSTITIAL NEPHRITIS OF INFECTIOUS ORIGIN?

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Mesoamerican nephropathy (MeN), a kidney disease of unknown origin, is an unrelenting epidemic, primarily in pacific coastal areas of Central America, resulting in over 20,000 deaths. MeN primarily affects young agricultural workers who lack traditional risk factors for kidney disease, and sugarcane workers in Nicaragua are disproportionately affected. MeN's etiology is a mystery, and no case definition of acute onset has been established. Our goal was to describe the acute clinical scenario of early suspect MeN cases. We conducted a prospective case investigation at a large sugar estate in Nicaragua. Physicians identified patients in the emergency room with decreased kidney function and completed case reports with clinical data and medical history. From Feb 2015-Feb 2016, 255 cases of acute MeN cases were identified, mostly male (90%) and young (median age 29yrs), and glomerular filtration rates (eGFR) were low (mean 48±15 ml/min/1.73m^2). The highest incidence was in June (16% of cases). Frequent symptoms were fever (55%), nausea/vomiting (65%), back pain (58%), headache (47%), and muscle debility (45%). Leukocytosis (75%), with neutrophilia (85%), was characteristic. Almost all had WBC (98%), epithelial cells (94%) and erythrocytes (82%) in urine. Few had history of hypertension (n=8) or diabetes (n=4). Based on this data, we defined an acute case of MeN as: a patient with unexplained impaired kidney function and leukocyturia and 2 or more of the following: (1)nausea/vomiting, (2)back pain, (3)headache, (4)muscle weakness, (5) fever, or (6)leukocytosis or neutrophilia. Our data suggests acute MeN involves systemic inflammation and may reflect acute interstitial nephritis, which can progress to chronic tubulointerstitial nephritis and CKD. Renal biopsy results will confirm diagnosis. We establish an evidence-based case definition of acute MeN to be used to identify new cases in Nicaragua and throughout the region, allowing targeted diagnostics to determine the etiology of MeN.

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SEVERE AND HIGHLY FATAL OUTBREAK OF HISTOPLASMOSIS AMONG TUNNEL WORKERS — DOMINICAN REPUBLIC, 2015

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Histoplasmosis is typically a self-limited illness in immunocompetent persons and can be acquired through inhalation of fungal spores from disturbed bat guano. In September 2015, Dominican Republic (DR) health authorities received reports of several tunnel workers hospitalized with a febrile illness suggestive of histoplasmosis. Outbreaks of histoplasmosis have never been reported in the DR. We investigated to confirm etiology and identify factors associated with severe infection. A case was defined as fever and ≥2 symptoms (headache, constitutional, cough, or respiratory difficulty) in a person who worked in the tunnels during July 30–September 2, 2015. We interviewed workers, reviewed medical charts, and tested serum and urine for *Histoplasma* antigen at the Centers for Disease Control and Prevention (CDC). Thirty-five workers used shovels and wheelbarrows to remove large amounts of bat guano from enclosed, unventilated tunnels without respiratory protection. Thirty (86%) workers had illnesses meeting the case definition, 28 (80%) were hospitalized, 9 (26%) required intensive care unit (ICU) admission, 6 (17%) were intubated, and 3 (9%) died. All were men and none were immunocompromised; median age was 30 (range: 18–62) years. The most common symptoms were fever (83%), cough (77%), and headache (70%). Median time from symptom onset to antifungal treatment was 6.5 days. All intubated patients developed ventilator-associated pneumonia. Eighteen (53%) serum and 13 (42%) urine specimens tested positive for *Histoplasma* antigen. The high case-fatality rate (10-fold higher than typically reported) likely resulted from exposure to a high inoculum of *Histoplasma* in the setting of inadequate respiratory protection, delays in treatment, and high rates of nosocomial infection. Clinicians in the DR should be familiar with the presentation of histoplasmosis to allow for timely recognition and appropriate treatment. The risk of histoplasmosis should be considered in creation and implementation of occupational health and environmental safety standards in the DR.

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PERFORMANCE CHARACTERISTICS OF THE WHATMAN FTA ELUTE CARD AND TAQMAN ARRAY CARD PCR ASSAY AS AN ALTERNATIVE METHOD OF STORAGE OF FECAL SAMPLES AND ENTEROPATHOGEN DETECTION AS PART OF THE TRAVELERS' DIARRHEA TREATMENT TRIAL

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Polymerase Chain Reaction (PCR) assays are increasingly used for pathogen detection in travelers' diarrhea (TD), but their use is limited in field settings due to on-site processing and storage requirements for fecal specimens. The Whatman FTA® Elute Card (FTA card) is an appealing alternative for stool collection, due to its ability to store nucleic acid for prolonged periods at room temperature. We evaluated the performance characteristics of the TaqMan Array Card, a quantitative, singleplex PCR (TaqMan PCR), when performed on diarrheal smears on FTA cards, compared to frozen stool samples. Subjects enrolled in a treatment trial for acute watery diarrhea provided a stool sample prior to treatment, a portion of which was smeared onto a FTA card. FTA cards and frozen stool samples were tested at a central lab, using the TaqMan PCR assay with a broad range of enteropathogen targets. A cycle threshold of 35 was used as the cut-off for positive detection. 153 paired FTA stool cards and frozen stool samples were stored for a median of 23 months (IQR 13-25) before testing. High detection rates were observed for frozen stool (79% [95% CI:73-86%]) and FTA cards (72% [95% CI:65-79%]). The most common pathogens detected in frozen stool were enterotoxigenic *Escherichia coli* (ETEC) (36%), enteroaggregative E coli (EAEC) (36%), enteropathogenic E coli (EPEC) (31%), Norovirus (14%), and Shigella/enteroinvasive E coli (7%). Co-pathogens were detected in 33% of samples, the most common being EAEC and EPEC. Sensitivity and specificity of the TaqMan PCR on FTA cards compared to frozen stool for common enteropathogens was as follows: ETEC (89% [95% CI:78-96%]; 99% [95% CI:94-99%]); EAEC(76% [95% CI:62-86%]; 93%[95% CI:86-97%]); EPEC: (75% [95% CI:60-86%]; 93% [95% CI:86-96%]). No significant decline in performance characteristics was noted with prolonged duration of FTA stool cards. FTA stool cards are a useful alternative to standard collection and storage methods in the field setting, allowing sample storage for several months at room temperature, and exhibiting good performance characteristics when tested with the TaqMan PCR.

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RESULTS FROM THE TRIAL EVALUATING AMBULATORY THERAPY OF TRAVELERS' DIARRHEA (TREAT TD) STUDY: A RANDOMIZED CONTROLLED TRIAL COMPARING THREE SINGLE DOSE ANTIBIOTIC REGIMENS WITH LOPERAMIDE

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A recommended treatment for non-inflammatory travelers' diarrhea (TD) is the combination of an antibiotic, usually a fluoroquinolone or azithromycin, and loperamide. However, adverse events, post-dose nausea with high dose azithromycin, effectiveness of single dose versus multi-dose regimens, limited effectiveness data in Africa, and emerging antibiotic resistance to front line agents, remain a concern impacting evidence-based recommendations. A randomized, double-blind trial was conducted at four sites in Afghanistan, Djibouti, Kenya and Honduras between September 2012 and July 2015. US and UK adults with acute non-inflammatory diarrhea were randomized and received single dose azithromycin (500 mg; 106 persons), levofloxacin (500 mg; 111 persons), and rifaximin (1650 mg, 107 persons) plus loperamide (labelled dosing). Volunteers maintained a symptom diary and were evaluated 1, 3, and 7 days after therapy. The primary efficacy outcome, clinical cure at 24 hours, was evaluated in a non-inferiority trial design (delta, -0.15) with both rifaximin and azithromycin compared to a levofloxacin standard. Time to last unformed stool (TLUS) was a secondary efficacy outcome in addition to safety and tolerability. Clinical cure at 24 hours occurred in 80.2% of the levofloxacin arm, compared to 78.3% and 74.8% in the azithromycin and rifaximin arms, respectively. Compared to levofloxacin, azithromycin was not inferior (p=0.0105). Non-inferiority could not be shown with rifaximin (p=0.033). At 48 and 72 hours, efficacy among regimens was equivalent. Median TLUS among all three arms was no different (azithromycin: 4.0 hours; levofloxacin: 5.6 hours; rifaximin: 5.6 hours). Treatment failures were uncommon (3.8%, 4.5% and 1.9% in the azithromycin, levofloxacin and rifaximin arms, respectively) (p=0.6). There were no differences between treatment groups with respect to post-dose nausea (overall 3.1%, p=0.44), vomiting (overall 1.2%, p=0.7) or other adverse events. Single-dose azithromycin (500 mg), levofloxacin (500 mg) and rifaximin (1650 mg) with loperamide were comparable for treatment of watery diarrhea.

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EFFICACY AND SAFETY OF A SINGLE-DOSE MEBENDAZOLE 500 MG CHEWABLE TABLET IN THE TREATMENT OF *ASCARIS LUMBRICOIDES* AND *TRICHURIS TRICHIURA* INFECTION IN PEDIATRIC PATIENTS: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, PHASE 3 STUDY

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A double-blind, multi-center study was conducted to evaluate efficacy and safety of a single dose of a new chewable, rapidly-disintegrating, mebendazole (MBZ) 500 mg tablet for the treatment of *Ascaris lumbricoides* and *Trichuris trichiura* in pediatric patients (1–16 years). The study had a screening phase (3 days), double-blind treatment phase (DBP, 19 days) and an open-label follow-up (OLP, 7 days). A total of 295 patients, excreting eggs of *A. lumbricoides* and/or *T. trichiura*, were randomized (1:1) (age: mean [SD] =7.8 [3.18] years) and received MBZ or placebo on day 1 in the DBP. All patients were administered MBZ on day 19 (the start of OLP) post repeat stool microscopy analysis. At baseline, 167 patients were infected with A. lumbricoides, 243 with *T. trichiura* and 115 were infected with both. Cure rates (primary efficacy endpoint, end of DBP) were significantly higher in MBZ group vs. placebo group for *A. lumbricoides* (% [95% CI], 83.7% [74.2%; 90.8%] vs. 11.1% [5.2%; 20.1%], p<0.001) and *T. trichiura* (33.9% [25.6%; 42.9%] vs. 7.6% [3.5%; 13.9%], p<0.001). Egg reduction rates (secondary efficacy endpoint) were also significantly higher in the MBZ group vs. placebo for *A. lumbricoides* (97.9% vs. 19.2%; p<0.001) and *T. trichiura* (59.7% vs. 10.5%; p<0.001). There were no deaths or serious treatment-emergent adverse events (TEAEs). Comparable rates and low incidence of TEAEs were reported in the DBP between MBZ (9/144 [6.3%]) and placebo (8/140 [5.7%]) with none of the individual TEAEs being reported in >2 patients in either group. Abdominal pain (n=1), abdominal distension (n=2), and rash (n=1) were the only TEAEs considered possibly related to MBZ treatment. During OLP, the total TEAEs incidence was 2.5% with diarrhea (n=2), abdominal pain (n=1), and vomiting (n=1) considered possibly related to MBZ. All TEAEs resolved spontaneously. In conclusion, a single 500 mg chewable tablet of MBZ was found to have an adequate safety profile and was more efficacious than placebo in the treatment of *A. lumbricoides* and *T. trichiura* in pediatric patients. This new formulation will enable treatment of young children who have difficulty swallowing a tablet.

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ILLNESS AMONG MIGRANTS TO CANADA: SURVEILLANCE REPORT FROM CANTRAVNET SURVEILLANCE DATA, 2015

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Due to ongoing political instability and conflict in many parts of the world, migrants are increasingly seeking asylum and refuge in Canada. We examined demographic and travel correlates of illnesses among migrants to Canada to establish a detailed epidemiologic framework of this population for Canadian practitioners. Data on ill returned Canadian travellers presenting to a CanTravNet site between January 1, 2015 and December 31, 2015 were analyzed. During the study period, 2415 ill travellers and migrants presented to a CanTravNet site, and of those, 519 (21.5%) travelled for the purpose of migration. Sub-Saharan Africa (n=160, 30.8%), southeast Asia (n=84, 16.2%), and south central Asia (n=75, 14.5%) were the most common source regions for migrants, while the top specific source countries, of 98 represented, were the Philippines (n=45, 8.7%), China (n=36, 6.9%), and Vietnam (n=31, 6.0%). Compared to non-migrant travellers, migrants were more likely to have a pre-existing immunocompromising medical condition (p<0.0001) and to require inpatient management of their illness (p<0.0001). Diagnoses such as TB (n=263, 50.7%), viral hepatitis (n=80, 15.4%), nationally notifiable diseases (n=380, 73.2%), and HIV (n=11, 2.1%) were greatly over-represented in the migrant population compared to non-migrant travellers (p<0.0001). Among cases of TB in the migrant population, 82% (n=216) were latent, and 18% (n=47) were active. Compared to non-migrant travellers, migrants were more likely to present with a serious, communicable infectious disease, potentially complicated by an underlying immunosuppressing condition. These differences highlight the divergent health care needs in the migrant population, and underscore the importance of surveillance programs to understand their burden of illness.

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MATERNAL PARASITIC INFECTIONS ALTER INFANT ANTIBODY RESPONSE TO PNEUMOCOCCAL IMMUNIZATION

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Vaccine-preventable diseases remain a significant cause of early childhood mortality in developing countries, despite concerted efforts to improve vaccine coverage. One reason for this discrepancy may be the impact of prenatal exposure to parasitic antigens on the infant's developing immune system. Our goal in this study was to investigate the effect of maternal parasitic infections on the infant immune response to early childhood vaccines. 580 pregnant Kenyan mothers were enrolled in the study and tested at prenatal visits for malaria, soil transmitted helminths, *Giardia lamblia*, *Strongyloides stercoralis* and *Schistosoma haematobium* infection. The infants received the 10-valent *Streptococcus pneumonia* conjugate vaccine (PCV), *Haemophilus influenzae* type B (Hib) and *Diphtheria* toxoid (DT) vaccines at 6, 10, and 14 weeks of age. Serum was collected from cord blood, 10 and 14-weeks, 6 months, and every 6 months following through the second year of life. A multiplex fluorescent bead assay determined IgG concentrations to HiB, DT, and the ten PCV serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. Total parasitic infection incidence among mothers in the study is high, with 373 of 580 (64.2%) participants having at least one parasitic infection during pregnancy and 75 participants (13%) with 2 or more infections. The most common infections were hookworm (19.7%), *Plasmodium falciparum* (16.2%), *S. haematobium* (11%), and *Trichuris trichiura* (10.5%). In preliminary analysis using a mixed linear model comparing infection status to log (antibody concentration), we are able to see a 37% higher concentration of PCV 9V antibody in children born to mothers with infections (p=0.016) compared to uninfected mother/child pairs. *S. haematobium* infection was associated with significantly higher concentrations in 4 PCV antigens: 1 (3.9 fold higher, p=0.0006), 7 (2.3 fold higher, p=0.004), 9V (2.3 fold higher, p=0.002), and 18C (6.8 fold higher, p<0.0001). More testing is ongoing with this study, including more samples from later time-points, which will provide a more detailed analysis of the dynamics of first and second year responses to vaccine antigens.

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POPULATION GENOMICS OF *WUCHERERIA BANCROFTI* FROM ARCHIVED SAMPLES USING SELECTIVE WHOLE GENOME AMPLIFICATION

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Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. Until recently, Wb has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Our previous work has shown it is possible to concentrate Wb microfilaria from an infected blood sample and produce high quality genomic sequence. Here we expand upon our previous studies in Papua New Guinea to include three more endemic areas of Wb infectivity: Mali, Kenya, and Haiti. We utilize a new method of whole-genome amplification to generate whole-genome sequence data from archived DNA, blood, and PBMC samples containing microfilaria of Wb. Using this method, we have generated whole-genome data for 42 single microfilaria isolates from Haiti (9), Mali (11), Kenya (10), and PNG (12). We report on thousands of unique single nucleotide polymorphisms (SNPs) and identify genes that are highly variable among localities. We utilize discovered SNPs to: i) elucidate the historical context of admixture between endemic areas, retracing the possible routes of Wb migration, ii) root the gene trees of Wb to identify the species origin, and iii) reconstruct the historical demography by whole-genome coalescent models. Our results provide a new context for studying Wb biology and may implicate biological complexities that will hinder elimination. Our methods advance the study of Wb genomics by allowing the sequencing of archived Wb samples. This new data source, when paired with WGS from recent times and epidemiological data, provides a post-hoc method of hypothesis generation. These hypotheses can then be used to predict how different regions infected with Wb may respond to ongoing elimination pressure.

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POST-GENOMIC EMPIRIC IMMUNOMIC ANALYSES IDENTIFY NOVEL BIOMARKERS FOR ACTIVE *ONCHOCERCA VOLVULUS* INFECTION

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Onchocerciasis is a neglected tropical disease that has been targeted for control and elimination through mass drug administration of ivermectin. Currently, surveillance tools have relied on xenomonitoring and Ov16-based IgG4 antibody tests, the latter having sensitivities ranging from 70-80% across most endemic countries. As program goals have shifted from disease control to elimination of *Onchocerca volvulus* (Ov), additional tools may be needed that can be used in both surveillance and in the certification of elimination of onchocerciasis. Empiric analyses of the stage-specific Ov transcriptomes resulted in the identification of 398 proteins that favor exposure and propensity for eliciting antibody responses. These proteins were expressed, gridded on protein arrays and screened for isotype-specific (IgG1, IgG3, IgG4, IgE) responses using 52 individual sera from Ov-infected and appropriate control individuals. Multivariate analyses of isotype reactivity level and infection status resulted in the identification of 15 'Ov-specific' proteins that had significantly higher IgG4 responses ($p < 0.0001$, ANOVA) in infected individuals compared to control sera. The top 5 candidate proteins (OVOC10469, OVOC3261, OVOC10602, OVOC5127 and OVOC11950) were each expressed in mammalian cells as fusion proteins and tested in a luciferase immunoprecipitation system that allowed for the rapid identification of *O. volvulus* - (but not related filarial parasite-) specific targets of IgG4 reactivity. Among these 5 potential biomarkers 3 showed sensitivities ranging between 80-90%, with specificities that approached 100% when tested with a large panel (n=400) of well-characterized sera. When coupled to the IgG4 responses to Ov16, the IgG4 responses to any one of these 3 biomarkers achieved close to 100% sensitivity with no apparent loss of the 100% specificity seen individually. Additional optimization is being performed to configure the next generation of point of care immunoassays. These data provide an important and practical example of how "immunomic" analyses in the post-genomic era can rapidly provide solutions to very practical problems.

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NOVEL BIOMARKERS FOR THE IMMUNE-BASED QUANTIFICATION OF *LOA LOA* MICROFILARIAE AND THE DIAGNOSIS OF LOIASIS

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Some individuals infected with high levels of *Loa loa* (Ll) microfilariae (mf) are known to experience fatal post-ivermectin severe adverse events that have led to the interruption of ivermectin-based mass drug administration programs in some regions of Central and West Africa. Thus, tools that can accurately identify "at risk" individuals (with high Ll mf loads) at the point of contact (POC) are of critical need. To identify potential biomarkers that could be used as the basis of immunologically-based POC assays, we first conducted transcriptomic analysis of the Ll mf and used these data to provide a framework for identifying proteins (through LC-MS/MS analysis) present in the secretory/excretory (E/S) products of Ll mf from *in vitro* cultures. In addition, proteomic analyses of individual plasma (n= 10) and urine (n=6) of Ll-infected individuals compared to uninfected controls were also performed. Of the 12,200 transcripts expressed by Ll mf, 1,166 proteins were found in the Ll mf E/S products. Some of the mf E/S proteins were specifically detected in the urine (n=205) or the plasma (n=25) of Ll-infected individuals. Through a bioinformatics-filtering pipeline, we identified 28 potential Ll mf-specific biomarkers. Among these 28, 2 mf-specific antigens (LOAG_14221 and LOAG_15846) were detected in plasma of Ll-infected patients with little to no reactivity to sera from patients infected with *W. bancrofti* or *O. volvulus*. Interestingly, levels of LOAG_14221 in mf-positive Ll infected patients were positively correlated to measured mf densities in the corresponding blood ($r = 0.6$ and $P = 0.0007$), and LOAG_14221-based assay showed a sensitivity of 76% and a specificity of 78% compared to gold-standard microscopy. Thus, we are in the process of creating additional reagents to configure a quantitative POC rapid immunoassay for LOAG_14221 antigen in plasma and urine as a surrogate for Ll mf quantification using standard techniques.

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ALLERGIC SENSITIZATION UNDERLIES HYPER-REACTIVE ANTIGEN-SPECIFIC CD4+ T-CELL RESPONSES IN COINCIDENT FILARIAL INFECTION

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Among the various hypotheses put forward to explain the modulatory influence of helminth infection on allergic effector responses in humans, the IL-10 induced suppression of Th2-associated responses has been the leading candidate. To explore this helminth/allergy interaction more fully, parasite- and allergen--specific T cell responses CD4+ T cell responses in 12 subjects with filarial infections and coincident allergic sensitization (Fil+A+) were compared these to the responses to 3 appropriate control groups [Fil-A- (n=13), Fil-A+ (n=12), Fil+A- (n=11)]. The most important findings revealed that FIL+A+ had marked ($p<0001$ for all cytokines) increases in parasite antigen-driven Th2 (IL-4, IL-5, IL-13), Th9 (IL-9) and the regulatory (IL-10) cytokines when compared with Fil+A-. Moreover, using multiparameter flow cytometry, filarial parasite antigen induced a marked increase in not only the frequency of CD4+ T cells producing IL-4, IL-5, IL-2 and TNF-α in Fil+A+ when compared to Fil+A- patients but also in the frequencies of polyfunctional Th2-like (CD4+IL-4+IL-5+ and CD4+IL-2+IL-4+IL-5+TNF-α+) cells. The Th2-associated responses seen in the Fil+A+ group was correlated with serum IgE levels ($p<0.01$, $r=0.5165$ for IL-4; $p<0.001$, $r=0.5544$ for IL-5; and, $p<0.001$, $r=0.4901$ for IL-13), levels of circulating eosinophils ($p<0.0116$, $r=0.5656$) and their degranulation/activation products [major basic protein ($p<0.001$, $r=0.7353$) and, eosinophil derived neurotoxin ($p<0.01$, $r=0.7059$)]. CD4+ responses to allergen were not different (to a large extent) among the groups. Taken together, our data suggest that filarial infection drives an augmented parasite antigen-specific T cell response characterized by a Th2-dominated immune response that largely is pro-allergenic. This response, while possibly able to limit parasite burden, may be responsible for the induction of parasite-associated (but pro-allergic) pathology.

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SCHISTOSOMA MANSONI INFECTION IMPAIRS REPRODUCTION IN MICE

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Schistosomiasis is a neglected tropical disease, endemic in 76 countries, that afflicts more than 240 million people. The impact of schistosomiasis on infertility may be underestimated according to recent literature. Extracts of *Schistosoma haematobium* include estrogen-like metabolites termed catechol-estrogens that down regulate estrogen receptors alpha and beta

in estrogen responsive cells. In addition, schistosome derived catechol-estrogens induce genotoxicity that result in estrogen-DNA adducts and cause hormonal imbalance. We now hypothesize the induction of infertility in individuals infected with *S. mansoni* also through an hormonal imbalance. The aim of this study was to study infertility in mice infected with *S. mansoni*. Female mice infected with *S. mansoni* and noninfected female controls, male mice infected with *S. mansoni* and noninfected male controls were mated during 8 months. Gestational length and, number of pupps were studied. Animals were euthanized and their ovaries, uterus and testes were examined histopathologically. Infected females had shorted gestational length than controls. Births of infected females were not synchronous as in controls. The number of pupps was decreased in infected females in comparison to controls. Ovaries, uterus and testes of infected mice showed definite structural damage. No ova, worms or specific granulomata were detected in infected mice in organs other than liver and spleen. To our knowledge this is the first study addressing *S. mansoni* infection associated infertility. It is concluded that schistosomiasis has an important metabolic effect leading to reproduction disorder in infected animals. These results together with histopathological findings with absence of egg in all examined ovaries, uterus and testicular sections emphasize the possible role of hormonal imbalance in the pathogenesis of such lesions. The changes observed could be due to catechol-estrogens associated with schistosomiasis.

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THE EFFECTS OF PRAZIQUANTEL ON THE TEMPORAL INTERACTION BETWEEN THE HELMINTH PARASITE *SCHISTOSOMA MANSONI* AND ITS MURINE HOST

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After penetrating the skin of its mammalian host, *Schistosoma mansoni* schistosomula migrate via the bloodstream to the liver and mesenteric veins where males and females mature and pair to produce fertile eggs. While many of these eggs are excreted in the feces, a number become lodged in the liver where they drive granuloma formation that causes much of the pathology associated with the disease. Praziquantel (PZQ) is the only drug available for treatment of schistosomiasis. Although the drug kills sexually mature worms it is unable to cure infection because it is ineffective against sexually immature juvenile worms 2-4 weeks after infection. The molecular basis of juvenile resistance to PZQ remains unknown and here we investigate the potential role of ATP Binding Cassette (ABC) transporter genes in the differential response of adult and juvenile *S. mansoni* to PZQ treatment. Expression data for 16 *S. mansoni* genes including 9 ABC transporters over the treatment period will be shown. Our data suggests there is significant differential expression of transporters between *S. mansoni* adult versus juvenile in response to PZQ. In addition, we have employed next generation RNA sequencing technology (Illumina) together with the Lumenogix data analysis platform to examine the differential expression of mouse hepatic genes during *S. mansoni* infection. *S. mansoni* infected mice were treated with a lethal dose of PZQ over 4 consecutive days beginning on either day 25 (juvenile *S. mansoni*) or day 32 (adult *S. mansoni*) post infection. Infected liver tissue was excised and total RNA extracted. The differential expression of immune, fibrotic, and inflammatory genes and pathways will be reported and correlated with egg deposition and granuloma formation in the murine liver.

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DENGUE VIRUS: THE CIRCULATION OF FOUR SEROTYPES IN AN ENDEMIC REGION, DURING NINE SEASONS: SINGULARITIES ON EPIDEMIC DYNAMICS AND GENETIC DIVERSITY

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Dengue virus (DENV) is a Flavivirus of great importance in public health, especially in tropical regions that present favorable environment for mosquito vector development. This study presents a molecular surveillance of dengue occurred in São José do Rio Preto, São Paulo State, Brazil, during nine epidemiological seasons (August 2005 to July 2014). Patients with typical symptoms of dengue who were attended in the public health system had blood samples collected for DENV detection. A total of 1,774 samples were DENV positive. They were identified 511 (27.80%) DENV1, 202 (11.38%) DENV2, 494 (27.84%) DENV3 and 567 (32.96%) DENV4. DENV serotypes circulation can be described as it follows: DENV1 was mainly detected from 2009 to 2012; DENV2 was detected from 2008 to 2012; DENV3 was identified in 2006 but no longer detected after 2007 and finally, DENV4 was detected 2011 onwards. The four dengue serotypes have been detected, representing a hyperendemicity scenario. Phylogenetic reconstructions of 4 serotypes were conducts from 81 complete genome 74 complete sequences of the envelope gene (E Protein). The genotypes circulating were: DENV1 genotype V, DENV2 Asian/American genotype, DENV3 genotype III and DENV4 genotype II. DENV1 strains are from two different lineages, with specific amino acids for each lineage and two dates of introduction: 2008 up to 2014 (MRCA estimated to 2006) and 2010 up to 2012 (MRCA estimated to have existed in 2008). Two subgroups of DENV2 were founded, with specific amino acids changes for one: First group that include 2008 strains (MRCA dating probably in 2004) and other group with strains from 2006, 2011 up to 2014 (MRCA dating probably in 2006. DENV 3 and DENV4 showed one lineage each one and the serotypes circulating are the same as described previously, on Brazil. The co-circulation of multiple serotypes resulted in competition, and genetic diversity. Dengue surveillance is important to understand the mechanisms of introduction and extinction of strains and replacement of serotypes, genotypes or lineages.

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EVALUATION OF THE HEALTH-RELATED QUALITY OF LIFE OF CHILDREN WITH DENGUE AND MALARIA IN WESTERN KENYA

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Malaria and dengue are leading causes of mortality and morbidity in sub-Saharan Africa. Although acute disease symptoms can be clinically recognized, the effects on daily living associated with disease due to either or both infections are still unclear, especially in a pediatric population where disease burden is the greatest. The goal of this study was to investigate the effect of dengue virus (DENV) and/or malaria infection on health-related quality of life (HrQoL) in children in Western Kenya. We used the Pediatric Quality of Life Inventory (PedsQL), a modular

instrument, to assess health-related quality of life (HrQoL) among children, aged 2-18 (n = 171), who presented with fever to one of two health centers in Western Kenya. The PedsQL questionnaires were administered to subjects and their parents in their native Luo language. Blood samples from child with acute febrile illness were assayed for malaria parasitemia by light microscopy or by rapid diagnostic testing cards, and for DENV viremia by RT-PCR. Cases of DENV infection were also identified based on anti-DENV IgG seroconversion by ELISA between blood samples obtained at time of presentation and at one month follow up. The mean PedsQL score for all febrile children was 87.3 (95% CI 85.6 to 89.1) at the time of presentation. By the one month follow up visit, the mean score increased to 94.9 (95% CI 93.5 to 96.4, p<0.001 by paired T-test). The increases by the convalescent visit were also observed for groups of children who had malaria (mean score 88.5 to 96.1, p<0.001) or who had febrile illness that was neither malaria nor DENV (mean score 87.7 to 96.3, p<0.001). For children who were infected with DENV, or who had concurrent infection with both DENV and malaria, the increase in scores was less pronounced; the mean score increased from 84.7 to 91.1 (p=0.3) and 83.6 to 90.7 (p=0.07) for children with DENV mono-infection or DENV/malaria co-infection, respectively. Data are continuing to be collected as part of our ongoing study. However slower recovery in the PedsQL scores of DENV-infected children may indicate that consequences of DENV infection may have longer-lasting effects than previously believed.

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MOLECULAR CHARACTERIZATION OF TWO MAJOR DENGUE OUTBREAKS IN COSTA RICA

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Dengue virus (Flavivirus; Flaviviridae) is a re-emerging arthropod-borne virus with worldwide circulation transmitted mainly by *Aedes aegypti* and *Ae. albopictus* mosquitoes. Since the first detection of its main transmitting vector in 1992 and the invasion of DENV-1 in 1993, Costa Rica has faced dengue outbreaks yearly. In 2007 and 2013 Costa Rica experienced two of the largest outbreaks in terms of total and severe cases. In order to provide genetic information about the etiologic agents producing these outbreaks we conducted phylogenetic analysis of viruses isolated from human samples. A total of 23 DENV-1 and DENV-2 sequences were characterized. These analyses signaled that DENV-1 genotype V and DENV-2 American/Asian genotype were circulating in those outbreaks. Our results suggest that the 2007 and 2013 outbreak viral strains of DENV-1 and DENV-2 originated from nearby countries and underwent in situ microevolution.

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ARTHROPOD EXOSOMES AS NOVEL TRANSMISSION BLOCKING STRATEGIES FOR VECTOR-BORNE PATHOGENS

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Molecular determinants and mechanisms of arthropod-borne flavivirus transmission to the vertebrate host are poorly understood. The transmission strategies used by flaviviruses to exit arthropods and infect human host were envisioned as best approaches to develop transmission-blocking vaccines against molecules or determinants that facilitate pathogen transmission. Research in my laboratory has shown that both tick and mosquito-borne flaviviruses uses exosomes, the small membranous extracellular vesicles for transmission from arthropods to human host. Our studies have revealed that arthropod derived exosomes are important means of communication and transmission between the vector and the vertebrate host. We have found that Langat virus (LGTV), a flavivirus member closely related to tick-borne encephalitis virus and mosquito-borne dengue viruses are transmitted from vector to the vertebrate host through exosomes. The exosomes containing LGTV and dengue viruses were viable, secured and highly virulent in all tests such as re-infection kinetics, trans-migration, inhibitor studies and viral plaque formation assays suggesting exosomes as favorable modes of transmission. Both matured virions and replicative forms of arthropod-borne flaviviruses were found to be using exosomes for transmission. Our data also showed that arthropod derived exosomes facilitated infection of human cells that eventually produced exosomes loaded with flaviviruses. Our current efforts are focused on understanding the molecular mechanisms/associated signaling cascades and virus conformations in exosomes derived from arthropods and arthropod cells. Overall, our studies would suggest the arthropod derived exosomes as novel transmission blocking strategies for treating flavivirus infections.

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PLASMODIUM FALCIPARUM CO-INFECTION MODULATES DENGUE DISEASE SEVERITY

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Severe dengue virus (DENV) disease is an important cause of childhood mortality worldwide. Due to lack of surveillance, the burden of DENV infection in Sub-Saharan Africa is unknown, but likely underestimated. Further, since the DENV mosquito vector shares a geographic distribution with that of the *Plasmodium falciparum* (*Pf*) vector, concurrent infection with both pathogens can occur. Although DENV/malaria co-infection is increasingly recognized in other parts of the world, reports of DENV/*Pf* co-infection are sparse in Africa due to under-recognition of DENV disease. Consequently, little is known about the disease manifestations of DENV/*Pf* co-infection, particularly in children, who are known to bear a larger burden of both diseases. The purpose of the present study is to bridge gaps in our understanding of DENV/*Pf* co-infection disease. As part of our ongoing study on arboviral infection in Kenyan children, we enrolled children who presented with fever of unclear etiology to one of four centers located in Kisumu County in western Kenya, and Kwale County on the Kenyan coast. To date, 579 blood samples from febrile children (mean age 4.3-years) have been tested for both DENV RNA by RT-PCR and malaria by light microscopy. 333 (58%) were positive for *Pf*. 73 (13%) samples were positive for DENV. 33 (49%) of the DENV-positive samples also were positive for *Pf*. Children with DENV/*Pf* co-infection or *Pf* mono-infection were older than DENV mono-infected children (mean age 5.1 and 4.8 years vs. 3.4 years, respectively, p=0.008). DENV/*Pf* co-infected and *Pf* mono-infected children also had higher fevers at presentation than did DENV mono-infected children (mean temperatures of 39.0 and 38.8 vs. 38.5 degrees C, respectively, p<0.01). Compared with *Pf* mono-infected children, body aches, joint tenderness, and splenomegaly were observed more frequently in DENV mono-infected children, but less frequently in DENV/*Pf* co-infected children. These preliminary findings suggest that concurrent infection with *Pf* may exert a modulatory effect on clinical DENV disease. Further investigation of DENV/*Pf* co-infection will yield insights important for clinical care.

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LOWER T CELL APOPTOSIS IN THE SECOND INFECTION WITH HETERO-SEROTYPE DENV

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The available evidence suggests that dengue virus-specific T lymphocytes and cytokine storm play a pivotal role in the immunopathogenesis of plasma leakage. Investigations are underway to identify the immune profiles associated with increased or decreased risk for severe disease. In this study, CD14+ cells from the peripheral blood mononuclear cells (PBMCs) of patients who recovered from DENV-1 infection were infected with DENV-1 or DENV-2 and co-cultured with memory T cells. We found that secondary infection with DENV-2 suppresses the cell reproductive capacity but forms more cell clones and more functional cells to produce more proinflammatory factors (IFN-γ, TNF-α, IL-6, IL-8, IL-12 and IL-17) and less regulatory cytokines (IL-10, TGF-β) which results in higher viral replication compared to secondary infection with DENV-1. Memory dengue virus-specific T cells which are induced in a primary dengue virus infection are reactivated by the heterologous serotype of dengue virus and antigen-presenting cells (APCs) during a secondary infection. Dramatically, less apoptosis and more continuous activation of T cells in secondary infection with hetero-serotype DENV were observed. This discovery which has not been reported previously may be the reasonable and vital interpretation for the cytokine storm and severe symptoms observed in secondary infection with DENV. In summary, secondary infection with hetero-serotype DENV elicits the relatively pathological immune response while secondary infection with homologous-serotype DENV induces the relatively protective immune response by activation-induced cell death (AICD) of T cells.

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PREDISPOSING SECOND IN ADULT DENGUE PATIENTS

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Dengue, a major public health problem in the world, is now endemic in more than 100 countries. It is estimated to have 390 million Dengue infections with 96 million cases worldwide. Mortality rate can be high ranging from 0.1% to 5%. Bleeding is a common complication in Dengue which can even lead to death if not detected and treated early. Therefore, identifying predictors of bleeding would be very valuable as such patients could be closely monitored to detect and treat bleeding early. A prospective Case Control Study was conducted to determine the incidence of bleeding, type of bleeding and possible predictors of bleeding. All patients admitted to Dengue Management Unit at the National Institute of Infectious Diseases, Colombo, Sri Lanka for a period of four months from 1st of July 2014 were included in the study. Dengue infection was confirmed by NS1 antigen or Dengue Specific IgM antibodies. These patients were followed up to see the development of bleeding, possible effects of bleeding and the need of blood transfusion.There were 1000 patients with confirmed Dengue infection with 546 males and 454 females. Age ranged from 12 to 86 years. (mean 31 yrs.) 56.2% (n=562) had DF; 43.8% (n= 438) had DHF. 332(33.2%) had some degree of bleeding; major bleeding in 17.0%, minor bleeding in 15.9%; 67.1% had no bleeding at all, other than petechial bleeding. 81(8.1%) needed therapeutic blood transfusions. Major bleeding was significantly more common ($p<0.05$) in those who had severe vomiting, postural dizziness, abdominal pain and hepatic tenderness and those who had NSAIDS. Females had more bleeding than men ($p<0.05$). Obese patients had a higher risk of having bleeding ($p<0.05$), but not overweight patients(BMI 23-27). Patients with platelet counts less than 50,000 per cmm were at a higher risk of having bleeding as well as DHF patients. This study identifies predictors which would put Dengue patients at high risk of bleeding. This will enable clinicians to monitor such patients carefully to detect and to treat bleeding promptly thereby reducing morbidity and mortality.

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PREVALENCE OF DENGUE AND CHIKUNGUNYA VIRUS INFECTIONS IN NORTHEASTERN TANZANIA: A CROSS SECTIONAL STUDY AMONG PARTICIPANTS PRESENTING WITH MALARIA-LIKE SYMPTOMS

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Abstract In spite of increasing reports of dengue and chikungunya activity in Tanzania, limited research has been done to document the general epidemiology of dengue and chikungunya in the country. This study aimed at determining the sero-prevalence and prevalence of acute infections of dengue and chikungunya virus among participants presenting with malaria-like symptoms in three communities with distinct ecologies of north-eastern Tanzania. Cross sectional studies were conducted among 1100 participants (aged 2-70 years) presenting with malaria-like symptoms at health facilities at Bondo dispensary (Bondo, Tanga), Hai hospital (Hai, Kilimanjaro) and TPC hospital (Lower Moshi). Participants who were malaria negative using rapid diagnostic tests (mRDT) were screened for sero-positivity towards dengue and chikungunya Immunoglobulin G and M using ELISA-based kits. Participants with specific symptoms defined as probable dengue and/or chikungunya by WHO were further screened for acute dengue and chikungunya infections by PCR. Out of a total of 1100 participants recruited, 91.2% (n=1003) were malaria negative by mRDT. Out of these, few of the participants (<5%) were dengue IgM or IgG positive. A total of 381 participants had fever out of which 7.9% (30/381) met the defined criteria for probable dengue, though none (0%) was confirmed to be acute cases. Chikungunya IgM positives among febrile participants were 12.9% (49/381) while IgG positives were at 3.7% (14/381). A total of 69.0% (263/381) participants met the defined criteria for probable chikungunya and 4.2% (11/263) were confirmed by PCR to be acute chikungunya cases. Further analyses revealed that headache and joint pain were significantly associated with chikungunya IgM seropositivity. In north-eastern Tanzania, mainly chikungunya virus appears to be actively circulating in the population. Continuous surveillance is needed to determine the contribution of viral infections of fever cases. A possible establishment of arboviral vector preventive control measures and better diagnosis of pathogens to avoid over-treatment of other diseases should be considered.

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NATURAL ANTIBODY RESPONSES TO THE CAPSID PROTEIN OF DENGUE

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The capsid (C) protein is a structural protein of the dengue virus (DENV) that encloses its genetic material. Few previous studies reported antibody responses to DENV C protein during natural infection. To further understand this, we studied natural antibody responses against C protein of all four DENV serotypes. Antibody responses of sera obtained from dengue seropositive healthy volunteers (DENV1 n=8: DENV2 n=8: DENV3 n=5 and DENV4 n=8) from Sri Lanka, were screened against an array of 14 peptides, representing the entire C protein sequence of DENV2, using

ELISA assay. The peptides are of 15- to 18-amino acids (aa) in length, with 10 aa overlaps. Three peptides P1 (2-18 aa), P11 (79-95 aa) and P12 (86-101 aa) showed positive responses (cut-off = mean OD of 8 negative controls + 3 standard deviation) to sera from individuals infected with all four DENV serotypes. For P11 and P12, 100% of sera from each serotype were positive, whereas for P1, 100% sera from DENV1, 3 & 4 and 86% sera from DENV2 were positive. These peptides are located on N and C terminal regions (1-40 and 70-100 respectively) which are characterized to be highly hydrophilic, surface accessible and flexible regions. According to IEDB conservancy analysis, the overall conservancy % values across the four serotypes of the three positive peptides: P1, P11 and P12, are 44%, 64% and 58% respectively. Two peptides P6 (39-56 aa) and P10 (71-89 aa) were positive only against the sera of individuals who had been infected with DENV2 and DENV4 (100% positive). Remaining nine peptides P2 (8-25 aa), P3 (16-32 aa), P4 (23-40 aa), P5 (40-48 aa), P7 (47-63 aa), P8 (54-71 aa), P9 (62-79 aa), P13 (93-110 aa) and P14 (100-114 aa) did not show significant positive antibody responses. Out of those: P5, P7 and P8 are located in a hydrophobic region and therefore not likely to be potentially antigenic. These results further provide evidences for DENV C protein being immunogenic in natural infections.

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COCULTURE OF ENDOTHELIAL CELLS AND MONOCYTES AS A POTENTIAL MODEL TO STUDY DENGUE PATHOGENESIS AND SCREEN COMPOUNDS WITH THERAPEUTIC POTENTIAL

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Plasma leakage is one of the main signs of complication of dengue virus (DENV) infections and it is related to the disease severity. The lack of animal models representing satisfactorily the pathophysiology of dengue fever in humans has been limiting the advances in understanding the disease mechanisms as well as the development of drugs and vaccines for dengue management. Many studies have been shown that monocytes are one of the main cells responsible for immune response to DENV, producing mediators that interact with endothelia increasing vascular leakage in humans. Therefore, the aim of this work was to stablish a model for assessing the *in vitro* vascular permeability using endothelial cells co-cultured with monocytes infected by DENV. Monocytes (THP-1 cells) were infected with DENV-2 strain ACS 46, at MOI of 0.1 and 1, and put in contact with endothelial cells (HUVEC) monolayers through apical or basolateral side of transwell inserts. As controls, monocultures of HUVECs were infected likewise. UV inactivated virus and supernatant of mock infections were also tested. The endothelial barrier function was evaluated by measuring the Transendothelial electrical resistance (TEER). Data were compared by one-way ANOVA/Tukey's test with $p<0.05$. Results show that the coculture system, infected at MOI of 0.1 , presented significant lower TEER values in comparison to the infected monocultures of endothelial cells, as well as the cocultures infected with UV inactivated virus or supernatant of mock infections, after 24 h of basolateral contact. No differences were observed at the same conditions with apical contact. At MOI of 1, we found no difference between mono- or cocultures. These results indicate that the infected coculture more efficiently interfered with the endothelial barrier function *in vitro* at low MOI (0.1), being a potential mimicry model of plasma leakage triggered by dengue infections. The proposed system will allow us to screen of compounds with therapeutic potential and study the interference of different immunomediators on vascular permeability in order to better understand the pathogenic factors associated with severe outcomes.

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EFFICACY OF RUPATADINE IN THE TREATMENT OF ACUTE DENGUE INFECTION

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Our previous studies showed that platelet activating factor (PAF) was a potent mediator of vascular leak. Therefore, we proceeded to investigate the efficacy of rupatadine which is a PAF receptor blocker in patients with acute dengue infection. We conducted a phase II, open label, randomized placebo controlled trial to determine the safety of rupatadine in patients with acute dengue, the efficacy of rupatadine in preventing or reducing vascular leak and to determine its efficacy in reducing complications associated with acute dengue. The study was carried out in 3 arms: rupatadine 40mg daily, rupatadine 10mg daily and the placebo. The patients were examined and laboratory parameters were measured at least twice a day to detect any complications and fluid leakage. Daily ultrasound scans were done from the day of admission to determine the presence and the quantity of fluid leakage. 138 patients were recruited on day 4.8 of illness (SD±0.55) with 44 receiving 40mg daily rupatadine, 44 receiving 10mg daily rupatadine and 44 receiving placebo. Both rupatadine 10mg and 40mg were found to be safe and did not cause any increase in adverse effects when compared to the placebo. The proportion of individuals who developed either pleural effusions or ascites (22.7%), were similar in all 3 arms. None of the patients given rupatadine 40mg developed bleeding manifestations, while 2 (4.5%) in the 10mg and 5 (11.4%) in the placebo arms developed significant bleeding manifestations. None of the patients in the rupatadine 40mg arm developed organ dysfunction, while 1 (2.3%) patient in 10mg arm and 3 patients (6.8%), in the placebo arm developed liver dysfunction. Those given rupatadine 40mg daily had less reduction in the platelet counts and less elevation of liver transaminases when compared to the 10mg rupatadine and the placebo arm. Rupatadine appears to be safe in patients with acute dengue infection. Although rupatadine did not reduce the proportion of individuals who develop fluid leakage when given on day 4-5 of illness, it appears to reduce complications associated with dengue. However, it will be important to confirm these findings in larger studies.

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COMPARISON BETWEEN DENGUE AND CHIKUNGUNYA BY CBC AT THE HOSPITAL OF THE NO. 2 POLICE OF THE CITY OF GUAYAQUIL PERIOD 2015

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Introduction Dengue and chikungunya are similar in their classical forms of clinical presentation, especially present as febrile syndrome and in areas where both viruses can circulate simultaneously confused clinically and even coexist in the same patient, also transmitted by the same vector. It is important to reach a differential diagnosis in patients with febrile illness as dengue carries a worse prognosis. Objectives This study aims to define and compare the clinical and laboratory associated with each condition in a hospital population who came to the hospital for febrile syndrome Police features N.2 Methodology A retrospective cross-sectional study. 150 patients were compared to positive 150 Chikungunya IgM positive patients Dengue IgM. Laboratory variables were extracted through medical records of patients and laboratory records Health Center, then compared by measuring association adjusted odds ratio obtained by logistic regression. Software used STATA version 14.1 for Mac. results The parameters of

the blood count which most were associated with Chikungunya were leukopenia with an OR 3.5 CI (1.63-7.47) p = <0.000, OR 7.14 IC low Hemoglobin (1.71-29.85) p = <0.000. however not decreased hematocrit IC OR 0.58 (0.27-1.23) p = 0.15 and OR 1.05 plaquetopenia IC (0.41-2.65) p = 0.9 In the case of Dengue low hematocrit OR 1.71 CI (0.8-3.63) p = 0.15, OR 0.13 low hemoglobin IC (from 0.03 to 0.58) p = 0.016, OR 0.94 plaquetopenia IC (0.37-2.38) p = 0.9, leucopenia OR IC 0.28 (0.13 to 0.61) p = <0.000. In the distribution by age was evident that CHIKV only present in the 80 years Dengue change in the most affected range was 20-40 years but was not significant p = 0.38 GPT transaminase CHIKV presents an OR 0.26 IC (0.05-1.22) p = 0.06, OR 3.84 Dengue the IC (0.81-18.05) in the case of GOT in CHIKV OR 0.47 IC (0.10-2.20) p Dengue = 0.33 and OR 2.10 IC (0.45-9.79) p = 0.23 Discussion We found a clear distribution of laboratory values that associate low hemoglobin disorders and leukopenia with Chikungunya, the platelet count and hematocrit was indistinct between the two diseases. T.

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A CLINICAL GRADING AND DECISION SUPPORT SYSTEM FOR DENGUE VIRAL INFECTION AND ACCOMPANYING COMORBIDITIES USING SYNDROMIC SURVEILLANCE IN THE HOSPITAL SETTING

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Dengue fever has increased its geographical spread across non-endemic areas. Therefore, timely surveillance and clinical decision-making before, during, and after outbreaks of dengue are highly important to controlling epidemics. While dengue has not been endemic to Taiwan, outbreaks have occurred annually in southern Taiwan for the vast majority of the past two decades. Therefore, Taiwan's health authorities have employed a system of both passive surveillance and serological surveillance for the detection of dengue. However, as detection of early cases may prove to be challenging using current methods, a more sophisticated form of is preferable for the early detection of cases of dengue, especially as Taiwan's case population is one of the oldest in the world and complicated with multiple co-morbidities such as diabetes and cardiovascular, renal, and liver disease. Extending previous studies from this group, this study attempts to establish a clinical grading and decision support system based around a concept of syndromic surveillance, drawing from a hospital database of confirmed cases of dengue in southern Taiwan and its complete set of electronic health record information to determine the significance and contribution of accompanying co-morbidities on the clinical presentations of dengue infections. The resultant data provide helpful insight into the formulation of clinical guidelines for early detection and management of dengue infection, particularly for severe, complicated cases in adults. Syndromic surveillance in an area non-endemic for dengue such as Taiwan may also prove applicable for other non-endemic nations also under the threat of dengue fever outbreaks.

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INVESTIGATING DENGUE VIRUS INFECTION AS A CAUSE OF FEVER IN KENYAN CHILDREN

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Dengue virus (DENV) is endemic in more than 100 countries around the world and causes 400 million infections yearly. 16% of DENV disease is predicted to occur in Africa, but due to lack of surveillance, the burden of DENV in the Africa remains uncertain. The objective of this study is to describe the incidence of DENV infection in febrile children in coastal and western Kenya. We are enrolling children presenting with acute fever to any of 4 health centers located in Chulaimbo and Obama Children's Hospital in western Kenya, and Msambweni and Ukunda on the Kenyan coastal region. To investigate whether DENV causes febrile illness in Kenyan children, clinical data and blood samples are collected at presentation and at one month follow up and tested for DENV IgG by ELISA. Out of 180 paired serum samples tested to date, 1 (0.6%, 95% CI 0.01 to 3.1%) male subject aged 2 years old seroconverted from Chulaimbo. Based on preliminary results the incidence of DENV infection is 56 per 10,000 cases of febrile illness in Kenyan children.Further ongoing testing of 1000 paired samples will measure the true incidence of DENV infection in this region. These data will be helpful to clinicians since dengue symptoms are non-specific and lack of awareness leads to missed opportunities for community education and vector control and prevention. Without further investigation, our knowledge of the real impact of DENV in the region is severely limited.

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A HUMANIZED MOUSE MODEL FOR STUDYING HUMAN IMMUNOLOGY AND PATHOGENESIS OF DENGUE VIRUS INFECTION

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The limitation in our current understanding of dengue disease pathogenesis may be attributed to the lack of an ideal animal model. To address this, we have utilized a mouse strain, DRAGA, that is immunocompromised and transgenically expresses Human Lymphocyte Antigen (HLA) molecules. We hypothesize that these mice are capable of sustaining dengue virus (DENV) infection and will generate a human immune response to DENV. We conducted experiments to determine if DRAGA mice can support DENV replication, sustain DENV infection, develop clinical signs of disease, and produce a humoral and cellular immune response to DENV. Mice were injected subcutaneously in the right flank with 1x106 pfu of DENV-1 Western Pacific 74, and monitored for several weeks for clinical signs (clinical score, temperature, weight loss). Blood was sampled at various time points for viremia determination by a CDC DENV-1 RT-PCR assay. Measurements of anti-dengue antibody titers (IgM and IgG) in serum samples were done at various time points using two-fold serial dilutions of sera in an in-house ELISA. Cell mediated immune responses in spleens of humanized mice was measured by IFN-γ ELISPOT assay at the time of euthanasia, and organs were harvested for detection of virus. Mice demonstrated relevant clinical symptoms. Clinical scores (including rashes, hunching posture, lack of movement) increased for all five mice resulting in all five mice being euthanized before day 80. Kaplan-Meir results indicate a difference in outcome for infected mice compared to controls (P=0.0011, Log-Rank Test). Viremia was detected in all mice. At the time of euthanasia one mouse demonstrated a strong cellular immune response to DENV non-structural 1 glycoprotein by ELISPOT following stimulation by peptide pools (P=0.0013, Unpaired t test). No other cellular immune response was observed. This mouse also demonstrated an IgM humoral immune response. This model has the potential to represent a powerful small animal model for the preclinical testing of experimental vaccines and become a critical capability for advancing candidate dengue vaccines to human trials.

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EVALUATING INTERNET-BASED COMMUNICABLE DISEASE BIOSURVEILLANCE METHODS FOR VECTOR BORNE DISEASES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Internet-based vector-borne disease (VBD) surveillance methods using 'big data' sources such as Google and Twitter have recently been developed. These methods may offer complementary real-time VBD surveillance, but appraisal of their performance and validity is essential for their improvement and possible implementation. We performed a systematic review and meta-analysis to assess the performance of VBD internet-based surveillance methods and the quality of evidence supporting them. We included studies that predicted population-level VBD activity with search-engine, social media and other forms of internet data and that were validated against a reference source of public health data. We searched MEDLINE, EMBASE and Web of Science databases in addition to reviewing bibliographies. Study quality was assessed using a framework covering documentation, analysis reproducibility and external validation. Studies measuring performance by correlation between internet-predicted and reported VBD trends underwent meta-analysis, weighted by effective sample size. Subanalyses were performed to explore heterogeneity. Of 2476 non-duplicate studies, 14 met eligibility criteria, of which 11 examined dengue, and the remainder malaria, leishmaniasis, Lyme disease or other arboviruses. Two completely satisfied quality criteria across all domains and six underwent external validation. Ten were included in the meta-analysis, yielding a summary correlation r = 0.69 (95% CI 0.67 - 0.70) with large heterogeneity between subgroups of pathogens, internet data type and disease endemicity. Internet-based VBD prediction performed best for malaria (r = 0.92) and dengue (r = 0.73); and with data derived from Wikipedia (r = 0.89) and Twitter (r = 0.70). Internet-based surveillance is overall moderately accurate for detecting trends in VBD yet may perform better for certain pathogens, internet-data sources and disease burdens. Variable study quality warrants further rigorous study in this field.

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COMPLEMENTARY USE OF CRYO-ELECTRON MICROSCOPY AND MASS SPECTROMETRY TO ASSESS THE MATURITY OF SANOFI PASTEUR DENGUE VACCINE VIRUSES

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The Sanofi Pasteur dengue vaccine demonstrated significant efficacy in phase III studies and is now licensed in several countries. The vaccine is composed of four recombinant, live, attenuated viruses based on a yellow fever 17D vaccine backbone, each expressing the pre-membrane (prM) and envelope (E) genes of one of the four dengue virus serotypes. Dengue is a flavivirus, which are small, enveloped, icosahedral, positive-single strand RNA viruses. The glycoprotein shell consists in 180 copies each of an envelope (E) and membrane proteins (prM/M, prM being M precursor). In immature particles, 60 trimeric spikes extend from the particle surface, each of them consisting of 3 prM:E heterodimers, conferring the "spiky" morphology to the virion as observed by cryo-electron microscopy. Maturation process occurs during transport through the Trans-Golgi where E undergoes conformational changes triggered by low pH, after which prM is eventually cleaved by furin, a host protease. The final mature particle presents 90 E homodimers on its surface and usually presents a "smooth" morphology, while it may also present a "bumpy" structure depending on the temperature and serotype. Maturity plays a critical role in dengue virus infectivity but it remains unclear if it is linked to some extent to immunogenicity and eventually protection triggered by vaccine viruses. To first address this question at the protein level, we have developed a mass spectrometry based method (LC-MS) to quantify specifically prM, pr and M proteins and estimate the mean maturity of the 4 vaccine viruses that were part of clinical phase III studies. Virus maturity was also analyzed by cryo-electron microscopy at the particle level. Percentage of spiky (immature), smooth/bumpy (mature) and mixed (partially mature) viral particles were determined. Results from these two orthogonal methods were in good agreement and showed a significant maturity for all batches and serotypes used in Phase III studies. In this regard, it does not appear that differences in serotype-specific efficacy observed in these trials could be linked to differences in maturity for the corresponding serotypes.

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A STATISTICAL APPROACH TO ESTIMATE DENGUE VIRUS INFECTION HISTORIES, INCLUDING BROADER CRITERIA FOR INAPPARENT INFECTIONS

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The four dengue viruses (DENV1-4) cause 100 million symptomatic dengue cases and an estimated 300 million inapparent infections each year. We explored current assumptions used to identify inapparent infections and describe a statistical approach to create probabilistic infection histories for children in the Nicaraguan Pediatric Dengue Cohort Study (PDCS), ongoing since 2004. Inapparent infections were identified by a ≥4-fold increase in antibody titers as measured with the Inhibition ELISA (IE) between annual samples. Controlling for year, we found that the proportion of children with symptomatic or inapparent infections decreased with higher pre-infection IE titers. Further, there was a significant inverse relationship between pre-infection IE titer and the magnitude of IE titer increases the following year. These observations suggest that children with high pre-infection IE titers may control infection without observable disease *or* large increases in IE titer, making them an important group for studying protection. We also observed that the number of boosts (≥2-fold increases in IE titer) correlated with epidemic cycles: the largest proportion of children had boosts before or after the year with the highest DENV incidence, with the lowest proportion of boosts observed in years of transition in epidemic dominance of one DENV type to another. Consistent with this observation, children with symptomatic infections were more likely to have an increase in IE titer the next year if that year had high incidence of symptomatic dengue, even if the dominant circulating DENV type matched their previous infecting type. Based on these observations, we are estimating probabilistic infection histories for all children in the PDCS with a Bayesian approach that accounts for infections that cause <4-fold IE titer increases as well homologous reinfection. From this model, we estimate the infection probability per child per year and repeatedly sample the cohort with these probabilities to create DENV infection histories. We then use these infection histories to test for determinants of protection against symptomatic dengue and severe disease.

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SUSCEPTIBILITY OF NEOTROPICAL PRIMARY BAT CELL LINES TO DENGUE INFECTION

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Dengue Virus is the most widespread arboviral disease that affects humans worldwide. Bats have been identified as carriers of emerging viral zoonoses and proposed as possible Dengue reservoirs, since viral RNA/NS1 and/or antiviral antibodies have been detected. Yet, Dengue experimental inoculation of Artibeus bat species failed to show dengue replication. Also, a putative organ for virus replication in bats is still not identified. Therefore for testing *in vitro* susceptibility of bat cells for dengue infection, we established primary bat embryonic cells from diverse organs of three different bat species (Artibeus jamaicensis, Molossus sinaloae, and Desmodus rotundus). We observe a serotype-, organ-, and bat species-specific dengue susceptibility of infection, though virus replication in all cases is limited. Only Molossus-derived fibroblasts, kidney, liver, and intestine cells sustained poor dengue serotype 1 replication, though at 96 hpi replication is controlled by bat-specific mediators. Dengue does not replicate efficiently in cell lines derived from the other bat species. Therefore, Artibeus and Desmodus bats may unlikely serve as dengue reservoirs. Nevertheless, to elucidate if Molossus bats play a role in dengue replication, ecological or *in vivo* experiments must be performed, however with an appropriate dengue serotype.

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EFFECTS OF COMMUNITY STRUCTURES AND ENVIRONMENTAL HETEROGENEITIES ON THE SPREAD AND PERSISTENCE OF DENGUE

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Dengue, a multi-strain vector-borne tropical disease, presents a substantial and increasing burden on global public health. The interactions between the virus, its mosquito vectors and the human host are complex and only partially understood. Immune competition between dengue's four antigenically related serotypes (DENV1-4) together with the dependencies of vector ecologies on environmental attributes, such as temperature, rainfall, and host density, introduce strong spatio-temporal heterogeneities, resulting in irregular epidemic outbreaks and asynchronous oscillations in serotype prevalence. Local and global human movement have been implicated as important drivers of dengue epidemiology across space and time and further create the conditions for the geographic expansion of dengue into new habitats. However, the effects of demographic and ecological structures on dengue epidemiology have not yet been explored in detail. To this end, we constructed a stochastic individual-based transmission model that explicitly includes spatio-temporal heterogeneities in host and vector population sizes and further incorporates complex community structures and connectivities between sub-populations. The model's more realistic meta-population formulation allows for the exploration of the effects of environmental heterogeneities on dengue incidence, in addition to the identification of critical community size and connectivity necessary for the emergence and spread of dengue virus. Our results will thus help to better understand the spatio-temporal epidemiology of dengue and assess the risks of further geographic expansion.

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DEVELOPMENT OF ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY ASSAYS FOR DENGUE VIRUS

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Dengue virus (DENV) infection affects US military personnel deployed to endemic areas. DENV infection leads to a number of host immune responses, one of which is antibody-dependent cell-mediated cytotoxicity (ADCC). The aim of this study is to develop and standardize assays to assess serum ADCC activity. We first examined the percentage of antigen opsonization and level of natural killer (NK) cell degranulation using CEM-NKR-DC-SIGN cells as targets and normal human PBMCs as effectors. CEM-NKR-DC-SIGN cells were infected with DENV for 3 and 24-hours. The infected cells were treated with a dengue immune and a control naïve serum at five-fold dilutions and then with a secondary antibody, PE-labelled goat anti-human IgG Fc. The opsonization assessment indicated similar levels of DENV Ag expression on the surface of target cells at both 3- and 24-hours. The degranulation experiment was done by co-incubating the effectors and the opsonized targets for 2 hours and consequently staining the cultures with an antibody cocktail (APC-CD56, PerCP-CD3, FITC-CD107a, and PE-CD16). The expression of CD107a on CD3-CD56+ suggested a significant increase in degranulation of NK cells against target cells infected for 24-hours but not 3 hours for all four serotypes. This could be due to possible increase in expression of different viral antigens on the cell surface at 24-hour. Additionally, cells infected by DENV for 24 hours may express certain stress factors and certain NK cell activation ligands which could potentially increase functional activity of NK cells. ADCC is a mechanism that leads to lysis of infected cells; therefore it is important for viremia control during an infection. Hence we are working towards developing a non-radioactive lysis assay and further standardizing these assays for future application for clinical research and vaccine development.

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SEROCONVERSION TO DENGUE AND CHIKUNGUNYA IN IMMUNOLOGICALLY NAÏVE ADULTS IN ST. KITTS

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Ross University School of Veterinary Medicine (RUSVM) is located on St. Kitts and Nevis, a popular tourist destination in the Lesser Antilles in the Caribbean. Dengue and chikungunya are endemic on St Kitts and students attending RUSVM for their 2 ½ years of study principally come from areas where these diseases are not present. Studying the rates of exposure of the students to the viruses and the risk factors involved would provide valuable epidemiological data for students and tourists visiting areas where dengue and chikungunya are endemic. The aim of the prospective study is to determine the survival time and risk factors in naïve adults visiting an endemic area. Student volunteers attending RUSVM at one of the three intakes between September 2014 and May 2015 were recruited for the study. Whole blood was collected from consenting volunteers when they enrolled in the study and every 4 months subsequently. Plasma was separated and tested with a DENV IgM and IgG antibody capture ELISA developed at the Centers for Disease Control and Anti-Chikungunya Virus ELISA IgM test and Anti-Chikungunya Virus ELISA IgG test - (Euroimmun, Lübeck, Germany). Plaque reduction neutralization (PRNT) following standard methods is also being performed to confirm the dengue serology results. A total of 161 of the 218 volunteers were sampled within 4 weeks of arrival to the island with 76 (47%) already showing IgG antibodies to dengue virus at titers of 2 or higher. Of these, 27 reported they had not previously visited any dengue endemic areas which would indicate that seroconversion may have occurred very shortly after arrival on St Kitts. In

the subsequent testing of the students we found 21 (10%) seroconverted against dengue virus with an average time to seroconversion of 24 weeks. The rate of seroconversion between sampling periods was 1.35 per 10 individuals. One student (1%) seroconverted against chikungunya within 2 weeks of arrival while another 4 (2%) seroconverted subsequently with an average time to seroconversion of 25 weeks. None of the seropositive students reported classical signs of dengue or chikungunya. (The confirmatory PRNT results for dengue are pending).

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FLUORESCENTLY LABELED FLAVIVIRUSES TO TRACK ANTIGEN-SPECIFIC B CELLS

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We recently developed alexa fluor labeled dengue viruses (AF DENV) to evaluate frequencies of antigen-specific memory B cells in the peripheral blood of immune individuals. We used two serotypes of AF DENV together on PBMC from children in Thailand undergoing acute primary or secondary DENV-1 infections to determine whether patterns emerged on antigen-specific B cells that reflected their exposure or clinical diagnosis. Brightly labeled AF DENV serotype specific and cross-reactive B cells were identified in PBMC from all subjects. Frequencies of AF-DENV+ class switched memory B cells (IgD-CD27+ CD19+ cells) reached up to 8% during acute infection and early convalescence. In a number of subjects, AF DENV labeled B cells expressed high levels of CD27 and CD38 during acute infection, characteristic of plasmablasts, and transitioned into memory B cells (CD38-CD27+) at the early convalescent time point. Our analysis of total B cells and AF DENV+ B cells revealed higher activation of memory B cells early during acute secondary infection suggesting reactivation from a previous dengue infection. AF DENV are useful reagents to identify differences in the phenotype of subsets of antigen-specific and cross-reactive B cells during and after natural dengue infection and vaccination.

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A CASE OF DENGUE ENCEPHALITIS CAUSED BY DENV-4

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We describe a case of dengue encephalitis in a primary dengue infection caused by DENV-4. A 62 year old Chinese woman, previously well, presented with a four-day history of fever, headache, postural giddiness, vomiting and exertional dyspnoea. She had not travelled out of Singapore. Clinical evaluation on admission (day 4 of illness) revealed temperature of 38.2oC, blood pressure 140/80 mmHg, pulse rate 92/min, physical examination was otherwise unremarkable. Total white cell count was 1.9 x 109/L (neutrophils 1.2 x109/L), haemoglobin 13 g/dL, haematocrit 39.5%, platelet count 106 x 109/L, ALT 218 u/L and AST 155u/L. Renal function was normal. Dengue NS1 antigen was positive, dengue IgM and IgG were negative. Chest radiograph was normal. She developed confusion and expressive aphasia on day 6 of illness. Neurological exam was unremarkable with no focal deficit. Blood cultures were negative and there was no pyuria. Cerebrospinal fluid (CSF) analysis revealed a cell count of 62 cells/uL (97% lymphocytes), red cell count 9 cells/uL, protein 2.33 g/L and glucose 3.5 mmol/L (serum glucose 5.9 mmol/L). CSF bacterial culture, antibody (measles, mumps), and polymerase chain reaction (PCR) for CMV, HSV, VZV, Toxoplasma, Enterovirus were negative. Magnetic resonance imaging of the brain was normal. CSF dengue PCR was negative but IgM and IgG were positive. Serum dengue PCR was positive for DENV-4. She was treated with intravenous acyclovir till PCR results were available, and made a complete recovery at day 11 of illness. The proposed case definition for dengue encephalitis includes (1) fever, (2) acute cerebral involvement, (3) positive dengue IgM or PCR on serum and/or CSF, and (4) exclusion of other causes of viral encephalitis, encephalopathy as demonstrated in our patient. Prior case series of dengue encephalitis have been associated most commonly with DENV-2, DENV-3 and occasionally with DENV-1. ,3 Although DENV-4 has been reported to potentially cause encephalitis, those cases were fatal with multi-organ involvement and dengue haemorrhagic fever , . This is the first known case of DENV-4 encephalitis with complete recovery.

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PROFILING ANTIBODY RESPONSES TO DENGUE NS1 IN VACCINE STUDIES AND NATURAL HUMAN INFECTIONS USING PEPTIDE MICROARRAYS

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A major challenge in dengue vaccine development is that cross-reactive anti-dengue virus (DENV) antibodies can be protective or potentially enhance disease via antibody-dependent enhancement. DENV nonstructural protein 1 (NS1) has long been considered a vaccine candidate, and we have shown that NS1 from all 4 DENV serotypes protects against challenge in a mouse model of lethal vascular leak syndrome. Conversely, we found that DENV NS1 by itself triggers vascular leak *in vivo* and *in vitro* and increases disease severity during infection. Here, we evaluated survival to challenge in the lethal DENV vascular leak model in mice immunized with NS1 combined with alum, Monophosphoryl Lipid A (MPLA) + AddaVax, or Sigma adjuvant system (SAS) + CpG DNA. We characterized antibody responses to NS1 using a microarray with 20-mer peptides (overlapping by 15 amino acids [aa]) from prM, E, and NS1 of DENV-1, -2 and -3. We compared these antibody profiles to those of mice immunized with ovalbumin (OVA) plus adjuvant and mice infected with a sublethal dose of DENV2. Mice immunized with OVA or NS1+alum were not protected, whereas immunization with NS1+MPLA/Addavax or NS1+SAS/CpG or prior infection with DENV2 resulted in 100% survival but exhibited distinct antibody responses to NS1 peptides. We identified two DENV-2 NS1 peptides (D345 and D347) that were recognized strongly by NS1+MPLA/Addavax-immunized mice and DENV-infected mice; analogous peptides in DENV-1 and -3 were similarly recognized. In addition, immunization with NS1+MPLA/AddaVax induced antibodies specific NS1 epitopes that were not observed following DENV2 infection (e.g, D356). In parallel, we found that human sera from natural DENV infections reacted to the same D345 and D347 peptides but did not react to D356. We mapped these epitopes onto the NS1 crystal structure and found that D345 and D347 mapped to aa 101-130 in a surface-exposed flexible loop in the wing domain. D356 (aa 156-174) is in the putative membrane-binding "greasy-finger" region of NS1. These data identify antibody responses to NS1 vaccination that target specific epitopes and may help prevent NS1-mediated pathogenesis.

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POLYCLONAL ANTIBODY RESPONSES TO SEROTYPE-SPECIFIC NEUTRALIZING EPITOPES IN NATURAL DENGUE VIRUS INFECTIONS

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The development of an effective and safe dengue vaccine relies on identification of neutralizing epitopes from all four dengue virus (DENV) serotypes. Strongly neutralizing DENV serotype-specific human monoclonal antibodies (hmAbs) target quaternary epitopes spanning multiple E protein monomers and are only preserved in intact virions. Among hmAbs with these properties is 5J7, which is a DENV3 serotype-specific hmAb. Given the potential role of the 5J7 epitope as a determinant of the DENV3 type-specific neutralizing response, we previously created a partial functional transfer of the DENV3 5J7 epitope into a DENV4 background. The recombinant virus, rDENV4/3, is neutralized by human DENV3-immune sera, indicating the functionality of this epitope. Here, our study aims to explore the recognition of the hmAb 5J7 epitope in the epidemiological context of an endemic setting. Specifically, we are assessing the proportion of 5J7 epitope-specific neutralizing antibodies following primary DENV3 infection and determining the kinetics and magnitude of the response over time. We first analyzed primary DENV3 sera from 24 individuals enrolled in a longitudinal dengue hospital-based study in Nicaragua, which allowed us to track the proportion of the DENV3 type-specific neutralizing response attributable to the 5J7 epitope at two time-points post-infection (3 and 18 months). The neutralizing titers (NT_{50}) to the rDENV4/3 virus and its parental viruses (DENV3 and DENV4) were obtained via a flow cytometry-based neutralization assay using human U937-DC-SIGN cells. We also measured the cross-reactive DENV4-directed antibody responses that recognize the rDENV4/3 virus at 3 and 18 months post-infection. Further studies will evaluate how the DENV3 type-specific neutralization tracks with the 5J7 epitope using samples from our Nicaraguan cohort study over multiple years and following second and third DENV infections. Overall, this project contributes to increased understanding of the antibody response in natural DENV infections and has direct implication for design and evaluation of dengue vaccine candidates.

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ENDURING A DENGUE/ZIKA EPIDEMIC IN RIBEIRAO PRETO, BRAZIL

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Dengue fever (DF) is the most important arbovirus infection in the world. Our busy hospital emergency room (ER) located in an endemic DF region in Brazil has been facing large DF outbreaks every year. In 2016, a new virus (Zika) was introduced to our region. We described how we dealt with both epidemics in the setting of not having Zika serology and reserving Zika RT- PCR for pregnant women (PW). The patient care flow was based upon in the Lean Methodology that has a focus in process improvement with the identification and elimination of everything that does not add value. We followed the Brazilian Health Ministry Guidelines for dengue care; the focus was prompt detection of alarm signs, vigorous endovenous hydration for high hematocrit and low platelet count, oral hydration for every patient at the ER and daily or every other day returns based upon the patient risk to develop severe DF. We established a flow for PW presenting with a rash and/or suspected DF (SDF); they would have daily returns, samples would be sent to Zika RT- PCR testing and dengue testing, and after disease resolution, they would be followed at a high-risk pregnancy clinic (HRPC). From January 1 to April 10, 2016, the total # of SDF patients was 29, 841, 43% of the total # of ER patients (69,777); the peak of SDF patients was seen on February (12,668). Only 101 patients (0.34%) of SDF were admitted to the hospital; there was one death of a woman with adrenal insufficiency. Eight hundred thirty seven NS1 tests were done, with 120 (14%) positive. One hundred twenty six PW presented to the ER with rash and/or SDF; six were NS1positive, 23 with DF IgM; 22 (17% of total) had Zika RT-PCR results released by the State Reference Laboratory for Zika (13 positive and 9 negative). There were two spontaneous abortions in both groups; 91 women (72%) are still seen at the HRPC with no report of microcephaly so far. DF disrupts ER routines in our region; it is possible that many SDF patients had in fact Zika but because tests were not available except for PW, all were treated as SDF. Health services in our region need to be prepared to endure dengue and Zika epidemics in the next years.

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ACCESSING HEALTH CARE IN VENEZUELA: A COMMUNITY BASED STUDY ON HEALTH CENTER ATTENDANCE FOR DENGUE AND FEVER

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Dengue is a major public health problem in Venezuela. Timely health centre (HC) attendance is crucial in reducing mortality and severity of dengue. The health care system in Venezuela comprises a public and a private sector. The public system includes the traditional primary/secondary (Ambulatorios), and tertiary level HCs (Hospitals) and is usually free of charge. To improve limited access to care for the poorer, a parallel public health care system ("Mission Barrio Adentro") was set up in 2003. We assessed the intended HC attendance in the case of fever and suspected dengue in an urban area of high dengue transmission. Between September 2013 and February 2014 a crosssectional household survey was performed in Maracay, Venezuela. Intended HC attendance and the perceived barriers in the case of fever and dengue of adults and parents/guardians of children were assessed. Data was collected through structured questionnaires from 105 individuals. We show that people would visit several different HCs if needed, and that the health preferences differed throughout the community. The most frequent first choice of health centre was an Ambulatorio, in the case of fever (n=82; 78.8%) and dengue (n=84; 80.8%). Several economic, ethnic, logistic, and quality aspects influenced the preference to access the HCs. Individuals preferred to first attend traditional HCs as they trusted the care given at these institutions, but a barrier was the lack of treatment supplies. Although the lack of supplies was mentioned to a lesser extent in the case of the parallel HCs, people reported not to trust the medical staff, nor the diagnosis and treatment given in these HCs. Furthermore, the private care, which was considered best, was mainly accessible for those with a health insurance. A higher education (fever/dengue: p=0.001/p=0.001) and a nonmanual occupation (fever/dengue: p=0.007/p=0.016) were associated with more intended private HC attendance. Access to care in Venezuela is currently a complex situation where individuals need to juggle between the different available public and private HCs in order to obtain proper/timely care and medical supplies.

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IDENTIFICATION OF CLINICAL AND LABORATORY PARAMETERS THAT DISTINGUISH BETWEEN DENGUE AND NON-DENGUE ILLNESS WITHIN THE FIRST 72 HOURS OF FEVER

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As of March 2016, the prospective multicentre IDAMS study has enrolled 7178 out-patients aged ≥ 5 years in 8 countries across Latin America and Asia. Participants with non-specific febrile illness are enrolled within 72 hours of fever onset and followed daily with a common protocol, a key objective being to identify simple clinical and/or laboratory parameters that distinguish between dengue and other febrile illness (OFI) thus leading to an evidence-based case definition. Laboratory diagnosis relies on an algorithm including PCR, NS1, and IgM seroconversion, all performed following strict protocols. We will analyse clinical and laboratory parameters at enrolment as predictors for confirmed dengue by multivariable logistic regression and flexible classification algorithms. The full analysis will be carried out stratified by age group, day of illness and country/ continent, and subsequently pooled if appropriate. To improve model prediction we will also include changes between enrolment and the following day, thus basing the assessment on two time points. Data is currently available on 5078 Asian participants originating from Vietnam, Malaysia, Cambodia, Bangladesh and Indonesia, with data from Latin America (Brazil, Venezuela, and El Salvador) due to be added after the study closes in June 2016. In the preliminary pooled analysis, adjusting for age group and day of illness at enrolment, the presence of skin rash and skin flush, anorexia, dizziness, diarrhoea and conjunctival injection at enrolment was associated with a diagnosis of dengue. The strongest association was found for skin bleeding and low platelet count at enrolment. The presence of sore throat, cough, and rhinitis was associated with OFI. However, more than 20% of patients with these symptoms had laboratory confirmed dengue, highlighting the role of dengue in the differential diagnosis when upper respiratory tract symptoms are present. The findings of the full analysis will be presented and are expected to have an important impact on diagnostic algorithms for dengue in settings where confirmatory laboratory testing is not possible.

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THE ASSOCIATION BETWEEN DENGUE PRE-EXISTING ANTIBODY ON ZIKA VIRUS INFECTION IN THP-1 MONOCYTES CELL LINE

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Antibody-dependent enhancement of infection (ADE) is postulated as one factor contributing to dengue severe disease such as dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS). Pre-existing antibody against heterotypic dengue serotypes binds to the virus but does not neutralize it. Thereby, enhancing DENV infection of Fcγ receptor bearing cells increasing the number of viral infected cells and the amount of virus produced. DENV has routinely showed cross-reactivity in serological assays with Zika virus (ZIKV) and co-infections of DENV and ZIKV have been reported. Here we report the effect of pre-existing DENV antibody on the ability of three different strains of ZIKV (African and Asian genotypes) to infect THP-1 monocytes cell line. The mean amino acid sequences homology between DENV and ZIKV showed 42.9%, 42.5% and 57.0% identical for capsid, prM and E regions, respectively. The growth of ZIKV in the presence of pre-existing DENV antibody was higher than that in the absence of DENV antibody. Immune mediators involved virus infection were also investigated. ADE-induced ZIKV infection had higher production of IL-12, a pro-inflammatory cytokines than in the absence of DENV antibody. Down-regulation sensors including RIG-I and MDA-5 are waiting to be elucidated. As DENV vaccines have been licensed in endemic areas of both DENV and ZIKV, an understanding of the pre-existing DENV antibody effect on other flavivirus is necessary.

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PREPARATION OF STANDARD ZIKA VIRUS (ZIKV) IGM FOR SEROLOGICAL DIAGNOSIS OF ZIKV INFECTION

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Laboratory diagnosis of acute Zika virus (ZIKV) infection during the febrile phase of illness currently depends on detection of the virus by RT-PCR. A specific IgM serological assay for ZIKV has yet to be developed. Here we describe the development of a specific anti-ZIKA IgM ELISA for the diagnosis of acute ZIKV infections. To develop a specific anti-ZIKA IgM antibody to use as a positive control for the assay, the ATCC strain of ZIKV was subcutaneously injected into a Rhesus monkey at a dose of 5x106 PFU. Blood was collected from the monkey daily from 0 to 12 days after infection and on days 15 and 30. Viremia was determined by RT-PCR and IgM/IgG antibody kinetics was determined by EIA form blood collections. The data show ZIKV was detected by PCR on days 1, 2, and 3 with the highest peak occurring on day 2. ZIKA specific IgM was detected starting on day 6 and continued through day 30 with a peak from days 10 to 15. To determine the specificity of the assay we tested known positive cases of DENV, JEV and ZIKV to establish the amount of cross-reactivity from previous infections. The following samples were used to test the specificity of the ZIKA ELISA: 9 cases of primary DENV, 10 cases of secondary DENV infection, 10 cases of JEV infection, 3 cases of ZIKV infection and 7 cases of negative specimens. The cut off value for the anti-ZIKV IgM/IgG EIA was set at ≥40 EIA units. The results showed that primary DENV infection and samples testing negative for other flaviviruses tested negative for anti-ZIKA IgM. However, 1 out of 10 secondary DENV infections and 2 of 10 JEV infections tested positive for ZIKV IgM most likely due to cross-reactive antibodies. These data indicate that detection of acute ZIKV infections in patients previously exposed to flavivirus could produce false positive results under certain conditions but should be useful in detecting ZIKV in patients with primary flavivirus infections.

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CO-CIRCULATION OF ZIKA, CHIKUNGUNYA AND DENGUE VIRUSES DURING DENGUE OUTBREAK IN SUMATRA, INDONESIA

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Arthropod-borne viruses (arboviruses) are known to cause significant public health problems throughout the world. Indonesia has been affected by the arboviral diseases such as dengue for decades with frequent epidemic cycles. In 2015, dengue cases increased significantly in Jambi municipality in Sumatra with the number doubled from previous year. To understand the dynamic of the disease, we conducted dengue molecular study in Jambi. Sera were collected from dengue-suspected patients and dengue diagnosis was performed using NS1, IgG/IgM, and RT-PCR detection. Of 210 dengue-suspected patients, 107 were confirmed dengue based on NS1 and RT-PCR. All four dengue virus (DENV) serotypes were detected with DENV-1 as the predominant serotype (66%). To determine the disease etiology of the 103 dengue-negative cases, we screened the samples using RT-PCR for other viruses which include flavivirus and alphavirus families. Among them, we detected eight cases were infected by Chikungunya viruses (CHIKV) and one by Zika virus (ZIKV). All viruses were successfully isolated and propagated in tissue culture. The clinical manifestations of the Chikungunya and Zika patients were mild and mimicking dengue symptoms. To determine the genotypes the viruses, we sequenced the Envelope, E1, and NS5 genes of DENV, CHIKV, and ZIKV, respectively. Phylogenetic analyses revealed the DENV-1 viruses belonged to Genotype I, DENV-2 was of Cosmopolitan genotype, DENV-3 as Genotype I, and DENV-4 belonged to Genotype II. The CHIKV isolates were grouped as Asian genotype, while the ZIKV was classified as Asian lineage. Our finding demonstrates the co-circulation of multiple arboviruses during dengue outbreak in Indonesia. This co-circulation will likely to contribute to a large neglected disease burden and causes misdiagnosis and underreporting of these diseases. It is essential that systematic surveillance be implemented to evaluate and monitor the distribution these arboviruses infections and its potential public health problems in Indonesia.

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FROM DENGUE TO ZIKA—TAIWAN'S EXPERIENCE

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Dengue and Zika viruses involve common vectors - *Aedes* (Ae.) aegypti and Ae. albopictus - both widespread in Taiwan. While the epidemics of dengue in Kaohsiung and Tainan in 2015 have been the largest and most severe, one imported Zika case from Thailand was detected in January 2016, indicating that future public health threat of Zika virus can't be ignored. Epidemiology of dengue in Taiwan involves three types: (1) sporadic cases in Taipei where only Ae. albopictus are present, (2) an epidemic type in Tainan with a lower ratio of *Ae. aegypti* /Ae. albopictus, and (3) DENV-endemicity in Kaohsiung with higher ratio of *Ae. aegypti*/ Ae. albopictus. The specific aims of this study were: (1) to compare epidemiology of dengue in Taipei, Tainan, and Kaohsiung, (2) to investigate the impact of daily meteorological factors on mosquito populations and dengue cases, and (3) to address recommendations for prevention and control measures. Among 43,784 total laboratory-confirmed dengue cases in 2015, 43,419 were indigenous cases (99.16%). Most of them were in Tainan (22,777 cases, 52.4%) [predominantly dengue serotype 2 virus (DENV-2)] and Kaohsiung (19,784 cases, 45.6%) [predominantly DENV-1 in the beginning but subsequently turning to DENV-2]. Taipei had 128 confirmed cases, in which 70 were indigenous DENV-2 cases (0.2% of total in Taiwan), with 21 cases from Kaohsiung, and 46 cases from Tainan. About one month after typhoon Soudelor hit, dengue cases of DENV-2 peaked in Tainan, while Kaohsiung still had mostly DENV-1 cases. Interestingly, after the 3-month window period of cross-protection of different serotypes, DENV-2 cases started to climb and peaked about one month after typhoon Dujuan. Taipei, however, with Ae. albopictus only, was least impacted by the typhoon. In conclusion, *Ae. aegypti* plays the most crucial role in dengue transmission, so reducing the population of *Ae. aegypti* is the key element in preventing dengue and Zika infections. The lessons from dengue epidemics in southern Taiwan in 2015 provide an important future direction for the elimination of Ae. aegypti.

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POTENTIAL USE OF SALIVA SAMPLES FOR DIAGNOSIS OF ZIKA VIRUS INFECTION

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Zika virus (ZIKV) is a mosquito-borne flavivirus first isolated in Uganda, in 1947. Since then, sporadic cases of human ZIKV infections were reported in Africa and Asia, but the first ZIKV outbreaks occurred in the last decade, in the Pacific Ocean region. Late in 2014, large outbreaks of acute exanthematous illness (AEI) were reported from various Northeast states of Brazil, and, in April 2015, ZIKV was identified as the etiologic agent. ZIKV diagnosis is challenging, because serological methods is not specific as a consequence of IgM cross reactivity between Flaviviruses. Currently, molecular techniques, such as conventional or real time reverse transcriptase–polymerase-chain-reaction (qRT-PCR), are the most used methods to diagnosis ZIKV. ZIKV RNA is usually detected by RT-PCR in serum samples, but use of alternative samples has already been described. ZIKV RNA has been found in saliva in concomitance with either blood or urine. There are also a few studies describing viral RNA amplified only from saliva. The objective of this study was to investigate the potential use of saliva samples as an alternative for diagnosis of ZIKV infection. In June 2015, nine patients assisted in an emergency health unit of Salvador, Brazil due to an AEI suggestive of ZIKV had both saliva and serum samples collected after two to five days of symptoms onset. Samples were subjected to RNA extraction using the QIAamp Viral RNA Mini Kit (QIAGEN) and ZIKV qRT-PCR described by Lanciotti et al (2008) using the QuantiTect Probe RT-PCR Kit (QIAGEN). Zika RNA was detected in four of nine samples of saliva (Ct values < 38.5). All serum samples were negative. Our findings coincide with that of prior studies and suggest that qRT-PCR performed in saliva samples may have greater sensitivity compared to serum. In addition, obtaining saliva is easier than serum, particularly in newborns or in remote places where medical facilities are lacking. Further studies with larger number of specimens are needed to confirm our findings, but given the current evidence we suggest that in situations where a blood sample cannot be collected, the use of saliva should be considered.

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THE REEMERGENCE OF ZIKA VIRUS: FROM ARTHROPOD VECTOR TO POSSIBLE SEXUAL TRANSMISSION

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Zika virus, first described in central Africa in the late 1940s, has attracted considerable attention in recent months. Over more than six decades, researchers have carefully described its arthropod-mosquito vector, its pathogenicity, and its structure. During the past year, however, numerous studies have suggested the possibility of non-arthropod transmission via sexual intercourse. Indeed, health authorities have cautioned women in high-risk areas to avoid pregnancy because of the risk of fetal microcephaly. Sexual transmission of Zika virus, of course, is quite plausible. Clearly, a number of viral pathogens, most notably HIV, can be transmitted via sexual intercourse. This study will analyze what is—and has been—known about sexual transmission of Zika virus. It will also examine ongoing public efforts to prevent its transmission.

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SERODIAGNOSIS OF ZIKA IN TRAVELERS RETURNING FROM FLAVIVIRUS-ENDEMIC REGIONS

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Zika virus (ZIKV) has disseminated throughout Latin America and is a major public health concern due to an association with adverse pregnancy outcomes such as miscarriage and microcephaly as well as an increased incidence of Guillain-Barré syndrome. Many of the urgent needs (including diagnostics, surveillance, vaccine development, and pathogenesis studies) in responding to this epidemic hinge on a clear and detailed understanding of the human antibody (Ab) response to ZIKV. It is well known that humans produce flavivirus cross-reactive Ab following natural infection and that these responses can confound interpretation of serology during a secondary flavivirus infection - a highly relevant phenomenon in Latin America where dengue (DENV) seroprevalence can exceed 90%. Serodiagnosis is particularly essential in the ZIKV epidemic as the window for diagnosis by molecular methods is narrow and the majority of new infections are asymptomatic. To address these issues and develop critical tools for study of humoral immunity to ZIKV, we recruited travelers reporting potential exposure to arbovirus infection and tested serum samples for reactivity to DENV and ZIKV. Depending on an individual's travel history and country of origin, we find a variety of patters. Some sera samples bind and neutralize a single virus; others demonstrate broad cross-reactivity. Several primary ZIKV infections were identified in those reporting an acute febrile illness during or after travel to Brazil or Colombia in 2015. This well-characterized set of sera serve as a standard of comparison for novel serodiagnostics that we are developing and deploying to ZIKV-endemic regions. Human monoclonal Ab derived from memory B cells of participants with primary ZIKV infection will be used to map binding determinants of ZIKV-specific and flavivirus cross-reactive Ab and define epitopes targeted by neutralizing Ab.

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GUILLAIN-BARRÉ SYNDROME OUTBREAK - BAHIA STATE, BRAZIL, 2016

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In mid-2015, reports of Guillain-Barré syndrome (GBS) increased in certain regions of Brazil. These reports coincided with the introduction and rapid spread of Zika virus in Brazil, and geographic areas with the highest reports of Zika-like illness and GBS overlapped. The Brazil Ministry of Health and CDC performed an investigation to identify risk factors and potential infectious pathogens associated with GBS. We conducted a case-control investigation in the Salvador metropolitan area, Brazil. We defined GBS cases according to the Brighton Collaboration criteria. Two controls matched by age group were randomly selected from the same neighborhoods as the cases using modified WHO cluster survey methodology. We conducted in-person interviews to obtain risk factor and exposure (environmental, food/water) histories in the 2-month period prior to GBS-case onset. Of 77 suspected GBS case-patients, 50 (65%) met Brighton case definition criteria. The incidence of GBS during April-July 2015 was approximately 12-times higher than expected. Among 41 enrolled GBS case-patients and 85 controls, there were no differences in demographic or exposure data. A higher proportion of GBS cases compared to controls reported an antecedent illness (88% versus 21%, P<.01), with rash and conjunctivitis being reported by 71% and 56% of GBS case-patients, respectively, versus 39% and 22% of the controls (P<.01). Arboviral testing is underway for serum samples collected from all cases and controls. Our investigation identified increased incidence of GBS case-patients occurring in tight geo-temporal clustering in the Salvador area during mid-2015. Many GBS case-patients reported an exanthematous illness during a time of recognized Zika transmission in Salvador, suggesting a possible association between GBS and Zika virus infection. Further surveillance for GBS, additional case control studies, and refined Zika virus laboratory diagnostics are needed to substantiate this possible association.

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THE DIFFERENTIAL IMPACT OF YELLOW FEVER VACCINE ACROSS TRANSMISSION CYCLES: ACCOUNTING FOR HERD IMMUNITY IN THE FACE OF ZOONOTIC TRANSMISSION

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Yellow Fever (YF) is a mosquito-borne flavivirus infection, its major burden is concentrated in sub-Saharan Africa. Two transmission cycles co-exist: a sylvatic cycle in non-human primates causing zoonotic spill-over infections in humans and an urban cycle perpetuated in human populations, with intermediate transmission among both humans and non-human primates also playing a role. The relative contribution of these cycles to the human burden of YF is not well understood. After a period of re-emergence, implementation of large mass vaccination campaigns started in 2006 in the most affected West African countries. Mathematical modelling is currently used to inform these vaccination activities. However, vaccine impact estimates differ depending on assumptions about transmission cycles. In urban transmission, herd immunity effects increase the impact of vaccination compared to the sylvatic cycle. To address this issue, we constructed two alternative versions of a model estimating YF burden,

assuming either 100% zoonotic or 100% inter-human transmission. Both models were fitted to reported outbreaks from 1984-2013 using environmental and demographic variables across the endemic zone of Africa, and were calibrated with serological survey data. Over the 1984-2013 period, both models estimated a very similar cumulative YF burden across Africa. However temporal trends in the burden differed between both models. Notably, the inter-human model estimated a lower burden than the zoonotic model for the period corresponding to recent vaccination activities (2006-2013). Over this period, vaccination was estimated to have prevented 4.5x105 (95% CI: 1.5x105 - 10.4x105) deaths according to the zoonotic and 21x105 (95% CI: 8x105 - 45x105) according to the inter-human model. This corresponds to an estimated 2.2 (95% CI: 0.7 - 5.0) and 10.0 (95% CI: 4.0 - 22) deaths averted per 1,000 vaccine doses, respectively. Integrating herd immunity into the model thus strongly influences vaccine impact estimates. Further efforts to assess the relative contribution of both transmission cycles are needed.

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FULL GENOME SEQUENCING OF ZIKA VIRUSES USING A TARGETED AMPLIFICATION APPROACH

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The recent outbreak of Zika virus highlights the importance of having access to sensitive molecular tools for diagnosis and identification of circulating viral strains. Next-generation sequencing, and shotgun sequencing in particular, are powerful tools that can be used to generate complete viral genomes even in the absence of any a priori knowledge about the virus of interest. As such, shotgun sequencing is usually one of the first tools utilized to generate complete viral genomes from clinical samples. Unfortunately, viruses in clinical samples can be present in small amounts, and as such, these "low viral load" samples are often plagued by high signal-to-noise ratios that prevent the generation of complete viral genomes. One way around this issue is to culture the virus in order to increase viral load. However, passaging viruses through laboratory cell lines can have the unintended consequence of introducing mutations that are not observed in the wild. Here, we report a set of 22 Zika-specific primers that can be used to generate overlapping amplicons by PCR to cover the entire Zika genome even in samples with low viral load. Given that PCR amplification efficiency is directly related to template concentration and to amplicon size, samples where Zika is present in low abundance can be fully covered by as many as 11 overlapping segments (of about 1 KB each), whereas samples with high Zika abundance can be fully covered by as few as 5 (of about 2.5 KB each). An added advantage of this targeted amplification approach is that although the overlapping amplicons can be used as starting material for next-generation sequencing libraries, sequencing can also be completed using Sanger methods. As such, this approach may be useful for epidemiological surveillance in low resource settings that may not have access to the latest next-generation sequencing technologies.

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ZIKA VIRUS PERSISTENCE IN BODY FLUIDS AMONG PATIENTS WITH ZIKA VIRUS INFECTION IN PUERTO RICO

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Little is known about the presence or duration of Zika virus (ZIKV) in body fluids other than blood. Understanding the presence and duration of ZIKV detection in urine and saliva may facilitate diagnostic testing. Information about ZIKV in semen and vaginal secretions is urgently needed to inform prevention messages to prevent sexual transmission. Moreover, the relationship between development of anti-ZIKV antibodies and the presence and duration of ZIKV in body fluids other than blood has not been described. To investigate the persistence of ZIKV in body fluids and its relation to immune response, we are conducting a prospective cohort study of patients with laboratory-confirmed ZIKV infection. Patients with rash, fever, arthralgia, or conjunctivitis that present to an out-patient clinic or hospital emergency department in Ponce, Puerto Rico will be offered participation in the study. For those that consent, demographic and clinical characteristics will be collected in addition to blood, nasopharyngeal, and urine specimens. Patients in which ZIKV nucleic acid is detected by RT-PCR in any specimen will be invited for follow-up, in which blood, saliva, and urine specimens will be collected; among participants 21 years and older, semen or vaginal secretions will also be collected. All specimens will be tested for the presence of ZIKV nucleic acid by RT-PCR, and positive specimens will be further tested for virus isolation to evaluate the presence of infectious virus. Each body fluid will be collected on a weekly basis for 4 weeks and biweekly thereafter until two consecutive negative RT-PCR results are obtained from all specimens. Irrespective of RNA detection, body fluids will also be collected for RT-PCR at 2, 4, 6 and 9 months to investigate intermittent shedding. All blood specimens will also be tested by anti-ZIKV IgM and IgG ELISA. Results will be used to update relevant counseling messages and recommendations from the CDC. At the conference we will present preliminary results regarding the antibody response and RNA persistence in body fluids. Only results for body fluids for which sufficient sample size and follow-up will be presented.

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EPIDEMIOLOGIC INVESTIGATIONS OF GUILLAIN-BARRÉ SYNDROME INCLUDING CASE-CONTROL INVESTIGATION TO DEFINE ASSOCIATION WITH ZIKA VIRUS INFECTION — PUERTO RICO, 2016

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Guillain-Barré syndrome (GBS) is a post-infection autoimmune disorder characterized by motor weakness, sensory abnormalities, and/or autonomic dysfunction due to peripheral nerve damage. In February 2016, Puerto Rico Department of Health (PRDH) reported its first case of GBS with evidence of Zika virus (ZIKV) infection. Given reported increased GBS incidence in ZIKV-affected regions, PRDH aimed to calculate GBS incidence, implement a GBS surveillance system, and investigate the association between GBS and ZIKV infection. Medical records at 8 hospitals for patients admitted with suspicion of GBS during 2012-2015 were evaluated using Brighton Collaboration criteria for GBS diagnostic certainty. Of 140 patient records reviewed, 61 (44%) met the confirmed case definition (Brighton levels 1-3). By applying the proportion of confirmed cases to 2013 Puerto Rico medical insurance claim data, 2013 incidence was estimated at 1.6 cases per 100,000 population. For GBS surveillance, neurologists and hospital infection control staff report suspected cases using a case report form and submit specimens for ZIKV diagnostic testing by reverse transcription-polymerase chain reaction (RT-PCR) and anti-ZIKV immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA). During January 1-April 11, 2016, 14 suspected GBS cases were reported, of which 6 (43%) had anti-ZIKV IgM antibodies in serum. Of these, median age was 42 years (range = 32-68) and 3 (50%) were male. All 6 patients were residents of ZIKV-affected municipalities in eastern Puerto Rico. A prospective case-control investigation is underway whereby cases are matched to two controls by age and location of residence. Data on behaviors, exposures, and recent illnesses are collected; RT-PCR and IgM ELISA is conducted on blood specimens, and RT-PCR on urine and saliva specimens. Results of the case-

control investigation are pending. The 2013 GBS incidence provides a baseline to assess trends in GBS cases reported to PRDH during ongoing ZIKV transmission. The case-control study will prospectively recruit cases to better define the association between GBS and ZIKV infection.

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CHARACTERIZATION OF ZIKA VIRUS INFECTIONS AND THE POTENTIAL EFFECT OF PRIOR DENGUE VIRUS EXPOSURE IN CHILDREN IN MANAGUA, NICARAGUA

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The first evidence of Zika virus (ZIKV) emergence in the Americas was documented in Northeast Brazil in May 2015. Since then, 35 countries and territories in the Americas have reported autochthonous transmission of ZIKV. In Nicaragua, the first case was reported in January 2016, and by April 2016, 130 cases had been identified by the national surveillance of the Ministry of Health. We are currently analyzing the epidemiology and clinical presentation of Zika in a prospective, community-based pediatric cohort study of dengue and chikungunya in Managua, Nicaragua. This study, ongoing since 2004, follows ~3,500 children aged 2-14 in a low- to middle-income area of Managua. Suspected Zika, chikungunya, and/or dengue cases and cases with undifferentiated fever are screened for ZIKV infection. Blood, saliva and urine samples are collected from suspected cases during the acute phase and at convalescence. Screening is conducted by real-time RT-PCR using the CDC protocol and a triplex ZIKV-CHIKV-DENV (ZCD) assay developed at Stanford University and validated in Nicaragua. From January to April 2016 (the dry season is March-May), 8 Zika cases were identified in the cohort (5 female and 3 male). Median age at presentation was 11 years (range: 7-14). One case required hospitalization. Six participants were DENV-naïve and two had had a previous DENV infection. As ZIKV transmission increases in the next rainy season, we will describe the natural history of ZIKV infection in our study populations and detection of ZIKV in blood, saliva and urine. In particular, clinical follow-up will continue after the acute phase, and participants requiring hospitalization and/or neurological examination will be transferred to our study hospital. We will also study potential immune enhancement between DENV and ZIKV infections. In our cohort, we will be able to compare ZIKV infection outcomes in participants with no, one or >2 previous DENV infections. Finally, as current serological assays cannot easily differentiate DENV and ZIKV, in particular if the patient has had a previous flavivirus infection or vaccination, we are developing and assessing new serological methods.

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THE SEASONAL INFLUENCE OF CLIMATE AND ENVIRONMENT ON YELLOW FEVER TRANSMISSION ACROSS AFRICA

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Yellow fever (YF) is a vector-borne, viral haemorrhagic fever endemic to Africa and Latin America with 90% of the burden occurring in Africa, transmitted primarily by *Aedes* spp, with *Ae. aegypti* the main vector for urban YF. Mosquito life cycle and viral replication in the mosquito heavily depend on climate indicators, particularly temperature and rainfall. We aimed to assess whether seasonal variations in climatic factors are associated with the seasonality of YF reports. We constructed a temperature suitability index for YF transmission, capturing the temperature dependence of mosquito life cycle and viral replication within the mosquito. We then fitted a series of generalised linear models to a dataset of YF reports across Africa, taking into account location and seasonality of occurrence for seasonal models and using the temperature suitability index, rainfall and the Enhanced Vegetation Index (EVI) as covariates alongside further demographic indicators. Model fit was assessed by the Area Under the Curve (AUC), and models were ranked by Akaike's Information Criterion. The seasonal interaction between temperature suitability and rainfall explained the seasonal and geographical patterns of YF occurrence well (AUC = 0.84), although models also including EVI performed significantly better (AUC = 0.88, $p < 0.001$). Despite the lower performance of the interaction of temperature suitability and rainfall compared to the EVI as a predictor of YF reports, the former offers a mechanistic explanation for the spatio-temporal variability and therefore enhances our understanding of the factors influencing YF transmission intensity. The description of seasonality in the transmission of YF opens up the possibility for the development of "early-warning" systems for outbreaks, which could facilitate the allocation of resources and guide vector-control programmes particularly if the insights gained here are combined with seasonal weather forecast data.

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ZIKA VIRUS: KNOWLEDGE GAPS AND VACCINE RESEARCH AND DEVELOPMENT CHALLENGES

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The recent emergence of Zika virus as a cause of severe disease has mobilized public health agencies, as well as commercial organizations, to embark on efforts to develop new approaches for combating this infection. Zika, a mosquito-borne flavivirus, has only recently been associated with severe disease in humans, including a variety of neurological complications that include microcephaly, congenital cerebral embryopathy and Guillain-Barré syndrome associated with an unprecedented spread of the virus. These links prompted the World Health Organization to list Zika as a "Public Health Emergency of International Concern" and the US CDC has now declared that Zika is a cause of microcephaly and other severe fetal brain defects. As exemplified during the recent Ebola outbreak, international and regional collaborations will be required to advance our understanding of Zika and to accelerate vaccine research and development. Potentially significant challenges for vaccine R&D have been identified, including; unknown incidence rates for clinical complications; while neutralizing antibodies will likely mediate protection, the level required is not yet known; the complexities of symptomatic and asymptomatic infection leading to clinical trials needing to demonstrate efficacy against disease/viremia and possibly fetal transmission for registration, and the development of diagnostic assays that avoid cross-reactivity with other flaviviruses. Sanofi Pasteur has initiated vaccine research and development activities to rapidly proceed through preclinical assessment into clinical evaluation. Assay optimization, animal model development and process improvements, as well as further disease understanding through potential surveillance of subjects participating in the follow up of the dengue efficacy study in Latin America, constitute the current focus of our work. Sanofi Pasteur's experience in licensed flavivirus vaccines will be leveraged against Zika vaccine development. Available results and progress will be presented.

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CO-INFECTION STUDIES BETWEEN WEST NILE VIRUS AND CULEX FLAVIVIRUS DETERMINE AN INHIBITORY EFFECT ON WEST NILE VIRUS INFECTION

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Vector-borne diseases remain a major public health concern as new diseases are emerging and previously controlled diseases are now resurging. West Nile virus (WNV) is a mosquito-transmitted flavivirus. Since its introduction in North America in 1999, it was spread rapidly to Central and South America. In Argentina, WNV was isolated in 2006; however, evidence of circulation by neutralizing antibodies in birds was detected by 2004. Still, human cases are scarce; interactions between vector, virus, and environment could be playing a role to explain it.The high rate of prevalence of Culex flavivirus (CxFV), an insect-specific flavivirus, detected in Argentina in previous studies, determined the importance to develop co-infection studies with WNV. For that purpose, *in vitro* studies were conducted in order to assess the potential of CxFV for blocking WNV infection. Co-infection assays using WNV and CxFV with different multiplicity of infection (MOI) in C6/36 cells from *Aedes albopictus,* were performed during 7 days. WNV titers were analyzed by plaque titration on 12-well plates of Vero cells.Results indicated that concurrent infection with CxFV resulted in a significant reduction in virus production of WNV. An inhibition of 100 fold reduction for WNV in presence of CxFV was detected. Reduction was statistically significant at 1 days post infection (dpi) and at 7 dpi using MOI of 0.1 for both viruses. Interesting, when MOI of CxFV was 10 or 100 times higher than MOI of WNV, reduction was statistically significant for every dpi. These results are in concordance with previous studies from other authors; co-infection resulted in suppression at higher total MOI, as a result of competition since the degree of resource depletion increases with MOI. This highlights that CxFV could be interfering in transmission of other flaviviruses of medical importance such as WNV. These results could explain, in part, the low level of transmission of WNV in Argentina. However, it will be necessary *in vivo* assays in order to evaluate this hypothesis. The results that are presented remark the potential interaction between flaviviruses.

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WEST NILE VIRUS INFECTION IN HUMAN AND MOUSE CORNEA TISSUE

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The purpose of this study was to determine the *in vitro* and *ex vivo* susceptibility of human corneal cells to West Nile virus (WNV) infection and evaluate the ability of the virus to disseminate to the corneas of infected mice. Human corneal epithelial cells were challenged with WNV, incubated for 1 to 6 days, and tested for evidence of WNV infection. Viral RNA and antigen were detected at every time point and the virus reached a peak titer of 2.5 x 10^7 plaque-forming units per milliliter (pfu/ml) at 3 days post-inoculation (PI). Corneas procured from donors were incubated in culture dishes containing WNV for 1 to 5 days and tested for evidence of WNV. Viral RNA and antigen were detected and the virus reached a mean peak titer of 4.9 x 10^4 pfu/ml at 5 days PI. Mice were inoculated intraperitoneally with WNV, and their eyes harvested at 2, 5, and 8 days PI and tested for evidence of WNV. Viral RNA was detected in anterior segment tissues in 4 of 9 systemically infected mice as early as 2 days PI. We conclude that human corneal cells support WNV replication *in vitro* and *ex vivo*, and WNV may disseminate into the corneas of experimentally infected mice. These findings indicate that corneal transmission cannot be ruled out as a novel mode of human-to-human WNV transmission and additional experiments should be conducted to assess this risk further.

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INTEGRATING SATELLITE REMOTE SENSING AND MOSQUITO SURVEILLANCE TO PREDICT HUMAN ARBOVIRAL DISEASE

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Mosquito-borne diseases present both novel and persistent challenges to public health, and predicting human risk is a perpetual, difficult exercise in integrating various streams of data. While remotely sensed environmental data (e.g. temperature and moisture) are readily available and are relevant to mosquito ecology, they do not directly capture other essential factors such as the immunity status of reservoir hosts. Mosquito surveillance data (e.g. infection status) are better measures of these hidden aspects of the disease transmission cycle, but are less accessible and more difficult to incorporate into operational forecasts of human disease. We present a flexible framework for predicting a mosquito-borne disease in humans, combining large volumes of meteorological data and sparse measurements of mosquito infection status. The approach was applied to reported human West Nile virus (WNV) infections in South Dakota, USA, 2004-2016. Data from NASA's North American Land Data Assimilation System (NLDAS) characterized temperature, precipitation, etc. on a daily basis. Early-season mosquito infection status data, measured in relatively few of the state's counties, were used to estimate influences not perceptible remotely. A statistical model of human WNV based on a parsimonious set of covariates achieved excellent discriminatory and predictive power, and yielded a straightforward description of human risk. Namely, long-term, interannual trends in human disease were explained by early-season mosquito infection status, and short-term variations by temperature. Early-season predictions for 2015 and 2016 are compared to observations. Scenarios for 2017, conditioned on predictive climate models, are discussed.

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WEST NILE VIRUS SUSCEPTIBILITY OF AMERICAN SINGER CANARIES: A LABORATORY MODEL OF A HIGHLY SUSCEPTIBLE AVIAN SPECIES

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In North America, West Nile virus (WNV) was first detected in 1999 in the New York area, and has since spread across the continental U.S., southern Canada, Mexico, Central America, and South America. Significant mortality, caused by WNV, was recognized in corvids (ravens, crows, magpies, and jays), and population declines have been recorded in corvids, as well as other avian species. Experimental challenge of birds with WNV has demonstrated susceptibility ranging from low - no mortality following challenge and WNV viremia unlikely to infect feeding mosquitoes - to high -frequent mortality and viremia very likely to infect feeding mosquitoes. Using wild-caught birds the extreme of this range might be observed with doves and corvids, with house sparrows filling the mid-range of susceptibility. Using wild-caught birds to examine species susceptibility to WNV has several disadvantages including difficulty of capture, presence of potential co-infections, such as avian malaria, and the potential for stress hormone release due to confinement at a research facility. We examined the susceptibility of mature American singer canaries (*Serinus canaria*) for WNV and found the species to be highly susceptible to the virus. Birds were inoculated with 10^5, 10^2, and 10^1 plaque forming units of WNV and mortality was observed in all birds by 5 days post inoculation. Viremia was quantitated by referring RT-PCR Ct scores to a standard curve and was comparable to the level of viremia that developed in American crows (*Corvus brachyrhynchos*) experimentally infected with WNV. Using a plaque assay, WNV was quantitated in tissues

obtained from diseased birds that were euthanized and was similar to viral load reported in WNV challenged American crows. Histopathology revealed lesions in a variety of tissues with liver, spleen, and kidney most severely affected. Immunohistochemistry (IHC) staining was pronounced in spleen and kidney sections. Brain and heart tissue were unremarkable on histopathology, and IHC stained sections were negative. American singer canaries provide a useful laboratory model to study the effects of WNV on birds highly susceptible to the virus.

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POLYMORPHISMS AT THE NS3-249 LOCI ARE ASSOCIATED WITH ALTERED VECTOR COMPETENCE OF *CULEX PIPIENS* FOR LINEAGE 2 WEST NILE VIRUSES

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Lineage 2 (L2) West Nile viruses (WNV) have undergone recent geographic expansions, resulting in outbreaks in eastern and southern Europe. Most notably, L2 WNV outbreaks have occurred annually in Greece since 2010. Genetic analysis of mosquito pool isolates made annually from Greece since 2010 have demonstrated the presence of a conserved L2 genetic variant containing a proline at NS3-249. This same genetic mutation has previously been associated with increased viremia and mortality in American crows (*Corvus brachyrhynchos*) infected with lineage 1 WNV strains. In order to evaluate if NS3-249 polymorphisms can alter L2 vector competence phenotypes, three parental L2 isolates (with NS3-249H) were compared to a Greek isolate from 2010 (NS10; NS3-249P) in *Culex pipiens* and *Cx. quinquefasciatus* mosquitoes. A lower infection rate was observed for *Cx. pipiens* orally exposed to NS10 compared to the three parental L2 isolates (NS3-249H); however, a similar lower infection rate for NS10 wasn't observed in Cx. quinquefasciatus mosquitoes. NS10 also exhibited reduced dissemination and transmission rates compared to SA89, a South African L2 (NS3-249H). To assess whether the NS3-249 loci is a determinant of vector competence in *Cx. pipiens*, vector competence of SA89, NS10 and corresponding mutants generated containing reciprocal substitutions at the NS3-249 loci (His vs Pro) was assessed. A higher infection rate was observed with the NS10-His mutant compared to wild type NS10. No differences in rates of dissemination or transmission were observed. Reciprocally, no difference in infection rate was observed for the SA88-Pro mutant relative to WT SA89; however, dissemination and transmission were lower. Increased avian replication could offset the negative impact of the NS10 Pro mutation observed on mosquito infectivity. In contrast, a loss in dissemination and transmission efficiency observed for the SA89-Pro mutant likely precluded maintenance of this mutant. As such, these data indicate the potential role mosquitoes play in restricting the emergence potential of high avian fitness variant due to reciprocal fitness effect in mosquito vectors.

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EVALUATION OF DIFFERENT VIRAL ENRICHMENT METHODS FOR WEST NILE VIRUS RNA EXTRACTION IN BRAIN

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Physical virus enrichment is thought to increase sensitivity and enhance detection of viral diversity, but these techniques are limited using brain tissue. Physical virus enrichment is especially useful for deep sequencing investigations. The hypotheses that elimination of host brain RNA will enhance sensitivity of viral detection and render undetectable virus available to sequence was investigated. Specifically, since West Nile virus (WNV) causes grave clinical signs in the face of low to undetectable virus, the aim of this research was to develop repeatable methods for detecting and generating WNV sequences from archived brain tissues of horses in which virus was either not detected or in limited quantity. Different WNV viral RNA extraction methods and host RNA separation methods were investigated by conducting artificial inoculation of WNV into horse brain. Twenty-one horse brains were collected and small pieces of brain from each were inoculated with WNV NY99 strain (low passage to maintain diversity) and each of them underwent eight different RNA extraction protocols. The protocols utilized low-speed centrifugation, syringe filtration, and nuclease treatment with combinations of these. The WNV viral RNA was analyzed using real-time PCR targeting WNV Envelope (e) protein and equine G3PDH. Deep sequencing was also performed using Illumina® platform. Real-time PCR results showed that the more enrichment applied to a sample, the less viral and host RNA was obtained. Data obtained for sequencing showed that more enrichment treatments resulted in more reads. Although calculation of single nucleotide polymorphism and diversity is necessary for full analysis, these results demonstrate that additional steps utilized for extraction of brain do not enhance detection of WNV. Methods for phylogenetic and phylodynamic studies in RNA viruses need to be tissue specific.

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DEVELOPMENT OF A LIVE ATTENUATED WEST NILE VIRUS VACCINE

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West Nile Virus (WNV) is an emerging human neurotropic pathogen that targets the central nervous system (CNS). Recent work has shown that inserting a single copy of a target for brain-expressed microRNAs (miRNAs) in the 3' noncoding region (3'NCR) of the flavivirus genome abolished virus neurovirulence in the mature mouse CNS. Furthermore, studies showed that introducing mir-124a target sequence into a flavivirus genome completely abolished neuroinvasivness in immunodeficient mice. Here, we developed a chimeric WNV / Dengue 4 virus vaccine candidate containing mir-124a targets in the junction of E-NS1 genes and in 3' non-coding region. miRNA targeted WNV / Dengue 4 was attenuated in 3-day old Swiss mice infected IC and did not cause neurologic disease in type I interferon receptor deficient mice infected intraperitoneally with10^4 PFU. To determine if miRNA targeted WNV /DEN4 is immunogenic and protects against challenge with wild type WNV, we inoculated C3H mice intraperitoneally with 10^5 PFU. Virus induced a high level of serum WNV-specific neutralizing antibodies in each immunized animal 28 days following inoculation. Moreover, 100% of immunized mice survived the lethal WNV challenge. We conclude that our developed virus has potential to be used as a live attenuated vaccine candidate against WNV disease.

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CLINICAL FEATURES OF ADMITTED CHILDREN WITH LABORATORY-CONFIRMED EBOLA VIRUS INFECTION DURING THE 2014-2015 EBOLA OUTBREAK IN GUINEA

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Background:Ebola outbreak in 2014 in West Africa is the largest in history and children are the most vulnerable. In this study, we aimed to understand the clinical characteristics of admitted children with confirmed Ebola virus infection. Methods: We have analyzed the data from 30447suspected or probable cases were admitted in ETC in Guinea. Among them 3062 were EVD confirmed between January 2014 and May 2015. Results:9155 were children less than 16 years and 518 of them were confirmed for EVD in Guinea. We observed that the main symptoms

were fever (92%), fatigue (85.7%)and anorexia (75.3%). The lethality rate were 82.9% and 55.8% for less 5 and 6-16 age groups respectively. age les than 5 and hospitalization less than 7 days were associated with death. Conclusion: We observed that the risk of getting the Ebola disease was significantly lower in children under 5 years. In contrast, the fatality rate was a higher rate in the same group (82.9 %).

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HEPATITIS E SEROPREVALENCE AMONG BLOOD DONORS IN RWANDA

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Hepatitis E Virus is mainly transmitted by the fecal-oral route but other routes have been reported. It usually causes self limited disease but can cause fulminant hepatitis in pregnant women. The prevalence of hepatitis E viral infection among Rwandan adults has not been reported previously. We aimed to determine the seroprevalence of Hepatitis E Virus among adult Rwandans, its relationship with pork consumption and other risk factors. A cross-sectional survey was conducted between November and December, 2014 on 309 blood donors in Rwanda. A subsequent nested case-control study assessed exposures in seropositive cases and seronegative controls. Hepatitis E Virus testing was performed by detection of anti- Hepatitis E Virus IgG antibodies. Demographic data and information about risk factors for Hepatitis E Virus infection were recorded. The average age was 30.6 years with a male to female ratio of 4:1. 54% were farmers .The overall Hepatitis E Virus seroprevalence was 13.3%. Rates of anti- Hepatitis E Virus positivity were lower in the Eastern Province and Kigali city than the Southern Province (p=0.01 and p=0.003, respectively). An association was found between pork consumption and Hepatitis E Virus seropositivity (p=0.04).The rate of pork consumption positively correlated with the rate of anti-HEV seropositivity (p=0.01). The analysis did not show an association between Hepatitis E Virus seropositivity and the source of drinking water, status of drinking water , exposure to animals or exposure activity. Therefore Hepatitis E Virus seroprevalence among blood donors in Rwanda is high. Anti- Hepatitis E Virus IgG seropositivity is likely associated with pork consumption and may be more prevalent in some regions of Rwanda.

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SINU VIRUS, A NOVEL ORTHOMYXOVIRUS RELATED TO MEMBERS OF THE THOGOTOVIRUS GENUS, ISOLATED FROM MOSQUITOES IN COLOMBIA

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During the past decade, many novel insect-specific viruses have been isolated from characterized in mosquitoes and phlebotomine sandflies. These insect-specific viruses are widely distributed geographically and represent a number of different virus taxa, including Togaviridae, Flaviviridae, Bunyaviridae, Rhabdoviridae, Mesoniviridae, Reoviridae and Birnaviridae. Here we report a novel insect-specific orthomyxovirus, designated CoB 38d, isolated in C6/36 cells from mosquitoes collected northwestern Colombia. Genome sequencing of CoB38d revealed the presence of a hexa-segmented RNA virus (Segments 1 to 6). Genetic analysis of each RNA segment demonstrated the presence of six distinct ORFs encoding for the following genes: PBS (Segment 1), PB1, (Segment 2), PA subunit (Segment 3), envelope glycoprotein gene (Segment 4), Nucleoprotein (Segment 5), and Membrane gene (Segment 6). Multiple sequence alignment, using all RNA segments of CoB 38d, revealed low nucleotide and amino acid identity (<50%) with all other members of the Orthomyxoviridae family. Phylogenetic analysis using the polymerase subunit 1 (PB1) amino acid sequences showed that the isolate is most closely related to members of the Thogotovirus genus. Based on the geographic origin of the isolate and phylogenetic analyses, we propose the name of Sinu virus (SINUV) and show that it is a new member of the family Orthomyxoviridae, and possibly a new genus within the family.

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PREDICTING THE GEOGRAPHIC SPREAD OF THE 2014-2016 WEST AFRICA EBOLA VIRUS DISEASE OUTBREAK

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Between 2013 and 2016 West Africa experienced the most geographically extensive outbreak of Ebola virus disease (EVD) recorded to date. It affected all districts in Sierra Leone and Liberia and most of Guinea, resulting in more than 11,301 deaths among 28,603 reported cases. The outbreak spread from its origin in Meliandou, Guinea by movements of infected individuals across the region. Understanding how human mobility influenced the transmission dynamics of this epidemic is important in planning responses to future outbreaks. Using empirical data on human mobility, we model the effect of human movement on the geographic diffusion of the outbreak, as well as on the dynamics of the growth and decline phases of the epidemic within and between each country's districts, to identify areas that were the main exporters and importers of disease transmission. We identify considerable spatio-temporal heterogeneity in transmission that is driven by human mobility both locally and between regions. We show that by incorporating a range of different human mobility models we improve predictions of both spatial spread and the prediction of the epidemic's trajectory. These results provide a robust approach to predicting the geographic spread of future outbreaks. Such models are crucial information for surveillance and control strategies both in preparation for, and in response to, future contagious disease outbreaks.

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SEVEN YEARS OF INFLUENZA SURVEILLANCE IN PRAMONGKUTKLAO HOSPITAL, THAILAND FROM 2009 TO 2015

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Influenza poses a constant threat to military forces around the world due to close living quarters and other environmental factors. The Virology department, AFRIMS conducted flu surveillance at Pramongkutklao Hospital, Thailand in order to assess this threat. Patients who presented with ILI were considered for enrollment. Two respiratory specimens were collected from each consented patient, a nasal swab for performing rapid testing and throat swab for RT-PCR testing / virus isolation. From 2009 to 2015, a total of 6263 patients with ILI were enrolled. A total of 4526 patients were negative and 1736 patients were found to be positive for Flu by rapid testing. Through RT-PCR testing, 2045 (32.6%) of the specimens were found to be positive for Flu. Of the positives, 1309 (64%) tested positive for Flu A (H1, H3) while 735 (36%) tested positive for Flu B viruses. Of Flu A subtypes, A/H1N1pdm contributed 577 (28.2%) of all Flu A positives while A/H3 accounted for 732 (35.8%). A subset of RT-PCR positive specimens were tested by virus isolation and sub-typed by HAI. From 2045 specimens cultured 779 (38%) yielded virus and 139 (6.8%) specimens were positive for A/H1N1pdm, 223 (11%) specimens were positive for A/H3, 216 (10.5%) specimens were positive for B/Victoria Lineage and 174 (8.5%) were positive for B/Yamagata Lineage. There are 26 (1.3%) Flu A specimens that could not be subtyped. All HAI sub-typing results correlate with RT-PCR results. For the entire surveillance period, specimens positive for A/H1N1pdm peaked in 2010, accounting for 307 (15%) of the specimens evaluated. Specimens positive for A/H3 were most commonly found in 2015, 222 (10.8%) and those positive for Flu B were very common in 2010 and 2014, accounting for (9.8% and 9.3%) of evaluated specimens, respectively. It was found that some of patients testing positive for influenza by RT-PCR in each year had received the seasonal Flu vaccine (within the last year). The vaccine efficacy was between 70% and 86%, except in 2014, where the level of protection was 28%. Most of the patients who received Flu vaccination one year prior to 2014 acquired Flu B, with incidence as high as 44.5%.

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PERSISTENCE OF IMMUNE RESPONSE IN EBOLA ZAIRE SURVIVORS FORTY YEARS AFTER INFECTION

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Ebola virus (EBOV) is a zoonotic filovirus that can produce highly lethal disease in humans. Presentation is non-specific, characterized by vomiting, diarrhea, fever and bleeding. The first reported outbreak of Ebola Zaire (ZEBOV) occurred in 1976 in the northwestern part of the Democratic Republic of Congo in the Yambuku village (DRC, former Zaire). While there is no licensed EBOV targeted vaccine or treatment, there is a growing body of evidence that offers hope for finding ways to pharmacologically mimic or boost the natural resistance some individuals have to EBOV. To better understand long-term humoral immunity following EBOV infection, we obtained blood from 12 remaining survivors from the Yambuku outbreak to determine if antibodies to ZEBOV were retained 40 years post initial infection. Serum samples from survivors were screened and analyzed using Human Anti-Zaire Ebola Virus Glycoprotein (GP) IgG ELISA Assay kits (Alpha diagnostic International, Inc) in Kinshasa, DRC. Manufacturer procedures were followed for all incubation and washing steps. Cutoffs were calculated using the provided calibrator 1 and subtracting the background optical density (OD). OD values at 450nm (OD450) were recorded. Results demonstrate that samples obtained 40 years post infection contain virus specific antibodies. We identified 5 positive subjects (OD ≥ Cal 2.5OD), 5 weak positives subject (≥Cal 1.0 OD and <Cal 2.5 OD) and two negative subject (<Cal 1 OD). These results provide insight into the duration of humoral immune response against ZEBOV and open the door to in depth characterization of the immune mechanism against EBOV as well as further studies are needed to provide more details on long term sequelae of the Ebola virus disease.

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ASSESSMENT OF MISEQ NEXT GENERATION SEQUENCING PROCEDURE FOR VIRAL PATHOGEN DETECTION IN SOUTHEAST ASIA

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Southeast Asian (SEA) countries have recently reported an increase in the detection and spread of emerging diseases. Currently, Next Generation Sequencing (NGS) is becoming an important tool for detection and analysis of emerging diseases. NGS is a reliable and accurate method of detection for emerging viral pathogens and provides critical information during outbreaks and routine disease surveillance. The Department of Virology, AFRIMS, has developed standard operating procedures and viral panels for performing NGS to assess the ability of laboratories to achieve reliable, accurate and rapid turnaround times for the detection of viral pathogens. The viral panels consist of the following commonly found viral pathogens circulating in Southeast Asia: Zika virus (ZIKV), dengue viruses (DENV) serotype 1-4, influenza A/pdmH1N and A/H3N2, and influenza B Victoria and Yamagata. Laboratories were trained to follow the SOPs and given the viral panel to perform NGS sequencing and pathogen identification. The laboratories were unaware of the viruses included in the panel before performing sequencing and analysis. A total of four different laboratories from three different countries including Thailand (2 laboratories), Cambodia, and Philippines were trained and given viral panels for sequencing. The panels were tested by 21 laboratory personnel from the different laboratories using the MiSeq® (Illumina) sequencing platform. All 21 laboratory personnel correctly identified the viral pathogens represented in the panel. Suggesting results from different locations and personnel are highly reproducible after laboratory technicians are trained to follow NGS sequencing SOP.

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ACCEPTANCE OF THE EBOLA VIRUS VACCINE BY THE COMMUNITY IN GUINEA

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The rVSV-ZEBOV vaccine was tested as Prevention means to Ebola virus through a vaccine trial led by the WHO in the region of Lower Guinea between April and July, 2015. The preliminary results of this vaccine trial showed that the rVSV-ZEBOV vaccine was safe and effective in the prevention of the Ebola virus disease ten days after its administration. With the prospect of moving to scale up for a large part of the population, a study on acceptability of the vacccine was conducted to identify possible challenges. Interviews were conducted on the basis of a standard questionnaire administered to 209 people in Coyah, Forécariah, Kindia and Dubreka. Quantitative datas were entered in the Epidata version 3.1 software and then exported to the software Stata 13 (Stata Corp., Texas, USA) for analysis. Descriptive statistics and chi square test at 95% confidence interval were used to measure the association of selected variables in the study. Nearly 78% of the people interviewed agreed to be vaccinated and 73% of those interviewed were willing to encourage their close ones and relatives to take this vaccine. Nevertheless, 22% of the survey participants expressed doubts or had mixed feelings about the vaccine. The main concerns raised in the qualitative interviews conducted focused on the quality of the vaccine, the side effects of the vaccine, fear of being contaminated by Ebola during vaccination exercise and lack of adequate information. Knowledge of the existence of the new vaccine was higher in areas where the community was involved in response activities (Coyah and Forecariah) than in the areas where the community was not involved (Dubreka and Kindia). Similarly the willingness to be vaccinated (p= 0.003) or encourage relatives to do the same were higher in the prefectures of Forécariah and Coyah compared to the Prefectures Dubreka and Kindia (p = 0.011).The results of this study show that there is need for public information on the new Ebola vaccine, especially in prefectures where acceptance is low, by involving local authorities, community leaders and community-based organisations to conduct outreach sensitization outreaches.

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COMPARISON OF CULTURE, SINGLE AND MULTIPLEX REAL-TIME PCR FOR DETECTION OF SABIN POLIOVIRUS SHEDDING IN RECENTLY VACCINATED INDIAN CHILDREN

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Poliovirus identification in clinical and environmental samples is a crucial component of the global polio eradication program led by the World Health Organization (WHO). Although culture is considered the gold standard for poliovirus detection from stool samples, real-time polymerase chain reaction (PCR) has emerged as a faster and more sensitive alternative. In this study, we evaluated the performance of culture in the L20B cell line and two real-time PCR assays (for single poliovirus types and a one step multiplex real-time PCR) in the detection of Sabin poliovirus from stool samples of recently vaccinated Indian children. A random set of 80 stool samples from infants vaccinated with trivalent OPV (tOPV) at 6 weeks of age was selected for the study. The samples were collected as part of a clinical trial (CTRI/2012/05/002677) evaluating supplementation with zinc and/or probiotics to enhance the immune response of oral rotavirus vaccine and tOPV in Indian infants which was conducted at Vellore, India, between July 2012, and February 2013. Of the 80 stool samples tested, 55 (68.75%) were positive by culture. In contrast, 61 (76.25%) and 60 (75%) samples were positive for poliovirus by the single and one step multiplex real-time PCR assays respectively. If culture in L20B cell line is considered the gold standard for poliovirus detection, the sensitivity of singleplex and multiplex real-time PCR were 94.5% and 92.7% respectively, while the specificity was 64% for both the PCR assays. If the PCR methods are considered the gold standard for detection, the sensitivity of culture was 85% and the specificity varied from 84.2% to 80%. The quantity of virus as estimated by Ct values differed between culture positive and negative samples. Culture positive samples had significantly lower Ct values than samples that were negative in culture, ($p<0.05$), except for Sabin 3 detection by multiplex real-time PCR where the difference did not reach statistical significance. To conclude, the two real-time PCR assays for detection of single or multiple Sabin polioviruses in stool samples from vaccinated children were more sensitive than culture.

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THE PREVALENCE OF HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES AMONG WOMEN WHO PRESENTED FOR ROUTINE PAP SMEAR TEST IN THE UNITED ARAB EMIRATES

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Cervical cancer is the second most common cancer that affects women worldwide. Some genotypes of human papillomaviruses cause genital infection and can lead to cervical cancer. Therefore, the aim of this study was to determine the prevalence of HPV genotypes among women who presented themselves for a routine Pap smear test in the United Arab Emirates. A total of one hundred three (n=103) samples were collected between April-October 2011 and January-October 2012. Pap smear method was used to detect the abnormal cytology results of HPV. Then, initial test which is Digene hc2 was used to detect high/low risk HPV and DNA was extracted from these samples .After that, PCR was used to amplify the desired gene of HPV and the specific genotypes of HPVs were identified by an automated sequencing. One patient was excluded from the study because of incomplete data and a total of 102 were used for the final analysis. Based on the collected data, 39 (38.2%) samples out of 102 samples were positive for HPV DNA and 63 (61.8%) samples had negative results for HPV DNA. Meanwhile twenty seven (26.5%) samples were detected positive for HPV with PCR as well as Pap smear method. However, twenty (42.6%) samples were identifies positive for HPV with Pap smear method; were negative for HPV DNA by PCR. Among 102 samples; HPV types were identified as following; 4.9% samples with HPV-16, 3.9% samples with HPV-6 and 3.9% samples with HPV-53. The result illustrated that the most prevalent high risk HPV type among women in the United Arab Emirates was HPV-16. In conclusion, the high prevalence of carcinogenic HPV in our study, screening the normal population of women in the United Arab Emirates is recommended by both Pap smear and HPV DNA tests to avoid any false positive or negative results. The genotyping is essential for the decisions by the national government to support vaccination initiatives.

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INFLUENZA, DENGUE, CHIKUNGUNYA AND MULTI-DRUG RESISTANT BACTERIA SURVEILLANCE IN A PHILIPPINE TERTIARY HOSPITAL

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USAMD-AFRIMS has collaborated with the V Luna General Hospital since 2008 in emerging and re-emerging disease surveillance including antimicrobial resistance. Influenza and dengue virus was identified by real-time and conventional Polymerase Chain Reaction (PCR) respectively. Bacterial identification and antibiotic susceptibility testing was conducted using the Microscan Walkaway 40 Plus System. Mycobacterium tuberculosis was identified using the GeneXpert (Cepheid). From Feb 2008 - Mar 2016, 950/2,896 (33%) of collected respiratory specimens were positive for influenza: 307 FluA/H3; 325 Flu A/pdmH1; 289 Flu B; 11 Flu A/H1. 224/552 (44%) specimens collected from Nov 2012 - Feb 2016 were positive for dengue virus with the following serotypes detected: 69 DENV-1, 71 DENV-2, 61 DENV-3 and 41 DENV-4. 11/112 (10%) of clinically diagnosed dengue cases were confirmed to be chikungunya after PCR testing for both dengue virus and chikungunya virus. From Aug 2013 - Dec 2015, 28.3% (45/159) of Klebsiella spp. isolates and 2.7% (4/146) of *Escherichia coli* isolates were identified as imipenem resistant. 39.5% (30/76) of Acinetobacter spp. and 19.5% (30/154) of Pseudomonas aeruginosa isolates were resistant to all antibiotics in the Microscan negative breakpoint combo 30 and 34 panels. 70.4% (95/135) of Staphylococcus aureus isolates were methicillin resistant. 22% (18/81) sputum samples collected from Mar 2015 - Feb 2016 were identified as Mycobacterium tuberculosis and 22% (4/18) of the M. tuberculosis isolates were rifampicin resistant. This paper describes a research collaboration focusing on monitoring for emerging & re-emerging diseases in a tertiary military hospital setting. This information is crucial to define disease burden as well as to strengthen disease surveillance to better characterize and improve early detection and containment strategies.

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SEROPREVALENCE OF EBOLA VIRUS AMONG HEALTH CARE WORKERS IN THE TSHUAPA DISTRICT, DEMOCRATIC REPUBLIC OF CONGO

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Ebola virus disease (EVD) is caused by a zoonotic filovirus infection that can be highly lethal in humans. Since 1976, a total of seven confirmed EVD outbreaks have occurred in the Democratic Republic of Congo (DRC). Health care workers (HCW) are at particularly high risk of EVD infection given the high titers of virus in bodily fluids and lack of compliance with precautions to prevent exposure either due to lack of knowledge, training or equipment. Therefore, we conducted a serosurvey among HCWs attending a workshop in the Tshuapa district's capital, Boende, DRC. Field collection occurred in September 2015. Interviews and blood specimens were collected from all consenting individuals. Serum samples from 70 HCWs based in12 health zones in Tshuapa were screened for Ebola virus zaire (ZEBOV) GP Ig detection using Human Anti-Zaire Ebola Virus Glycoprotein (GP) IgG ELISA Assay kits (Alpha diagnostic International, Inc.) in Kinshasa, DRC. Among the 70 health care workers, 43% (n=30) were seropositive for ZEBOV GP IgG, 49% (n=40) were seronegative, and 21% (n=17) were indeterminate. Among those seropositive, 68% (n=34) had participated in 1 or more EVD outbreaks of which 41% (n=14) had used Personal Protective Equipment (PPE). Among all HCWs, only 10% (n=6) suspected that they had become infected with EVD, however none were confirmed through testing. Our findings suggest that some Tshuapa HCW's may be highly exposed to EBOV. While our estimate of seroprevalence is higher than other studies, this may be explained by HCW participation in the 2014 Boende outbreak or by asymptomatic exposure in an endemic region. Regardless, increased biosafety training is needed to prevent transmission in HCWs.

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SUSCEPTIBILITY OF BAT CELL LINES R06E AND R05T DERIVED FROM THE EGYPTIAN FRUIT BAT TO PATHOGENIC HUMAN VIRUSES

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Recent surveys have found that bats harbor viruses from at least 24 viral families and are suspected reservoirs for many emerging human pathogenic viruses. While the variety of viruses identified in bats is staggering, many have been detected only by serology or molecular techniques, with the isolation of virus particles being a challenge in existing cell lines. Dr. Ingo Jordan's group at ProBioGen, Germany developed the R06E and R05T primary cell lines as potential tools for the propagation of bat viruses. These cultures are adenovirus E1 immortalized cell lines derived from Rousettus aegyptiacus fruit bat fetal cells. Both cell lines have been verified by BEI Resources to be of R. aegyptiacus origin through cytochrome c oxidase subunit 1 (CO1) barcoding. Select viruses from BEI Resources were passaged in R06E and R05T to evaluate infectivity and the initial host cell line used for the propagation of the stock virus was run as a control for optimal growth. The tissue culture infectious dose (TCID50) was determined for each passage and cytopathic effect was documented. The first two viruses tested were the Sindbis (Togaviridae) and Tacaribe (Arenaviridae) viruses, which are known to be found in bats. Both bat cell lines demonstrated cytopathic effects comparable to those observed in Vero E6 control cell line. Susceptibility to a particular virus was observed in cells that did not exhibit a significant decrease in viral titer after three passages. Testing is underway for cell line susceptibility to additional virus families, including Coronaviridae (Middle East respiratory syndrome virus) and Flaviviridae (Zika virus). The verified pure culture R06E and R05T bat cell lines support the growth of zoonotic viruses detected in both humans and bats, and can be used to study crossover between human and bat pathogens. Future studies are required to demonstrate whether these cells support the isolation and propagation of bat viruses that are currently detected by molecular methods only. These cell lines should support further research in bat immunology and virus-host interactions and are available through NIAID's BEI Resources.

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ETIOLOGY OF ACUTE FEBRILE ILLNESSES AMONG PREGNANT WOMEN FROM THE SENTINEL ENHANCED DENGUE SURVEILLANCE SYSTEM (SEDSS) IN PUERTO RICO

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Acute febrile illnesses (AFI), characterized by undifferentiated fever, present a risk for pregnant women due the level of suppression of the immunological system during pregnancy. Fever during pregnancy has been consistently associated with congenital anomalies, including neural tube defects, craniofacial malformations, and cardiac anomalies, with 1.5-3 fold increased risk after febrile illness in the first trimester. There is no published evidence that describes the etiology of AFI during pregnancy in Puerto Rico, an island with endemic transmission of arboviruses such as dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) viruses. Our study aims to describe the infectious agents and outcomes of AFI during pregnancy by trimester of participants from the Sentinel Enhanced Dengue Surveillance System (SEDSS), a facility-based epidemiologic platform established in southern PR. Pregnant women with fever of <7 days were enrolled from May 7, 2012 to May 6, 2015. Clinical data was collected and specimens were obtained and tested by RT-PCR and immunodiagnostic methods as appropriate for DENV-1-4, CHIKV, Leptospira species, enteroviruses, influenza A/B viruses, and other respiratory viruses. A total of 152 pregnant women were enrolled in SEDSS, half (46%) of which were aged 20-24 years. A pathogen was identified in 93 (61%) cases: 56 (37%) had CHIKV, 23 (15%) influenza A/B virus, 6 (4%) DENV, 5 (3%) other respiratory virus, 2 (1%) enterovirus and 1 (1%) co-infected. When analyzing by trimester of pregnancy, one (1.1%) DENV and 2 (2.2%) CHIKV cases were found during the first trimester; 3 (3.2%) DENV and 25 (26.9%) CHIKV during the second; and 2 (2.2%) dengue and 26 (28%) CHIKV during the third. All DENV cases were diagnosed between 2012 (n=4) and 2013 (n=2) and all CHIKV cases between 2014 (n=53) and 2015 (n=3). In conclusion, the surveillance system was able to identify pathogens associated to AFI during pregnancy. The most recent CHIKV outbreak contributed the majority of the cases presenting in pregnant women. Of interest are the outcomes of the mother and newborns, for which analysis is currently being done and will be presented.

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LEISHMANIA INFECTION IN SANDFLIES IN A CUTANEOUS LEISHMANIASIS FOCUS IN GHANA

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The distribution and *Leishmania* infection in sandflies was examined in the endemic cutaneous leishmaniasis (CL) communities of the Volta region, Ghana. CL was first report in 1999 by the Ghana Health Service in the Ho, Hohoe and kpando municipality. Since the first outbreak of the disease, there have be increasing reports of the disease in various villages in the Volta Region of Ghana. In this study, to identify natural infection by *Leishmania* sp. in insect vectors of CL, entomological survey was performed in three endemic communities (Dodome Awiasu, Dodome dogblome and Lume atsyame) in the Volta region. From October 2012 to February 2013, a total of 4219 female sandflies were captured with CDC light traps and dissected for morphological identification. It was observed the 20(0.5%) female sandflies were identified from the genus *Phlebotomus* and 4199 (99.5%) from the *Sergentomyia*. To determine leismania infection in female sandlies, DNA was extracted from pools of sandfly species ranging from 1 to 25 dissected females. In considering the pools of individual sandfly species, *Leishmania* sp. infection of 0.0384% was detected in a pool of 7 (5.7%) *S. africana* female sandflies out of 122 pools using PCR. This is the first report of natural infection by *Leishmania* sp. in *S. africana* in Ghana. This observation that *S. africana* are naturally infected by *Leishmania* sp., suggest that the sandfly species might play a role in the transmission of cutaneous leishmaniasis within the Volta Region of Ghana.

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MEGALOPYGE OPERCULARIS: THE STING THAT KEEPS ON STINGING, 1798-2016

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Megalopyge opercularis (Order: Lepidoptera) is known commonly by a number of monikers, most notably the "wooly asp" and the "puss caterpillar." First described by the pioneering English lepidopterist, James Edward Smith in 1798, the Megalopyge opercularis quickly gained a reputation for its painful "urticating sting." In the more than two centuries that have passed since Smith first described the Megalopyge opercularis, a steady flow of studies has made it one of the most widely studied of the "urticating lepidoptera." This presentation will examine reports on Megalopyge opercularis since the late 18th century. Particular attention will be paid to their place within the historical development of medical entomology.

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ECTOPARASITE INFESTATION OF A HOSPITAL DUE TO NESTING CLIFF SWALLOWS

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The Cliff Swallow (*Petrochelidon pyrrhonota*) is a migratory passerine that constructs conical mud nests in colonies during the summer throughout North America. A large colony of swallows was established in the window eaves of a hospital. In July of 2015 the Infection Prevention Committee was informed of unusual "bugs" found in patient rooms. Two avian ectoparasites were identified, *Argas cooleyi* (argasid tick) and *Oeciacus vicarius* (swallow bug). Initial surveillance revealed ectoparasites in twenty-two locations (hallways, entrance points, and patient rooms) correlating with the presence of swallow nesting sites. Eradication of the ectoparasites was executed over a six-month period which included insecticide application and re-caulking of window sills throughout the structure. However, the swallows were not disturbed during their nesting period as required by the Migratory Bird Act and by late November of 2015 the birds had migrated. Removal of the abandoned nests (n=267) soon followed. Installation of bird netting in locations of prior and/or potential nesting sites completed the project. Since the discovery of this infestation, bimonthly surveillance took place to assess the ectoparasite burden. Decreasing numbers were recorded with each month and by the end of December 2015 no more ectoparasites were discovered. Argasid ticks are known to opportunistically feed on humans in the right circumstances. The collected ticks (n=237) were tested for the presence of human blood by an immunohistochemical test specific for human glycophorin A (surface protein on red blood cells). The results indicated that human blood was present in some of the collected ticks. The nesting of Cliff Swallows on man-made structures, including medical facilities, may

facilitate an accompanying ectoparasite infestation as demonstrated in this case. Argasid ticks are known vectors worldwide in the transmission of arboviruses and bacterial (*Borrelia* and *Rickettsia* species) pathogens that can infect both animal and human hosts. This unusual ectoparasite infestation involved two arthropods whose domiciles needed to be removed in order to eradicate them.

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FIRST DETECTION OF *LEISHMANIA TROPICA* IN NATURALLY INFECTED *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE) IN NORTH SINAI, EGYPT

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Cutaneous Leishmaniasis (CL) is endemic in the Middle East and prevalent in the Sinai Peninsula, Egypt. Cutaneous Leishmaniasis is caused by *Leishmania major* or *L. tropica. Leishmania major* is transmitted by a bite of female *Phlebotomus papatasi* (*P. papatasi*), the principal sand fly vector throughout the Mediterranean basin, Middle East, Central Asia, and East Africa. *Leishmania tropica* occurs widely in Israel, Jordan Valley, and the Negev Desert. Personnel participating in the peace keeping in the Sinai-based Multinational Force and Observers (MFO) camps, are at risk to sand fly bites. Herein we describe surveillance of *Leishmania* disease vectors in the Sinai. Vector surveillance was conducted at multiple MFO remote sites using the Centers for Disease Control and Prevention (CDC) light traps. Species identification was done based on the morphological characters. *Leishmania* species was detected by amplification of the small-subunit ribosomal RNA (ssu rRNA) gene. *Phlebotomus papatasi* (Scopoli) comprised 999 (97%) of 1,030 collected sand flies. Out of 354 tested *P. papatasi* females, 106 (29.9%) of 354 were positive for *Leishmania* ssu rRNA gene by Real-time PCR. Restriction fragment length polymorphism (RFLP) typing of the internal transcribed region (ITS1) of ssu rRNA amplicons from positive samples confirmed *L. tropica* and *L. major* fingerprints. Twenty of 106 (19%) females captured were infected with *L. tropica,* while 86 (81%) females were infected with *L. major.* This study reports the first detection of *L. tropica* in *P. papatasi* from Sinai, Egypt. The abundance of infected and non-gravid *P. papatasi* suggests a vectorial capacity of this species to transmit *L. tropica*. This work emphasizes the value of systematic survey of *Leishmania* reservoir hosts and vectors.

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DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR ASSAY FOR IDENTIFICATION OF *PHLEBOTOMUS* SAND FLY SPECIES INVOLVED IN *LEISHMANIA* TRANSMISSION

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Phlebotomine sand flies (Diptera, Psychodidae, Phlebotominae) are the principal vectors of *Leishmania* parasites. Phlebotomine vectors are significant threats to public health in areas such as North Africa, the Mediterranean basin and the Middle East. Of 800 sand fly species published, 10% are competent vectors of Leishmaniasis. Identification of sand flies in *Leishmania*-endemic areas is paramount. Morphological sand fly identification, a process reliant on discernment of delicate features, is time consuming and requires taxonomic expertise. Molecular-based identification methods provide an alternative approach to morphological identification; however few studies have developed PCR techniques to discriminate between specific sand fly species. Here we describe the development of a multiplex TaqMan real-time PCR assay for the detection of three *Leishmania* vector species (*Phlebotomus papatasi*, *Ph. sergenti*, and *Ph. alexandri*) in a single reaction. DNA was extracted from individual *Phlebotomus* sandfly species that have been maintained in continuous laboratory colonies. We design three species-specific primers and TaqMan probes targeting a consensus region of 18S ribosomal DNA gene using sequences retrieved from the GenBank in addition to other in-house sequences generated from individual *Phlebotomus* specimens (target species) and *Sergentomyia* specimens (non-target species). To evaluate the sensitivity of the assay serial dilutions of the three target species in mixed-pools were prepared at ratios ranging from 1:10 to 1:50. Mixed-pools of target to non-target species were used to determine the specificity of the assay. This TaqMan multiplex real-time PCR method identified *Ph. papatasi*, *Ph. sergenti*, and *Ph. alexandri* effectively, and demonstrates a higher sensitivity up to a dilution of 1:20. Mixed pools of target and non-target species showed the assay to be 100% specific. The present study provides a quick and reliable one-step multiplex assay for the identification of *Leishmania* vectors in field-caught sand flies where pools of vector and non-vector species share the same ecological niche.

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IMMUNE MODULATING MOLECULES OF *SARCOPTES SCABIEI*

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The ectoparasitic mite *Sarcoptes scabiei* uses a variety of mechanisms to evade its host allowing a parasitic colony to become established. One of these strategies involves modulation of the innate and adaptive immune responses in the host skin. Having previously sequenced the scabies mite genome and characterized > 200 of the proteins produced by this mite, we are now seeking to identify the molecules that this mite uses to immunomodulate the function of fibroblasts and endothelial cells of the microvasculature of the host dermis. Several proteins were selected for study and their genes were chemically synthesized and cloned into appropriate vectors for expression in *Escherichia coli.* Fusion proteins were purified and used to challenge normal human dermal fibroblasts (NHDF) and microvascular endothelial cells (HMVEC). Two of these clones stimulated the secretion of IL-6, IL-8, and GCSF by NHDF and of IL-6 and GM-CSF by HMVEC in a dose dependent manner. These responses are similar to the cellular responses to whole scabies mite extract suggesting that these molecules may be among those used by the mite to protect itself from the host. Neither protein bound antibody from the serum of any scabies infested hosts tested suggesting that they are able to evade the host's adaptive immune system as well.

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CONCEALED ANTIGENS AS VACCINE TARGETS FOR CONTROLLING TRIATOMINES, VECTORS OF CHAGAS DISEASE

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Controlling triatomine (Hemiptera:Reduviidae) bugs is an important aspect of managing the spread of *Trypanosoma cruzi*, the causative agent of Chagas disease, for which no vaccine is currently available. Triatomine control methods depend on insecticide use and environmental management. Insecticide resistance and the ecology of some triatomine species, that tend to persistently re-invade domestic and peridomestic structures following the application of these control methods, may require additional control approaches. The development of a triatomine vaccine to target the insect vector would provide an additional prevention and control method. Vaccination of animal hosts with triatomine antigens would cause the hosts' immune system to produce antibodies that would be ingested in a blood meal. Many proteins within the triatomine salivary glands and midgut are critical to the acquisition and digestion of obligatory blood meals. Thus, inhibiting one or more of these proteins using ingested antibodies could interrupt important biological processes required for triatomine survival. To this end, the selection, recombinant expression and

vaccine evaluation of twelve Rhodnius prolixus (Hemiptera:Reduviidae) midgut protein targets are discussed as well as the concept of "exposed" and "concealed" antigens.

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OVIPOSITION ATTRACTANTS OF *PHLEBOTOMUS PAPATASI* - PRELIMINARY RESULTS

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Phlebotomine sand flies transmit protozoan parasites (*Leishmania* spp.), bacterial (*Bartonella bacilliformis*), and viral pathogens. An alternative approach to the traditional delivery of an insecticide to the vector is to bring the vector to the insecticide using an attractant. In the context of controlling vector-borne disease, oviposition-site attractants are expected to be highly effective because they target gravid females that are responsible for transmission of the pathogen and amplifying vector populations. Decomposing organic matter is the main food source for sand fly larvae. We therefore hypothesize that gravid sand flies are differentially attracted in a dose-dependent manner to a blend of fecal- and microbially-derived chemical cues associated with the decomposition of fecal material, as well as to signals from eggs and larvae which indicate suitable oviposition sites. Our overall goal is to develop and optimize an attractive blend of semiochemicals that would function as a lure for oviposition-site seeking sand fly females using *Phlebotomus papatasi* (vector of old-world cutaneous leishmaniasis) as a model system. We will apply an integrated interdisciplinary approach including behavioral, electrophysiological, and microbiological studies to address the following specific aims: (1) Identify the most attractive and oviposition stimulating conspecific stages, rearing medium, and saprophytic microbes; (2) Isolate and identify oviposition attractants and stimulants from the most attractive conspecific stage, rearing medium, and microbial isolates; and (3) Develop an optimal blend of oviposition attractants and stimulants and evaluate it at the micro- and meso-scales. This proposed study introduces several novel and innovative approaches including: (1) Application of an integrated approach including behavioral, electrophysiological, analytical and microbiological investigations; (2) Study a neglected aspect of oviposition - the role of saprophytic fungi as indicators of suitable oviposition sites; (3) Evaluate the effectiveness of the optimized blends at the scale of meters using a wind-tunnel.

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RNASEQ OF WILD CAUGHT SAND FLY *PHLEBOTOMUS CHINENSIS* FROM SICHUAN, CHINA

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Sandfly *Phlebotomus chinensis* is responsible for the transmission of visceral leishmaniasis in China. In this study we conducted RNA-seq for head and body of both male and female sandflies to determine the transcriptional differences between females and males, head and body. Wild caught sandfly specimens were collected in an endemic region in Sichuan, China. cDNA libraries of male heads and male bodies, female heads and female bodies were prepared for RNAseq using Illumina paired-end sequencing technology. Approximately 128 million clean reads were assembled into 32,628 unigenes with an average length of 1,235 bp, an N50 of 2,551 bp, and an average GC content of 47.88%. All unigenes have good hits against Nr, 26,601 (46%) have Swiss-Prot hits, 5,244 (19.15%) have KEGG annotation. Furthermore, a total of 256,890 SNPs were identified. Transcriptomic comparisons between head vs. body as well as males vs. females revealed ~5,000 unigenes that were deferentially expressed between these samples. Our RNAseq data provide a comprehensive transcriptomic resource for Ph. chinensis, and will facilitate further studies on genetic and genomics. The genome of *Ph. chinensis* has been sequenced. Efforts are now underway to assemble and annotate the genome.

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TOXICITY OF PLANT EXTRACTS AND PYRETHROIDS TO CATTLE TICK, *BOOPHILUS* SPECIES (IXODIDA : IXODIDAE)

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Ticks transmit protozoan, bacterial, rickettsial and viral pathogens to both human and animals worldwide leading to enormous economic losses to the livestock industry. However, the indiscriminate use of synthetic acaricides for tick control has contributed to the development of resistance in tick population. There is an urgent need to search for effective and safe alternative means of control. A laboratory evaluation was therefore carried out to determine the toxicity of aqueous extracts of two plants (*Azadirachta indica* and *Annona muricata* leaf) and two synthetic pyretheroids (deltamethrin and lambda-cyhalothrin) to tick species of cattle (*Boophilus* species). Wild adult ticks collected from cattles around Amansea, Awka, Anambra state, Nigeria were exposed to the acaricides using adult immersion test. Five concentrations of the plant extracts (500, 250, 125, 62.5 and 31.2µL/mL) and synthetic pyrethroids (50, 25, 12.5, 6.25 and 3.12µg/mL) were used to immerse 10 active adult *Boophilus* ticks. Each concentration was done in duplicate and replicated thrice and a control was included. Mortality post exposure was monitored and recorded at 24, 48, 72 and 96 hours and data was analysed using log-probit regression analysis. Results showed that dose-related mortality responses were observed at different time intervals. Mortalities of 100% and 76.7% respectively was recorded for *A. indica* and *A. muricata* at 50% concentration while exposure to deltamethrin and lambda-cyhalothrin resulted in 86.7% and 76.6% mortality respectively at 50µg/mL concentration. LD_{50} values for adult *Boophilus* ticks were 5.36% and 14.40% for *A. indica* and A. *muricata* while that of deltamethrin and lambda-cyhalothrin were 6.00µg/ml and 2.25µg/ml respectively. The LT_{50} values were 63.66 and 119 hours for *A. indica* and *A. muricata* and 66.69 and 76.13 hours for deltamethrin and lambda-cyhalothrin respectively. The study suggests that botanical extracts could serve as a good alternative to synthetic acaricides.

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A BANDIT MODEL TO OPTIMIZE ENTOMOLOGICAL SURVEYS

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Although vector-control campaigns have prevented considerable morbidity and mortality from vector-borne diseases, there is increasingly a need to find strategies that accurately and efficiently identify foci of transmission based on limited information. Currently, control strategies rely on systematic screening for infestations and require time- and resource-intensive surveillance, while less resource-intensive reactive control strategies are often inadequate to detect the emergence or re-emergence of disease vectors. We propose and test a novel spatial search

strategy. Our strategy uses a multi-armed bandit algorithm to select among a number of proposed search areas each day based on results from previous days, and ultimately localizes searches to the most heavily infested areas. As an online optimization strategy, it obviates the need for preliminary surveys and responds to changing conditions experienced by the field teams. Furthermore, as a bandit strategy, the algorithm is designed to optimally balance between exploiting high-prevalence areas and exploring unknown areas that may have a high infestation burden. We investigate the properties of this strategy using simulation studies and apply it to retrospective data from several recent vector-control campaigns in Arequipa, Peru.

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VECTOR CONTROL USING LONG-LASTING INSECTICIDAL NETS AGAINST VISCERAL LEISHMANIASIS IN BANGLADESH

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Vector control takes on a role as important part in controlling the diseases transmitted by arthropods such as leishmaniasis. Visceral leishmaniasis (VL) is one of the major public health problem in Bangladesh, but the most appropriate vector control measures is still a matter of debate. Here, the efficacy of permethrin treated long-lasting insecticidal nets (LLINs), Olyset® and Olyset® Plus against field collected sand flies was evaluated in a VL endemic area in Bangladesh. Sand fly bioassays were conducted according to the WHO-approved cone test methodology with modification. A major species of tested sand flies (91.28%) was *Phlebotomus argentipes*. Sand flies were introduced into a plastic cone attached with a piece of Olyset® or Olyset® Plus for 3 min and mortality and knock down rate were continuously recorded until 24 hrs after the exposure. Approximately 20-25 sand flies were used in each set, and the tests were repeated 4 times. The mortality of sand flies recorded on 24 hrs was 100% in Olyset® Plus group while that mortality of sand flies in Olyset® group was 83.63% (corrected mortality = (% test mortality – % control mortality) / (100 – % control mortality) × 100). The knowledge, attitude and practice of people live in endemic area about VL are also essential in order to propose successful vector control strategies. Therefore, questionnaire-based survey was also demonstrated to know whether the vector control using LLINs is sustainable application for the people lived in endemic are or not. The questionnaire was consists of three sections, socio-demographic characteristics, knowledge of VL and history of VL, and perceptions of VL vector control. Based on the analysis of 1393 households, the knowledge, attitude and practice of people live in endemic area about VL were relatively low. Though utilization of LLINs is promising, its ownership is noticeably low. Vector control using permethrin treated LLINs can be one of a potential tool for reducing the morbidity rate of VL in endemic area in Bangladesh. This works was supported by Science and Technology Research Partnership for Sustainable Development.

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EXPERIMENTAL SUSCEPTIBILITY OF *LUTZOMYIA LONGIPALPIS* TO DIFFERENT SPECIES OF *LEISHMANIA*

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In order to better understand the epidemiology of leishmaniasis and the parasite transmission it is important to develop studies on the *Leishmania*-vector interaction. The knowledge of the process of the sand fly-parasite interaction can contribute for new control strategies of a disease that until now has no effective vaccine a and limited range of drugs for the treatment. In nature, some sandfly species show remarkable specificity to transmit an exclusive *Leishmania* species (non-permissive vector) while others can be vectors of more than one parasite species (permissive vector). However, in in the laboratory some sandfly like *Lutzomyia longipalpis*, exclusive vector of *L. chagasi infantum*, can be experimentally infected by different parasite species. Here, we analyzed in details the development of four *Leishmania* species: *Leishmania major, L. amazonensis, L. braziliensis* and *L. chagasi* in the *Lu. longipalpis*, a New World sandfly. Experimental infections were conducted using three parasite doses (4x107, 2x107 and 1x107) achei que fossem 4 doses mixed with mouse blood. Our data demonstrated that *Lu. longipalpis* is capable of sustaining infection by *L. infantum chagasi, L. amazonensis* and *L. major*, but not by *L. braziliensis*, which only developed infection when the vector ingested a high dose of parasites and even so, with low parasitic density. We also showed that infection rates of the *Lu. longipalpis* are correlated with the amount of ingested parasites. This study characterizes important aspect of the *Lu. longipalpis* vector competence.

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PRELIMINARY CHARACTERIZATION OF POTENTIAL SAND FLY VECTORS IN LEISHMANIASIS AND BARTONELLOSIS ENDEMIC AREAS IN THE PERU-ECUADOR BORDER

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Leishmaniasis and bartonellosis are known to be endemic in the Peru-Ecuador border, yet information about the sand fly vector species incriminated in transmission of both diseases is lacking. A major bartonellosis outbreak was reported in the Peruvian side of this region in 2013-2014 with 622 cases reported in Piura department and 159 cases in Cajamarca province. Concurrently, 417 and 85 leishmaniasis cases were reported in Piura and Cajamarca, respectively. The goal of this study was to characterize the sand fly fauna and potential bartonellosis and leishmaniasis vectors in sites with the highest prevalence of both diseases in this region: Lalaquiz (Piura; 1027 m.a.s.l) and Namballe (Cajamarca; 1197 m.a.s.l). Sand flies were collected in July 2015 and January 2016 using standard CDC light traps, CDC blue LED traps, CDC UV traps and Mosquito Magnet trap. A total of 464 adult *Lutzomyia* sand flies were captured; 22 from Lalaquiz (5%) and 442 from Namballe (95%). Standard CDC light trap and Mosquito Magnet trap collected the highest proportion of sand flies in Lalaquiz (45%) and Namballe (59%). The most abundant sand fly species collected from Lalaquiz were *Lu. castanea* (23%) and *Lu. shannoni* (23%); other species included *Lu. ayacuchensis*, *Lu. gomezi* and members of the Verrucarum and Micropygomyia Groups. *Lutzomyia maranonensis* (38%), *Lu. robusta* (35%), and *Lu. castanea* (27%) were recorded in Namballe. Differences in sand fly species composition between sites may be related to ecological factors. Our preliminary results suggest that different species could play a role in leishmaniasis and bartonellosis transmission at study sites. In Lalaquiz, *Lu. ayacuchensis* and *Lu. gomezi* are potential vectors of both diseases, while in Namballe, the potential vectors are *Lu. maranonensis* and *Lu. robusta*. Future studies will include assessing *Leishmania* and *Bartonella* infection rates to further characterize potential regional vectors of both diseases and contribute entomological risk information for military and civilian populations in the Peru-Ecuador border.

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NUTRITIONAL STATUS, FREQUENCY OF HUMAN BLOOD MEALS, AND *TRYPANOSOMA CRUZI* INFECTION OF *TRIATOMA DIMIDIATA* IN YUCATAN, MEXICO

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In Yucatan, the causative agent of Chagas disease, *Trypanosoma cruzi*, is transmitted by the hematophagous bug *Triatoma dimidiata*. In order to evaluate human/vector/parasite contact, and clarify the parasite cycles of transmission in the region, we are currently assessing by PCR the presence

of vertebrate and human blood meals, and the presence of the parasite in *T. dimidiata* specimens collected in sylvatic and domestic/peridomestic ecotopes in 3 rural communities of Yucatan. At this time, 222 bugs (100 males, 113 females, and nine 5th instar nymphs) have been tested for infection with *T. cruzi*. Of them, 33% were infected; infection prevalence did not differ significantly between males (33%, 33/100) and females (35%, 39/113) (χ^2=0.05, p=0.8); 22% of nymphs (2/9) were found infected. Infection rate of sylvatic bugs (45%, 34/75) was significantly higher than infection rate of (peri)domestic bugs (27%, 40/147) (χ^2=7.4, p=0.007). This may be due to different blood feeding sources between sylvatic and (peri)domestic bugs. Vertebrate blood meals were detected in 45/62 (73%) bugs tested. Of the 45 detected blood meals, 10 (22%) were human blood meals. All human blood meals were detected in (peri) domestic bugs. Thirty percent of human-fed bugs were infected with *T. cruzi*, confirming the risk of parasite transmission to human. Cloning and sequencing is now been used to identify the different vertebrate blood meal sources, and possible associations between *T. cruzi* infection and the different vertebrates. At this time, *Homo sapiens* (human), *Canis lupus familiaris* (dog), and *Zenaida macroura* (Mourning dove) have been identified. This work will help us to better understand the feeding behavior of *T. dimidiata* and to accurately describe the parasite transmission cycles occurring in Yucatan, Mexico.

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THE *LUTZOMYIA LONGIPALPIS* MICROBIOTA ASSOCIATED WITH INFECTION BY *LEISHMANIA INFANTUM CHAGASI*

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Lutzomyia longipalpis is the main vector of *Leishmania chagasi*, which is a protozoan transmitted by sandfly bite. During blood feeding on infected vertebrates, sandflies can ingest the parasites. In the midgut, the parasites interact with the native microbiota. This interaction may contribute to the metabolism of the insect providing resistance against natural enemies and parasites and also, affects the immune and reproduction. The microbiota diversity in sandflies is well known but their role on parasite infection is still not clear. Our aim was to identify the microbiota role associated with *Leishmania* infection in the sandfly *L. longipalpis*. The microbiota diversity was determined by metagenomic method using next generation sequencing (NGS) of the 4 sandfly experimental groups: sugar-fed, blood -fed, infected-blood fed and gravid. We used the multivariate method NMDS to analyze the results. It was observed that the two groups of sandflies that were fed on blood separated from the other two other groups. This observation was firstly based on the abundance of bacterial family profiles. However, the Multivariate Analysis using PCA showed the similar profile. Throughout these analysis, it was possible exclusively identify in the blood-fed sandflies the bacteria family Xanthomonadaceae. Distinctly, in the infected-blood fed sandflies, it was identify the following families: Enterobacteriaceae, Enterococcaceae, Bacteroidaceae, Coxiellaceae, Flameovirgaceae, Deferribacteraceae, Glycomycetaceae. Our results demonstrated that the bacterial community in blood-fed differs from infected blood-fed *L. longipalpis*. In vivo studies are necessary in order to show how bacteria interfere in the growth of the parasite.

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A REVERSE TRANSCRIPTASE PCR ASSAY FOR THE IDENTIFICATION OF *DIROFILARIA IMMITIS* INFECTIVE (L3) LARVAE IN MOSQUITOES

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Dirofilariasis, a mosquito-transmitted disease, is caused by parasitic nematode worms from the genus *Dirofilaria*. *Dirofilaria immitis* is principally associated with infections of domestic canines and felines but it is also capable of infecting a wide-range of mammalian species including humans. Current molecular assays for detecting *D. immitis* DNA in mosquitoes by PCR are unable to differentiate between infected mosquitoes that contain any stage of parasite DNA and infective mosquitoes that harbor third-stage larvae (L3), the larval stage capable of establishing infections in canines and other mammals. *D. immitis* has the potential to be a major public health concern due to its distribution in tropical, subtropical, and temperate regions around the world. However cases of human dirofilariasis are likely under reported due to a dearth of knowledge concerning the epidemiology and risk factors associated with this disease. The ability to identify mosquitoes that are both competent vectors of *D. immitis* and could infect humans (based upon known host feeding preferences), could play an important role in estimating transmission risks to humans. We have developed a detection assay for *D. immitis* in a model system using the mosquito, *Aedes aegypti*, based on a conventional RT-PCR assay that detects an L3-activated gene transcript. Potential L3 genes were identified using bioinformatics tools and were screened by RT-PCR using *D. immitis* stage-specific mRNA libraries as templates. Candidate genes were screened for stage-specific expression using RNA isolated from both *D. immitis* infected and uninfected mosquitoes. This L3 specific gene combined with a *D. immitis* specific control gene, expressed in all vector-stage filarial larvae, can be utilized to accurately identify mosquito species capable of transmitting this zoonotic disease.

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ARE THE POPULATIONS OF *TRIATOMA INFESTANS* FROM SANTIAGO DEL ESTERO, ARGENTINA, RESISTANT TO PYRETHROIDS

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Under the vector surveillance and control program for *Triatoma infestans* that Mundo Sano has in place since 2002 in the urban and surrounding rural areas of Añatuya (Santiago del Estero Province), monitoring of insecticide resistance was implemented in 2014. The objective was to evaluate the susceptibility of *T. infestans* samples from this area to the pyrethroid deltamethrin, active ingredient found in the most widely used formulations for triatomine control. Bioassays were performed following the World Health Organization protocol for the evaluation of insecticidal effect on triatomines. First instar nymphs obtained in the laboratory from adult insects collected in the field (F1 generation) were used. Mortality was evaluated 24 hours after topical application of the discriminant dose (2 ng/insect). The CIPEIN and La Pista (resistant to deltamethrin) strains were used as negative and positive controls, respectively. Possible resistance was considered when survival was observed in at least one insect in two of every three independent assays. Percent mortality for the different collection sites were: El Desvio 90%; Barrio Sportivo, 75%; Miel de Palo, 70%; Lote 47, 38%. These results suggest that some of the populations evaluated present early-stage resistance to deltamethrin. New assays will be conducted in order to quantify this phenomenon with greater precision.

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ANOPHELES COUSTANI, AN IGNORED SUPER VECTOR OF ARBOVIRUS AND *PLASMODIUM*

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Human malaria cases increased in Madagascar despite the use of indoor residual spraying and long lasting insecticide-treated bed nets. Vectors diversity and abundance over the year explained the persistence of malaria. Amongst 7 *Anopheles* malaria vectors including An. funestus and

An. gambiae s.s., *An. coustani* attracted the attention. During the past few years and in different seasons, *An. coustani* was regularly found positive with *Plasmodium* sp. in different ecotypes.. It was quite surprising to find this mosquito species naturally infected either by *P. falciparum* or *P. vivax*, and sometimes also with a co-infection *P. falciparum* / *P. vivax*. Moreover, during the Rift Valley Fever (RVF) outbreaks occurred in Madagascar in 2008 and 2009, *An. coustani* was also detected positive with other two species, Culex antennatus and An. squamosus/cydippis. This study aims to highlight the exact role played by *An. coustani* facing the Plasmodium and RVF virus transmission. To achieve this objective, experimental infections were developed in a high malaria prevalence area. In parallel, study on vector competence of *An. coustani* and *Cx. antennatus* to RVF virus was carried out in laboratory. We suspect that *An. coustani* played, and still playing an important role in malaria transmission whereas 14.1% of *An. coustani* and 7.23% of *Cx. antennatus* were able to transmit RVF virus in laboratory. The role of *An. coustani* in transmission of human *Plasmodium* and RVF virus was highlighted in field and in laboratory. These findings coupled with its tendency to bite on animal with an opportunistic to human confirmed its medical and veterinary importance. Otherwise, this species was found involved in transmission of *Wuchereria bancrofti* and West Nile virus in Madagascar and in transmission of Zika virus in Africa. Thus, specific attention of the biology study of this species must be carried out in order to control it and to determine its role during inter epidemic period.

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SCREENING THE MIDGUT BACTERIAL COMPOSITION OF TWO COLOMBIAN FIELD-COLLECTED MALARIA VECTORS FOR BIOCONTROL STRATEGIES

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Malaria constitutes a relevant problem of public health in Latin America with more than 300,000 cases confirmed every year in Brazil, Venezuela and Colombia. Although the number of deaths has decreased worldwide, the dramatic increase in insecticide-resistant *Anopheles* populations has led to search for alternative strategies to diminish or eliminate malaria vector populations. Recent studies have shown that some bacteria present in the mosquito microbiota have important negative effects on the sexual stages of the parasite within the mosquito midgut, as well as in vector survival. However, little is known about the microbiota of Latin American anopheline mosquitoes and its significance for parasite inhibition. Therefore, the purpose of this study is to characterize the midgut microbiota composition of two main Latin American malaria vectors, *Anopheles darlingi* and *An. nuneztovari*, collected in two malaria-endemic regions of Colombia. Metadata generated for adult mosquitoes, larvae and breeding sites by Illumina sequencing is currently under analysis. In addition, preliminary data resulting from culture-dependent methods shows interesting differences in the diversity profiles in the bacterial community of female adult mosquitoes of the two localities and species. A combined analysis of both culture-dependent methods and high throughput sequencing will help to reveal a detailed composition of the midgut microbiota of these two main vectors and elucidate potential candidates for future vector biocontrol strategies.

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DISTRIBUTION AND HABITAT CHARACTERIZATION OF *ANOPHELES* LARVAE IN FOUR COMMUNITIES IN THE PERI-IQUITOS REGION OF AMAZONIAN PERU

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Malaria control focused on drug treatment and insecticide-impregnated nets, or insecticide residual spraying, is increasingly compromised by insecticide resistance in many endemic regions. One consequence is renewed interest in the reduction of adult vector populations by targeting aquatic immature stages. Aquatic stages determine abundance, dynamics and fitness of mosquito adults, directly affecting malaria transmission. To evaluate the characterization and distribution of anopheline breeding sites, collections are being carried out in two riverine sites, Lupuna and Santa Emilia, and two highway sites, Triunfo and Nuevo Horizonte during the rainy season (Jan-Feb; April-May) and the dry season (Aug-Sept). Sampling of breeding sites is conducted using a standard dipping technique within a 1 km radius of the center of each village. Quantification includes relative abundance of larvae and Larval Index (LI) (larval density). Breeding sites will be sampled and analyzed for: concentration of nitrates and nitrites, alkalinity, pH, temperature, conductivity, salinity, turbidity, water movement, algae, density of surrounding vegetation, relative shade, and emergent light and canopy coverage. In Jan-Feb, 2016, of 32 sampled breeding sites, *Anopheles* larvae were detected in 5. Positive breeding site types were fishpond, stream margin, pond and swamp. Of 255 *Anopheles* larvae collected, An. darlingi represented 37% (96 larvae). In Lupuna 2 breeding site types were identified: stream margin (larvae=45) and swamp (larvae=65); in Santa Emilia, only pond was positive (larvae=71). In Nuevo Horizonte, larvae were found exclusively in fishponds (larvae=51); in Triunfo only pond was positive (larvae=51). Mean quantitative characteristics of the positive breeding sites were: pH = 5.5, conductivity = 29.9 ppm, salinity =4.1%, and turbidity = 20 JTU. Collected larval stages were: I = 32, II = 66, III = 92, IV = 59, and 6 pupae. Descriptive characteristics were partial shade, moderate water movement, and moderate submerged and emergent aquatic vegetation. These characteristics can determine presence of *Anopheles* larvae in the habitats.

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CHARACTERIZATION OF LARVAL HABITATS OF *AEDES AEGYPTI* IN KENYA

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Aedes aegypti, the principal vector for dengue and other emerging arboviruses, breeds preferentially in various man-made and natural container habitats. In the absence of vaccine, vector control is the primary means to reduce the incidence of dengue. Effective vector control depends on a good understanding of larval and adult vector ecology of which little is known in Kenya. Twenty sentinel houses in each of four study sites (in western and coastal Kenya) were assessed for immature mosquito incidence once a month for a period of 20 months (May 2014

to December 2015). All water-holding containers in and around the households were inspected monthly for immature *Ae. aegypti* mosquitoes. A total of 19,249 containers were inspected from Chulaimbo (6929) and Kisumu (6927) in the west, and from Msambweni (2689), and Ukunda (2704) on the coast. Of these, only 5.8%, 5.3%, 5%, and 6.6%, respectively, were positive for *Ae. aegypti* immatures. In all four sites, significantly more positive containers were located outdoors than indoors (χ^2= 712.4, DF=1, P<0.001). A total of 12,547 *Ae. aegypti* immatures were collected from these containers, which comprised 13 container types. More than 40% were from buckets, tires, and water-tanks, which produced 49% (1,245/2,530) of the pupae in the western and coastal study sites combined. Tanks, buckets, drums, and flowerpots were the key indoor containers, producing > 80% (92/108) of the pupae. Key outdoor containers in the coast were tires, tanks, buckets, and basins which accounted for 57% (1,316/1,965) of pupae, while pots and tires were the only key containers in the western region producing 71% (329/457) of pupae. Coast region produced significantly more *Ae. aegypti* immatures than the western region (Kruskal-Wallis, χ^2 = 179.8, DF=1, P< 0.0001). These results indicate that *Ae. aegypti* breeding habitats are abundant outdoors and are diverse both in the coast and western regions of Kenya. However, only a few containers are responsible for majority of the production. Targeting source reduction efforts towards these productive containers may be a cost-effective way to reduce dengue transmission in these regions.

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XENOMONITORING OF *WUCHERERIA BANCROFTI* INFECTION BEFORE AND AFTER MASS DRUG ADMINISTRATION IN DREKIKIER, PAPUA NEW GUINEA

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Xenomonitoring using entomological techniques is useful for measuring filarial infection in human populations indirectly through mosquito collections. Since the introduction of mass drug administration (MDA) and vector control programs, microfilaria prevalence in human populations is decreasing to thresholds where transmission may no longer be sustainable. The human landing catch is the standard used in calculating transmission indices where large numbers of mosquitoes can be collected to estimate disease prevalence and monitor transmission of *Wuchereria bancrofti* filarial worms in post-MDA programs. In this study, we monitor bancroftian infection in mosquitoes pre- and post-MDA using 2014 and 2015 collections from East Sepik, Papua New Guinea. Monitoring was conducted in three representative villages of high and moderate transmission zones within the Drekikier District. In the two representative villages of high transmission, 293 samples were collected in 2014, and 448 samples were collected in 2015. In the representative village of moderate transmission, 43 samples were collected in 2014, and 61 samples were collected in 2015. The samples were collected using human landing catch conducted from 6pm to 6am each night for six nights before and after one round of MDA. Results show 1.5 times more infection in the moderate transmission zone than in the high transmission zone pre-MDA but 0.6 times lower in post-MDA. This could be explained by a roughly 7-fold higher man biting rate in the high transmission zone compared to the moderate transmission zone (24.4±3.6 vs. 3.6±1.5 in 2014 and 74.7±14.8 vs. 10.2±2.9 in 2015). Sample DNA was extracted, run using conventional PCR and viewed through gel electrophoresis to find the mosquito infection rates. The mosquito infection rates of *W. bancrofti* decreased in both high (10.8%±0.21% to 2.6%±0.07%, p<0.0001) and moderate (16.3%±1.7% to 1.6%±0.4%, p=0.0082) transmission zones. Xenomonitoring of mosquito collections done before and after one round of MDA correspond with decreases in human infection by light microscopy (28.8% to 9.0%, p< 0.0001) in one representative village after one round of MDA.

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ASSESSING THE SPATIAL HETEROGENEITY OF MALARIA VECTORS IN THE CONTEXT OF INCREASING VECTOR CONTROL INTERVENTIONS

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Environmental variation across a landscape naturally contributes to spatial heterogeneity in malaria risk, yet the coverage of malaria interventions is increasing dramatically across many endemic regions. Novel geostatistical methods to investigate the spatio-temporal heterogeneity of malaria are increasingly available in open-source software. Investigating the spatio-temporal heterogeneity of malaria vectors is critical for understanding how transmission patterns change in the context of increasing intervention coverage. In this study we investigated the spatio-temporal heterogeneity of host-seeking adult mosquitoes in a region of southern Malawi where malaria interventions are being intensively scaled-up. We used geostatistical methods to efficiently sample houses for mosquitoes at a sub-district level. Host-seeking mosquitoes were collected using Suna traps on a continuous, rolling basis for one year. We used model-based geostatistics to account for spatial correlation in assessing the determinants of mosquito distribution, and to map spatial variation in the abundance of mosquitoes. Preliminary findings suggest clear spatial patterns in mosquito abundance. The results provide a basis for determining the spatio-temporal heterogeneity of malaria in the context of a community-based, vector-control intervention trial.

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BITING BEHAVIOR OF *ANOPHELES ALBIMANUS* IN ARTIBONITE, GRAND'ANSE AND NORD-EST, HAITI

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Hispaniola is the only island in the Caribbean where malaria transmission still occurs, with Haiti having a higher number of malaria cases than the Dominican Republic (DR). While both Haiti and DR are committed to eliminating malaria by 2020, no studies on mosquito behavior have been conducted on the island since 1988. We conducted studies to determine human exposure to *Anopheles albimanus* bites indoors and outdoors during the night in three different regions in Haiti: Artibonite, Grand'Anse and Nord-Est. Mosquitoes were collected by all-night human landing catches to determine man-biting rates and the timing of bites. *A. albimanus* showed preference to bite more often outdoors than indoors in all three sites: the outdoor-to-indoor biting ratio (O/I) was 1.10 (95% CI: 1.03 – 1.18) in Artibonite, 1.72 (95% CI: 1.59 – 1.87) in Grand'Anse and 1.88 (95% CI: 1.13 – 3.92) in Nord-Est. However, when weighting the biting rate against the time people reported being in bed, there was a higher exposure of mosquito bites to people indoors than outdoors in Artibonite (weighted O/I = 0.11; 95% CI: 0.11 – 0.13) and Grand'Anse (weighted O/I = 0.51; 95% CI: 0.41 – 0.61) but not Nord-Est (weighted O/I = 1.36; 95% CI: 0.57 – 3.40). These results suggest that indoor mosquito control methods such as indoor residual spraying or insecticide treated bednets may be effective in preventing malaria transmission in some areas

of Haiti where most people are indoors during the early evening. However, outdoor vector approaches may still be required to support malaria elimination efforts in Haiti.

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CHARACTERIZING TEMPERATURE IN LOCAL MALARIA TRANSMISSION ENVIRONMENTS

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The key mosquito and parasite life history traits that combine to determine malaria transmission intensity are all affected by temperature. To understand transmission ecology, therefore, it is important to determine the range of microclimatic temperatures experienced by malaria vectors in the field. This study was conducted in 6 rural villages of Sundargarh District in Odisha, India. Within these villages, data loggers were used to record microclimates in multiple locations and structures. These microclimate data were then used to drive a malaria parasite development model to compare predictions of parasite extrinsic incubation period (EIP) under local conditions (we use EIP here as an illustrative trait to explore the biological consequences of variation in local microclimate). Mean temperatures and temperature variation differed between resting sites within the transmission environments. Mean temperatures were around 2°C higher inside asbestos roofed houses than in outdoor vegetation, with tiled houses and cattle sheds intermediate to these extremes. Diurnal temperature variation was much greater outdoors compared with variation measured within domestic dwellings. Exploring the effects of temperatures on malaria parasite development rate revealed negligible differences between mircohabitats during the warmest times of the year (i.e. EIPs of around 10 days irrespective of environment). However, under cooler times the predicted EIPs varied substantially, being 10 or 20 days longer in vegetation, tiled houses or cattle sheds, than asbestos houses. Moreover, for large parts of the year, the EIPs predicted for the specific microhabitats differed to those predicted using weather station data. The current study reveals that microclimates can vary substantially between habitats within local transmission environments. Small differences in microclimate can potentially lead to large differences in life history traits. Measuring the range of conditions available to mosquitoes within local transmission settings should provide a more robust characterization of transmission ecology than remote weather station data.

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MEASURING SPECIES - AND REGION-SPECIFIC MARKERS OF MOSQUITO BITES BY SMALL PEPTIDE ARRAYS

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Knowledge about human exposure to mosquitoes can help target interventions to those at greatest risk of malaria and other vector-borne diseases. Mosquito trapping estimates local vector populations, but implementation of large scale collection programs is difficult, and mosquito presence alone does not indicate who is being bitten. Measuring antibody responses to mosquito salivary antigens is a potential strategy to estimate exposure to specific vectors, locations and risk groups. Two *Anopheles* salivary antigens, SG6 and cE5, have been shown to elicit a humoral immune response in humans that can be measured by ELISA to indicate recent exposure to mosquito bites. However, due to the conservation of these genes, ELISAs have limited ability to discriminate between exposures to different vector species. We developed a small linear peptide array to measure antibody responses to individual epitopes along the full length of six mosquito salivary proteins each from six *Anopheles* species. The arrays were probed with sera from West African children and adults, Southeast Asian adults with acute falciparum malaria, and North American adults, allowing us to observe differences in the antibody binding profiles of people to antigens from geographically separated mosquito species. Southeast Asian adults reacted most strongly to peptides from Southeast Asian mosquitoes and West African sera reacted most to peptides from African mosquitoes, while North American adults had the lowest seroreactivity to all six *Anopheles* species. From these data, we identified a set of peptides that can differentiate between the sera of people bitten by mosquitoes from different regions. The set of mosquito proteins on the array can expand to include more salivary proteins and mosquito species, allowing us to build a panel of peptides to measure human exposure to diverse vectors of a variety of mosquito-borne diseases. These peptides could potentially be used to develop a point-of-care test to estimate human exposure to mosquitoes allowing for better targeting of vector-borne disease interventions.

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ENVIRONMENTAL FACTORS INFLUENCING *ANOPHELES DARLINGI* POPULATION DYNAMICS AND MALARIA TRANSMISSION IN ZUNGAROCOCHA, LORETO, PERU

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A sustained increase in the number of malaria cases has been observed in Peru since 2012 with the majority of cases reported in Loreto, northern Amazon, where *Anopheles darlingi* is the dominant malaria vector. Examining *An. darlingi* population densities, age structure, and natural *Plasmodium* infection rates in this malaria endemic region is essential for malaria transmission risk assessment. Moreover, the impact of environmental factors on *An. darlingi* abundance and malaria transmission could further contribute to understand malaria transmission patterns. This study aimed to estimate *An. darlingi* seasonal abundance, age structure, and *Plasmodium* infection rates; and correlate with local environmental parameters and malaria cases reported by the Ministry of Health in Zungarococha, a malaria-endemic community in Loreto. *An. darlingi* densities were estimated by protected Human Landing Catch performed outside local homes from 1800h to 0600h for a 3-day period each month from June 2014-March 2016. A subset of *An. darlingi* females (30%) was dissected to determine parity status and remaining specimens were stored for *Plasmodium* spp. detection. Local temperature, humidity and rainfall were simultaneously recorded. A total 15,904 *An. darlingi* females were captured; 5,123 were dissected. *An. darlingi* densities ranged from 0.5 to 164 mosquitoes landing per person per hour, with the highest densities (>100) recorded in June 2014 (111), January-March 2015 (123-127), July 2015 (117) and January 2016 (164). Parous was the predominant ovarian stage in mosquitoes dissected (>60%) during density peaks. The number of malaria cases reported monthly ranged from 62-285, with highest numbers (>150 cases) in June-July 2014 (175-214), July-September 2015 (152-157), and Feb-March 2016 (157-285), a few weeks after mosquito population density peaks and heavy rainfall. *An. darlingi* densities shows a positive association with rainfall events. Results are discussed in terms of the impact of environmental factors on vector population and malaria transmission dynamics, and will help guiding vector control strategies in the region.

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ASSOCIATION OF AUTOCIDAL GRAVID OVITRAPS WITH REDUCED RATES OF CHIKUNGUNYA VIRUS INFECTION IN PUERTO RICO

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There are currently no effective and sustainable interventions to prevent infections with viruses transmitted by *Aedes* mosquitoes. Since 2012, four communities in Puerto Rico have been participating in a field trial of a recently developed autocidal gravid ovitrap (AGO). After 3 AGO traps were placed in >85% of homes in two intervention communities, adult *Ae. aegypti* mosquito populations were reduced by ~80% compared to two non-intervention communities. The introduction of chikungunya virus (CHIKV) to Puerto Rico in May 2014 provided an opportunity to determine if AGO traps were associated with CHIKV infection rates in humans and mosquitos in these communities. To estimate the seroprevalance of CHIKV infection in intervention and non-intervention communities, 377 houses were randomly selected. Participating household members provided a blood specimen and completed a questionnaire on demographics, recent illnesses, and mosquito avoidance practices. Serum specimens were tested by IgG ELISA to detect historic CHIKV infection. During November 2015 and February 2016, a total of 233 (62%) households from the four communities agreed to participate. Mean age of participants (53 years) was greater than that of all eligible residents (49 years). Mean age of participants from intervention communities was not significantly different from those from non-intervention communities. Among 152 and 175 participants from non-intervention and intervention communities, historic CHIKV infection was detected in 69 (45%) and 40 (23%) participants, respectively. The observed two-fold difference in the prevalence of CHIKV infection in intervention compared to non-intervention communities may be associated with the lower measured mosquito densities in communities where AGO traps are present. Additional analyses are being performed to adjust anti-CHIKV antibody prevalence with respect to sampling design, community differences, and participation rates. These findings may implicate AGO traps as an effective and sustainable community intervention to prevent infections transmitted by *Ae. aegypti* mosquitos.

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BACTERIAL COMPOSITION OF LARVAL BREEDING SITES OF AFRICAN *AEDES AEGYPTI* AND ITS EFFECT ON VECTORIAL CAPACITY

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Like other animals, insects establish symbiotic associations with microbial communities that shape their individual phenotype and fitness. In particular, the native gut bacteria of insect vectors can modulate their immunity and susceptibility to human pathogens. Compared to vertebrates, insects have more labile gut bacterial associations that are under strong influence of the environment. Thus, habitat-related differences in bacterial communities could mediate an environmental influence on vector-borne pathogen transmission. We investigated whether differences in the bacterial communities of larval breeding sites may drive variation in vectorial capacity of the mosquito *Aedes aegypti*, a major vector of dengue, Zika, and chikungunya viruses. In Sub-Saharan Africa, *Ae. aegypti* larvae develop both in domestic habitats such as human-associated containers and in sylvatic habitats such as rock pools or tree holes. Comparison of natural sylvatic and domestic breeding sites in Gabon by metataxogenomics revealed contrasted bacterial communities in the water and, to a lesser extent, in the midgut of adult *Ae. aegypti* emerging from these breeding sites. To test whether exposure to different bacteria during larval development may differentially affect adult vectorial capacity, we created gnotobiotic larvae using a selection of four bacterial isolates from the natural breeding sites in Gabon. Mono-association with *Enterobacter*, *Salmonella*, *Arthrobacter*, or *Rhizobium* bacterial isolates during larval development resulted in significant differences in pupation rate. In addition, larvae exposed to the *Arthrobacter* isolate had larger bacterial loads in adult midguts pre and post blood meal, showed decreased antibacterial activity in adult hemolymph, and were less susceptible to dengue virus infection. No differences in adult lifespan were detected between the different gnotobiotic treatments. Together, our results provide the proof of principle that habitat-related differences in larval exposure to bacteria can drive variation in adult mosquito immunity and vectorial capacity.

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THE IMPORTANCE OF HUMAN POPULATION CHARACTERISTICS IN MODELING MOSQUITO VECTORS: A COMPARATIVE ANALYSIS OF MODEL COMPONENTS

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The current Zika virus epidemic in the Western hemisphere is representative of the confluence of global climate change and infectious disease expansion, and vector modeling represents a pertinent and timely method to analyze the environment associated with Zika-carrying mosquitoes. Among many mosquito species distribution models, there are varying opinions on which variables are most predictive and, consequently, should be included in modeling efforts. While climate variables (e.g., mean temperature, mean precipitation) are routinely included, some argue that human population dynamics, in the form of population density and socioeconomic status, should also be included. This project aimed to test the importance of including human population characteristics by modelling the Zika virus vector *Aedes aegypti* in the Southeastern United States with climate variables, population density, and poverty characteristics. A. aegypti occurrences, global climate data, and population characteristics were obtained from publicly available sources and sampled at a resolution of 2.5 arc-minutes. Data pre and post-processing was completed in ArcMap 10.3 and models were created in Maxent v.3.3.3k. Four models were developed for this project: a climate-only model, a climate and population density model, a climate and poverty model, and a combined model with climate, population density, and poverty. Models were evaluated by comparing test and training area under the curve metrics, omission and commission errors, and variable jackknifing results. The climate-only model performed poorly compared to models with human population characteristics. The combined model was the best fit, though the model with climate and population density had a lower commission rate (21.0% and 20.6%, respectively). Jackknife results for the full model showed that population density was the most significant contributor to the model. This research indicates that more consideration should be given to human population characteristics when modelling mosquito habitats.

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URBAN MICROCLIMATE AND DENGUE VECTOR COMPETENCE OF THE INVASIVE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*

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Vector-borne diseases have increased in urban environments in recent decades. Incidence of vector-borne disease is often unevenly distributed across urban landscapes, which are themselves comprised of a diversity of landscape features that can modify thermal microclimates. Because mosquitoes are small ectotherms, their growth, survival, and reproduction are all sensitive to fine-scale variation in microclimate. Additionally, microclimate during the larval stage can affect life history traits of adults, a phenomenon known as carry-over effects. The majority of studies assume temperature to be the most important factor driving carry-over effects, enabling research to be done in the lab. However, laboratory research is unable to fully incorporate realistic field microclimate conditions experienced by mosquito vectors. To further explore the effect of larval microclimate on Ae. albopictus vector competence, we conducted a semi-field experiment examining vector competence across urban, suburban, and rural sites in Athens, GA. *Aedes albopictus* larvae were reared in the field across the three treatments, and offered a DENV-2 infectious bloodmeal post-eclosure, to measure the effect of microclimate on dengue infection, dissemination, and transmission. Information on vector competence was then incorporated with mosquito life history, potential larval habitat density, and adult abundance data collected from the same sites, to better characterize risk of dengue through vectorial capacity across the urban gradient.

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A TRADE-OFF BETWEEN DRY SEASON SURVIVAL LONGEVITY AND HIGH WET SEASON NET REPRODUCTION EXPLAINS THE PERSISTENCE OF *ANOPHELES* MOSQUITOES

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Plasmodium falciparum malaria remains a leading cause of death in tropical regions of the world. Despite efforts to drive transmission down, rebound epidemics associated with the persistence of malaria vectors have remained a major impediment to local elimination. One area that remains poorly understood is how *Anopheles* populations survive long dry seasons to re-emerge following the onset of the rains. We developed mathematical models to explore the impact of different mosquito survival strategies on the dynamics of the vector population. We show that mosquitoes have different lifestyles between the wet season and the dry season. Their ability to persist is attributed by their propensity to exploit the wet season (fast and high reproductive output), but then mitigate the effects of the dry season (longevity and aestivation). We demonstrate that aestivation is a population rescue strategy that makes ecological vector population extinction difficult, while wet season high reproductive output buffers the population against dry season potential extinction. We show that both longevity/aestivation and high wet reproduction allow persistence of the mosquitoes, and can reproduce patterns observed in field data from the Sahel region. Our results demonstrate the importance of practical ecological methods to control vectors in the dry and wet seasons if malaria transmission is to be interrupted.

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DENGUE FEVER AND *AEDES AEGYPTI* RISK IN THE GALAPAGOS ISLANDS, ECUADOR

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Dengue fever, chikungunya and zika virus, transmitted by the *Aedes aegypti* mosquito, are emerging infectious diseases in the Galapagos Islands of Ecuador. In 2014 we conducted a pilot study on two islands (Puerto Ayora (PA) on San Cristobal and Puerto Baquerizo (PB) on Santa Cruz) to assess *Ae. aegypti* abundance, key larval habitats and household risk factors for dengue infection. We surveyed 100 households (50 per island) in high-risk areas. Adult *Ae. aegypti* were collected inside and outside the home using prokopack aspirators, larval indices were determined through container surveys, and heads of households were interviewed to determine demographics, housing conditions, and knowledge, attitudes and practices regarding dengue. Multimodel selection methods were used to identify best-fit logistic regression models to explain the presence of *Ae. aegypti* and self-reported prior dengue infections. We found that 24.3% of PB and 14% of PA participants self-reported a prior dengue infection. The best-fit model to explain prior infection indicated higher risk for people who frequently traveled between the islands, households that experienced interruptions in the piped water supply, and heads of households with salary above the minimum wage. Adult *Ae. aegypti* were collected from 14% of PB and 4% of PA houses; other adult mosquitoes collected included *Culex quinquefasciatus* and *Ae. taeniorhynchus*. Significantly more PB homes than PA homes had containers with *Ae. aegypti* juveniles ($p = 0.012$; PB House Index = 20, Breteau Index = 26; PA House Index = 6, Breteau Index = 6). The characteristics of the predominant *Ae. aegypti* larval habitat were 55-gallon water storage drums, located outdoors, uncovered, shaded, and filled with tap-water. The best-fit model to explain the presence of *Ae. aegypti* indicated higher risk in households that used tanks for water storage and in households that perceived that dengue prevention was difficult. These findings provide the region's public health sector with key information for conducting dengue and zika control campaigns, and highlight the importance of local socio-ecological studies to understand dengue risk.

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DYNAMICS OF INGESTED *ENTEROBACTER* SP. IN THE GUT OF MOSQUITOES THROUGHOUT LIFE CYCLE

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There is a symbiotic relationship between the mosquito and its gut microbial residents. Taxa in genus Enterobaacter are commonly present in the gut of various mosquitoes. But few data were available regarding the dynamics of bacteria from being ingested to being egested. In this study the strain Ag1 of Enterobacter sp. was tagged with green fluorescent protein (GFP), and its dynamics were tracked in the gut of both *Anopheles gambiae* and An. stephensi. GFP tagged bacteria were provided to mosquitoes in the sugar meals for two days. Introduced bacteria were visualized by imaging individual gut under a fluorescent microscope. The bacterial intensity was estimated by average number of fluorescent colonies per gut. In the sugar fed guts, the prevalence of bacteria

decreased over time from 100% to 37% in *An. gambiae* on day 6 post ingestion and 30% in An. stephensi on day 6 post ingestion. At this time point, mosquitoes were given a blood meal, which brought prevalence back to ~70%. The decline of bacteria in the gut is correlated with the presence of GFP-bacteria in the feces, indicating that the bacteria were discharged through defecation. Enterobacter was favored in the blood fed guts, and proliferated well for two days, then starting being egested with digested wastes in feces. The data indicate that the bacteria are able to reside in the gut but the length of stay varies individually. Moreover, when gravid mosquitoes laid eggs in water, Enterobacter cells in feces entered into aquatic habitat. GFP bacteria were detected in the culture of larval gut homogenates of larvae and emerged adults. Evidently, Enterobacter transstadially passed through metamorphosis from larvae to adults. Mosquito associated bacteria can cycle from generation to generation through fecal-oral route.

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DRIED BLOODSPOTS FROM PAIRED HUMAN AND ENGORGED MOSQUITOES TO DEMONSTRATE THE FEASIBILITY OF XENOSURVEILLANCE IN WEST AFRICA

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Xenosurveillance is a novel technique that utilizes mosquito bloodmeals to noninvasively survey human populations for infectious diseases. The technique takes advantage of the host-seeking and blood-feeding behavior of *Anopheles gambiae* mosquitoes. Two villages in Northern Liberia were enrolled in to our study, and human dried bloodspots were collected during the initial enrollment period. The villages were subsequently sampled for indoor resting engorged mosquitoes on a rotating schedule for the following two weeks. Bloodfed mosquitoes collected from inside homes had their midgut contents expelled onto FTA cards for further testing. RNA from both human and mosquito samples were eluted, extracted, pooled, and subjected to Next Generation Sequencing. Bioinformatic analysis revealed the presence of GB virus C in both human and mosquito samples collected in the same home. A total of 15 and 28 reads aligned to West African strain of GB virus C, respectively. This resulted in a combined coverage of 40% of the genome. These matched results indicate that our technique can reliably detect genetic signatures of human viruses in mosquito bloodmeals. This study demonstrates the feasibility of Xenosurveillance in a field setting.

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ADVANCING ARBOVIRUS SURVEILLANCE BY ASSESSING THE EFFICIENCY OF VECTOR AND DISEASE MONITORING PROGRAMS

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Mosquito surveillance is a necessary component of public health for assessing human health risks for a plethora of transmittable diseases. Vector presence, abundance, and infection rates are needed to effectively manage efforts in mosquito control operations. Over the past few years, mosquito surveillance and control have become increasingly important as endemic diseases resurface, as happened West Nile virus (WNV) in 2012 in the United States, and new diseases emerge, as is currently occurring with Zika virus. However, mosquito collection and testing to monitor these diseases can become financially burdensome, especially in developing countries and sparsely population regions. Using WNV surveillance data from South Dakota, our study looks to determine whether the current level of mosquito and arbovirus surveillance can be reduced while maintaining a similar capacity to predict risk to humans. Mosquitoes were captured and tested for WNV in 27 counties for varying numbers of years from 2003 to 2015. The minimum infection rate was calculated for every county in every reported week, and the maximum MIR (MMIR) for every county-year combination was estimated in those county-years. Analysis of variance was used to determine if any significant differences could be detected among the MMIR for county-year combinations. Our model suggests that there are some persistent differences in MMIR between counties ($p = 0.01245$). A Tukey multiple comparison test showed only two counties to have a significant difference in their mean MMIR. However, MMIR correlated well with human cases, and was an effective predictor of total yearly risk. In a set of regressions, we considered whether any county could serve as a proxy for other counties or the state as a whole. We found that no individual county's MMIR was more informative than statewide MMIR. The results suggest that maintaining broader spatial coverage across the state is likely preferable to oversampling individual counties.

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COMPARATIVE SUSCEPTIBILITY BETWEEN FIVE *AEDES AEGYPTI* POPULATIONS TO FOUR DENGUE VIRUS SEROTYPES

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Dengue is the most important arboviral-infection affecting humans and in the America, its main vector is *Aedes aegypti*. There are multiple factors that are key to the successful spread and maintenance of the dengue virus cycle in nature: vertebrate host biology, circulating viral strain, vector susceptibility towards the virus. The last one, vector competence, may vary according their geographic distribution, even within the same city. Therefore our aim is assessing the existence of vector competence differences between five *Ae. aegypti* populations collected throughout the city of Manaus considering four dengue virus serotypes. *Ae. aegypti* eggs from five locations within the city of Manaus, Amazonas, Brazil were collected and reared under laboratory conditions (temperature 26°C and a relative humidity of 80%). Individuals from each mosquito population were then challenged simultaneously to the tested serotypes by membrane feeding assay. Fourteen days post infection, the mosquitoes were dissected separating the head from the body. Viral RNA extraction was performed with the QIAamp Viral RNA Mini Kit, whereas the detection and quantification of viral RNA were performed with the Power SYBR Green Kit Step-1. We then calculated the infection rate (IR) and dissemination rates (DIR), as well as the vector competence(VC) for each population. To date, our analyses showed significant differences regarding DENV-1 IR than have variation 25 to 100% and DIR range of 50 to 95%, as well in VC with a range of 12,5 to 95% between the five *Ae. aegypti* populations. Despite the high rates of infection, there was no significant difference with DENV-2 in all populations with IR varying from 90-100%, DIR 100% and VC varying from 90 to 100%. The results also showed DENV-4 with the similarity among populations, with IR ranging from 85 to 100%, DIR varying from 80 to 100% and VC with a range of 70 to 94.7%. This study we will be able us to further understand the vector- virus interaction and the dynamics of dengue transmission in a local urban context.

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HUMAN EHRLICHIOSIS AND ANAPLASMOSIS IN NORTHEASTERN THAILAND

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Human Monocytic Ehrlichiosis (HME) and human granulocytic anaplasmosis (HGA) are emerging, tick-borne rickettsial diseases. In Thailand, the first HME cases were first reported in 1997.A prospective etiologic study of patients with acute undifferentiated fever (AUF) was conducted in Maharat Nakhon Ratchasima hospital, northeastern Thailand. Serological tests for *Ehrlichia chaffeensis* and *Anaplasma phagocytophilium* IgM and IgG antibodies using commercial immunofluorescence antibody assay were performed in 70 AUF patients who tested negative for scrub typhus, murine typhus, spotted fever group, Q fever, leptospirosis, dengue, malaria, and bloodstream infection. The result showed seroprevalence (IgG) of *E. chaffeensis* and *A. phagocytophilium* in 37.1% (26/70) and 21.4% (15/70) patients, respectively. Eleven (15.7%) patients were IgG positive for both *E. chaffeensis* and *A. phagocytophilium* antigens. Additionally, 5 (7.1%) cases had positive *E. chaffeensis* IgM antibody titer at 1:64. Three confirmed HME cases were diagnosed based on clinical compatible illness and single serum of *E. chaffeensis* IgG titer ≥ 1:256. Most of them had common clinical (fever, myalgia, headache) and laboratory findings (elevated liver enzyme, thrombocytopenia). Complications developed in two patients: septic shock and acute renal failure. Two patients, one of which had acute renal failure, received ceftriaxone and doxycycline and both of them fully recovered. Another patient with septic shock received intravenous ceftriaxone and chloramphenicol and subsequently died. In conclusions, HME and HGA should be included in the differential diagnosis of Thai AUF patients and prompt doxycycline treatment is likely to improve outcome.

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DISTRIBUTION AND MOLECULAR CHARACTERISTICS OF *RICKETTSIAE* FOUND IN TICKS ACROSS CENTRAL MONGOLIA

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Little is known regarding tickborne diseases in Mongolia. A total of 1,497 adult unfed ticks; 261 *Ixodes persulcatus*, 795 *Dermacentor nuttalli*, and 441 *Hyalomma asiaticum,* were collected from the environment and off livestock returning from pasture, across a vertical stretch of Central Mongolia spanning from China to Russia (Selenge, Tov, & Dornogovi aimags). Ticks were then separated into genus specific pools (n=299), by sample location, containing ~5 ticks each. After extraction of DNA and RNA, nested polymerase chain reaction (PCR), reverse transcription-PCR (RT-PCR), and quantitative real-time RT-PCR (qRT-PCR) were conducted to detect rickettsia bacteria and tickborne encephalitis virus (TBEV). Assays yielded pool detection rates of 92.5% (49/53) and 1.9% (1/53) of *I. persulcatus* pools testing positive for *Candidatus Rickettsia tarasevichiae* and TBEV respectively, while *Rickettsia raoultii* was found in 72.8% (115/158) of *D. nuttalli* pools. Both *R. tarasevichiae* and *R. raoultii* are recognized as emerging tickborne diseases, with this being one of the first reports of *R. tarasevichiae* in Mongolia. Given that *R. tarasevichiae* shares the same vector (*I. persulcatus*) as TBEV, and may present with severe atypical spotted fever group (SFG) rickettsia-like symptoms, Mongolian physicians treating suspected cases of TBEV should include *R. tarasevichiae* infection in their differential diagnosis and consider prescribing antimicrobial therapy.

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SEROPREVALENCE OF *RICKETTSIA* AND *ANAPLASMA* EXPOSURE IN HUMANS AND LIVESTOCK ACROSS A CENTRAL STRETCH OF MONGOLIA

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Tickborne diseases (TBD) are suspected to be a major cause of illness in Mongolia, although the extent of which remains unknown. Therefore, to better understand the burden of TBD in Mongolia, serosurveillance focusing on anaplasma and rickettsial infections was carried out through an international collaboration between US and Mongolian researchers from September 2014 to October 2015. Samples were collected from 388 nomadic herders, 867 goats, 871 sheep, 367 cattle, and 216 horses, across three provinces (Selenge, Tov, Dornogovi). Serum samples diluted 1:50 in sterile 1X PBS were placed on IFA slides coated with *R. rickettsii* and *A. phagocytophilum*, and examined using a fluorescent microscope. The overall seroprevalence of anaplasma and rickettsia in human samples was 136/365 (37.3%) and 73/374 (19.5%), respectively. Anaplasma and rickettsia seroprevalence rates in livestock were 1,120/2,370 (47.3%) and 478/2342 (20.4%). Such high rates of exposure might possibly be attributed to the increased risk of tick bites associated with a nomadic lifestyle, where both herder and livestock spend most of the day traveling from pasture to pasture. Significant differences in detection rates by sample species and region were observed for both pathogens of interest, indicating a higher prevalence of exposure of TBD in the central and northern provinces of Tov and Selenge, compared to that of the Gobi region of Dornogovi. These findings lay a framework for much needed future tickborne disease research in Mongolia, while providing valuable information to veterinarians, clinicians, and policy makers currently involved in ongoing TBD control efforts.

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RAPID DIAGNOSIS OF *ANAPLASMA PHAGOCYTOPHILUM* WITH P44 DIPSTICK ASSAY

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Anaplasma phagocytophilum is tick-borne bacterial zoonosis, infects into and destroys host blood cells. Human patients show influenza-like symptoms with fever, headache, myalgia, and malaise. Human granulocytic anaplasmosis (HGA) is 3rd most common tick-borne infections in US behind Lyme disease and Rocky Mountain spotted fever. Commercial IFA kit has been used for diagnosis of canine or horse Anaplasmosis, but other animal reservoirs, such as cattle, deer, goats, have not been developed until now. Dipstick for detection of *A. phagocytophilum* antibody will be useful for early control of *A. phagocytophilum* because of on-site applicability. Therefore, dipstick kit for reservoir animals is urgently needed for early diagnosis of animal anaplasmosis. Recombinant surface protein p44 of *A. phagocytophilum* was produced by *E. coli* expression system and the immunological specificity was confirmed by Western blotting with positive control serum. Three types of dipstick kit for *A. phagocytophilum*

antibody detection were designed and compared with their reactivity, which were sandwich, indirect I using anti-bovine IgG, and indirect II using protein A. And then the reactivity of test line was also read by gold reader. Recombinant p44 protein was expressed from *E. coli*, and used as dipstick antigen. The protein size was 44 kDa and the specificity was confirmed by Western blotting. Indirect I and II could differentiate positive and negative serum, and the discriminatory power of indirect II was superior to those of other types. Dipstick kit for *A. phagocytophilum* will be useful for early detection of *A. phagocytophilum* infection at on-site farm and helpful for early control of zoonotic anaplasmosis at animal level.

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RAPID DIAGNOSIS OF *EHRLICHIA CHAFFEENSIS* WITH P120 DIPSTICK ASSAY

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Ehrlichia chaffeensis is tick-borne bacterial zoonosis, infects into and destroys host blood cells. Human patients show influenza-like symptoms with fever, headache, myalgia, and malaise. Human monocytic ehrlichiosis (HME) is 4th most common tick-borne infections in US behind Lyme disease, Rocky Mountain spotted fever, and HME. Commercial IFA kit has been used for diagnosis of human or canine Ehrlichiosis, but those for other animal reservoirs, such as deer, goats, and cattle, has not been developed until now. Antibody detection dipstick kit to *E. chaffeensis* infection will be useful for early control of *E. chaffeensis* by early detection at on-site farm. Therefore, dipstick kit for reservoir animas is urgently needed for early diagnosis of animal ehrlichiosis. Recombinant surface protein p120 of *E. chaffeensis* was produced by *E. coli* expression system and the immunological specificity was confirmed by Western blotting with positive control serum. Concentration of gold particle and dilution factor of serum were determined by Dot assay with recombinant p120 protein and positive control serum. Recombinant p120 protein was expressed from *E. coli* and used for dipstick antigen. The protein size was 43 kDa and the specificity was confirmed by Western blotting. Optimal concentration of gold particle in Dot assay was OD7 and that of serum dilution 2X dilution. Deer positive serum showed clear band on test line, and there was no reaction band to deer negative serum. Dipstick kit for *E. chaffeensis* will be useful for early detection of *E. chaffeensis* infection in on-site farm and helpful for early control of zoonotic ehrlichiosis at animal level.

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UTILIZE ALL FOR HEALTH. AN OBSERVATIONAL STUDY

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Human is the only attribute to blush good innermost emotions healthy norm of daily living activities. Human mutual cooperation, share events, happiness and benefiting the human as a whole less tendencies toward disease, deaths. Happiness more choices, more demand and increasing supply goods decreasing diseases, deaths, disparities among human. Health contribution is susceptible to sustainable variable environment. The total gross world product (GWP) is approximately US$107.5 trillion reflect purchasing power parity the per capita GWP was approximately US$16,100 in 2014. An in-depth review was performed to emerging idea on the role of the global health investors in health service utility. The articles and other documentation were then summarized to assess what is known about the investor's sector's role in global health as well as gaps in the literature. In conclusion, society health contribution system reduces the health care cost serve the wellbeing of humanity the final key to health for all. The health utility is a total multidisciplinary and multi-sectoral approach is emerging, leading to sustainable global health. It is suggested to promote and practice global health collaboration education, promotion activities among sectors to raising the health status of societies to accepting globalization and prosperous future societies.

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USING AN INNOVATIVE TELEHEALTH MODEL TO SUPPORT PROVIDERS IN GEOGRAPHICALLY DISPERSED AREAS WHO DELIVER CARE TO HIV-POSITIVE PREGNANT WOMEN

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Human Immunodeficiency Virus (HIV) remains a global pandemic, with mother-to-child transmission (MTCT) persisting in many areas due to inadequate access to early diagnosis and prenatal care. These barriers to HIV management are most pronounced in rural areas where resources are often limited, both in the United States and abroad. As many rural primary care providers do not receive a high volume of HIV patients, they often face challenges in maintaining familiarity with current HIV guidelines, including those related to MTCT. Frontier AETC ECHO is an innovative telehealth program that offers longitudinal teaching and mentorship as well as remote consultation to community HIV practitioners with the goal of strengthening capacity of the HIV workforce, supporting high-quality HIV care, and disseminating best practices in HIV medicine. This virtual peer-to-peer support network connects community HIV providers and a multidisciplinary team of specialists at the University of Washington across vast distances and employs real-time, case-based discussion and lectures to educate and support providers in low-resource and rural settings. Here, our goal is to assess the impact of the Frontier AETC ECHO program on provider management of HIV in pregnancy through two means: 1) reviewing cases of HIV in pregnancy presented by community practitioners to the ECHO network, and 2) a survey of community providers who regularly participate in ECHO. The survey assessed providers' knowledge, comfort level, and local resources for managing HIV in pregnancy. More than half of participants responded. All patient cases had the successful outcome of prevention of MTCT of HIV. Patient and provider-based outcomes support that ECHO is an efficient tool for supporting management of HIV in pregnancy, with providers reporting increased knowledge as a result of both presenting and observing cases on ECHO and many providers reporting they would be refer their patient to another provider if ECHO support were not available. These results demonstrate the potential of this unique model for application to other prevalent healthcare issues in rural and low-resource settings.

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COMMUNITY-BASED SCREENING FOR CARDIOVASCULAR RISK USING A NOVEL MOBILE HEALTH (MHEALTH) TECHNOLOGY IN RURAL KENYA

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An increasing burden of non-communicable diseases, including cardiovascular disease (CVD), in low- and middle-income countries demands innovative approaches. In remote, resource-constrained settings, models of laboratory-based, physician-supervised management are not sustainable. Community health workers (CHWs) can provide primary health care, yet many lack necessary background, training and skills. Communication technology, such as mobile health (mHealth) has the potential to augment CHW capacity. We hypothesized that mHealth could be used to identify individuals at high CVD risk in remote communities with poor access to health clinics who would benefit from education and pharmacologic interventions. We designed and implemented a novel mHealth tool based on principles and data from the WHO Package of Essential Noncommunicable Disease Interventions for Primary Health Care. Our "two-way" mobile phone application collects and centrally stores SMS

text message data entered by a CHW on a subject's age, gender, smoking, diabetes, and systolic blood pressure, and returns as SMS text message the category of 10-year CVD risk: "green" <10%; "yellow" 10 to 140mmHg. The prevalence of hypertension was similar in men and women (22/97 [23%] men vs 26/126 [20%] women, p=0.74). Only 2/223 (0.9%) of subjects reported a history of diabetes. The estimated 10-year risk of CVD event was <10% ("green") in 218/223 (98%) and 10-20% ("yellow") in 5/223 (2.2%). All subjects received immediate feedback on their risk profile, counseling on cessation of tobacco use, healthy diet and exercise, and referral to the a nearby clinic for follow-up for patients with elevated CVD risk. Acceptability of the mHealth application, and of CHW CVD risk screening by subjects was excellent. We have developed an mHealth tool that can be used by CHWs to screen for CVD risk factors, demonstrating proof-of-concept in rural Kenya.

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DOMESTIC IMPLEMENTATION OF THE INTERNATIONAL HEALTH REGULATIONS: BRINGING THE WORLD OF HEALTH SECURITY HOME

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Domestic Implementation of the International Health Regulations: Bringing the World of Global Health Security Home The IHR is an agreement between all WHO Member States, 196 countries, to strengthen public health preparedness and response capacities globally. Under the IHR member states are required to establish core capacities to detect, assess, report and respond to public health emergencies. These core capacities allow for the assessment and notification of public health emergencies with potential international impact to World Health Organization (WHO) through the international network of IHR NFPs. In the United States, the Division of International Health Security (DIHS) in the Office of the Assistant Secretary for Preparedness and Response (ASPR) coordinates, and is a critical component of, the three-part U.S. NFP. In this role, DIHS advises the federal government on international IHR policies, develops domestic polices and processes to address IHR obligations, coordinates IHR-related communications and technical exchanges with international partners and domestic stakeholders, and leads efforts on bilateral policy and capacity-building exchanges with foreign NFPs. State, local, tribal, and territorial (SLTT) public health professionals play an integral role in the fulfillment of U.S. obligations under the IHR Specifically, the US NFP works closely with federal departments and agencies who support SLTT reporting and response networks (e.g., CDC, FDA, DOI, USDA) to ensure communication of IHR-relevant events to WHO and international partners. Additionally, the US NFP uses SLTT self-assessments associated with the federal Public Health Emergency Preparedness cooperative agreement and the Hospital Preparedness Program to inform annual assessments of domestic IHR capacities and reporting to WHO. Attendees of this Learning Session will have the opportunity to discuss management of the US NFP, better understand the policies and processes central to the U.S. NFP, and consider how capacities at the state and local level support U.S. IHR obligations and, as a result, reinforce domestic and international health security.

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IMPLEMENTATION OF INTEGRATED POINT-OF-CARE TESTING FOR HIV SYPHILIS MALARIA AND ANAEMIA (IPOC) IN ANTENATAL CLINICS IN WESTERN KENYA

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Prevalence of HIV, syphilis, malaria, and anemia is high among pregnant women in western Kenya; testing for these is currently part of the country's antenatal recommendations. However, implementation of the full antenatal testing profile is limited because not all tests are done in smaller facilities, requiring women to be referred to distant labs. We conducted an implementation study to introduce point-of-care testing for HIV, syphilis, malaria and anemia (iPOC) in small rural health facilities in western Kenya. Seven small facilities without screening tests were purposively selected to introduce iPOC in December 2014. Antenatal nurses received a week of training. All 4 point-of-care tests were provided. For 8 months, 588 exit interviews were done to assess testing uptake and quality of care. Comparison of ANC register data before and after iPOC was done to assess uptake increase. On-site observations of testing for quality control were done at 3, 6, 9 and 14 months. To determine the perception of the ANC experience with iPOC, 12 focus group discussions were held among women who had attended ANC recently. In-depth interviews on iPOC delivery were also done with program stakeholders and 16 iPOC nurses. Overall implementation success was assessed using 5 indicators: program acceptability, adoption, appropriateness, feasibility and fidelity. We assessed the impact of iPOC on operations using discrete event simulation (DES) modelling to compare system outputs with and without iPOC. Over a 4-week period in August 2015, clients attending MCH services at these facilities were observed and their process times taken to construct flow pathways and parameters to input into the model using WITNESS software. DES model outputs will include time to complete testing, waiting times, duration of activities, staff and resource impacts, disease diagnosed, process bottlenecks, and cost impacts in clinics with and without iPOC intervention. This study will give insight into the implementation of iPOC from the client, provider and health system perspective for future program delivery. The study will be completed in August 2016 and results will be presented.

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CROWDSOURCE REPORTING OF INFECTIOUS DISEASES DURING A DISASTER: A NOVEL TECHNOLOGY APPROACH

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Crowdsourcing of patient text data related to infectious diseases such as viral respiratory is a viable option for reporting of cases in the event of a natural disaster such as floods and hurricanes. The challenge during a natural is that access to the Internet is limited and traditional websites for logging infectious reports may not be operational. The data gathered from this process will be kept confidential and used as planning data and tracking cases for healthcare provider resources in a community. A personal cloud server is a solution to host a data collection system that will crowd source infectious disease data for a community such as gender, age of patient, patient history and medications, etc. Community citizens, public employees, healthcare providers can input data to the wireless personal cloud infrastructure via a smartphone or a laptop. These personal cloud servers can be co-located in storm-protected buildings with a back-up power source and be connected to a wireless wide area network (WAN). Even in the absence of general Internet connectivity, a user just has to be in the range proximity of the wireless WAN and be able to

upload their text data to the personal cloud server. After regular Internet is restored to the community, an administrator can download the text data from the personal cloud server to a larger database for cartographic and statistical analysis. The outcome of this project is to identify the user and technical requirements and human interface & security challenges for connecting several connecting personal cloud servers to a larger cloud-based database and creating a strategy for implementing, testing and evaluating this process in a real-world natural or man-made disaster. In onclusion, local healthcare administrators will find this system a viable alternative to reporting of infectious diseases during a natural disaster. The continuous reporting of infectious diseases at ground zero level is important for planning, monitoring and tracking of these cases. This novel approach allows for the uninterrupted collection of infectious disease text data for later analysis.

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EBOLA VIRUS DISEASE PREPAREDNESS AND RESPONSE STRATEGIES IN ETHIOPIA AND LESSONS LEARNED FROM WEST AFRICAN COUNTRIES

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Ebola Virus Disease Preparedness And Response Strategies In Ethiopia Lessons Learned From West African Countries Ebola virus disease has claimed more than 11,000 lives during the current epidemic in West Africa making it the deadliest outbreak since Ebola was discovered in 1976.On 8 August the World Health Organization declared Ebola outbreak as a public health emergency of global Concern. The Ebola outbreak in West Africa is unprecedented in terms of its geographical scope. It began in Guinea in early 2014 and quickly spread to the neighboring countries. Inadequate health infrastructure and overall fragile health system has fueled rapid spread of the outbreak in West Africa. The severity of the outbreak is exacerbated by lack of understanding about the disease by communities and lack of experience among health-care workers .Lack of adequate treatment facilities, rumor, fear and stigma has in turn led families to keep sick patients at home, risking further spread of the virus. Resistance to proposed response measures as well as traditional burial practices further aggravated high transmission of the outbreak with devastating economic and livelihood implications. Ethiopia by the virtue of its geographic position and being political capital of Africa and being a major transport hub in the east Africa region is very prone to the deadly virus due to importation from Ebola riddled countries. Hence it has swiftly enhanced its domestic preparedness to respond in a predictable manner in the event of Ebola outbreak .It has employed state of the art screening measures at main entry ports and has also been engaged in fighting the outbreak at its source by sending medical teams to Ebola-stricken nations. This paper reviews the current Ebola preparedness and response strategies in Ethiopia, challenges and crucial lessons learned from West African countries and map out the strategies that are in place for improvement preventing future outbreaks.

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ASSESSMENT ON COMMUNITY BASED HEALTH INSURANCE IMPLEMENTATION IN RURAL ETHIOPIA

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Ethiopia is among developing country with more than 90 million populations. The country is highly depending on external development partner to fiancé the health need of this huge population.Since the presence of donors' resource is unpredictable, inadequate and unsustainable, the health sector is doing its best to bring sustainable domestic financing through implementing health care financing reforms (HFR). Community based health insurance (CBHI) is one of the reforms under implementation at piolet level in rural part of the country. This research is aimed to assess the implementation of CBHI and understand dropout, enrollment, re-enrollment, regional disparities and service uptake to inform the scale up strategy of the program. Both quantitative and qualitative methodology used to generate evidences. Around 1600 household surveyed in the piolet and control districts. Four rounds of survey data from same households, with same survey instrument and at the same season applied to understand the trends of key CBHI implementation indicators aforementioned above with econometrics model. It is found that the uptake of the scheme has been 41% of the target households in 2012 and this has increased to 58% in 2015. Membership renewal is more than 80% of the initially enrolled households. The Ethiopian scheme enrollment and retention rates are impressive as compared to the experiences of other African countries. In terms of uptake, there are substantial differences across the pilot regions. It is found that Amhara is the best preforming region with coverage rate of 68% while Tigray is the lowest one with 49% uptake rate. Variations in the extent of ownership and commitment from local administration bodies to concerted mobilization effort during the defined renewal time frame, waiting time, renewal timing, and allocation of targeted subsidies for indigent groups contributed to the differences in the coverage of the scheme across the pilot regions. Drop out decision does not significantly relate with socioeconomic status.

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WEAK COLD CHAIN CAPACITY CAN COMPROMISE IPV INTRODUCTION IN SUB-SAHARAN AFRICA: A CASE STUDY FROM CAMEROON

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In 1988, the World Health Assembly resolved to eradicate poliomyelitis by the year 2000. Since then, tremendous strides have been made towards this goal and the incidence of polio has fallen by more than 99%. As part of the endgame strategy, the World Health Organization recommended countries to introduced at least one dose of inactivated polio vaccine (IPV) by 2015 into their routine immunization schedules. However, there is emerging evidence that weaknesses in cold chain systems can hamper successful introduction and roll out of IPV in low income countries. In Cameroon, for instance, over half of country's stock was damaged prior to IPV launch. In this paper, we explored the reasons that led to this loss and the potential implications. We collected data from central, regional, district and health facility levels on IPV stock status, functional status of cold chain equipment, temperature monitoring practices and IPV coverage rates. Following the decision to introduce IPV, Cameroon imported a total of 840,000 doses of IPV. Nearly 500.000 of these doses were damaged at a central level. An additional 18,000 doses were damaged in one region. The primary causes for this loss were dysfunctional cold chain equipments, limited staff training and weak temperature control practices amongst others. The estimated financial loss was US$452,000. This loss, alongside challenges in importing additional doses because of limited global production capacity, resulted into prolonged stock out in many health facilities, which turn affected national coverage rates. With limited global IPV production capacity, weak cold chain systems can affect the availability and adequacy of IPV for routine immunization. This, in turn, may affect IPV coverage rates and may ultimately compromise polio eradication efforts.

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COMMUNITY HEALTH VOLUNTEERS' USE OF MOBILE PHONES TO REPORT MASS DRUG ADMINISTRATION (MDA) DATA

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There is growing evidence of the potential of mobile phones to support public health programmes. Community health volunteers (CHV) who play a major role in mass drug administration (MDA) for Neglected Tropical Diseases Control Programmes (NTDP) are being considered as potential aides in improving data reporting from the community level. Little is known about technology adoption for MDA reporting by the CHVs working with NTD programmes. This study sought to determine CHVs willingness to use mobile phones to report MDA data and factors associated with their intention to use. This was a mixed methods study with data collected during two consecutive annual LF MDAs in two districts in Ghana. A structured questionnaire was used to collect data on socio-demographic characteristics, mobile phone use experience and readiness to adopt mobile phones for MDA data reporting. Readiness was measured using the Unified Theory of Acceptance and Use of Technology model. CHVs were trained to submit summarised MDA reports by SMS in the first year in both districts and USSD in year two in one district. Overall mobile phone ownership was 99% while 33% had text messaging experience. About 31% ($\chi 2=2.67$, $p<0.05$) submitted their MDA report using the mobile phone. The model explained very little about the CHVs behavioural intention (R2= 0.17). Willingness to submit text was high however actual submission was low. Factors associated with the low response were the CHVs perception of effort needed to send the text, social influence, poor network quality and texting experience. Factors such as age and gender did not have significant effect on their intention to use and actual use. General enthusiasm for MDAs should be reignited among CHVs. National policies on network quality improvement will provide enabling environment for implementation of such technology.

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COST-EFFECTIVENESS OF DENGUE VACCINATION IN FIVE SOUTHEAST ASIAN COUNTRIES

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In 2015, the first dengue vaccine was licensed in several endemic countries, initiating a valuable new control tool. Decisions about its use in public programs depend on anticipated health benefits, costs, and cost-effectiveness. To inform policy discussions in Asia, we used a transmission model calibrated with data collected during vaccine efficacy Phase III trials. Costs of vaccine administration, procurement, and dengue treatment were based on publications and reports. Each vaccine dose was projected to cost $2 for vaccine delivery plus $20 for vaccine procurement. Our base case assumed that a 3-dose vaccination program would be offered to all 9 year-old children each year, plus a 4-cohort initial catch up (10-13 year-olds), phased over 3 years and achieving 80% coverage. Our base case expressed costs in 2013 US dollars from a health system perspective, conducted 100 simulations with a 30-year horizon to account for variability in dengue transmission and uncertainty on vaccine efficacy, measured health impacts in disability-adjusted life years (DALYs), and assessed cost effectiveness as $/DALY averted. Our base case results indicated that vaccination would save from $0.11 (Vietnam) to $1.72 (Malaysia) in annual per capita dengue treatment costs and would reduce dengue-related DALYs by 26% (Thailand) to 32% (Malaysia). Cost effectiveness ratios, expressed as multiples of each country's per capita gross domestic product (GDP), were: Indonesia (0.17), Malaysia (-0.13), Philippines (0.56), Thailand (0.20), and Vietnam (3.57). In Malaysia, the vaccine is cost saving. Using WHO benchmarks of 1 and 3 times per capita GDP, the vaccine is highly cost-effective in Indonesia, Philippines, and Thailand (being below the most stringent benchmark), but is not cost-effective in Vietnam from a health system perspective. Cost effectiveness results were similar for other vaccination programs (0 to 8 catch-up cohorts) and coverage rates (50% to 80%). The consideration of a societal perspective, increasing dengue incidence, dengue's adverse impacts on tourism, and rising real incomes and health care costs further favor the case for vaccination.

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COMMUNITY PERCEPTIONS OF HYPERTENSION IN LOW-RESOURCE SETTINGS IN THE DOMINICAN REPUBLIC

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The bateys (sugar cane towns) of La Romana, Dominican Republic are home to a large number of Haitian migrant workers. Due to their poor living conditions and barriers to accessing care, the health status of batey residents is severely compromised. Although efforts have been made to improve the health outcomes of batey residents, the discontinuity and lack of culturally appropriate care significantly reduces the effectiveness of these efforts. This study describes the perceptions of hypertension (HTN) among batey residents, to aid in developing a protocol that improves quality of care provided in the bateys. The goal of this study was to assess participants knowledge of HTN and HTN care. Data was collected using surveys administered verbally (N=81) using an interpreter fluent in Creole and Spanish. Of those surveyed, 54 were female and 27 were male, with 59 participants aged 40 or older. Results showed that batey residents had learned about HTN from various sources including local clinics, hospitals, foreign medical teams and other batey residents. Headache (n=18), dizziness (n=15), shortness of breath (n=10), and weakness (n=7) were commonly reported symptoms of HTN. Additionally, most respondents were aware of the nearest clinic, with a majority (n=62) accessing clinics by taxi. Finally, batey residents viewed financial cost (n=24), a lack of clinicians in the bateys (n=20), and underdeveloped transportation (n=10) as the most significant barriers to accessing HTN care. Our findings reveal that males are underserved by existing models of HTN care, as many work during the day when mobile clinics visit the batey. Results also suggest that an optimized protocol should focus on bringing care closer to the bateys to increase access. Finally, there is a need for coordinated education and treatment programs between the different care providers in batey communities to ensure sustainable and continuous care. Overall, these results shed insight on how chronic disease protocols can be successfully implemented in low-resource settings to improve health outcomes.

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PEACE AND POST-CONFLICT HEALTH: NEONATAL AND MATERNAL MORTALITY AFTER CIVIL WARS WITH DIFFERENT ENDS

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The outbreak of "peace" after civil war is characterized by deeply entrenched grievances, mistrust, and insecurity. Violence often persists at high levels as the beneficiaries of resilient war economies shift to alternative means of maintaining profit and power. Institutional violence disproportionately affects different segments of society as the quality of peace may be dictated by a conquering victor who may seek retribution or well-armed warlords invited to the negotiating table whose

interests may only be the legitimization of territorial and economic gains achieved through battle. This study tests the assumption that diplomatic negotiations at the end of war protect the health and interests of vulnerable, non-combatant populations such as women and children. We examined neonatal and maternal health in societies after civil war whereby neonatal mortality rates and maternal mortality ratios are compared between three peace types: a peace imposed by the victor, a negotiated peace between capable warring parties, and no peace/continued war. The Uppsala Conflict Data Program Armed Conflict Dataset was queried with UNDP neonatal mortality rates (NMR) and maternal mortality ratios (MMR) for five years after conflict termination in Africa, South Asia, and South America between 1990 and 2013. Health data were annualized to net change year-on-year relative to health at the point of peace declaration. Health outcomes by peace type were compared by Mann-Whitney U-tests at α=0.05 with 95% confidence intervals. We found that NMR fell by 1.8 and 1.9 deaths per 1,000 births in VP and NP, respectively, with no statistical differences observed; we found that MMR fell by 35.5 and 55.6 deaths per 100,000 births in VP and NP, respectively, with no statistical difference observed. Although a statistical difference could not be appreciated at five years, concerning trends in MMR between different peace outcomes were observed and suggest a difference in the quality of peace for women.

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MANAGEMENT OF DENGUE HOSPITALIZATIONS IN BRAZIL DURING AND OUTSIDE EPIDEMIC PERIODS: INSIGHTS FROM DATA MINING

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Brazil reported 1.5 million dengue cases in 2015, more than any other country. Most of the Brazilian population rely exclusively on the publicly funded health care system SUS (Sistema Unico de Saude). All hospitalizations paid for by SUS are registered in a publicly available database (SIH/SUS). We used the information included in this database to assess the implication of the epidemic nature of dengue on the management of dengue hospitalisations during and outside epidemic periods. Over 2008-2015, the SIH/SUS database describes 92 million admissions in 15,002 departments of 4,293 hospitals, of which 354k are admissions associated to a dengue diagnostic. For the analysis, hospital departments were classified as "High Dengue Activity" (HDA) departments or "Low Dengue Activity" (LDA) departments using the following criteria for HDA departments: at least 200 dengue admissions over 2009-2015 and a maximum dengue patient load (daily bed count) exceeding 20% of the estimated bed capacity of the department. Over 2009-2015, 43% of admissions occurred in 274 HDA departments, while the remaining 57% dengue cases were managed in 4,070 LDA departments. The maximum level of dengue patient load in the 274 HDA departments was notably high, at 43% on average and up to 62%. The overall patient load indicated that these HDA departments operate near or even sometimes beyond their maximum capacity during dengue epidemic periods. The systematic analysis of admissions to HDA departments using data mining tools also led to the identification of subgroups showing an excess of mortality during dengue epidemic periods. These subgroups are associated to dengue-related diseases and case management characteristics. We also observed a larger proportion of cases managed in LDA departments during dengue epidemic periods. Finally, we observed a higher dengue case fatality rate in LDA departments than in HDA departments (0.9% and 0.4% respectively; OR=2.1 (1.9-2.3)).This analysis highlights the consequences of the seasonal aspect of dengue on the management of dengue hospitalization in Brazil and some implications for dengue-related mortality.

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BALANCING RISK AND COSTS IN CONTROLLING THE INTERNATIONAL SPREAD OF INFECTIOUS DISEASE OUTBREAKS WITH ACTIVE MONITORING

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The 2014-2015 west African Ebola outbreak was an unprecedented global public health emergency that underscored the ease with which pathogens can spread in today's interconnected world. To improve rapid identification and evaluation of individuals at risk for Ebola, some countries actively monitored of individuals who were returning from Ebola-affected regions. A common policy was to have individuals under active monitoring make daily contact with local health authorities each day for 21 days after their last potential exposure. Active monitoring has shown promise as a tool in preventing and responding to outbreaks of pathogens that pose a grave threat to public health. It may also play an important role in containing outbreaks of emerging pathogens that could rapidly spread throughout a population. We developed a framework for evaluating the risks and costs associated with active monitoring using. As a case study, we analyzed new data on the cost of the Ebola response in New York City and existing data on the incubation periods of Ebola, MERS-CoV, and smallpox. Our analysis provides empirical evidence that could inform the design and implementation of future active monitoring programs. For example, to tolerate the level of absolute risk implied by Ebola active monitoring programs, our model would suggest that for both MERS-CoV or Smallpox, a duration of active monitoring could be set at less than 2 times the median incubation period for each disease. Furthermore, for low-risk and high-risk individuals, our model provides estimates of how long individuals should be monitored to minimize the expected cost of the monitoring program. This model could be used to help guide future assessments and data collection on active monitoring programs. It also provides a pathway for data to inform the design of active monitoring programs for the next global epidemic.

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UNDERSTANDING RESEARCH ACTIVITY IN THE HEALTH SECTOR OF UZBEKISTAN: IMPLICATIONS FOR HEALTH RESEARCH CAPACITY STRENGTHENING

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Health research capacity building between high income countries and low income countries is being recognized as an important field in achieving health equity. Evaluating the research capacity is considered to be one of the key principles in achieving good practices in research capacity building. This study sheds light on the current research capacity in health sector of Uzbekistan. Bibliometric analysis was applied to examine the publications trends in health research to map the Uzbekistan research production along with the key research topics. A search strategy was built to retrieve journal articles from the Web of Science (WOS) and PubMed Medline from 1991-2015. A total of 430 articles were identified and 321 articles were analyzed after exclusion criteria were applied. The number of articles trended upward with accentuated growth during 2000 to 2010 of 180 articles, six times than the earlier decade (1991-2000). 62.7% of the articles were related to communicable diseases, newborn, maternal, and nutritional causes and 26.8% of the articles reported on non-communicable diseases while only 9.5% of articles reported research on health systems. In total, 60.4% of papers involved international collaborations with institutions in USA(26.8%), Japan(13.4%), and Russia (10.3%) followed by multilateral organizations such as WHO and MSF. Uzbek authors in international collaborations published in high impact

factor journals (average of 2.46) while studies reporting of only Uzbek authors published in low impact factor journals (average of 0.205). Health research output in Uzbekistan has increased since independence but Uzbek authors face difficulty in making their research visible in international community without international collaboration.

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RESIDENT-DRIVEN TELEDERMATOLOGY IN HAITI: A SYMBIOTIC PARTNERSHIP IN DERMATOLOGIC EDUCATION AND HEALTH

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It is important for residents to pursue relevant exposures during training for their intended career niche. I created a resident-driven teledermatology program in Haiti with the dual-goal of providing care to patients lacking access while broadening resident exposure in a tropical underserved population. 59% of Haitians live on less than $2 a day with a life expectancy of 63 years. Up to 20% of outpatients in the tropics have a skin complaint. Infrastructure and health services are limited in Haiti, but internet access and cellular service is present throughout the country-making teledermatology a more practical approach to see patients. The foundation for this program was built upon multiple medical aid trips to Haiti working alongside local Haitian Dermatologists. The partnerships developed with local practitioners helped to create the foundation for this program through patient evaluation, referrals, follow up, and treatment. A donated used cameraphone held by a local nurse offers a portable method to take clinical images which can be emailed at any time ("store and forward telemedicine")- ideal in an area with sporadic internet connectivity. A patient questionnaire provides relevant information without the time & cost of a physician visit. Recommendations are made within 48 hours by volunteer residents, overseen by attending physicians, and conveyed to local health providers. Donations cover the costs of labs, biopsies, or medications for patients who cannot afford them. Referrals are provided to those requiring additional care in-person. From mycetomas to varicella and an increased rate of genodermatoses (due to founder effect in an island population), the educational value for residents is vast. Dengue and Chikungunya viruses appeared in the Caribbean a full year before cases appeared in Florida and as we anticipate the spread of Zika virus our residents have already become clinically familiar through Haiti. Establishing a telemedicine program is an inexpensive, reproducible and feasible endeavor for residents seeking niche experience in the field of global medicine while providing healthcare to those most in need.

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AN INNOVATIVE TRAVEL MEDICINE APPROACH USING MHEALTH TECHNOLOGY TO DESCRIBE HEALTH RISKS TO TRAVELERS

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Emerging mHealth technology shows great potential in more accurately and completely documenting travel itineraries and modeling health and disease risk patterns of travelers, including accidents/injuries, infectious and other non-communicable disease outcomes. Our study aims to address several major shortcomings in travel health research by using a smartphone application to collect detailed information on health behaviors, symptoms and accidents, and environmental risk factors during travel. In partnership with the Swiss Federal Institute of Technology (ETH) Wearable Computing Lab, the Epidemiology, Biostatistics, and Prevention Institute at the University of Zürich have developed a novel data collection instrument and analysis concept: a smartphone application that collects data on 1) travelers' exact itinerary and environmental conditions using passive GPS localization, and 2) a daily-self report questionnaire on health risk behavior, accidents, and symptoms while traveling. A prospective cohort of 107 travelers planning travel to Thailand between January and June 2015 was recruited from the travel clinics of Zurich and Basel in Switzerland. Of the 101 recruited travelers that went to Thailand, 75 (74.3%) answered at least 1 questionnaire during travel, 10 (9.9%) had technical difficulties, and 16 (15.8%) dropped out or were lost to follow-up. Travelers filled out a median of 12.0 surveys during their trip (range: 1-30), corresponding to a median completion rate of 85.0% days of travel. Questionnaire completion rates were best for shorter trips, with a survey completed on average 94.8% of days for trips less than 10 days. Non-infectious disease health outcomes were common, with 22.7% of travelers experiencing an accident, 49.3% mental distress, and 14.7% an animal bite. Use of a smartphone application to collect health information is technically feasible and acceptable among the traveler population, minimizes recall bias, and greatly increases the quality and quantity of data collected during travel. MHealth technology shows great potential for innovation in the field of travel medicine.

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ASSOCIATION BETWEEN IMPROVED HOUSING CHARACTERISTICS AND MALARIA PREVALENCE IN CHILDREN UNDER FIVE

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In the past decade, malaria vector control strategies in sub-Saharan Africa have focused on the use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) in combatting malaria. House construction using modern, impermeable materials also improves vector control. Past studies have examined the association between household characteristics and malaria through meta-analysis or localized surveys; however, there has been few studies using nationally-representative population-based surveys. This study examined 24 Demographic Health Surveys (DHS) and Malaria Indicator Surveys (MIS) with data on malaria parasitemia status in children under five and household characteristics such as type of flooring, wall, and roofing materials. Logistic regression was used to assess whether improved flooring, wall, and roofing types were protective against malaria after controlling for ITN use, IRS spraying in the past 12 months, household wealth status, age of child, sex, and malaria endemicity in survey specific analyses as well as meta-analysis. Results of the country-specific analyses showed a significant protective effect of an improved roof on malaria infection in children in 10 of the 24 surveys included (from Benin, Burundi, Cameroon, Mali, Malawi, Nigeria, Senegal, Tanzania and Uganda). Results of the meta-analysis show a protective effect of improved roof (metal, wood, ceramic tiles, cement, and shingles) on malaria infection in children under five (OR=0.82; 95% CI: 0.74-0.92). Marginally significant protective effects of improved walls (baked bricks or cement blocks) and improved floor (not earth, sand or dung) on malaria infection in children under five was also found (OR = 0.91; 95% CI: 0.82-1.00) and (OR=0.95; 95% CI: 0.86-1.04, respectively). Results corroborate findings from other studies that show housing as an important risk factor for malaria. Findings suggest that investments in improved housing may contribute to sustainable development goals by conferring protection against malaria as well as other socio-economic benefits.

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THE IMPROVING MALARIA CARE (IMC) PROJECT'S CONTRIBUTION TO FOLLOW UP A PILOT TO USE RAPID DIAGNOSTIC TESTS (RDTS) AT THE COMMUNITY LEVEL IN BURKINA FASO

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Early and correct case management of malaria in health facilities and at the community level is among the priorities of Burkina Faso's National Malaria Control Program (NMCP). In line with this initiative, the NMCP piloted use of Rapid Diagnostic Tests (RDTs) by Community Health Workers (CHWs) to confirm malaria cases in the three health districts of Kaya, Saponé and Nouna between 2013 and 2015. With PMI support, follow-up visits were organized to document best practices, as well as challenges, on RDT use by CHWs that could serve as lessons learned for scale-up. During follow-up visits, malaria commodities management (supply, storage and use) at the community level was examined, use of RDTs was assessed, and implementation at the community level was discussed with all actors at regional, district, health facilities, and community levels. The team examined the monitoring/supervision processes at all levels, used a check list on malaria commodities management, and employed a questionnaire for each type of actor. Both qualitative and quantitative data have been collected. A total of 108 persons were contacted including 32 CHWs, 42 community leaders and 34 health care providers and managers. Findings revealed frequent stock-outs of RDTs and artemisinin-based combination therapies, non-payment of stipends to CHWs (a demotivator) and insufficient supervision of CHW by health teams. From the community perspective, 66% of community leaders were satisfied with their CHW's work (diagnosis and treatment of uncomplicated malaria and referral of severe cases to health facilities). However, 46% of community leaders complained of frequent stock-outs and unanimously agreed on the importance of regular payment of premiums to CHW. Follow up of the pilot was valuable in obtaining community, CHW and health worker perspectives for improving the program. While the community finds the program acceptable, its sustainability will require that solutions be found for stock-outs, non-payment, and insufficient supervision before scale up takes place.

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AVAILABILITY OF ORS, ZINC, AND AMOXICILLIN AMONGST PUBLIC FRONTLINE WORKERS IN UTTAR PRADESH, INDIA: A KEY DETERMINANT TO REDUCING UNDER FIVE MORTALITY DUE TO CHILDHOOD DIARRHEA AND PNEUMONIA

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In India, diarrhea and pneumonia contribute to around 12% and 23% of all under five deaths. Under the National Rural Health Mission, Government of India's flagship initiative to provide quality health care to rural population, accredited social health activists (ASHAs), the community-based workers, have been key players for the provision of health services in Indian villages. ASHAs have received a number of trainings in the past to strengthen their skills in the management of childhood illnesses. Despite the trainings and widespread presence of ASHAs, the decline in the mortality rates due to childhood diarrhea and pneumonia has been slow. 473 ASHAs sampled as per a predetermined randomization protocol were interviewed face-to-face from six districts of Uttar Pradesh to understand their treatment recommendation practices and proportion of diarrhea and pneumonia cases seen by them. Additionally, a medicine audit was undertaken to check the availability of ORS, zinc, and amoxicillin. On average, ASHAs assessed less than one case of diarrhea and pneumonia in the last 7 days. 72% recommended both ORS and zinc, 16% ORS alone, and 0.2% zinc alone, and none recommended amoxicillin. Of those ASHAs that did not recommend ORS and zinc, non-availability was mentioned as the main reason for non-recommendation. During the medicine audit, 43% and 17% had a stock of ORS and zinc respectively, and none had amoxicillin. Significantly low number of diarrhea and pneumonia cases seen by ASHAs means that there is little opportunity for them to consolidate and refine their skills. Also, a high proportion of ASHAs recommended ORS and zinc for diarrhea, but did not have ORS or zinc in their drug kits. Lack of amoxicillin in their drug kits was a significant factor in not recommending amoxicillin for pneumonia. The success of government rural health programs is dependent on ASHAs and their ability to cater to the health needs of the rural population. Ensuring adequate stocks of ORS, zinc, and amoxicillin with ASHAs is crucial to accelerating the decline in diarrhea and pneumonia mortality rates.

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COMPREHENSION OF SURGICAL INFORMED CONSENT IN HAITI

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Informed consent has long been considered an essential requirement of surgical care in the United States. However, little is known about the use of informed consent on international surgical trips. Since 2008, a multi-disciplinary team from Emory University has partnered with Hospital St. Therese in Hinche, Haiti to provide surgical care. An informed consent tool has been developed and evaluated by the Emory team to prepare patients for surgery. All patients at Hospital St. Therese scheduled for surgery by the Emory team (n=52) received a dual video and written informed consent both translated into Creole describing the procedures, risk and benefits of both surgery and anesthesia. Procedures performed were primarily inguinal herniorraphy and open prostatectomy. Following the informed consent, patients completed a survey translated into Creole evaluating their understanding of and satisfaction with the procedures using a tablet app both before (n=48) and after surgery(n=47). Prior to surgery, 91% of patients were able to correctly identify their surgical procedure. The majority of patients were able to identify the most common risks of surgery including pain (85%), bleeding (80%) and infection (70%). 98% of patients were satisfied with the informed consent process and 91% of patients would have their operation again at discharge. Our survey revealed that our ability to obtain informed consent was limited by language barriers despite the use of translators (61%) and poor literacy (54%). We plan to refine our informed consent process to better address these challenges in the future. The results of our survey demonstrate that an informed consent tool can aid in preparing patients for surgery but that communication barriers inherent to the setting of international surgical trips should be considered in the development of successful informed consent tools.

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LONG-LEAD EL NIÑO FORECAST INFORMATION TO SUPPORT PUBLIC HEALTH DECISION MAKING: APPLICATION TO DENGUE EPIDEMICS IN ECUADOR

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El Niño Southern Oscillation (ENSO) is a high-impact climatic phenomenon causing substantial changes in the weather worldwide. It leads to floods or droughts in certain regions of the globe, damaging agriculture and marine ecosystems, and increasing the risk of infectious disease epidemics such as dengue epidemics in some tropical countries. Therefore, ENSO forecasts could help authorities to plan in advance of imminent disasters, to mitigate the risk, and to protect vulnerable communities. A structural time series model, which uses a state space approach and explanatory variables relevant to the El Niño (EN) evolution, has been developed and tested to predict sea surface temperature (SST) in the equatorial Pacific Ocean (in the Niño 3.4 region). The model configuration is specifically tailored to forecast EN at long lead times of 24 months or more, going well beyond the traditional ¨spring barrier¨ of ENSO prediction. The forecasting scheme provides information about the amplitude of the events, their duration, and the peak time of the SST. This information could be used to support decision making, especially in tropical and subtropical countries, which are directly and severely affected by the anomalous temperature and precipitation rates that occur during and after El Niño events. Certain diseases are particularly sensitive to climate extremes. For example, a previous study found that the timing and magnitude of dengue outbreaks in El Oro province in Ecuador were associated with El Niño events. In this study, long-lead forecasts of equatorial Pacific SST (i.e. the Niño 3.4 index) are used within a dengue prediction model, to assess the extent to which dengue epidemics can be predicted well in advance. The ENSO forecasting model could have helped to predict the dengue epidemic that occurred in the region in 2010 as early as 30 months ahead. Thus, long-lead ENSO forecasts could be incorporated into dengue prediction models, to enhance the development of a dengue warning system for Ecuador and other tropical and subtropical countries sensitive to the ENSO phenomenon.

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DUAL PERSPECTIVES ON STIGMA: REPORTS OF EXPERIENCED AND ENACTED STIGMA BY THOSE AFFECTED AND UNAFFECTED BY PODOCONIOSIS

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Disease-related stigma is a public health concern steadily gaining global attention. Evidence consistently shows that an individual's attribution of disease cause can prompt or justify interpersonal stigma. However, few studies have explored causal beliefs about inherited disease and their influence on stigmatizing behaviours in low and middle income countries (LMICs). The study was conducted in 2013, in six communities 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. Two versions of the enumerator-administered survey were created, with measures assessed in parallel on "experienced" stigma for the affected and "enacted" stigma for unaffected household respondents. Mean levels of reported enacted stigma were slightly lower (2.0, SD 0.7) than experienced stigma reported by affected respondents (2.2,SD 1.1). Males consistently reported significantly lower levels of experienced and enacted stigma than females, $p<0.0001$. Beliefs that podoconiosis was hereditary were significantly and positively associated with reported levels of experienced stigma among affected respondents and enacted stigma for unaffected respondents ($p<0.001$). There was no association between levels of stigma experienced by affected households with corresponding levels of enacted stigma reported by the neighbouring unaffected households. In conclusion, if stigma reduction interventions are to be successful, culturally-tailored, gender inclusive and innovative health education programs are required, directed at the general community as well as at patients.

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DOES HAVING HEALTH INSURANCE RELATE TO MEDICATION USE FOR HYPERTENSION IN THE DOMINICAN REPUBLIC?

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Hypertension, a major contributing factor to mortality worldwide, can be reduced by the use of antihypertensive medications. Studies within high-income countries have found that having health insurance increases the odds of use of medication among those with hypertension. However, there has been little examination of influence of health insurance on hypertension management in low and middle-income countries (LMIC) where hypertension affects a larger number of people. This study examined one LMIC, the Dominican Republic (DR), which has high rates of hypertension and a population incompletely covered by health insurance. This study investigated the relationship between having health insurance and recent use of medication among those diagnosed with hypertension using data from the 2013 Demographic and Health Survey (DHS) for the DR. Among survey participants who had been told by a health professional that they had hypertension, 42.3% of men and 42.7% of women reported taking antihypertensive medication in the two weeks preceding the survey. On bivariate analysis, women, but not men, were more likely to report having taken medication if they had health insurance. Within a multivariate model, having health insurance significantly increased the odds of medication use. Being a women and older age also increased the odds of medication use in the model. A sex by health insurance coverage interaction term was not significant. The wealth indicator demonstrated a more complex relationship with those in households classified in the poorest quintiles having higher medication use than those in the poorer quintile; this despite lower insurance coverage for the poorest. Perhaps non-insurance based targeted intervention efforts for the poorest may have extended coverage. This study is limited secondary to the truncated age range for women (15-49 years) available from the DHS. Future research should investigate types and quality of care provided for persons with hypertension for a fuller age range, with consideration of both health insurance coverage and wealth strata.

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LOW COST, IMAGING BASED DEVICE FOR PERFORMING A WHITE BLOOD CELL COUNT AND 3-PART DIFFERENTIAL AT THE POINT OF CARE

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The white blood cell (WBC) count and differential is an important laboratory diagnostic test. However, current methods for performing a WBC count and differential have high associated costs and infrastructure requirements and are therefore not available in low resource settings. There is a particular need for affordable tools to rapidly measure the WBC count and differential at the point of care in such settings. To meet this need, we have developed a portable device and low cost disposable cartridge that can be used at the point of care to perform a WBC count

and 3-part differential using 10 uL of blood obtained from a finger prick. The device is battery powered, can be deployed at the bedside, and produces results in under 5 minutes. The cartridge contains a microfluidic channel in which acridine orange is pre-dried. Blood from a finger stick is drawn into the channel via capillarity and WBCs are fluorescently stained with acridine orange, staining nuclei green and granules red without any sample processing. An all plastic fluorescence microscope is housed within the device, and a series of three images is captured and automatically analyzed to report the WBC count and the percentages of granulocytes, monocytes, and lymphocytes to the user. This is achieved by a novel image analysis program that identifies the WBCs in each field of view and classifies them into the WBC subtypes based on the red and green pixel intensity within each cell. The device has been recently tested with finger prick samples from 91 oncology patients at Lyndon B. Johnson Hospital in Houston, TX with WBC counts ranging from $2.7x10^3$ to $30.4x10^3$ WBCs/uL. Preliminary analysis of the results shows a strong linear correlation between the number of WBCs in the image field of view and the WBC count (R-squared 0.96). Further, the differential data also correlates strongly with the true values with an overall R-squared value of 0.92. We estimate at production scale the device can be developed for under $800 and the cartridge for less than $0.25, increasing access to this important diagnostic measurement to settings that currently cannot determine a patient's WBC count and differential.

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ADAPTING AND ENHANCING MALARIA INFORMATION SYSTEMS IN COUNTRIES ENTERING PRE-ELIMINATION

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There has been substantial progress in addressing sub-Saharan Africa's malaria burden. As countries reduce transmission and move from malaria control to pre-elimination, strong health management and information systems (HMIS) become critical to provide routine data to monitor progress, identify rebounds, and tailor new approaches for residual foci transmission. To ensure HMIS in sub-Saharan Africa are prepared to perform these roles in countries with the potential to move to pre-elimination, MEASURE Evaluation conducted a study to systematically measure the functionality of HMIS and to identify supporting factors. The study comprised a systematic literature review of HMIS performance assessments with a special focus on malaria. From an initial 1,581 peer-reviewed articles on HMIS in Africa, information was extracted and synthesized from a final 25 along the following subthemes: indicators of data quality, elements of well-functioning HMIS, supportive context for HMIS, and comprehensive measures of HMIS performance. The literature review revealed no uniform approach for assessing and improving the performance of HMIS and little guidance on how to adapt and enhance HMIS to keep them functional for countries at various points on the malaria control-to-elimination continuum. HMIS strengthening approaches generally focused on human capacity and participation, data quality and completeness, and a country-led enabling environment for policy and planning. The results of this desk review will be supplemented with in-country work to develop country case studies and a toolbox to help countries entering pre-elimination learn from the experiences of countries further along the continuum to adapt and strengthen routine data capture.

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THE PRIORITY REVIEW VOUCHER AS A MEANS OF ADDRESSING GLOBAL HEALTH PRIORITIES

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Neglected tropical diseases (NTDs) remain important sources of morbidity, mortality and disadvantage in low and middle-income countries, and yet the development and registration of new treatments for these diseases is rare. Funding to support the research and development of new medicines for these diseases is directly correlated with their low potential for return on investment, and remains insufficient to consistently meet regulatory standards. To drive this drug development, in 2007 the United States Congress created the priority review voucher (PRV) to reward sponsors who successfully register medicines to treat specified NTDs. The PRV is saleable to any company wishing to gain priority review of their new treatment, regardless of indication, creating a market that has yielded up to US$350 million per PRV. Although three new NTD treatments have been approved under the scheme, concerns have been expressed that sponsors benefitting from PRV sale are not bound to ensure treatment access and that the program does little to drive new NTD drug development. Medicines Development for Global Health (MDGH), a non-profit biotechnology company, partnered with the Global Health Investment Fund (GHIF), a U.S.-based social impact investment fund, to develop and register moxidectin for the treatment of onchocerciasis. MDGH is the first company to attract venture capital investment for a new treatment for a NTD on the basis of the PRV program. With limited commercial return, the investment in moxidectin would have been previously inconceivable, particularly as the registered treatment option for onchocerciasis (ivermectin) is donated. GHIF and MDGH, both with constitutionally enshrined global health objectives, have entered into a legally binding commitment to use PRV funds to support equitable and sustainable access to moxidectin for onchocerciasis, as well as support further development of moxidectin for other NTDs. In conclusion, this is the first example of a program utilizing the PRV scheme to support NTD drug development and access, directly addressing the majority of concerns raised about the use of PRV scheme to achieve global health impact.

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EVALUATING LONG-LASTING INSECTICIDAL NET EFFECTIVENESS OVER TIME USING SENTINEL SURVEILLANCE NETWORK: EVIDENCE FROM MADAGASCAR

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The reduction of global malaria burden over the past 15 years can be attributed to an unprecedented scale-up of malaria control interventions, particularly through mass distribution campaigns (MDCs) of long-lasting insecticidal nets (LLIN). Three LLIN MDCs were implemented in Madagascar at the end of 2009, 2012 and 2015 (coverage ranges from 80% to 94%). While malaria decline in Madagascar suggests the impact of MCIs, instances of malaria outbreaks between LLIN MDCs (in the absence of routine continuous distribution channels) suggest that diminished LLIN coverage may exist or net effectiveness may decline faster than expected. We conducted a study based on sentinel surveillance at 17 sites to assess the relationship between the effectiveness of LLIN MDCs over time and malaria outbreaks identified in Madagascar from 2009 to 2015. The association was evaluated using Generalized Linear regression Model

(GLM) and survival analysis. An alert was defined as weekly malaria cases exceeding the 90th percentile value for the three previous consecutive weeks. The percentile value is not seasonally-dependent and calculated over the whole chronological series of a site. GLM analysis showed that compared to the first year after a LLIN MDC, the probability of a malaria risk alert at surveillance sites increased dramatically during the second year (OR 37.9, 95% C.I 15.9-123.7) and further increased during the third year (OR 54.2, 95% C.I. 22.6-177.5). The survival analysis showed that each year that followed an LLIN MDC was alert free. Within two years after LLIN MDC, 51.4% (18/35) of sites were affected by an outbreak, and 77% (27/35) after 2 years. Data from Malagasy sentinel surveillance were valuable in assessing the effectiveness of LLIN mass campaigns. We provide evidence that LLIN MDC prevent malaria outbreaks although over time the frequency of outbreak alerts increased. Approaches for continuous LLIN distributions to maintain high coverage between MDCs are likely necessary to accelerate malaria control in Madagascar. Further studies are needed to investigate other causes responsible for the increase in malaria alerts over time after LLIN MDCs.

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A FRAMEWORK FOR THE SYSTEMATIC EVALUATION OF SEVERE DISEASE SURVEILLANCE SYSTEMS: BANGLADESH AS A CASE STUDY

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The International Health Regulations (IHR) outline the core requirements to ensure the timely detection of public health threats of international concern. Assessing the sensitivity and representativeness of surveillance systems to detect these threats is crucial to quantify the capacity to detect outbreaks and to interpret case statistics. We propose a framework for the systematic evaluation of severe disease surveillance systems and apply it to assess severe neurological and respiratory diseases surveillance in Bangladesh at tertiary care hospitals. During 2009- 2013, all cases of severe neurological and respiratory diseases were identified in surveillance hospital catchment areas outside of the capital city using key informant and house-to-house surveys. We ascertained where cases had sought care. We then estimated the probability of the surveillance system detecting case-clusters of varying size by distance from hospitals using a statistical algorithm and compared characteristics of cases identified in the community to the subset that sought care at surveillance hospitals. An estimated 25% of severe neurological and 16% of severe respiratory cases residing at 10km from the surveillance hospital sought care at those facilities. Outbreak detection probabilities decreased with distance from the hospital such that a cluster of severe respiratory disease occurring 30km from the hospital would be detected 90% of the time only if it included >30 cases. Characteristics of cases from surveillance were largely representative of all cases, however, <5 year-old children and lowest socioeconomic neurological cases and severe respiratory cases aged ≥60 were underrepresented (absolute difference 19%, 13%, 16%, respectively). Our study identified weaknesses of this system in detecting small-to-medium sized outbreaks at >30km distance from surveillance sites, likely because of limited access to healthcare in rural areas. These findings highlight difficulties that low and middle income countries may have in meeting IHR requirements, despite considerable investment in hospital-based surveillance platforms.

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KNOWLEDGE, ATTITUDES AND PRACTICES (KAP) ASSESSMENT OF MALARIA INTERVENTIONS IN ZAMBIA

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Despite the rapid upscale of malaria control interventions, such as long-lasting insecticidal nets and indoor residual spraying, malaria remains a major source of morbidity and mortality in Zambia. A comprehensive understanding of community knowledge, attitudes and practices (KAP) is a crucial component for enhancing the uptake and use of current and novel malaria control interventions for sustained disease prevention. The overall objective of this study was to assess malaria-related KAP, as well as entomological factors associated with the use and acceptability of malaria vector control interventions in a select cohort of caregivers (n=75) within Luangwa and Nyimba districts, eastern Zambia. Specific aims focused on assessing acceptance and/or use of currently deployed malaria interventions in relation to: 1) socio-demographic factors (education, age and occupation); 2) knowledge of malaria disease and function of available interventions; 3) cultural attitudes regarding perceptions of disease risk, the barriers to and benefits of intervention use; 4) cultural practices (outdoor sleeping and cooking); and 5) indoor densities of mosquitoes in study households. Similar data were generated and analyzed for assessing potential acceptance and use of a novel intervention currently being evaluated for malaria control, specifically a spatial repellent. Methodologies included the use of questionnaires and in-depth interviews as well as mosquito collections using CDC light traps. Findings from this study are anticipated to benefit the Zambian Ministry of Health's malaria education and vector control campaigns in these study sites.

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SITUATIONAL ANALYSIS OF MENTAL HEALTH ON EBOLA VIRUS DISEASE (EVD) AFFECTED COUNTRIES' VULNERABILITY USING THE PRESSURE AND RELEASE MODEL

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Prior to the 2013 West Africa Ebola outbreak, there were high rates of Post-Traumatic Stress Disorder (PTSD), intimate partner violence, and poor psychosocial adjustment in Liberia, Sierra Leone, and Guinea as an aftermath of two decades of civil war and political instability. Yet a low level of economic development contributed to poor access to health care and a high unmet need for treatment, particularly for PTSD. We adapted Blaikie's Pressure and Release (PAR) Model for disasters to apply it to a situational analysis of mental health factors that contributed to the initially poor Ebola Virus Disease (EVD) response in West Africa. The PAR Model assesses the contribution of political, economic, and environmental vulnerabilities as root causes for socio-economic pressures that create unsafe conditions. We posited that systemic vulnerabilities in the three countries contributed to the epidemic: A traumatized population evidenced distrust for existing institutions, yielding low levels of cooperation with public health authorities early in the outbreak, resulting in a delayed response to EVD. As it is highly likely that the EVD outbreak has increased trauma and economic losses in a population already burdened with high rates of mental illness, there has never been a more crucial time to address mental health needs in West Africa to reduce societal vulnerability and decrease maladaptive responses to hazards inherent in the environment. Community based services focusing

on building psycho-social and economic resilience can rebuild trust and lessen poverty, supporting healthy individual and societal adaption to change. The PAR model provides an effective tool for situational analysis by highlighting vulnerabilities and suggesting interventions to mitigate or prevent future health-related or natural disasters.

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HIGH PREVALENCE OF DUFFY-NEGATIVITY AMONG INDIVIDUALS INFECTED WITH *PLASMODIUM VIVAX* AND *P. OVALE* IN NORTHWEST ETHIOPIA

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The Duffy blood group antigen (Fy) is expressed on the surface of red blood cells and encoded by the Duffy Antigen Receptor for Chemokines (DARC) gene. Until very recently it was considered to be essential as a portal of entry of the *Plasmodium vivax* parasite into human red blood cells. Recent data suggest that this theory may no longer hold true and that in some parts of the world, *P. vivax* infects and causes disease in Duffy-negative individuals. Ethiopia reports one of the highest *P. vivax* malaria burdens in the world and the role the Duffy blood group is playing remains poorly understood. A total of 122 randomly selected samples from *P. vivax*-positive individuals originating from a region of high malaria endemicity and host genetic diversity in northwestern Ethiopia were successfully genotyped at the -33rd (promoter region SNP) and the 125th (A/B SNP) nucleotide positions. Another 47 samples from *P. vivax*-negative individuals from the same region as well as 15 samples from Asian individuals were tested as controls. Eleven out of 122 (9.0%) *P. vivax* malaria patients tested Duffy-negative (FY*BES/*BES). The allele frequencies were 18.8, 34.8, 0, and 46.3% for FY*A, FY*B, FY*AES, and FY*BES, respectively. The majority of the test samples (91/122; 74.6%) were heterozygous in the promoter region with the most common (58/122; 47.5%) genotype being FY*B/*BES. With almost 50% (23/47) the proportion of Duffy-negative individuals was significantly (OR=9.67; P<0.001) higher among *P. vivax*-negative controls from the same region. Interestingly among a small subgroup (N=10) of *P. ovale*-positive controls the proportion of Duffy-negative individuals was 70%. Although the FY*BES/*BES genotype seems to confer a certain level of protection against *P. vivax* malaria in Ethiopia, this protection seems to be far from universal. The fact that the population of northern Ethiopia is genetically highly heterogeneous may allow for the circulation of *P. vivax* parasites in host populations that otherwise may not be able to sustain long term transmission of *P. vivax*.

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EXPRESSION OF *PLASMODIUM FALCIPARUM* GENES INVOLVED IN ERYTHROCYTE INVASION, IMMUNE RESPONSES AND CLINICAL OUTCOMES

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Red blood cell (RBC) invasion is a key step in the *Plasmodium falciparum*'s life cycle and many vaccine candidates are proteins involved in this process. However, redundancy in protein function leads to different pathways used to invade and therefore, to a high diversity in ligand expression, highlighting the need of studying gene expression profiles in field isolates. We hypothesize that differences in gene expression are associated with immune responses to the proteins expressed or with the clinical outcomes of the infection (severe (SM) / uncomplicated (UM) malaria). Therefore, we sought to study the expression of genes involved in the invasion process in clinical isolates from infected individuals in Mozambique (adults with UM n=50; children with UM n= 25 or SM n=25). Transcript levels of *P. falciparum* genes *pfrh1, pfrh2a, pfrh2b, pfrh4, pfrh5, eba175, eba140, aarp, ptramp, p41, cyrpa* and *ama1* were determined using quantitative Polymerase-Chain Reaction (qPCR) and IgG/IgM levels against the resulting proteins were measured by Luminex. Preliminary results show a negative correlation between antibodies to some proteins participating in one invasion pathway (SA dependent or independent) and expression of genes involved in the other: IgG to EBA175 PfF2 and *pfrh2a* expression; IgG to $PfRh2_{2030}$ and *eba140* expression; IgG to $PfRh2_{40}$ and *eba140* expression; IgM to EBA175 III-V and *pfrh4* expression; IgM to EBA175 PfF2 and *pfrh4* expression. Also, we report a negative correlation between *ptramp, eba140, pfrh2b, pfrh4, pfrh5* and age, while *p41* expression is higher in older individuals. When accounted for "immune pressure" we found *aarp* expression at higher levels with higher immune pressure, whereas *pfrh2a* and *pfrh4* expression was lower. We also found that *p41* negatively correlated with *pfrh5* (rho -0,49; p<0,0001) and positively correlated with IgG against PfRh5. Data analysis is still ongoing. The greatest impact of this work will be on vaccine development: the effectivity of a vaccine designed to target RBC invasion could depend on the expression of candidate genes, which could be influenced by immunity against the ligands, as we suggest in this study.

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A LAP-LIKE PROCESS AS A NOVEL IMMUNE MECHANISM DOWNSTREAM OF IFN-γ IN THE CONTROL OF THE HUMAN MALARIA *PLASMODIUM VIVAX* LIVER STAGE

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Interferon-gamma (IFN-γ) is a main regulator of immune functions and has been previously shown to induce *Plasmodium* liver stage elimination both in *in vitro* and *in vivo*. However, the molecular mechanism responsible for the restriction of *Plasmodium* liver stage downstream of IFN-γ remains uncertain. Recently autophagy, a newly described immune defense mechanism, was identified as a downstream pathway in response to IFN-γ in the control of intracellular infections. We, therefore, hypothesized that the killing of liver stage malaria parasites by IFN-γ may be through autophagy induction. Our results showed that while IFN-γ treatment of human hepatocytes activates autophagy, IFN-γ-mediated *P. vivax* liver stage restriction only requires downstream autophagy-related proteins Beclin 1, PI3K, and ATG5 but not the upstream autophagy-initiating protein ULK1. In addition, an enhanced recruitment of LC3 onto the parasitophorous vacuole membrane (PVM) and an increased colocalization of lysosomal vesicles with *P. vivax* compartments were observed in response to IFN-γ. Altogether, these data indicated that IFN-γ mediates the control of *P. vivax* liver stage by inducing a noncanonical autophagy pathway resembling that of LAP in which LC3 is directly decorated onto the PVM and mediates the fusion of *P. vivax* compartments with lysosomes resulting in the killing of the pathogens. Understanding hepatocyte response to IFN-γ during *Plasmodium* infection and the roles of autophagy-related proteins may provide an alternative strategy urgently needed for the elimination of this human malaria.

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ASSOCIATION OF HOST GENETIC POLYMORPHISMS WITH PROTECTION AGAINST SEVERE MALARIA IN UGANDAN CHILDREN

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Human genetic polymorphisms are associated with risk of severe malaria. We compared the prevalence of selected host polymorphisms in children aged 4 months to 10 years enrolled in a case-control study in Jinja District, Uganda. Healthy controls (HC) and children with uncomplicated malaria (UM) were matched by date and location with children with severe malaria (SM) diagnosed based on standard WHO criteria; 305 children were enrolled in each group. Malaria was diagnosed based on fever and a positive Giemsa-stained smear. Genes of interest were amplified from purified DNA, polymorphisms identified by standard methods, and prevalences compared by Fisher's exact test. We present preliminary results for the first 100 cases and controls. For two studied alleles, the prevalence of WT was significantly greater in children with SM compared to UM (α-thalassemia 3.7kb deletion 60% in SM vs. 42% in UM, p = 0.0193; CD36 T188G 84% in SM vs. 38% in UM, p<0.0001) or compared toHC (α-thalassemia 60% in SM vs. 43% in HC, p = 0.0290; CD36 T188G 84% in SM vs. 55% in UM, p<0.0001). Non-significant trends toward a greater prevalence of WT with SM were also seen for sickle hemoglobin (β-globin E6V 92% in SM vs. 86% in UM, p=0.25 and vs. 87% in HC, p=0.36) and ICAM1K29M (61% in SM vs. 55% in UM, p=0.46 and vs. 48% in HC, p=0.063), but not the G6PD A- genotype (80% in SM vs. 79% in UM, p=1.0 and vs. 83% in HC, p=0.58) or CD36 T1264G (85% in SM vs. 82% in UM, p=0.70 and vs. 82% in HC, p=0.70). Our results suggest associationsin Ugandan childrenbetween some previously studied polymorphisms and protection against severe malaria, and in particular suggest a marked protective effect of the CD36 T188G genotype. Analysis of our full study population and consideration of different clinical presentations of SM are underway.

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THE IMPACT OF PRENATAL MALARIA EXPOSURE ON THE VULNERABILITY OF OFFSPRING TO NEUROPSYCHIATRIC DISORDERS

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Each year ~125 million pregnant women are at risk for malaria infection. Malaria in pregnancy (MiP) has a profound impact on mother-child health, including delivery of low birth weight (LBW) infants. Even in the absence of LBW, epidemiological studies have shown a connection between maternal infections during pregnancy and increased susceptibility of offspring to neuropsychiatric disorders later in life. Moreover, immune activation in pregnancy has been shown to prime the offspring to psychological trauma, showing a synergistic effect with stress-induced neuropsychiatric disorders. Mental illness represents the leading cause of DALYs globally and exerts a huge burden in malaria-endemic areas, but the impact of malaria exposure *in utero* on long-term vulnerability to psychiatric diseases has not been reported. We hypothesize that prenatal exposure to MiP will increase the risk of neuropsychiatric disorders in offspring. We used the established mouse model of MiP with *Plasmodium berghei* ANKA (PbA). Uninfected offspring of dams infected with an inoculum of PbA that does not induce a birth phenotype, were subjected to stressors or control handling at peripubertal age. Adult behavioural outcomes were assessed using standardized neuropsychiatric tests, including prepulse inhibition, open field, and amphetamine hypersensitivity. MRI, neurochemistry and levels of neuroinflammation were monitored to define correlates of susceptibility. Recent work by our lab showed that interrupting pathways of complement activation (i.e. C5a) and dysregulated angiogenesis (i.e. decreased Ang-1) improved fetal outcome in the MiP mouse model. We will therefore compare the neuropsychiatric outcomes in α-C5a/rAng-1 treated and untreated offspring as putative interventions. This concept represents a paradigm shift in our current understanding of the risk factors for neuropsychiatric disorders and identifies PM as a potential modifiable risk factor. This has important implications for the cause and prevention of what are believed to be "non-communicable" diseases, and may shift global health priorities from costly rehabilitation to prevention.

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EARLY AND LATE *PLASMODIUM FALCIPARUM* GAMETOCYTES-SPECIFIC EXPRESSION FOLLOWING TREATMENT WITH CHLOROQUINE AND SULPHADOXINE/ PYREMITHAMINE

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Elevation of sexual stages of *Plasmodium falciparum* following anti-malarial treatment has been attributed to either induction of gametocytogenesis, or release of sequestrated gametocytes in deep tissues. Attempts to address this question have been impaired by the unavailability of markers to specifically detect and quantify expression of early stages gametocyte (I-III) in peripheral blood. We have established and validated RT-qPCR assays to detect and quantify genes specifically expressed in early gametocytes and used them alongside a known mature gametocyte-specific marker (*pfs25*) to monitor gametocytogensis following treatment of *P. falciparum* infections with CQ or SP. The density of early and mature gametocytes and its ratio to total parasite density pre-treatment (D0) were compared to that seen post-treatment on D7, D14 and D21 among patients treated with different drug regimens (CQ or SP) and among different parasitological responses (sensitive and resistant [RI and RIII]). Prevalence and density of early gametocytes as well as early gametocyte parasite ratio (EGPR) decreased following treatment (D7), paralleled to parasite density, among all treatment groups and parasitological responses. However, EGPR increased significantly on D14 irrespective of the treatment regimen or parasitological response, which suggests an enhanced gametocytogenesis. Density but not prevalence of mature gametocytes increased on D7 and D14 post-treatment, regardless of drug treatment regimen or parasitological response, suggesting release of sequestrated gametocytes that were not affected by treatment on D0. This study demonstrated evidences for increasing commitment to gametocytogenesis following anti-malarial therapy as well as possible release of sequestrated gametocytes.

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COMBATING ANEMIA WITH IRON SUPPLEMENTATION MAY INEVITABLY CAUSE A TRANSIENT INCREASE IN MALARIA RISK

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Epidemiological studies suggest iron deficiency protects against malaria and administering iron to iron-deficient individuals may increase malaria risk. This has generated much debate in the public health field about how to best distribute iron supplements to anemic populations in malaria endemic areas. Our previous laboratory work demonstrated decreased *Plasmodium falciparum* growth in iron deficient red blood cells (RBCs) and increased infection susceptibility in young RBCs and reticulocytes *in vitro*. Here, our objective was to comprehensively evaluate *P. falciparum*

pathogenesis in iron deficient children and pregnant women before, during, and after iron supplementation. We also sought to evaluate the hypothesis that malaria risk increases as erythropoiesis increases in response to iron supplementation. To do so, we investigated *P. falciparum in vitro* growth characteristics in RBCs from Gambians participating in iron supplementation trials. RBCs were collected from 135 children (ages 6-24 months; hemoglobin levels < 11 g/dL) and 165 pregnant women (2nd and 3rd trimester) before, during, and upon completion of 12 weeks of iron supplementation (12 mg or 60 mg daily, respectively). Using flow cytometry-based assays, we separately examined effects of iron deficiency and iron supplementation on overall parasite growth and merozoite RBC invasion. Our results demonstrate *P. falciparum* erythrocytic stage growth *in vitro* is low at baseline and increases during supplementation using RBCs from both the children and pregnant women. Additionally, we show parasite invasion is reduced in iron deficient RBCs from Gambian children and increases during iron supplementation. The elevated growth rates parallel increases in circulating reticulocytes and RBC mean corpuscular hemoglobin concentration, the kinetics of which correlate with increased erythropoiesis. We conclude malaria growth *in vitro* corresponds with elevated erythropoiesis, an inevitable consequence of iron supplementation. Our findings imply iron supplementation in malarious regions should be accompanied by effective preventative measures against falciparum malaria.

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CD155, THE POLIOVIRUS RECEPTOR, NEGATIVELY REGULATES IMMUNE PROTECTION TO *PLASMODIUM YOELII* 17XNL MURINE MALARIA

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CD155, the cell entry receptor for poliovirus, is a member of a large family of immunoglobulin-like molecules called nectins and nectin-like proteins. Recently, CD155 has been ascribed several important immunological functions. For example, CD155 expression on target cells is essential for natural killer and $CD8^+$ T cell mediated lysis of certain tumor cells. In addition, CD155 promotes Th1 development from naïve $CD4^+$ T cells and can significantly alter the humoral immune response to invading pathogens. We measured the effect of CD155 on the immune response and pathogenesis following infection with a highly virulent and avirulent malaria in mice deficient for the *CD155* gene. In the *Plasmodium berghei* ANKA murine model of experimental cerebral malaria (ECM), there was no significant difference in susceptibility to ECM or parasitemia between CD155-deficient mice and wild type (WT) controls. In contrast, loss of *CD155* resulted in a 2.8 fold decrease in peak parasite burden on day 10 post-infection in the *P. yoelii* 17XNL, self-resolving and non-lethal malaria, suggesting that CD155 hinders the immune clearance of *P. yoelii* 17XNL. Since CD155 can serve as a ligand for the CD96, CD226, and TIGIT receptors, functional studies are currently underway to determine which interaction is responsible for CD155 mediated regulation of *P. yoelii* 17XNL parasite burden. In addition, key differences in immune cell subsets measured by in depth flow cytometric analysis and the cytokine and antibody profiles determined by ELISA between WT and CD155 knockout mice over the course of a *P. yoelii* 17XNL infection will be presented.

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PLASMODIUM FALCIPARUM PARASITES THAT INFECT RETINOPATHY POSITIVE AND NEGATIVE CEREBRAL MALARIA CHILDREN HAVE SIMILAR TRANSCRIPT ABUNDANCE OF VAR GENES ASSOCIATED WITH SEVERE MALARIA

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An accurate diagnosis of cerebral malaria (CM) is important for understanding the pathogenesis of CM. Malarial retinopathy, which is seen in some (retinopathy-positive, RP) children but not others (retinopathy-negative, RN) with clinical CM (coma with *P. falciparum* on blood smear) has been associated with parasite sequestration in the brain and is considered a strong indicator of "true" CM. However, it is unclear whether RN CM is a severe non-malarial illness with incidental parasitemia or a less severe form of RP CM. P.falciparum erythrocyte membrane protein-1 (PfEMP-1) is an important virulence factor in severe malaria and expression of group A var genes and more specifically domain cassette (DC) 8 and DC13 PfEMP-1 have been associated with severe malaria. Characterizing var gene expression in both RN and RP CM children could help elucidate the role of P.falciparum in RN CM pathogenesis. In the present study we performed RT-quantitative PCR using degenerate primers amplifying var genes encoding for 13 different PfEMP-1 domains. Testing was performed on RNA isolated from whole blood of Ugandan children with RP CM (N=39), RN CM (N=35), severe malarial anemia (SMA, N=39) and asymptomatic children (AC, N=12). Transcript abundance of var genes encoding for DC8, DC13 and other group A domains (CIDRα1.7) was higher in children with severe malaria than in asymptomatically infected community children ($P<0.007$ for all). The prevalence of DC8 and DC13 high transcribers was similar between RP and RN CM children (74.4% vs. 68.5 %, P=0.58 for DC8 and 53.8% vs. 37.1%, P=0.15 for DC13). RN children had lower median transcript abundance for DC13 (median arbitrary units Tu 4.16, interquartile range (IQR) 1-11.12) than children with RP CM (median Tu 8.08, IQR 2.43-20.31, P=0.04) but similar to SMA (median Tu 1.11, IQR 1-10.51, P=0.43) and higher than asymptomatically infected children (median Tu 1, IQR 1-1, P=0.0009). In our cohort, the parasites infecting RN CM children have similar transcript abundance for var subtypes associated with binding and severe disease as compared to RP CM, suggesting an important role for P.falciparum binding in the pathogenesis of RN CM.

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CONTINUOUS DETERMINATION OF BLOOD GLUCOSE IN ADMITTED CHILDREN WITH MALARIA IN A RURAL MOZAMBICAN HOSPITAL

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Hypoglycaemia is a frequent complication among admitted children, particularly in malaria-endemic areas. We aimed to estimate the incidence of hypoglycaemia throughout the hospitalization in children with malaria. A simple pilot study to monitor continuously glycaemia in children aged 0 to 10 years, admitted with malaria in a rural hospital was carried out in Southern Mozambique. Continuous glucose monitors (CGMs) were inserted in subcutaneous tissue of the abdominal area, producing glycaemia readings every 5 minutes. Glucose was continuously monitored during a mean of 48 hours, in 74 children. All of them were admitted

with malaria (22 severe, 52 uncomplicated). Five children (6.8%) had hypoglycemia (<3.0mmol/l) on admission as detected by routine capillary determination. Analyzing the data collected by the CGMs, we detected hypoglycemia episodes (<3.0mmol/l) in 11/74 (14.9%) children, of which, 8 (10.8%) could be classified as severe (≤2.5mmol)%). No differences in age, sex, malnutrition status and duration of hospital stay were found among hypoglycemic and normoglycemic children. Anemia was more common in hypoglycemic children. Only one death happened (1/74, 1.4%) among a normoglycaemic child. Hypoglycaemia beyond admission in children with malaria appears to be much more frequent than what had been previously described. The clinical relevance of these episodes of hypoglycemia in medium or long term remains unclear.

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ASSESSING THE PROTECTIVE ROLE OF NEUREGULIN 1 (NRG-1) AGAINST HEME-INDUCED TROPHOBLAST APOPTOSIS AND FUSION DAMAGE

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Syncytial trophoblasts (ST) Fusion and conversion from the mononucleated to the syncytial state are mandatory for successful pregnancy. In placental malaria, *Plasmodium falciparum* infected erythrocytes (IE) bind to trophoblasts resulting in inflammation and pathology associated with poor pregnancy outcomes. The interaction between ST and IE is reminiscent of the interaction that occurs in the brain leading to cerebral malaria. However, the role of free heme in the maintenance of the integrity of the placental barrier and the effects of free heme on pregnancy outcome are unclear. Recent studies have demonstrated the cyto-protective role of Neuregulin 1 (NRG-1) in brain vascular endothelial and neuroglia cells during cerebral malaria pathogenesis. We hypothesized that apoptosis and fusion of trophoblast-derived choriocarcinoma cell line (BeWo) will be attenuated by NRG-1In the present study we determined the effect and molecular mechanisms of heme on the syncytial fusion triggered by forskolin using the BeWo cell line. Results of this study demonstrated that 1). Heme induces BeWo cells apoptosis which is attenuated in the presence of NRG-1 2). Heme induces apoptosis of BeWo cells through activation of STAT3/caspase-3/PARP and P73 signaling pathways. 3). BeWo cell fusion is reduced in the presence of heme, 4).Heme reduces mRNA expressions of cell-fusion related genes when forskolin induces syncytialisation, 5). Heme inhibits differentiation and fusion of BeWo cells through activation of STAT3 signaling pathways. 6)NRG-1 against heme-induced trophoblast apoptosis and fusion damage. In conclusion, we identified a nove mechanism responsible for the inhibition of trophoblast fusion by heme. In the absence of forskolin, heme induced apoptosis in BeWo cells via activation of STST3/caspase-3/APARP; while in the presence of forskolin, pSTAT3 was induced by heme to inhibit cell fusion. Our studies indicated that heme-induced pathways may be attenuated by NRG-1 and therefore could be potential drug targets in the prevention of heme-associted trophoblast cell apoptosis and fusion damage.

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PREVALENCE OF PFCRT, MOLECULAR MARKER OF RESISTANCE OF *PLASMODIUM FALCIPARUM* ISOLATED FROM PATIENTS WITH UNCOMPLICATED MALARIA IN DAKAR

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The malaria remains the major parasitic disease affecting for Human. Nearly 40 % of the world population lives in an area at risk of infection. In Senegal a new strategy called Seasonal Malaria Chemoprevention (SMC) is currently implemented in four districts in the center and the south of the country. However, the protective efficacy of these strategies depends heavily on the current level of resistance to Sulfadoxine-Pyrimethamine (SP) and its spread among populations of *Plasmodium falciparum*. Resistance of *P. falciparum* to this molecule is well established in East Africa and continues to spread westward. It is shown further that Amodiaquine (AQ) associated with SP in the SMC had the same action as Chloroquine (CQ) and malaria strains resistant to CQ became resistant to AQ. It is in this context that our work objective was to provide information on the impact of control strategies against malaria on molecular markers of resistance, this for a good orientation of policy making malaria burden and thereby strengthen malaria control programs. The specific objective was to re-evaluate the prevalence of mutations associated with resistance of *P. falciparum* to CQ after the abandonment of this molecule in the treatment of uncomplicated malaria in Senegal. Blood samples on filter paper were made in patients with uncomplicated malaria and treated with ACTs as ASAQ, Duo-Cotexcin and Coartem in two health districts in Dakar. We have identified mutations in the pfcrt gene by PCR- RFLP. Result In this study, men were more represented than women, 63% and 37% respectively. Pfcrt mutation gene is more pronounced among women within all treatment groups. Also our study showed return susceptible strains to Chloroquine (75% of wild strains), molecular analysis showed that among the 160 patients analyzed 25 % of patients had a mutation located on the pfcrt gene. In conclusion, this study showed a relatively high prevalence compared to WHO standards which provides for a maximum transfer of 10%. This is a sign of resistance to antimalarial *P. falciparum* face hence the need to implement the ACT resistance surveillance studies.

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A NOVEL CLASS OF METABOLIC REGULATORS MEDIATE FOSMIDOMYCIN SENSITIVITY AND RESISTANCE IN MALARIA PARASITES

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Drug resistance remains a significant challenge in malaria control. Isoprenoid synthesis via the methylerythritol phosphate (MEP) pathway is essential for parasite survival and is a proven antimalarial drug target. The phosphonic acid antibiotic fosmidomycin (FSM) is a well-characterized inhibitor of this pathway and is currently in Phase II clinical trials. In a forward selection for FSM resistance, we identify a loss-of-function mutation in PfHAD2, a member of the haloacid dehalogenase-like hydrolase (HAD) superfamily, as the resistance-causing mutation. Enzymatic characterization of PfHAD2 shows that it is a purine monophosphatase. Using metabolic profiling, we show that loss of PfHAD2 results in increased levels of MEP pathway metabolites, allowing the parasites to overcome pathway inhibition by FSM. PfHAD2 mutants also have a growth defect, allowing for selection of suppressors of PfHAD2-mediated resistance. We identify hypomorphic mutations in the glycolytic enzyme phosphofructokinase (PfPFK9) that suppress HAD2-mediated resistance. This points to PfHAD2 as a novel regulator of essential central carbon metabolism, and dysregulation of this metabolism mediates drug sensitivity. Our work represents a novel use of forward genetics in *Plasmodium falciparum* to better understand drug resistance, metabolism, and the function of this previously undescribed protein class. PfHAD2, PfPFK9, and other regulators of essential metabolism may function as future targets for antimalarials.

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PREVALENCE OF MOLECULAR MARKERS OF *PLASMODIUM FALCIPARUM* RESISTANCE TO SULPHADOXINE/ PYRIMETHAMINE IN CHILDREN WITH SICKLE CELL ANAEMIA AGED 6 TO 59 MONTHS IN BENIN CITY

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Sulphadoxine/Pyrimethamine (SP) for Seasonal Malaria Chemoprevention (SMC) or Intermittent Preventive Therapy (IPT) has been shown to be efficacious and effective in preventing malarial induced morbidities and deaths in infants and young children including those with sickle cell anaemia (SCA). However, mutations in *Plasmodium falciparum dihydrofolate reductase (pfdhfr) and dihydropteroate synthase (pfdhps)* genes, resulting in SP resistance pose a threat to its efficacy. Little is known about the prevalence of these mutations in malaria parasites infecting children with SCA, who are usually on proguanil as chemoprophylaxis (which has been found to be ineffective). To determine the pattern and prevalence of *pfdhps* and *pfdhfr* mutations in children with SCA infected with *P.falciparum*, we enrolled 146 (71 children with SCA and 75 children without SCA) children. Genomic DNA was extracted from one hundred and forty six filter paper bloodspots and point mutations at codons 431, 436, 437, 540, 581 and 613 of the *pfdhps* gene and codons 16, 51, 59, 108, and 164 of the *pfdhfr* gene were evaluated by nested Polymerase chain reaction amplification followed by direct sequencing. In children with SCA, the prevalence of *pfdhps* S436A, A581G and A613S mutations were 40(56.0%), 31(44.0%), and 30(42.0%) respectively; while children without SCA had a statistically significantly higher prevalence: 54(72.0%), 52(69.3%), and 53(70.7%) respectively (p=0.048 for A436S, p=0.002 for A581G, p=0.001 for A613S). The *pfdhps* K540E mutation and *pfdhps* double mutant haplotype (A437G+K540E), which confer significant resistance to SP were not found in this study. The prevalence of the emerging *pfdhps* mutant haplotype VAGKGS was significantly higher in the non-SCA group (p<0.001). The prevalence of *pfdhfr* triple mutant haplotype: N51I+C59R+108N was similar in both study groups. The deployment of SP for malaria chemoprevention in infants and young children with SCA can be explored in Nigeria as an alternative to proguanil. Parasites with mutations on the *pfdhps* gene are more likely to be found in children without SCA than in those with SCA.

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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. However, there are concerns that resistance to current drugs might emerge as has been reported along the Thailand-Cambodia border. Single nucleotide polymorphisms (SNPs) in critical alleles of Pfmdr1 gene has been associated with resistance to Artemisinin and its partner drugs. AL selects for K76 in Pfcrt and N86, 184F and D1246 in Pfmdr1 genes in recurring parasites compared to the baseline infections. Microsatellite analysis of loci flanking genes associated with antimalarial drug resistance has been used in defining the geographic origins, dissemination of resistant parasites and identifying regions in the genome that have been under selection. This study investigated evidence of selection in Pfmdr1 genotypes selected for by AL in treatment of malaria infections in Kenya and their genetic lineages. Parasites (n=252) from different regions in Kenya were assayed for SNPs at codons 86, 184 and 1246 and typed for 7 neutral microsatellites (NMS) and 13 microsatellites loci flanking (±99 kb) Pfmdr1 in *Plasmodium falciparum* infections. Full data sets of pure SNP genotypic data were obtained in 132 of the samples. Overall, the prevalence of N86 and D1246 was highest at 86.4% and 93.9% respectively. Single mutant NFD was the most prevalent haplotype at 44.7%, whereas the least prevalent were double mutants YFD and NFY both at 0.8%. The mean Expected heterozygosity (He) for NMS was 0.908 vs. 0.535 for the 8 closest MS indicating selection. Parasites carrying mutant alleles had reduced He compared to the NMS and wild type. Analysis of parasite genetic lineages showed that mutant parasites genotypes came from multiple genetic backgrounds. Data show a generally high prevalence of NFD and NYD, difference in genetic diversity between sites and little reduction of He in NYD and NFD from NMS. The reduction of He in YYD is however significant (P=0.0001). Data indicates parasites are evolving differently in response to AL drug pressure in the different regions suggesting the rate at which AL tolerance will develop in different regions of Kenya might vary.

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IN VITRO CHEMO-SENSITIVITY OF *PLASMODIUM FALCIPARUM* ISOLATES ON DAY 0 PRIOR TO TREATMENT DURING PHASE IIIB/IV CLINICAL STUDY IN BOBO DIOULASSO (BURKINA FASO)

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Artemisinin-based combination therapy (ACT) is used to treat all cases of uncomplicated malaria in Burkina Faso. In this study, 50% inhibitory concentration to determine the cut-off values for *in vitro* reduced susceptibility of Chloroquine (CQ), Dihydroartemisinin (DHA), Pyronaridine (PYD) and Piperaquine (PIP) for *Plasmodium falciparum* isolates from a phase IIIb/IV comparative 3-arm clinical study to assess the safety and efficacy of repeated administration of pyronaridine-artesunate, dihydroartemisinin-piperaquine or artemether-lumefantrine or artesunate-amodiaquine over a two-year period in children and adult patients with acute uncomplicated *Plasmodium* sp. malaria. *In vitro* susceptibility on day 0 prior treatments was assessed by the standard WWARN protocol. Each isolate was tested in triplicate by using pre-coated microplates with serial dilutions of CQ, DHA, PYD and PIP. The results were analysed on HNnonlin software and expressed as the 50% inhibitory concentration (IC50) or geometric mean IC50 (GMIC50). CQ IC50 values ranged from 25 to 464.89 nM, with a geometric mean of 63.34 nM. DHA IC50 values ranged from 0.33 to 12 nM, with a geometric mean of 1.16 nM. PYD IC50 values ranged from 1,250 to 25, 35 nM, with a geometric mean of 57.21 nM. PIP IC50 values ranged from 5 to 230.5 nM, with a geometric mean of 13.16 nM. In conclusion, around 70-90 % isolates were sensitive to CQ and DHA, but only 57% for PIP. Pyronaridine showed susceptibility of 14% on isolates tested. The risk of emerging resistance to ACT in West Africa makes necessary to continue monitor the susceptibilities of parasites to antimalarial drugs, particularly those used in combination with artemisinin derivatives.

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ROLE OF ANTIMALARIAL DRUG CONCENTRATION IN DE NOVO SELECTION OF DRUG TOLERANT *PLASMODIUM FALCIPARUM* PARASITE STRAINS *IN VITRO*

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Malaria is one of the most devastating infectious diseases, with the emergence of antimalarial drug resistance being of great concern. Availability of effective antimalarial drugs is quickly dwindling due to

different causes. Several factors have been attributed to drug resistance. However inadequate antimalarial drug concentration in blood plasma has been identified as a major cause. When exposed to a drug, sensitive parasites die while tolerant parasites survive leading to subsequent transmission. Genetic identity of these strains could give an answer to occurrences of treatment failure.This study seeks to establish how different antimalarial concentration can contribute in selection of drug tolerant parasite strains *in vitro*. It will utilize *Plasmodium falciparum* samples from an ongoing ethically approved clinical trial in holoendemic sites. Each admission sample (day 0) will be exposed to 70 nanomolar (nM) and 700nM dihydroartemisinin (DHA) for 6hrs and compared with the parasites in drug-free (DMSO) wells. The 700nM DHA is the optimum clinical relevant concentration while 70nM DHA is 10 fold lower sufficient to produce a change in malaria treatment. The DHA-exposed sample will be washed 3 times and re-cultured for 66 hours. Aliquot of 200 µL will be obtained from each of 28 samples recruited at 24 and 72 hour in culture respectively. Extraction of genomic deoxyribonucleic acid (gDNA) will be done using Qiagen kit. To determine selection after 24hrs and 72 hrs in culture respectively, fragment analysis will be run on 12 loci using capillary electrophoresis. Single nucleotide polymorphisms (SNPs) of Pfcrt and Pfmdr1 and will be determined using MassArray platform and Sanger sequencing. Categorical data for respective SNPs will be analyzed using Kruskal-Wallis test. Heterozygosity (He) and genetic differentiation at the 2 time points and concentration will obtain using GenAlex software. He values obtained will be used to determine selection. The findings from this study may reveal useful genetic information associated with tolerant *Plasmodium* strains that likely to become precursors for drug resistance.

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PHYSIOLOGICALLY RELEVANT EXPOSURE TO SYNTHETIC OZONIDE ANTIMALARIALS KILLS K13 WILDTYPE AND MUTANT *PLASMODIUM FALCIPARUM* MORE EFFECTIVELY THAN DIHYDROARTEMISININ

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Fully synthetic endoperoxide antimalarials, namely OZ277 (RBx11160, arterolane) and OZ439 (artefenomel), have been approved for marketing or are currently in clinical development. We undertook an analysis of the kinetics of the *in vitro* responses of *Plasmodium falciparum* to the new ozonide antimalarials, using a K13 mutant (artemisinin resistant) isolate from a region in Cambodia and a genetically matched (artemisinin sensitive) K13 revertant. We used a pulsed exposure assay format to interrogate the time-dependence of the response. Because the ozonides have different physicochemical properties to the artemisinins, assay optimization was required to ensure that the drugs are completely removed following the pulsed exposure. Like the artemisinins, ozonide activity requires active hemoglobin degradation. Short pulses of the ozonides were less effective that dihydroartemisinin; however when early ring stage parasites are exposed to drugs for time periods relevant to their *in vivo* exposure, the ozonide antimalarials are markedly more effective.

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HISTORICAL AND CURRENT PATTERN OF ANTIMALARIAL DRUGS USE IN THE EPIDEMIC PRONE AREAS OF WESTERN KENYA

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Management of malaria by artemisinin based combination therapy (ACTs) averts the disease sequel and limits infection propagation. This study aimed at examining the historical and current clinical prescription pattern, household use and availability of antimalarial drugs in Western Kenya. Multiple community and hospital based cross-sectional studies in three sites were done on types of antimalarial used and those prescribed respectively during ACTs drug policy implementation. Random selection of participants and review outpatient hospital registers together with the assessment of antimalarial drug availability in registered drug outlets were done. The hospital ACT prescription compliance was effectively done (100% (4042/4042) by 2015 but the adoption of the new drug policy had some rise and fall. Back in 2007 the prescription was 60% (6,363/10306) compliant before it dropped to 54.2% (712/1313) in 2010. The monotherapy antimalarial drugs use in households had been subsequently decreasing to 5.7% (86/1500) in 2015. Majority of users (90.5% (76/84) were from epidemic prone areas [$\chi 2=27.54$; df =2; $p<0.001$] and most were above 14 years of age (70/84 (83.3%) [$\chi 2= 56.08$; df=2; $p<0.001$]. In 2015 survey, majority (76.7% (227/296) of the under five years obtained their antimalarial drugs from Government hospitals while nearly half (42.5% (281/661) of the above 14 years of age obtained theirs from community drug outlets [$\chi 2= 126.13$; df =4; $p<0.001$]. The surveyed community drug outlets in 2015 found with high availability of both ACTs (100% (59/59) and 81.4% (48/59) of sulphadoxine-pyremethamine monotherapy. Compliance to ACTs drug prescription in Kenyan Government health facilities had been staggering along the course but currently effective. Similarly, the trend of household ACTs use had been improving with some rise and falls but the observed magnitude of monotherapies use might be even higher as their availability in community drug outlets still high. Continued monitoring of AMD use both in health facilities as well as in the community along with reinforcement of the endorsed efficacious drug use is highly recommended.

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PREVALENCE OF CHLOROQUINE AND ANTIFOLATE DRUG RESISTANCE MUTATIONS IN *PLASMODIUM FALCIPARUM* FIELD ISOLATES FROM TWO AREAS IN GHANA

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As a result of widespread drug resistance to chloroquine and the antifolates among *Plasmodium falciparum* parasites, artesunate combination therapies (ACTs) were introduced as first line drugs in Ghana in 2005. However, the establishment of ACT resistance in South East Asia is an ominous sign, since this region is notorious for the emergence of antimalarial drug resistance, with possible spread to the African region. To investigate the prevalence of chloroquine and antifolate drug resistant

parasites in Ghana, we tested for *P. falciparum* drug resistance gene mutations using Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP). *P. falciparum* isolates were collected from 203 children aged 2-14 years from two areas in Ghana with different transmission patterns, Navrongo and Kintampo, during the peak seasons in 2012-2013. The proportions of isolates with the *pfcrt* T76 mutation were 13.7% and 11.9% in Kintampo and Navrongo respectively. The overall prevalence rate of T76 mutation was 8.9% in the 2012-2013 period, which is significantly reduced when compared to data from the World Wide Antimalarial Resistance Network (WWARN) showing 76-100% rate from 2000-2005. For *pfmdr1,* the Y86 polymorphism was 10.4% and 17.5% in Kintampo and Navrongo respectively, with the overall prevalence in the combined population being 12.8% as against 51-75% in 2007-2008 as captured in the WWARN database. However, drug resistance mediating gene polymorphisms in *pfdhfr* (N108, 88.4%, I51, 80.6%; R59, 82.5%) *and pfdhps* (G437, 88.2%, E540, 1.2%) were comparable to data from 2000-2005 on *pfdhfr* (N108, 51-75%, I51, 51-75%; R59, 76-100%) and *pfdhps* (G437, 76-100%; E540, 1-25%) polymorphisms. The reduction in the *pfcrt* T76 genotype in the Ghanaian population is attributable to the re-expansion of the wildtype genotype K76 as result of the proscription of chloroquine as a first line antimalarial drug in 2004. However, the use of antifolates for intermittent preventive treatment of malaria in pregnancy and for seasonal malaria chemotherapy in infancy may explain the persistence of antifolate drug resistant gene polymorphisms at higher frequencies.

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CHANGES IN DRUG RESISTANCE-MEDIATING *PLASMODIUM FALCIPARUM* POLYMORPHISMS IN UGANDA OVER TIME

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Antimalarial drug resistance, mediated in part by known *Plasmodium falciparum* genetic polymorphisms, is of great concern. Chloroquine plus sulfadoxine-pyrimethamine (SP) was replaced by artemether/lumefantrine (AL) as the national treatment regimen, beginning in 2006. SP remains the standard-of-care to prevent malaria in pregnant women. We hypothesized that altered selective pressure for drug resistance would lead to changes in resistance polymorphisms in Ugandan parasites. We used ligase detection reaction-fluorescent microsphere assays to analyze 1486 samples from cross-sectional surveys of subjects in 2012, 2013, and 2015 in Tororo, Jinja, and Kanungu districts for polymorphisms in the putative drug transporters *pfcrt* and *pfmdr1* that impact on response to AL and other drugs, and polymorphisms in the folate genes *pfdhfr* and *pfdhps* associated with resistance to SP. The prevalence of pure WT sequences was markedly greater than seen in Uganda previously, and increased from 2012 to 2015 for *pfcrt* K76T (3.0% to 28.6%), *pfmdr1* N86Y (33.9% to 84.5%), and *pfmdr1* D1246Y (45.6% to 74.4%); combined WT/mixed genotypes increased in a similar manner. For antifolates, the prevalence of 5 mutations (*pfdhfr* N51I, C59R, S108N; *pfdhps* A437G, K540E) that have been common since initial studies over a decade ago remained high (generally >90% mixed/mutant). Of concern, two additional mutations that predict a greater level of resistance to SP appear to be emerging in Uganda, with the prevalence of mixed/mutant *pfdhfr* I164L increasing from 7.3% to 11.3% and of *pfdhps* A581G from 29% to 35% in Kanungu, and mixed/mutant *pfdhps* A581G also seen commonly in Jinja (21.0%) and Tororo (14.1%) in 2015. Our results demonstrate significant changes in the prevalence of transporter polymorphisms with increasing use of AL to treat malaria, persistent prevalence of 5 common antifolate mutations despite decreased use of SP to treat malaria, and the presence of additional antifolate mutations that predict high level resistance. Continued surveillance for drug resistance markers is an important priority.

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MUTATION ANALYSIS OF K13 GENE, PFMDR1 AND PFCRT ∫G V IN *PLASMODIUM FALCIPARUM* ISOLATES COLLECTED FROM WESTERN KENYA

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In Southeast Asia (SEA), mutations in K13 gene have been shown to be artemisinin resistance key determinant. Single Nucleotide Polymorphisms (SNPs) within the K13-propeller domain confer significantly higher ring-stage survival of the parasite *in vitro* and delayed parasite clearance *in vivo*, as reported previously. Although AL remains highly efficacious in Africa including Kenya, it is also associated with selection of SNPs in *Plasmodium falciparum* chloroquine resistance transporter gene (pfcrt) and *P. falciparum* multidrug resistance gene 1 (pfmdr1) in re-infections. The genotype associated with re-infection is K76 in pfcrt and N86, 184F and D1246 (NFD) in pfmdr1; K+NFD haplotype. In Africa however, data suggests K+NFD haplotype is selected for by AL, though no evidence linking this to artemisinin resistance. This study aims to investigate genetic markers for any polymorphisms found in western Kenya parasites associated with changes seen in clinical phenotype. K13 full gene was sequenced using previously published and designed primers. For Pfmdr1 gene, regions covering SNP 86, 184 and 1246 were amplified while for Pfcrt we amplified regions flanking the 72-76 haplotype region. Ring stage assay was performed and survival rates calculated. Pfmdr1 gene showed 95.7% wild, 58% mutant and 81.5% wild at codons 86, 184 and 1246 respectively. For haplotypes, K+NFD had highest frequency of 48.7%. K13 Full gene analysis showed 61 (84.7%) had non-synonymous SNPs in >5 locations out of which K189T dominated with highest frequency of (20.2%). Other SNPs include: Y493I (1.2%), A578S (1.9%) reported elsewhere while E433K (6), D464N (6), F483L (5) are uniquely found in western Kenya parasites (yet to be validated). Also, no variations in the number of a microsatellite repeat (ATA) corresponding to amino acid positions 137-142 of K13 was observed. No direct correlation seen between the mutations and the survival rates >10. It is evident that genetic determinant for higher survival rates to ACTs in African parasites is different compared to SEA parasites. K13 mutations described here including K189T are not linked to artemisinin resistance, as reported previously.

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HEPATOMEGALY IN ACUTE FALCIPARUM MALARIA IN NIGERIAN CHILDREN: BEFORE, DURING AND AFTER ARTEMISININ-BASED COMBINATION TREATMENTS

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Hepatomegaly or splenomegaly is common in childhood acute falciparum malaria but there is little evaluation of the risk factors, temporal changes and the disposition kinetics of the malaria-associated hepatomegaly following artemisinin-based combination treatments (ACTs). Liver and spleen enlargement below costal margin was detected by abdominal palpation and percussion and the liver size measured over a 6-week period. Changes in liver size was characterized using the rate of decrease or increase in size. Kinetics of the disposition of liver enlargement was evaluated using a non-compartment model. At presentation, hepatomegaly was significantly more common than splenomegaly in acutely malarious children (224 *versus* 129 of 786 children, $P < 0.0001$).

In a multivariate analysis, an age <5 years (adjusted odd ratio [AOR] = 3.1, 95%CI 2.2-4.6, P < 0.0001), duration of illness >2 days (AOR = 1.5, 95%CI 1.1-2.2, P = 0.02), core temperature >39ºC (AOR = 1.5, 95%CI 1.0-2.2, P = 0.03) and haematocrit <30% (AOR = 2.1, 95%CI 1.2-3.6, P = 0.01) were independent predictors of hepatomegaly or splenomegaly at presentation. The commonest temporal change following ACTs was ultra-rapid complete regression of hepatomegaly in over 93% of the children. Overall, mean hepatomegaly regression time (HRT) was 2.9 days (95%CI 1.9 – 3.8) and it was similar in artesunate-amodiaquine- and artemether-lumefantrine-treated children. Declines in hepatomegaly were monoexponential with overall estimated half-time ($t_{½hep}$) of 0.2 day (95%CI 0.2-0.3). HRT correlated significantly with $t_{½hep}$ (P < 0.0001). Bland-Altman analysis of 9.5 and 10 multiples of $t_{½hep}$ and HRT showed narrow limit of agreement with insignificant bias (P ≥ 0.06) suggesting both can be used interchangeably in the same patients. Young, febrile and anaemic malarious children with duration of illness >2 days, are at significant risk of hepatomegaly or splenomegaly at presentation. In acute childhood falciparum malaria, regression of hepatomegaly is rapid and is a first-order process following ACTs.

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GROWTH ADVANTAGE OF WILD-TYPE OVER DRUG RESISTANT *PLASMODIUM FALCIPARUM* GENOTYPES AMONG ASYMPTOMATIC CARRIERS IN ABSENCE OF THERAPY

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A large set of data, field and animal models, demonstrated a compromised fitness of drug-resistant malaria parasites in absence of chemotherapy. Here we extended the above observations, and quantitatively monitored drug resistant and sensitive parasite genotypes, within asymptomatic *Plasmodium falciparum* carriers, in absence of therapy, and determine their relative growth and ability to produce gametocytes. We test the prediction that drug resistant genotypes in absence of therapy are constrained by competition with co-infecting wild-type clones to maintaining low gametocyte production. We recruited 123 patients, in a seasonal malaria setting, where transmission occurs over a short period, and the rest of the year remains transmission-free. The patients were initially treated, and then monitored monthly throughout the long dry season. Relative abundance (RA) of wild- versus mutant genotypes of pfcrt and pfmdr-1 was determined using qPCR. Parasite and gametocyte densities were quantified by qPCR of 18s rRNA and RT-qPCR of pfs25 and pfs230, respectively. The densities of the mutant genotype of pfcrt and pfmdr-1 were high at enrolment and post treatment, however it decreased steadily over the dry season. The RA of wild-type of both genes increased substantially over the long dry and transmission-free period (pfcrt, p=0.009738; pfmdr-1, p=0.006867). Gametocytes density negatively correlated with the RA of wild-type pfcrt-CVMNK (p = 0.03044) but not with the wild-type pfmdr-1 86N (p= 0.06512). We provide the first direct evidence for within host growth advantage of wild-type strains of *P. falciparum*, in a therapy-free environment among asymptomatic carriers. Thus, in areas of seasonal transmission, the dry season can play an important role in harnessing susceptible parasites.

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ACTIVE MONITORING OF PHARMACOVIGILANCE AT COMMUNITY LEVEL DURING THE SEASONAL MALARIA CHEMOPREVENTION CAMPAIGN IN THE HEALTH DISTRICT KOLDA SENEGAL, 2015

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The scaling up of seasonal malaria chemoprevention campaign (SMC) began in Senegal in 2013 in four health districts with an extension in 2014 to cover four regions and over 600 000 children aged 3 months to 10 years. It is made in the form of local distribution campaigns / free mass administration of drugs Sulfadoxine-pyrimethamine (SP) + Amodiaquine (AQ) by a door to door strategy relying on the community network. The regions of Kedougou, Tambacounda, Kolda and Sedhiou meet the WHO criteria for eligibility. While the drugs used in SMC are generally considered safe and effective, they can cause adverse events that may be minor, moderate, or in very rare cases, severe. Spontaneous reporting is essential but not sufficient to assess any adverse effects related to the use of a drug. Its need to develop a community-based active pharmacovigilance through a cohort followed for SMC. The overall objective was to assess the ability of a strategy to strengthen the pharmacovigilance system and improve the reporting rate of adverse events in the context of SMC. The study took place in the health district of Kolda in southern Senegal during the season 2015 SMC for a total of three rounds (August, September and October) for 10,000 children benefiting SMC in three health posts district. In the same district, three other health posts targeting the same number of children were selected as controls. The side effects filings were received by the NMCP and processed by the pharmacovigilance center. In total 829 adverse events were reported for 743 consultations. More than half of the symptoms reported (591) were gastrointestinal disorders (71%). Other common symptoms are fever (24%). Accountability side effects made by the pharmacovigilance center and approved by the technical committee of experts shows 48% possible and probable for 28%.These results show a clear improvement compared to routine pharmacovigilance system implemented in other health districts. All results must be sent to Uppsala center UMC / WHO by vigiflow.

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HOT AND STICKY: THE EFFECT OF TROPICAL CONDITIONS ON THE STABILITY OF ARTEMISININ-BASED COMBINATIONS THERAPIES

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Poor-quality medicines, including falsified, substandard and degraded drugs, pose serious health concerns in malaria endemic countries as they contribute to the rise in drug resistance, can kill patients, and increase the public's mistrust of health systems. Substandard drugs are defined as those that contain either less or more than the acceptable dose (compliance with pharmacopeia tolerance limits) of stated active pharmaceutical ingredients (SAPIs) resulting from poor manufacturing practices, while degraded drugs are good quality formulations that have degraded on storage in the presence of heat, light and humidity. Guidelines are lacking distinguishing between substandard and degraded drugs in terms of markers of degradation. 'Forced degradation' was carried out on three common artemisinin-based combination therapy (ACT) brands and analytical methodology was developed to facilitate the classification of degraded drugs. This methodology was applied to ACTs purchased in Enugu, Nigeria that had been classified to be substandard (206 samples) and 18% of these were found to contain degradation products. We previously conducted a large-scale 'natural ageing' study (2,880 samples

of Coartem® and Winthrop®) to evaluate the long-term stability of ACTs in tropical climates, on-site in Ghana and in a stability chamber in London. Samples were aged in the presence and absence of light and removed from each site at regular intervals to measure loss of SAPIs over time as well as detect products of degradation. Loss of SAPIs in these naturally aged samples was 0 to 7% over 3 years (~12 months beyond expiry) with low levels of degradation products detected. ACTs that were found to be stable in tropical climates for periods up to and beyond their expiry dates were from WHO prequalified manufacturers, while those found to contain degradation products were from non-WHO prequalified manufacturers. Hence, presence of insufficient SAPIs, together with detection of degradation products, can be used to classify drugs as degraded.

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READMISSION TO HOSPITAL FOR WORSENED ANAEMIA IN MOZAMBICAN CHILDREN TREATED WITH INTRAVENOUS ARTESUNATE

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Readmission to hospital for worsened anaemia in Mozambican children treated with intravenous artesunate Parenteral artesunate is recommended as first-line therapy for severe malaria. While its efficacy is firmly established, data on safety are still incomplete and scarce among African children. We aim to assess and compare delayed (newly incident or worsening) anaemia rates in the first 30 days after parenteral treatment with Quinine or artesunate, after its introduction as first line policy. We also aim to identify risk factors associated to delayed anemia following artesunate iv treatment. We conduct a retrospective analysis for the period 2001-2015 using the outpatient and inpatient morbidity databases, and linking them with the demographic surveillance system ongoing in the Manhiça district, in Southern Mozambique. Recurrent hospital admissions or outpatient visits after a documented parenteral treatment for malaria will be analysed to determine whether the use of parenteral artesunate is associated with an increased risk of anaemia. Comparison of trends of this phenomenon (before and after the year when the switch from quinine to artesunate in first line policy occurred), in addition to the evaluation of other potential confounding factors, will allow an inference of the possible attribution to artesunate. We will present the results of this analysis, reviewing over 50,000 admissions during the study period.

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IMPROVING UPTAKE OF IPTP IN UGANDA THROUGH TEXT MESSAGING HEALTH WORKERS

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Malaria in pregnancy poses a risk to mother and child. The World Health Organization recommends intermittent preventive treatment in pregnancy (IPTp) for the prevention and control of malaria in pregnancy, typically provided through the focused antenatal care (ANC) package. Coverage of at least two doses of IPTp remains low in most countries implementing IPTp, despite generally high ANC attendance. Qualitative formative research conducted in 2013/14 concluded that supply-side issues are likely to account for the majority of missed opportunities for the provision of IPTp in Uganda. In particular, health workers' knowledge of IPTp guidelines was poor. To address this barrier, a pilot intervention was implemented in West Nile in 2015. This involved provision of classroom-based malaria in pregnancy training to selected health workers in two districts (n=24 per district). In one district only, all health workers involved in ANC provision (n=49) subsequently received 25 text messages reinforcing the training content. The study used a mixed-methods design to determine the impact of text messaging: i) a multiple choice knowledge questionnaire administered immediately after training (n=90) and six months after training (n=89), ii) calculation of IPTp coverage in participating health facilities (n=16) over six months pre- and post-training and iii) four focus group discussions with health workers and three in-depth interviews with district officials. Health workers who had received text messages in addition to training demonstrated better knowledge of malaria in pregnancy six months post-training compared to health workers receiving training only. IPTp coverage was also higher in facilities where health workers received text messages. Complementing classroom-based malaria in pregnancy training with text messages has the potential to improve health worker knowledge of and adherence to guidelines. Text messaging is inexpensive, well-received by health workers and does not disrupt service provision. This approach has potential applications to health worker training on other topics.

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EFFICACY OF THREE ARTEMISININ-BASED COMBINATION THERAPIES IN ANGOLAN CHILDREN WITH *PLASMODIUM FALCIPARUM* INFECTION, 2015

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Antimalarial resistance monitoring in Angola has recently garnered increased attention. Therapeutic efficacy studies (TES) from 2011-2013 in Luanda and from 2013 in Zaire Province showed efficacy of artemether-lumefantrine (AL) approaching the 90% threshold identified by the World Health Organization for artemisinin-based combination therapies (ACTs). In addition, a controversial case of malaria unresponsive to artemisinins was reported in a patient infected in Lunda Sul Province in 2013. In January-June 2015, investigators monitored the clinical and parasitological response of children with uncomplicated *Plasmodium falciparum* infection treated with one of three ACTs: AL, artesunate-amodiaquine (ASAQ), or dihydroartemisinin-piperaquine (DP). The study comprised two treatment arms in each of three provinces: Benguela (AL and ASAQ), Zaire (AL and DP), and Lunda Sul (ASAQ and DP). Participants were followed for 28 (ASAQ and AL) and 42 (DP) days. Samples from treatment failures were analyzed for molecular markers of resistance for artemisinin (K13) and lumefantrine (pfmdr1). A total of 475 children reached a study endpoint. Fifty-five treatment failures were observed: 4 early treatment failures, 41 reinfections, and 10 recrudescences. Excluding reinfections, the microsatellite-corrected efficacy at day 28 was 96.3% (95% CI: 91-100) for the AL arm in Benguela, 99.9% (95-100) for ASAQ in Benguela, 88.1% (81-95) for the AL arm in Zaire, and 100% for ASAQ in Lunda Sul. For DP, the corrected efficacy at day 42 was 98.8% (96-100) in Zaire and 100% in Lunda Sul. All treatment failures were wildtype for K13, but all AL treatment failures had pfmdr1 haplotypes associated with decreased lumefantrine susceptibility. The results suggest a parasite population sensitive to artemisinin derivatives in these three provinces. No evidence was found to corroborate the specific allegation of artemisinin resistance in Lunda Sul. The continued low efficacy of AL in Zaire might be due

to decreased susceptibility to lumefantrine, and further monitoring, particularly including measurement of lumefantrine blood levels during TES, is recommended.

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ACCELERATING THE DISCOVERY OF TRANSMISSION-BLOCKING DRUGS: HT SCREENING WITH A NOVEL *PLASMODIUM FALCIPARUM* FUNCTIONAL GAMETOCYTE ASSAY

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The last decade has witnessed unprecedented progress in reducing the incidence of *Plasmodium falciparum* malaria, especially in Africa where the burden of disease is greatest. Nevertheless, in order to achieve the ultimate goal of eradicating malaria, more effective tools will be required. These include efficacious vaccines and innovative antimalarial drugs with novel mechanisms of action displaying not only efficacy against resistant parasites but also transmission blocking potential. *P. falciparum* mature gametocytes are the parasite forms responsible for malaria transmission, thus our objective was the discovery of drugs with a novel mechanism of action, active against these stages, either by killing or functionally preventing their maturation to mosquito stages. For this purpose, we have developed a phenotypic HTS assay, which assesses the functionality and viability of *P. falciparum* stage V gametocytes by measuring the formation of female gametes. In this work, we present the screening of GSK compound collections. Over 400 hits were identified after a primary screening at 2μM and a subset of approximately 100 compounds representative of new chemical diversity were prioritized for further profiling. A selection of hits was assessed *ex vivo* in the standard membrane feeding assay and demonstrated complete transmission blockage. This new set of compounds may serve as starting points for future drug discovery programs as well as tool compounds for identifying new modes of action involved in malaria transmission.

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NEW ANTIMALARIAL FAMILY "RESISTANT TO RESISTANCE" DEVELOPMENT

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Malaria continues to be a major global disease still causing impermissible number of deaths. The effectiveness of current antimalarial therapy is under continuous threat through the spread of resistance. Consequently, there is an urgent need to replace those drugs compromised by resistance, as well as identifying potential novel therapies that offer significant advantages over the current standard of care. As result of the whole cell phenotypic screen conducted by GSK to identify TCAMS set of compounds, a number of lead optimization programs were initiated. One of them, pyrazine family, was progressed because the chemical novelty displaying exciting opportunities as antimalarial. The program led to the identification of balanced molecules with appealing biological profile. Pyrazine molecules demonstrated relevant activities against intra-erythrocytic asexual *Plasmodium* stages including multiple MDR laboratory adapted strains and clinical isolates. Pyrazines displayed a desirable rapid *in vitro* killing profile. This activity is accompanied by an oral efficacy characterized by a rapid parasite clearance in the *P. falciparum* mouse model. This rapid antimalarial activity is expected to maximize efficacy, achieving fast clinical resolution, and minimizing the in-patient window of opportunity for resistant parasite selection and dissemination. Notably, the series displayed an extremely low propensity to select resistance *in vitro*. The biological profile that the series offers seems to be associated to an unique mode of action not related to any of the known mechanisms tested, hence offering differentiation against all other antimalarials. The development of differentiated MoA antimalarials is of paramount importance to be able to develop de novo combination treatments, thereby ultimately avoiding the emergence of resistance. The overall properties of pyrazines constitutes a promising profile justifying further development and makes these assets suitable partners for any future combination treatment.

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IN VIVO STUDIES OF FITNESS OF DRUG RESISTANT *PLASMODIUM FALCIPARUM* DHFR MUTANTS USING *PLASMODIUM BERGHEI* TRANSGENIC PARASITES

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Drug resistance has hindered control efforts for malaria, where mutations to the target dihydrofolate reductase (DHFR) enzyme are responsible for resistance to pyrimethamine. Nonetheless, antifolates are key components of ACT's and used for intermittent preventive treatment in pregnancy. Understanding parasite fitness evolution is important for transmission. There is a lack of information about the cost of drug resistance mutations on parasite fitness, or the ability of resistant parasites to compete with sensitive strains and persist in the field. We studied how DHFR mutations under drug pressure influences survival of resistant alleles. We conducted competition experiments between *Plasmodium berghei* parasites in which endogenous dhfr was modified by gene replacement, with *P. falciparum* single (108 {1M}), double (59 and 108 {2M}) and triple (59, 108 and 164 {3M}) mutants. For each parasite line created, GFP and cherry RFP were used as reporters to aid parasite enumeration via flow cytometric analysis. All single infection and competition experiments were initiated with 1x106 parasites. Infections were followed up, with the treated groups administered 10-mg/kg pyrimethamine for 3 days, after which, parasitemia was assessed via counting of fluorescent parasites by a flow cytometer. In all experiments (both treated and non treated groups), single infection parasites grew better in mixed infections (competition). For all mutant lines in competition experiments, higher resistant parasites grew better in mixed infections when competing against lesser resistant mutants. 2M mutants grew better in single infections with drugs than without drugs. In all competition experiments 3M mutants grew better with or without drugs than other mutants. These findings support a model of parasite release and facilitation, whereby under drug pressure highly resistant parasites do better than less resistant parasites. Recognizing the costs of fitness will aid the development of optimal guidelines for the treatment and prevention of malaria.

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EVALUATION OF THE PERFORMANCE OF SD BIOLINE MALARIA AG *PF/PV* AND CARESTART™ MALARIA *PF/PV* COMBO TESTS FOR THE DIAGNOSIS OF MALARIA IN TWO MALARIOUS AREAS IN CENTRAL ETHIOPIA

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Early and accurate diagnosis of malaria followed by prompt treatment reduces morbidity and mortality in endemic regions. Presumptive

treatment of malaria is widely practiced where microscopy or rapid diagnostic tests are not readily available. Introduction of rapid diagnostic tests (RDTs) for the treatment of malaria in many low-resource settings need evaluation of their performance. This study evaluated the performance of two RDTs in two health centers from November-December, 2014 in Adam and Amaya. Oromia, Ethiopia. This study was undertaken to evaluate the diagnostic performance of SD BIOLINE malaria Ag *Pf/Pv* and CareStart™ malaria *Pf/Pv* Combo test relative to microscopy for the diagnosis of *P. falciparum* and *P. vivax* malaria in Ethiopia. In this cross-sectional study, patients who had malaria symptoms and visited two health facilities in Oromia Region were recruited. Thin and thick blood smears were prepared from finger prick and stained by 10% Giemsa. Microscopic examination was done under 100x magnifications for *Plasmodium* species identification and determination of parasitaemia. The two RDTs were performed as per the manufacturers instructionsA total of 547 febrile patients were diagnosed, of which 127 were microscopy positive for *Pf* (n=38) and *Pv* (n=85). The sensitivity, specificity, positive and negative predictive value of SD BIOLINE malaria Ag *Pf/Pv* test were 92.1%, 99.1%, 95.9% and 98.2%, respectively; and for CareStart™ malaria *Pf/Pv* Combo tests were 94.5%, 99.6%, 98.4% and 99.6%, respectively. In conclusion, the diagnostic performance of SD BIOLINE malaria Ag *Pf/Pv* test and CareStart™ malaria *Pf/Pv* Combo test were very good with respect to malaria microscopy.

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THE EFFECTS OF MALARIA SPECIES-SPECIFIC TREATMENT POLICY ON CASE MANAGEMENT AND DYNAMICS OF RESISTANCE MARKERS IN ETHIOPIA

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In Ethiopia, *Plasmodium falciparum* and *P. vivax* are co-endemic and virtually responsible for 60 and 40% of all malaria cases, respectively. Artemether-lumefantrine and CQ are first-line treatments for uncomplicated *P. falciparum* and *P. vivax* infections, respectively. The change from one drug to another, following the emergence of drug resistance, would alter the dynamics of sensitive and resistant parasite haplotype. Accurate diagnosis is, therefore, crucial for appropriate prescription of the correct drugs. A total of 1,094 patients were screened for malaria using microscopy and RDTs. Sets of microscopy and RDT-positive and negative samples were tested for confirmation using PCR. Anti-malarial drugs were prescribed for 9.3 and 20.0% microscopy- and RDT-based parasite negative patients, respectively, as confirmed by PCR. PCR detected the presence of *P. falciparum* DNA in 19.2% of microscopy- and RDT-negative samples. Of microscopy-positive *P. vivax* infections, 63.2% were proved *vivax* malaria by PCR. Of microscopy-positive *P. falciparum* infections, 71.4% were proved *falciparum* malaria by PCR. Of RDT-positive *P. falciparum* infections, PCR proved in 62.7% of them. Of RDT-positive *P. vivax* infections, 77.3% were proved vivax malaria by PCR. All parasites were carrying the *pfcrt* K76T mutant variants. The prevalence of parasites with the wildtype codon *pmdr*1 Y86N was 76.7%. Microscopy and RDT were insufficient at low density parasitaemia and hence misdiagnosis was significant. The use of molecular methods appropriate at field setting would help avoid over-prescription and under-diagnosis of malaria infections. While the presence of the mutant variant of *pfcrt* in the Ethiopian samples can be explained by continued use of chloroquine in Ethiopia for treatment of *P. vivax*, the selection of wild type *pfmdr*1 could be a consequence of using ACT for treatment of *P. falciparum.*

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PARASITOLOGIC CORRELATES OF *PLASMODIUM OVALE WALLIKERI* AND *PLASMODIUM OVALE CURTISI* INFECTION

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Plasmodium ovale is one species of human malaria that is prevalent in West Africa and can be challenging to diagnose due to clinically mild disease and low parasite burden. Consequently, conventional diagnostic tools, such as microscopy of blood films and rapid antigen-based diagnostic tests, have limited performance in detecting *P. ovale* infection. Furthermore, two genetically distinct sub-species of *P. ovale* exist: *P. ovale* curtisi (Poc) and *P. ovale* wallikeri (Pow). At present, it is unknown if the sub-species causing infection may affect clinical presentation or performance of malaria diagnostic tests. Therefore, we sought to investigate if parasite burden, morphological features, and pan-aldolase antigen-positivity differ between Poc and Pow. 49 *P. ovale*-positive, whole-blood specimens were identified from our malaria biobank, and DNA was extracted. Parasitemia and pan-aldolase antigen-positivity that were reported upon initial processing were obtained for analysis. Real-time PCR (qPCR) assays were conducted to confirm microscopy species identification, and quantify 18S rRNA gene copy number. Endpoint PCR of target regions and Sanger sequencing were conducted, and sub-species was determined by analyzing the 18S rRNA sequence. We compared reported parasitemia, parasite morphology, pan-aldolase antigen-positivity, and 18S gene copy number between the two sub-species. We identified 22 Poc and 27 Pow. There were no statistically significant differences between the two sub-species by parasitemia, 18S rRNA gene copy number, or pan-aldolase antigen-positivity. When comparing morphological features, we noted that all 8 *P. ovale* parasites without Schuffner's stippling were Pow while all Poc parasites had this feature (p=0.02). Poc and Pow do not differ significantly by parasite burden, although a lack of discernible Schuffner's stippling may be a feature specific to Pow. This is a novel finding, considering that the sub-species have been previously distinguished by their genotype alone.

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FALSE-NEGATIVE MALARIA RAPID DIAGNOSTIC TESTS IN RWANDA: IMPACT OF *PLASMODIUM FALCIPARUM* ISOLATES LACKING HRP2 AND IMPROVED MALARIA CONTROL

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Rapid Diagnostic Tests (RDTs) have become the focal point of the global approach to malaria control and are often used to determine whether persons with fever, chills or other symptoms of malaria are treated with antimalarials. Because false-negative RDTs can delay or prevent the effective treatment of this potentially fatal disease, it is essential to examine the factors that affect RDT performance. Recently, *Plasmodium falciparum* isolates lacking the *hrp2* gene have been identified as a cause of false-negative RDTs. However, despite the importance of this issue, there is a paucity of data on the frequency of these isolates. This study examined RDT sensitivity at sites with varying intensities of malaria transmission in Rwanda and used PCR to determine whether *hrp2* deletions were responsible for false-negative HRP2-based RDT results. Between April 2014 and April 2015, the study enrolled 9,219 symptomatic patients from 3 health centers in Rwanda and compared RDT results to microscopy for *Plasmodium* species as the gold standard. The overall slide positivity rates (SPR) were 53% at Rukara, 35% at Kibirizi and 10% at

Busogo. RDT sensitivity varied by month and site and was highest (94% [95% CI 92-95%.]) at Rukara, the site with the highest SPR. At Kibirizi, a site targeted by pre-elimination activities during this study, RDT sensitivity declined from 88% to 67% as the monthly SPR fell from 46% to 3%. For samples that were positive by microscopy but negative by RDT, PCR was performed to test for *Plasmodium* DNA and confirm the presence or absence of the *hrp2* gene. PCR analysis identified a total of 34 infections that were positive by PCR for *P. falciparum* but negative by RDT and PCR for *hrp2* (consistent with deletion of the *hrp2* gene). To the authors' knowledge, this is the first report of circulating *P. falciparum* isolates lacking *hrp2* in East Africa. However, this is not the first time that a decline in RDT sensitivity occurred following a decline in malaria transmission. Further investigation is warranted to assess the factors driving the decline in RDT sensitivity as malaria control improves.

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SNP-LAMP POINT OF CARE TEST FOR THE DETECTION OF ARTEMISININ-RESISTANCE IN THE FIELD

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Cases of artemisinin resistant malaria are rapidly increasing in South-East Asia. Even in some part of Cambodia, the proportion of the slow clearing parasites is now above 75%. Several mutations in the K13 propeller gene have been associated with 91.8% of slow-clearing parasites. Of these drug resistant mutations, C580Y and R539T constituted 85% of the resistant population in Cambodia. Recently,the C580Y mutation was also reported from Guyana, a South American country but not Africa. Monitoring resistance in the field site is important for the treatment and surveillance of resistant clones. Current methods to evaluate artemisinin resistance such as *in vitro* culture methods and DNA sequencing are time-consuming, reagent intensive, expensive and not applicable to the field setting. Point of care detection of K13 mutations will allow for correct antimalarial treatment choices as well as enhance surveillance. Loop mediated amplification (LAMP) has proven to be field adaptable in our hands. We now modify LAMP to detect the C580Y and R539T SNP. SNP-LAMP is based on the differential binding of amplification primers. Our strategy was to validate SNP-LAMP on bona fide resistant clones from Cambodia. Input template DNA and enzyme concentrations were varied to optimize amplificationof the mutant strain while not amplifying the wild type. Preliminary data is presented on the K13 SNP-LAMP assay for control strains, clinical isolates, and non- falciparum controls including *Plasmodium vivax*, *P. ovale* and *P. malariae*. Our data suggest the mutant K13 propeller gene can be specifically amplified within a range of 1000-10000 parasites/µL.Specificity is lost if parasitaemia goes over 10000/µL. Dilution of the high copy number sample can maintain the accuracy of the diagnosis. Further work is going on improve the minimum and maximum limit of detection by altering different sequences of loop primers.

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ASSESSMENT OF LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION (LAMP) METHOD FOR ASYMPTOMATIC MALARIA SCREENING IN THE PERUVIAN AMAZON SETTINGS

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In the Peruvian Amazon, asymptomatic malaria infections, with low parasite density, are common and has impact on maintaining malaria transmission. Within this context, molecular diagnostic tests would be more efficient than microscopy or RDTs to identify these cases; however, it requires well trained users and special equipment. LAMP is expected to be a point-of-care method that could be applied in rural communities with basic facilities and also an alternative for population screening aiming malaria elimination. In this work, we assessed the performance of LAMP to detect asymptomatic malaria infections compared with microscopy and validated by three different PCR protocols: nested PCR (nPCR), qPCR base on mitochondrial genome (qPCR-Pgmt) and qPCR base on 18S rRNA gene (qPCR-18S). 1173 subjects from eight communities along Alto Nanay river were screened by LAMP and microscopy and all asymptomatic individuals above 3 years old were enrolled. 58 (4.9%) were positive by microscopy and 259 (22.1%) were positive by LAMP (34 *Plasmodium falciparum* and 225 no-falciparum). All LAMP positive samples, 30% of negative samples and 7 positive controls with different parasitaemia were tested to evaluate LAMP sensitivity and specificity. Stratified analysis by parasitaemia level between LAMP and PCR showed a sensitivity of 100%, 94.8% and 93.1% against nPCR, qPCR-Pgmt and qPCR-18S, respectively, when parasitaemia was higher than 1 parasite/µL. However, with parasitaemias bellow 1 parasite/µL, LAMP sensitivity decrease to 77.8%, 86.6% and 69.9% against nPCR, qPCR-Pgmt and qPCR-18S, respectively. Our results suggest that LAMP is a good alternative for a point of care diagnostic and population screening, but its performance decays with samples with very low parasitaemia. To move towards malaria elimination in settings with very low transmission, LAMP performance should be improved, and the development of new tests capable of identifying very low parasite densities is required.

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VALIDATION OF DRUG SELLERS' MALARIA RAPID DIAGNOSTIC TESTS (MRDTS) RESULT RECORD AND FIELD MALARIA RDT PERFORMANCE AGAINST PCR ANALYSIS OF DUPLICATE BLOOD SAMPLES FROM UNDER-FIVE CHILDREN IN SOUTHWESTERN UGANDA

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Currently, the WHO recommends that every case of suspected malaria be confirmed by a diagnostic test. Use of malaria Rapid Diagnostic Tests (mRDTs) has been emphasized in communities and private drug outlets where more than 50% of fever case management occurs in malaria endemic LMICs. Due to limitations in mRDT sensitivity for efficient detection of low-density parasitemia or sub-microscopic infections, nucleic acid amplification techniques (NAATs) have been encouraged for epidemiological research and surveys to map sub-microscopic malaria infections in low-transmission settings such as South Western Uganda. Operator error, transport and storage conditions can also cause false negatives. On this basis, we are comparing test results from mRDT done by drug sellers in field conditions on under-five children in South Western Uganda against PCR test. A duplicate blood sample is picked using Whatman FTE card for additional test comparisons. Methods Duplicate samples from under-five children are collected using CareStartTM HRP2 Pf RDT cassettes and WhatmanTM 3MM filter paper at drug shops participating in the on-going AXEX study in cross-sectional study design. A total of 203 samples have been collected and analysis is ongoing at the Molecular Biology Lab at Makerere University. Proficiency testing on the PCR method was done. The method includes extracting *Plasmodium* DNA from blood spot collected on Whatman FTE card and mRDT cassette using Chelex method and Qiagen Blood DNA kit following manufacturer's instructions. The quantity and quality of the extracted DNA is assessed using a Nano Spectrophotometer and QIAexcel automated capillary electrophoresis. Results Results to be presented will include field mRDT performance when test is done by drug seller against PCR analysis. Sensitivity, specificity, positive and negative predictive values will be reported. Agreement between the two tests will also be calculated and evaluated using the kappa statistic. In conclusion, this study will provide additional empirical information on who to deploy mRDT alongside NAATs in screening and surveillance of malaria during country elimination efforts.

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HEALTH WORKER AND POLICY MAKER PERSPECTIVES ON USE OF INTRAMUSCULAR ARTESUNATE FOR TREATMENT OF SEVERE MALARIA AT HEALTH POSTS IN ETHIOPIA

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World Health Organization (WHO) recommends intravenous (IV) and intramuscular (IM) artesunate (AS) for management of severe malaria. The current national malaria treatment guidelines do not provide for use of IM AS at health posts (manned by health extension workers (HEWs)). Although WHO recommends use of pre-referral intrarectal artesunate, there are no WHO prequalified suppliers of intrarectal artesunate, and its use is limited to children under 6 years of age. We assessed the perspectives of health workers, and policy makers on the use of IM AS as a pre-referral and definitive treatment for severe malaria at health post level in Ethiopia. A qualitative exploratory study that employed in-depth interviews with 101 health workers from 60 health facilities, the Federal Ministry of Health, and development partners. All respondents were either health workers involved in the treatment of malaria, or in formulation of malaria policy. An interview guide was used. Data transcripts were translated into English, uploaded into Atlas.ti7 software, and coded. Thematic content analysis was employed. Key findings from this study are: (1) Provision of IM AS as pre-referral and definitive treatment for severe malaria at health posts could be lifesaving; (2) With adequate training, and provision of facilities including beds, health posts can provide definitive treatment for severe malaria using IM AS where referral is delayed or not possible; (3) Health workers at health centers and hospitals frequently use the IV route because it allows for co-administration of other drugs, but they find the IM route easier and propose it for HEWs; (4) The reasons commonly cited against the management of severe malaria using IM AS at community level were: Lack of capacity to manage complications and fear of irrational drug use; (5) use of IM AS at health post level will require evidence on safety and feasibility before policy shift. IM AS could be used at health posts to provide life-saving pre-referral, or definitive treatment when referral is delayed or not possible. Evidence on safety and feasibility of its use by HEWs is needed before policy change.

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EVOLUTION AND SPREAD OF "STEALTH" *PFHRP2* DELETIONS IN *PLASMODIUM FALCIPARUM* IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Deletions of the *Plasmodium falciparum hrp2* (*pfhrp2)* gene cause false-negative rapid diagnostic test (RDT) results and have been sporadically reported but never investigated systematically on a national population level. RDTs are a cornerstone of modern malaria control efforts; understanding the evolution of *pfhrp2* deletions is essential for ensuring their continued effectiveness. Using a nationally representative cross-sectional study of 7,137 children in the Democratic Republic of Congo, we investigated whether *P. falciparum* parasites with false-negative RDT results harbored deletions of *pfhrp2*. We employed polymerase chain reaction assays to identify *pfhrp2*-deleted parasites among those with false-negative RDTs and to examine microsatellite regions flanking the *pfhrp2* gene for population genetic analyses. We found that 4.3% (n = 117) of all *P. falciparum* infections country-wide were due to *pfhrp2*-deleted mutants, representing 14.9% of 783 parasites with false-negative RDT results. Bayesian spatial analyses identified two geographical clusters with significantly higher proportions of parasites harboring *pfhrp2* deletions. Population genetic analysis of these clusters revealed significant genetic differentiation between wild-type and *pfhrp2*-deleted parasite populations (G_{ST} = 0.021, $p \leq 0.00001$). In conclusion, *Pfhrp2*-deleted *P. falciparum* is a common cause of false-negative RDTs in the DRC. Use of RDTs as indicators for treatment may be exerting evolutionary pressure favoring the spread of "stealth" parasites, resistant to detection by currently used RDTs. To our knowledge, this is the first report of an outbreak of a pathogen that has mutated to elude detection by a diagnostic test.

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INTRODUCTION OF COMPETENCY BASED SELECTION CRITERIA FOR WHO EXTERNAL COMPETENCY ASSESSMENT FOR MALARIA MICROSCOPY

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In 2015 MalariaCare introduced a new selection criterion to the WHO External Competency Assessment for Malaria Microscopists (ECAMM) to determine the best-qualified candidates among those who met course entry requirements. We describe a competency-based modification to existing ECAMM course entry requirements that can be used to identify the best-qualified candidates for participation in WHO ECAMM courses. Pools of candidates were screened using existing WHO ECAMM course entry requirements. A second selection criterion was added based on satisfactory performance from a five-day pre-ECAMM refresher training course. Of the 119 participants included in the final WHO ECAMM data set, 103 (86.6%) were assessed prior to 2015 and did not participate in a pre-ECAMM course; however, 16 (13.4%) microscopists assessed in 2015 participated in a pre-ECAMM course and were selected for advancement to WHO ECAMM courses based on attainment of prescribed competency levels. Post-test pass rates for WHO ECAMM course components among microscopists not participating in pre-ECAMM courses were 82.5% for parasite detection (mean score = 89.8%), 26.2% for species identification (mean score = 62.5%), and 43.7% for parasite quantitation (mean score = 34.8%). Among participants who were subjected to the revised selection criteria, post-test pass rates for all 3 WHO ECAMM course components were 100.0%. Mean post-test scores within this participant pool were 97.6% (parasite detection), 90.2% (species ID), and 60.8% (parasite quant).Participants attending WHO ECAMM courses before 2015 were 3.7 times less likely to attain WHO certification, whereas all participants from the 2015 participant pool attained WHO certification based on their accreditation levels. WHO ECAMM course outcome (certification vs. non-certification) was not independent of participant selection criteria type, c^2 (1, N=119) = 35.87, p<0.0001. To identify the best qualified participants, our results suggest that course administrators may consider a second competency-based selection criterion based on satisfactory completion of a five-day pre-ECAMM refresher-training course.

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METHODS FOR IMPROVED NUCLEIC ACID-BASED DETECTION OF INTRAERYTHROCYTIC *PLASMODIUM* AND BABESIA PARASITES

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We have developed a novel nanoparticle-based sandwich hybridization assay (SHA) for the detection of *Plasmodium falciparum* (Pf) and *Babesia microti* (Bm) parasites without the need for amplification of target sequences in genomic DNA. A uniquely identifiable "barcoded" magnetic microbead and biotinylated silica nanoparticle are conjugated to either Pf or Bm specific 30-mer oligonucleotides corresponding to sequences of the 18S ribosomal gene. Parasite burden can then be quantified and analyzed upon the binding of an Avidin-PE fluorophore to the target capture complexes via a Bio-Plex 200 instrument. Probit analysis of 1 mL of parasite-spiked human blood revealed 95% detection thresholds of 62 and 122 p/mL for the Pf and Bm 18S bead sets, respectively. To further enhance assay sensitivity, we evaluated additional probe sets specific to novel high abundant targets for each pathogen (Pf EMP1 and Bm BMN) which dramatically improved detection of both parasites 4-30 fold. Utilizing these high copy probe sets against clinical blood samples has demonstrated 90-100% sensitivity. We also report on the development of PCR-based nucleic acid tests utilizing these same biomarkers for Pf and Bm parasites, detecting as few as 1 parasite/mL with a broad dynamic linear range allowing quantitation of parasite densities above 100 p/mL. Extensive data obtained during assay validation will be presented to highlight protocol-specific variables such as blood volume, reaction volume, and test sample volume that affect the sensitivity of both methods. For example, the performance of 18S target probes are dramatically improved when genomic DNA extracts are prepared from larger volumes of blood. Cumulatively, these data establish both the sandwich hybridization and PCR assays as sensitive pathogen detection platforms for diagnosis of chronically infected blood donors and provide useful insights on the development of general blood-based detection methods.

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COSTS AND AFFORDABILITY OF LAMP FOR MOLECULAR DIAGNOSIS OF MALARIA IN RESOURCE LIMITED, ENDEMIC SETTINGS

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Loopamp Malaria Pan/Pf Detection Kit (Eiken Chemical), based on the loop-mediated isothermal amplification of DNA (LAMP), is a simplified molecular method that offers PCR-equivalent diagnostic accuracy for the detection of low-density malaria parasitaemia and its speciation. Given its applicability in resource restricted field settings of malaria endemic areas, LAMP has considerable potential in shaping post-2015 strategies for global malaria control and elimination. We evaluated economic cost and affordability (in 2015 US$) of implementing LAMP as part of routine malaria control programs in two countries (Peru and Philippines) and compared it with that of smear microscopy and conventional PCR techniques. Per test costs were assessed based on a time and motion study that examined both direct and indirect resource inputs at various workload levels. Costs of LAMP implementation were calculated based on the laboratory workload and network data available in Peru. Per test cost of LAMP Pan or Pf followed an inverse exponential pattern against the workload levels with the cost stabilizing when at least 8 samples were processed per batch ($11-14). At highest workload levels (60 smears/88 PCR samples per batch), smear and PCR were $1.5 and $13 per test, but could cost as high as $7.5 (1 sample/batch) and $70 (4 PCR samples/batch). Implementing LAMP at 179 eligible laboratories (at least 1 to 5 samples per day) in Iquitos region in Peru, required $4.7 million per year for the first three years of implementation. Restricting implementation to 36 high workload laboratories (at least 12 samples per day), costs can be reduced to $2.1 million (contingency fund for malaria control in Peru for 2015 was $2.3 million). Though cost of LAMP can be competitive (vs. PCR), it will require considerable financial resources to achieve adequate coverage to meaningfully reduce time delays in malaria care. Likewise, cost-effectiveness or budget impact analysis evaluating various implementation strategies and their effects must be assessed to better guide policy decisions on future utility of LAMP for malaria control in high endemic, resource limited settings.

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FACTORS INFLUENCING THE USE OF MRDTS AMONG PRIVATE HEALTH PROVIDERS AND CONSUMERS AT THE KENYAN COAST

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In 2010, the Government of Kenya adopted the WHO test, treat and track policy which saw the introduction of malaria RDT testing in the public sector but limited support for the private sector. Fever seeking statistics indicate that 27% of people seek treatment in the private sector where availability of RDTs remains low at less than 20%. PS Kenya is implementing a fever case management project seeking to create a private sector market for increased access to and use of quality assured mRDTs. A qualitative study using In-Depth Interviews was conducted targeting private providers and patients in Kilifi, Mombasa and Kwale Counties. The study reached a total of 40 respondents i.e. 24 outlet personnel and 16 adult patients. Providers were trained and had 2 to 23 years' work experience. Patient interviews were conducted in private locations near but not affiliated with the health facilities. Interviews had no personal identifiers and were digitally recorded after obtaining interviewees' consent. All transcripts were analyzed and coded. Results showed that most providers reported using RDTs after training. They reported increased number of clients, ability to conduct out-of-office testing, reduction in workload /operation cost and patient preference and satisfaction. Concerns and challenges were 1) the lancets used were blunt and painful when pricking 2) inadequate buffer solution to conduct the test and 3) invalid results. Adoption of mRDTs depended on 1) trusted sources of the RDTs and type of brand 2) Purchase price for RDTs and costs to end-users. Overall, patients reported improved quality of care due to diagnosis before treatment and they liked seeing and interpreting results. In conclusion,Key factors that influence the use of the mRDT kit include ease of use of the kit, reliability of the test, reduction in provider work load and patient preference. Barriers include blunt lancets and inadequate amount of buffer- mRDT supply reliability, provider training and cost of mRDT to end user must also be addressed to ensure expansion of mRDT use in the private sector in emerging markets.

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EARLY POST-TREATMENT LEVEL OF HRP2 PREDICTS RECRUDESCENCE IN *PLASMODIUM FALCIPARUM* INFECTION

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Recrudescent infections following incomplete clearance of parasites are routinely reported for all classes of antimalarials. Traditionally, monitoring of response to antimalarial treatment has relied on serial blood microscopy. However, due to the limit of detection of microscopy, treatment failures are typically detected late, when the recrudescing infection has already reached a parasitemia high enough to cause symptoms. Stored longitudinal samples from 301 children enrolled in two therapeutic efficacy studies from 2013 and 2015 in Angola were analyzed, representing 243 participants with adequate clinical and parasitological response (ACPR), 39 with reinfection, and 19 with recrudescence. An ultra-sensitive bead-based Luminex immunoassay platform was used to quantify the concentration of the *P. falciparum* HRP2 protein in samples from follow up, ranging from 28 to 42 days. The HRP2 concentration on each day was normalized by the participant's HRP2 concentration on day 0, the first day of treatment. The concentration of HRP2 in participants with ACPR decayed exponentially after day 0, with a consistent decay rate of 6.5% (95% CI: 6.3-6.7) per day over the course of follow-up. Participants that ultimately suffered reinfections cleared HRP2 at an indistinguishable rate of 7.1% (6.4-7.8) per day prior to reinfection. In contrast, HRP2 concentration in participants that ultimately recrudesced behaved differently, increasing on average by 25% from day 0 to day 3, and was higher than in participants with ACPR or reinfections at all time points. The HRP2 concentration at day 3 was predictive of treatment outcome, with an area under the receiver operating characteristic curve of 0.86 (0.73-0.99). The remarkably consistent HRP2 decay rate in participants with ACPR and reinfections implies a common underlying biological clearance mechanism in the human body. Individuals that will ultimately fail treatment do not exhibit this same pattern of clearance, even in the absence of other indications of inadequate response to treatment. This raises the potential of using HRP2 concentration, particularly at day 3, to predict response to treatment.

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RELIABILITY OF RAPID DIAGNOSTIC TESTS TO ASSESS MALARIA TRENDS IN MADAGASCAR THROUGH A SENTINEL FEVER SURVEILLANCE NETWORK

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A sentinel fever surveillance network has been operational since 2007 in Madagascar. In all 34 sentinel sites, all febrile patients are tested with malaria rapid diagnostic tests (RDTs) for pan-LDH and pfHRP2, and data are monitored for diagnostically-confirmed malaria trends. Quality assurance of on-site RDT results are managed by the Institut Pasteur de Madagascar (IPM). Special attention is given to storage conditions and compliance with the manufacturer's instructions for RDTs. Results of RDTs stored at facilities are compared with results from the same RDT batch stored in ambient temperature <25°C and humidity <80% at IPM and also with microscopy. From January 2013 to December 2015, 33/34 sentinel fever surveillance sites were visited regularly throughout the country. There were no RDTs storage errors and no expired RDTs in stock at any sentinel sites. Most technicians (61/75, 81.3%) properly used RDTs in accordance with the manufacturer's instructions. The results of 1,638 febrile patients were used for quality assurance (3 invalid tests). Results of on-site RDTs and those stored at IPM were 99.8% concordant. Comparison with microscopy resulted in sensitivity of 92.5%, specificity of 97.1%, positive predictive value of 86.0%, and negative predictive value of 98.5% (n=1,635). These results indicate the reliability of malaria RDTs results from the fever sentinel sites. Thus, data collected at fever sentinel sites can be used by the National Malaria Control Program to better understand temporal and spatial trends in malaria transmission across Madagascar.

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COMPARISON BETWEEN A NOVEL COMMERCIAL ASSAY BASED ON LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AND PCR-NESTED ASSAY FOR MOLECULAR DIAGNOSIS OF *PLASMODIUM* PARASITES

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Currently, approximately 2 billion people still live in areas at risk for malaria, with disease morbidity surpassing 200 million cases and about 500,000 deaths per year. Standard methods for malaria diagnosis include microscopic examination of blood films and rapid diagnostic tests (RDTs). Conventional and real-time PCR techniques are more reliable than either microscopy and RDTs in identifying *Plasmodium* species accurately, however, these molecular methods are technically challenging and resource intensive making them generally restricted to reference laboratories. Recently, Illumigene®, a panel of simplified assays based on LAMP technology (Loop Mediated Isothermal Amplification), has been developed for the diagnosis of major public health impact infections. In 2016, two assays have been set up also for malaria diagnosis (Illumigene® Malaria and Illumigene® Malaria PLUS, Meridian Bioscience Inc, Cincinnati, OH, USA), in collaboration with the US CDC and the University Cheikh Anta Diop (Dakar, Senegal). These assays are commercially available as CE IVD. In clinical studies, they have proven to be extremely sensitive, detecting up to ½ parasite genome per sample. The objective of this study was to evaluate both "Malaria" and "Malaria PLUS" assays for malaria diagnosis in 50 patients from retrospective Italian imported malaria cases, infected with *Plasmodium falciparum*, *P. vivax*, P. malariae and the two subspecies P.ovale wallikeri and *P. ovale* curtisi. These new Illumigene® LAMP-based assays were able to detect all *Plasmodium* species (including *P. ovale* subspecies) at genus level, and no discrepancies were found between these assays and our in-house nested-PCR. Product from the assay sample preparation could be also used for *Plasmodium* speciation by conventional PCR approach. The hands on for these new assays showed to be extremely simple, rapid (results in less than one hour) and thus suitable for all microbiology and hematology laboratories. All these characteristics open up new opportunities for exploiting new molecular tools for malaria diagnosis in endemic and non-endemic countries.

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COVERAGE AND IMPACT OF THE WHO-FIND MALARIA RAPID DIAGNOSTIC TESTS (RDT) EVALUATION PROGRAM: SHAPING THE GLOBAL MALARIA RDT MARKET

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Rapid diagnostic tests for malaria (RDTs) play a critical role in malaria case management, with quality being a key factor for good adherence to test results. The WHO-FIND Malaria RDT Evaluation Programme now functions since 2007, comprising a pre-purchase performance evaluation

(Product Testing, PT) and a pre-distribution quality control of lots (Lot Testing, LT). A small-scale survey in 2011 showed that the market-share of good performing RDTs has increased since then, however large-scale and recent data were not available. More generally, the current knowledge on the global malaria RDT market is still limited and mostly based on general trends, with little information about procurement practices. In order to better document the global RDT market, and to evaluate the current impact of the PT- and LT Programmes, a large-scale survey has been conducted, gathering RDT sales and procurement data from 2011 to 2014 from a total of 32 manufacturers, 12 procurers and 68 national malaria control programmes (NMCPs). The RDT sales results highlight a concentration of the market around 3 manufacturers (86% of 2014 sales), and a confirmed market shift towards RDTs complying with WHO procurement criteria (from 83% in 2011 to 93% in 2014). Procurement data showed that 74% of the NMCPs procure only 'complying' RDT products, however there is a frequent overlap of different products and even product types (e.g. Pf-only and Pf-pan) in the same year and country (60% and 46% of countries, respectively). Importantly, the proportion of 'non-complying' or 'not-evaluated' products was found to be higher in the private health care sector than in the public sector (32% vs. 5%), and even increasing over time (from 22% in 2011 to 39% in 2014). An estimated 70% of the RDTs market is covered by the LT programme. The opinion about the PT- and LT Programmes has been positive overall, and the quality of RDTs was rated as one of the most important procurement criteria. In summary, this survey provides in-depth information on RDT sales and procurement dynamics, including the largely unknown private sector, and demonstrates how the WHO-FIND Programme contributes to shaping the RDT market.

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ANTIPLASMODIAL ACTIVITIES OF CHLOROQUINE AND ARTEMISININ IN COMBINATION WITH VERBASCOSIDE, A PHENYLETHANOID GLYCOSIDE FROM *STACHYTARPHETA CAYENNENSIS*

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Verbascoside (VB), a phenylethanoid glycoside has been shown to possess strong antioxidant and immune-stimulant effects. The present study was designed to evaluate the antiplasmodial effect of VB purified from Stachytarpheta cayennensis leaves and its combination with artemether (Art) and chloroquine (CQ) in established *Plasmodium berghei* berghei infection in mice. VB was obtained by successive column separation of a methanol extract of *S. cayennensis* leaves; identity and purity of the compound were established by nuclear magnetic resonance spectroscopy (proton, carbon), high performance liquid chromatography and differential scanning calorimetric thermal analysis. In antiplasmodial tests, mice were inoculated on day 0 with chloroquine - sensitive *P. berghei* berghei NK65 infected blood. On day 3, mice were grouped (n=5) and respective groups treated for five days with VB, combinations of VB and CQ, combinations of VB and Art, CQ alone, or Art alone; at a dose range of 1-25 mg/kg of body weight. During treatment, thin films of tail vein blood of the mice were prepared daily and assessed for parasitaemia. After treatment, survival time was also monitored for all the experimental groups. The results showed that VB alone possessed significant ($P<0.001$) intrinsic antiplasmodial activity and exhibited synergism in combination with CQ, as increasing doses of VB significantly ($P<0.05$, 0.01, 0.001) boosted parasite clearance and prolonged survival time compared to CQ alone. Co-administered VB and Art produced significant ($P<0.001$), rapid and sustained parasite clearance within the first 4 days of treatment compared to Art alone, but survival time was highest in the group that received Art alone and was reduced with increasing doses of VB given in combination with Art. VB also produced a rapid onset action as observed in its ability to elicit rapid parasite clearance when administered alone or with the low doses of CQ or Art administered as monotherapy. Thus verbascoside warrants further investigation as possible combination agent in antimalarial regimens for uncomplicated malaria.

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FLAVONE DERIVATIVES: A PROMISING NEW CLASS OF DRUGS ACTIVE AGAINST MULTI-RESISTANT FALCIPARUM MALARIA PARASITES

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Plasmodium falciparum malaria is the deadliest parasitic disease with 438.000 deaths in 2013. The emergence and the increasing proportion of *P. falciparum* parasites resistant to artemisinin derivatives, the most potent antimalarials, is a major concern in Southeast Asia. Fast acting drugs, with unaltered activity versus the current multi-drug resistant strains are urgently needed to replace artemisinins. Previously, traditional remedies such as Cinchona bark or Artemisia aerial parts led to the discovery of the most potent antimalarials, bearing out that Nature is still an incredible source of original compounds. Following this approach, we are developing new synthetic antimalarial agents based on the structure of an active natural product. We isolated a biflavonoid from *Campnosperma panamense* (IC50 = 480 nM *in vitro* on *P. falciparum* K1 multi-resistant strain), and developed novel simplified synthetic analogs (MR series) with improved pharmacological and pharmacokinetic profiles. One of these compounds, MR70, is strongly effective on *P. falciparum* early blood stage in less than 6 hours. Moreover, MR70 and its analog MR87, exhibit a partial *in vivo* antimalarial activity, reducing parasitemia by 35% and 70% respectively on day 4 in a murine model (*P. berghei* ANKA, 100 mg/kg for 4 days). The investigations of structure-activity relationship are still ongoing to further improve these results. As MR70 acts specifically on early ring stage, which has been associated to artemisinin resistance, we have assessed the *in vitro* susceptibility of Cambodian artemisinin-resistant isolates to MR70 and found no cross-resistance between MR70 and artemisinins. These findings make flavone derivatives a promising new class of antimalarials. Further investigation is needed to optimize MR70 activity and assess its efficacy against strains resistant to partner drugs, usually combined with artemisinin derivatives, like piperaquine, mefloquine, lumefantrine and amodiaquine.

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DISCOVERY OF NEW HERB EXTRACTS TO TREAT MALARIA

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Malaria caused by *Plasmodium* spp. is found worldwide in tropical and subtropical areas. It is estimated that about 3.2 billion people - almost half of the world's population - are at risk of malaria. Especially, *P. falciparum* can cause severe malaria because it multiples rapidly in the blood, and can thus cause severe blood loss (anemia), cerebral malaria, and death. Currently, there are several drugs, chloroquine, atovaquone-proguanil, artemether-lumefantrine, mefloquine, etc. to treat malaria. However, an appearance of drug resistance malaria parasite is getting the greatest challenges facing malaria control today. To overcome this matter, a development of novel malaria drug is necessitated. Therefore, we evaluated antimalarial effect of 10 herb extracts used to treat febrile

patients in South Korea. Among the herb extracts, 50 µg/ml of two extracts obtained from mushrooms inhibited *P. falciparum* growth *in vitro* culture. In addition, lipoxygenase fraction of the extracts especially inhibited *P. falciparum* growth *in vitro* culture. Moreover, the lipoxygenase from the extracts inhibited chloroquine-resistant and atovaquone-resistant *P. falciparum*. Further study about the target molecule of the lipoxygenase fraction will be needed.

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MODULATION OF THE ANG/TIE2 AXIS REDUCES PATHOLOGICAL VASCULAR LEAK AND ACUTE LUNG INJURY IN A MURINE MODEL OF SEVERE MALARIA (SM)

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Malaria -induced acute lung injury (MA-ALI) carries a high fatality rate despite the use of potent antimalarial therapies and optimal supportive care. Therapies targeting the underlying pathophysiology of MA-ALI 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 recent evidence implicates Ang-Tie2 axis disruption in ALI development in ICU settings. We hypothesise that the Tie2 receptor ligand Ang-1, Tie2 and its activation are required to prevent MA-ALI, and that pharmacological approaches using pro-Ang-1, and Tie2 activation strategies will reduce or prevent MA-ALI. Respiratory distress (RD) occurs in ¬16% of SM and results in 39% of deaths , and is associated with Ang-Tie2 axis dysregulation in children and susceptibility in mice . Genetic models of Tie2+/- and Ang-1-/- mice will be infected with PbA-infected erythrocytes. We will determine MA-ALI markers of vascular leak (i.e. Evans Blue assay, IgM), histology and physiological dysfunction (i.e. O2 saturation) in the lungs and endothelial activation markers in lung tissue/plasma. We will administer pro-Ang-1, Tie2 treatment strategies alone or in combination at parasitaemia onset and compare MA-ALI markers with littermate controls. Pilot studies indicate that genetic disruption of Ang-1 results in decreased survival ($p=0.0091$), and increased pulmonary vascular leak as determined by Evans Blue dye assay ($p<0.05$) and pulmonary fluid accumulation ($p<0.05$). Tie2 activation using AKB9785, a phosphatase inhibitor that selectively inhibits VE-PTP, reduces IgM accumulation in the lung and vascular leak ($p<0.05$). We will report mechanistic studies of ALI in our genetic models and intervention studies using both pro-Ang-1 and other Tie2 activating strategies. Pilot results indicate that Ang-1 is necessary for the prevention of MA-ALI. Increased Tie2 activation shows evidence of reduced pathological leak and improved survival. These findings support targeting the Tie2 pathway with pro-Ang-1 and Tie2 strategies as adjunctive therapy for MA-ALI.

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PHARMACOKINETICS AND PROPHYLACTIC EFFICACY OF EMULSION OF DECOQUINATE 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) emulsion injected into mice infected with *P. berghei* sporozoites. DQ nano-emulsion in 50% 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 emulsion doses for 2, 3, or 4 weeks prior to infection with *Plasmodium 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 large particle of nano-emulsion DQ (0.44 µm) at 120 mg/kg for 4 weeks and with small particle (0.18 µm) at 120 mg/kg lasted 2 weeks prior to inoculation. The 120 mg/kg IM emulsion dose was shown to be the minimal prophylactic dose required to provide full causal prophylaxis of malaria sufficient for a period of 2-4 weeks. A significant increase in the elimination half-life of the large particle DQ emulsion (632.15 hrs.) was achieved compared to that of the small particle DQ (494.47 hrs.). Similarly, the AUC of the large particle DQ nano-emulsion in plasma was observed to be 8,795 ng·h/ml, which is double the AUC observed for the small particle DQ emulsion (4,288 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 was 14.46 L/hr/kg, which is twice as fast as the clearance observed in animals treated with the microparticle DQ formulation (27.99 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 large particle of DQ emulsion provided a longer and more constant DQ release in the plasma, which resulted in a 1.8 fold longer drug exposure time above MIC. The prophylactic effect of the large particle emulsion observed in mice was shown to be 2 times longer than the small particle of DQ nano-emulsion.

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ANTIMALARIAL POTENTIAL OF KOLAVIRON, A BIFLAVONOID FRACTION, FROM GARCINIA KOLA SEEDS, AGAINST *PLASMODIUM BERGHEI* INFECTION IN SWISS ALBINO MICE

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To investigate the antimalarial potential of kolaviron (KV), a biflavonoid fraction from Garcinia kola seeds, against *Plasmodium berghei* infection in Swiss albino mice. The study consists of seven groups of ten mice each. Groups I, II and III were normal mice that received corn oil, KV1 and chloroquine (CQ), respectively. Groups IV, V, VI and VII were infected mice that received corn oil, CQ, KV1 and KV2, respectively. CQ, KV1 and KV2 were given at 10-, 100- and 200-mg/kg daily, respectively for three consecutive days. Results indicate that administration of KV1 and KV2 significantly ($P<0.05$) suppressed *P. berghei*-infection in the mice by 85% and 90%, respectively while CQ produced 87% suppression relative to untreated infected group after the fifth day of treatment. Also, KV2 significantly ($P<0.05$) increased the mean survival time of the infected mice by 175%. The biflavonoid prevented a drastic reduction in PCV from day 4 of treatment, indicating its efficacy in ameliorating anaemia. Significant ($P<0.05$) oxidative stress assessed by the elevation of serum and hepatic malondialdehyde were observed in untreated *P. berghei*-infected mice. Specifically, serum and hepatic malondialdehyde levels increased by 93% and 78%, respectively in the untreated infected mice. Furthermore, antioxidant indices, viz; superoxide dismutase, catalase, glutathione-s-transferase, gluathione peroxidase and reduced gluathione decreased significantly ($P<0.05$) in the tissues of untreated *P. berghei*-infected mice. KV significantly ($P<0.05$) ameliorated the *P. berghei*-induced decrease in antioxidant status of the infected mice. Overall, the study shows that kolaviron at doses of 100 and 200 mg/kg elicits potent antimalarial activity in *P. berghei*-infected mice.

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EVALUATION OF ANTIMALARIAL ACTIVITY OF CYCLOPENTANONE AND CYCLOHEXANONE ANALOGUES OF CURCUMIN IN *PLASMODIUM BERGHEI* INFECTED MICE

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Malaria is one of the world's most common and serious tropical diseases. Resistance of *Plasmodium* to available antimalarial agents has necessitated the need to develop new antimalarial drugs which would be efficacious against drug resistant strains of malaria parasites. The *in vivo* antimalarial activity of cyclopentanone (CP) and cyclohexanone (CH) analogues of curcumin as potential antimalarial agents was evaluated using *Plasmodium berghei* mouse model. Female Swiss albino mice (n=55) were infected with standard inoculum (1x107) of chloroquine resistant strain of *Plasmodium berghei* (ANKA) intravenously. Infected animals were randomly distributed into eleven groups of five animals each. Once daily dose of chloroquine (10 mg/kg), twice daily graded doses (50, 100, 200 and 400 mg/kg) of each of the curcumin analogues and artemether/lumefantrine (4 mg/kg artemether) were administered to the infected animals while the control animals (infected but not treated) received polyethylene glycol (PEG, 100%), the vehicle for drug delivery twice daily for three days. All treatments were orally administered for three days starting from 24 hours post infection. Thin blood smears were prepared from tail snips of the mice daily between days 4 and 7 and subsequently on days 9, 12, 14 and 21, and parasite count was estimated by microscopic examination of Giemsa-stained thin smears. The result of this study showed that there was no significant difference in the antimalarial activity of both analogues of curcumin ($p>0.05$) on day four. However, the cyclopentanone analogue of curcumin at a dose of 200mg/kg demonstrated a recordable suppressive antimalarial activity (75% suppression). Cyclopentanone and cyclohexanone analogues of curcumin appeared to have weak suppressive antimalarial activity. Further research into the pharmacological properties of these analogues is recommended.

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TARGET-BASED DRUG DISCOVERY IN MALARIA: A NEW WAY TO IMPLEMENT AN OLD STRATEGY

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Compared to phenotypic screening, target-based drug discovery allows for a more directed medicinal chemistry optimization and a better prediction of the safety risks. Despite these advantages, the target-screening strategy has clearly lagged behind phenotypic screening approaches in malaria drug discovery. This is probably because of two hurdles: the often found disconnect between enzymatic and whole cell activity and the lack of robustly validated targets in *Plasmodium*. Recent advances in our capacity to identify validated drug targets in *Plasmodium* might provide a way to increase the success rate of target-based approaches in the near future. The Tres Cantos Open Lab Foundation (TCOLF) has recently pursued this approach in cases where a high level of genetic and small molecule validation has been achieved. Among others, current TCOLF-funded projects are working on identification of compounds that inhibit plasmodial N-myristoyl transferase (NMT) and cGMP dependent protein kinase (PKG). Projects are implemented in collaboration with GSK and academic experts, aiming to combine the in depth biology knowledge with the possibility to find the best starting points for medicinal chemistry optimization within GSK diverse small molecule compound libraries. We will present progress to date in the quest to identify target-based antimalarials.

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PRE-CLINICAL ASSESSMENT OF DRUG-DRUG INTERACTIONS BETWEEN PRIMAQUINE AND BLOOD STAGE ANTI-MALARIAL AGENTS

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The liver stage anti-malarial activity of primaquine and other 8-aminoqinoline molecules are directly dependent upon bio-activation through Cytochrome (CYP) 2D6 metabolism. Primaquine metabolism through the CYP 2D6 pathway makes the drug prone to CYP 2D6 mediated drug-drug interactions with concurrent medications that are CYP 2D6 substrates/inhibitors. Primaquine therapy is typically accompanied by administration of blood stage anti-malarial agents that clear the blood stages of the parasite and resolve the clinical symptoms of malaria. Concurrent primaquine-blood stage anti-malarial therapies have the potential to interact through CYP 2D6 and alter pharmacokinetics anti-malarial activity. We sought to investigate these interactions using an *in vitro* primaquine metabolism assay and recombinant CYP 2D6. In this study, commercially available blood stage anti-malarial agents were evaluated for the potential to interact with primaquine. The inhibitory potential of the blood stage anti-malarials for CYP 2D6-mediated primaquine metabolism were assessed *in vitro*. The blood stage anti-malarial agents tested displayed a range of inhibitory activities on CYP 2D6-mediated metabolism of primaquine *in vitro* (IC50 ranges 2-523 μM). Quinine was the most potent inhibitor (IC50 ~ 2.98 μM) of CYP 2D6 mediated primaquine metabolism. Artesunate and the artemisinin class were the least potent (IC50s > 200 μM). The *in vitro* inhibitory data was then used to predict *in vivo* interactions using the AUCin/AUC prediction method. The results indicate that primaquine interacts with several blood stage anti-malarial agents through CYP 2D6. The clinical implications of these interactions are currently unknown and warrant further investigation.

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THE COMBINED IMPACT OF TRANSMISSION-BLOCKING INTERVENTIONS AND PRE-ERYTHROCYTIC VACCINES FOR MALARIA ELIMINATION

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Transmission-blocking interventions (TBIs) aim to eliminate malaria by interrupting transmission of the parasite between hosts and mosquito vectors. Accurate methods to assess TBI efficacy are key to ensure that the best candidate TBI drugs or vaccines progress to clinical trials. This is particularly vital for novel population assays (PA) where efficacy is measured over successive transmission cycles. We present a method for estimating TBI efficacy from PA data by fitting a hierarchical Bayesian model to multiple life stages of the parasite. This enables both host-to-vector and vector-to-host transmission to be density-dependent processes whilst accounting for stochastic fluctuations driven by superinfection and small sample sizes. This improves the precision of intervention efficacy estimates and demonstrates that TBI impact is not sufficiently captured by changes in prevalence alone because TBIs also suppress parasite density in secondarily infected hosts. Partially effective TBIs require multiple

generations before substantial reductions in prevalence are observed whilst immediately suppressing parasite density. This has valuable implications for assessing the performance of TBI candidates in field and clinical trials.

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NOVEL ELQ-300 PRODRUGS FOR ENHANCED DELIVERY AND SINGLE-DOSE CURE OF MALARIA

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In the effort toward global malaria eradication, the discovery of a single-dose cure would facilitate ease of drug distribution and limit propagation of drug resistance, two issues that burden the current state of malaria treatment. One class of *Plasmodium* cytochrome *bc1*-inhibitory compounds, the endochin-like-quinolones (ELQs), are extremely effective against the parasite and have been shown to deliver single dose cures. Specifically, ELQ-300 is a pre-clinical drug candidate that is effective against all life cycle stages of *P. falciparum* and against blood stage parasites at low nanomolar concentrations. ELQ-300 is curative with three sequential low doses in treatment of patent malaria infection in murine models, but its low aqueous solubility and high degree of crystallinity prevent the higher bloodstream concentrations necessary to achieve a single dose cure. To accelerate the clinical development of ELQ-300 we have employed a prodrug approach, attaching a variety of bioreversible groups to ELQ-300 to increase bloodstream drug exposure. We have synthesized over 30 prodrugs of ELQ-300, many with a significantly lower degree of crystallinity than ELQ-300, aiding in solubility while retaining antiplasmodial activity. We will present a number of the most effective prodrugs that show excellent antiplasmodial activity *in vitro* and are curative with a single low dose *in vivo*. In addition to full antiplasmodial and pharmacokinetic profiles, we will present optimal formulations of spray-dried dispersions that improve aqueous solubility and oral bioavailability of these highly effective prodrugs.

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PLASMODIUM FALCIPARUM CYCLIC AMINE RESISTANCE LOCUS, PFCARL: A RESISTANCE MECHANISM FOR TWO DISTINCT COMPOUND CLASSES

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The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and prevent the development of drug-resistance. As part of the Malaria Drug Target Identification Project efforts, we have adopted a chemogenomic approach to identify the targets of the most prominent compounds from chemically diverse libraries. Study compounds were selected based on availability, purity, potency in a multi-drug resistant isolate, and lack of known mechanism of action towards the mitochondrion or folate biosynthesis. Here we present studies of a drug-like compound from the Malaria Box, MMV007564, a novel antimalarial benzimidazolyl piperidine chemotype. To identify the genetic determinant of MMV007564 resistance, parasites were cultured in the presence of the compound to generate resistant lines. Whole genome sequencing revealed distinct mutations in the gene named *Plasmodium falciparum* cyclic amine resistance locus (*pfcarl*), encoding a conserved protein of unknown function. Mutations in *pfcarl* are strongly associated with resistance to a structurally unrelated class of compounds, the imidazolopiperazines, including KAF156, currently in clinical trials. Our data demonstrate that *pfcarl* mutations confer resistance to two distinct compound classes - benzimidazolyl piperidines and imidazolopiperazines. However, MMV007564 and the imidazolopiperazines – KAF156 and GNF179 – have different timing of action in the asexual blood stage and different potencies against the liver and sexual blood stages. Our results demonstrate that mutations in PfCARL mediate resistance to multiple chemical classes and might represent a common parasite drug-resistance pathway. Further characterization of PfCARL as a common drug-resistance pathway is underway.

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LEAD CANDIDATE SELECTION OF BROAD-SPECTRUM ANTIMALARIAL ACRIDONES

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We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against blood stage parasites. We have been successful in producing extremely potent new lead candidates with pico molar IC_{50} values against MDR resistant parasites, as well as full protection of liver stage infection at comparable dosage with primaquine. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY PHENOTYPE AND GENOTYPE DISTRIBUTION IN POINT-OF-CARE SETTINGS IN VHEMBE DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA

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South Africa is targeting malaria elimination by the year 2018. To facilitate transmission reduction, primaquine may be used as an additional chemotherapy to clear gametocytes, the transmissible stage of *Plasmodium*. Unfortunately, individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd) risk acute haemolytic anaemia if exposed to oxidant drugs, including primaquine particularly at high doses. A total of 248 subjects attending 6 primary health care facilities in Vhembe district, Limpopo province, South Africa were phenotyped for G6PDd using a commercial G6PDd test kit. Additionally G6PDd genotypes of the most common African forms G6PD A (A376G) and G6PD A- (G202A, A542T, G680T and T968C) were determined by polymerase chain reaction and restriction length fragment polymorphisms. There was 13.03% (33/248) G6PDd prevalence in Vhembe district as measured by the commercial testing kit. Of all males 18.2% (10/55) were G6PDd according to the commercial test, while females had a lower prevalence of 11.9% (23/193), Odds ratio 0.6088 (95% confidence interval 0.270 -1.371). The A376G/G202A genotype prevalence was 3.22% (6/248; 2.42% [N=6] male hemizygous and 0.80% [N=2] female homozygous). Heterozygous females were 12.90% (32/248) of participants. The A542T, G680T or

T968C variants were not detected in this locality. The sensitivity [95 % confidence interval (95 % CI)] and specificity of the commercial test kit to correctly identify G6PDd A- G202A deficiency were 90.38 % (95 % CI 85.54–94.03%) and 32.50% (95 % CI 18.57–49.13 %) respectively compared to genotyping. The agreement in test results was fair, Kappa value 0.246 (95% CI 0.090-0.413). There are low levels of G6PDd A376G/G202A mutations in the study locality. However, the poor specificity and agreement of the comercial G6PDd testing kit in comparison to the genotyping results call for development of additional robust field deployable point-of-care G6PDd test kits.

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SOCIETAL AND ENVIRONMENT CONTEXT OF PARENTS ACTING AS PREDICTORS OF SEVERE MALARIA IN CHILDREN UNDER 5 YEARS OF AGE ADMITTED IN KOUDOUGOU REGIONAL HOSPITAL, BURKINA FASO: A CROSS SECTIONAL STUDY

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Burkina Faso has a high incidence and death rate of severe malaria, especially for children under five years of age. Although the malaria elimination program is a cornerstone and high-priority public health project. Rating and understanding the various factors that contribute to the severity of malaria is useful in designing and conducting an effective strategy. In this study, factors associated with complicated malaria in Burkinabe children were investigated in semi urban city's hospital, Burkina Faso. Between Jun and September 2012, a cross-sectional study was used to test 510 children under 5 years of age (mean age: 32.23 months) admitted with suspected malaria. Each child was screened using two types of methods (test of diagnosis rapid and blood smear) to identify whether ill child had severe malaria focused on the criteria established by the World Health Organization. When a child was diagnosed with malaria, the relatives were interviewed by a trained nurse using a structured questionnaire to assess predicting determinants. A logistic regression using SPSS software version 17.0 was used to identify theses determinants of severe malaria and associated deaths. Of the 510 children having malaria, 203 (39.8%) had severe malaria. Most of the patients having severe malaria 86.2 % lived in rural areas. The main parental factors associated with severe malaria were delayed treatment [OR= 4.53, 95% CI = 1.76-11.65], low socioeconomic status [OR = 9.69, 95% CI (4.12-22.78)], a large household [OR = 2.28, 95% CI (1.50-3.47)], housewife [R = 16.39, 95% CI (2.18-122.9)], farmer [R = 19.39, 95% CI (4.61-81.44)]. The finding gathered from this study is one of the challenging and resources limited settings for emphasizing malaria elimination in children which remains a serious public health concern.Nevertheless understanding societal and environment contexts as possible predictors are still an important step towards the control of the disease. Improved health promotion, and encouragement to seek early care are urgently needed.

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IVERMAL: EFFICACY AND SAFETY OF HIGH-DOSE IVERMECTIN FOR REDUCING MALARIA TRANSMISSION - A DOSE FINDING STUDY

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Innovative approaches are needed to complement existing tools for malaria elimination. Ivermectin is a broad spectrum antiparasitic endectocide widely used for onchocerciasis and lymphatic filariasis control at doses of 150-200 mcg/kg. Ivermectin also has potent mosquitocidal properties, shortening the lifespan of mosquitoes that feed on individuals recently treated with ivermectin. However, the effect with the 150-200 mcg/kg dose is short-lived (6-11 days). Modelling suggests higher doses that prolong the mosquitocidal effects are needed for ivermectin to provide a significant contribution to malaria elimination. Ivermectin has a wide therapeutic margin, and previous studies have shown doses of 2,000 mcg/kg (i.e. 10x the FDA approved dose) are well tolerated and safe; the highest used for onchocerciasis is single-dose 800 mcg/kg. We are conducting a double-blind placebo-controlled, parallel-group, 3-arm, dose finding trial to determine the efficacy, tolerance and safety of 3-day courses of ivermectin 0, 300, 600 mcg/kg/day, when given in combination with standard 3-day course of dihydroartemisinin-piperaquine. We performed Monte Carlo simulations based on pharmacokinetic modelling to determine the dosing regimens to be tested. In our models, a dose of 600 mcg/kg/day for 3 days achieved similar median (5-95 percentiles) Cmax concentrations of ivermectin as single-dose 800 mcg/kg: 111 ng/mL (83-161) vs 108 (75-164), while increasing the median time above the MIC from 1.9 days (1.0-5.7) to 6.8 (3.8-13.4) days. The 300 mcg/kg dose was chosen at 50% to allow for a dose response. The clinical trial outcome of daily mosquito survival up to 28 days is assessed in laboratory-reared *Anopheles gambiae* s.s. populations fed on patients' blood taken at days 0, 2 (Cmax), 7 (primary outcome), 10, 14, 21, and 28 after the start of treatment. Safety outcomes include QT-prolongation and mydriasis. The sample size is 141 participants (47 per arm). Sub-studies include: (1) rich pharmacokinetics and (2) direct skin vs membrane feeding. The trial is ongoing in 6 facilities in western Kenya and will be completed in August 2016. Results will be presented.

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SPATIAL AND TEMPORAL VARIATIONS OF MALARIA RISK BETWEEN 2013 AND 2015 IN ZANZIBAR: A PRE-ELIMINATION SETTING

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The monitoring and evaluation of local transmission epidemiology to characterize malaria risk is essential for strategic planning of malaria elimination programs. Geographical Information System mapping techniques are a major set of tools for this approach to assess local time, spatial distribution and clustering of malaria cases in Zanzibar. In 2012,

the Malaria Case Notification system was developed to support individual case reporting. Each District Malaria Surveillance Officer (DMSO) was equipped with a tablet computer running a mobile application called Coconut Surveillance. Once a DMSO is alerted of a new case, he or she is guided through an active case response protocol by Coconut Surveillance. Additional case data are entered into the tablet at the facility and household. Each household member is tested and new cases are treated immediately. Coconut Surveillance uses the Geographical Positioning System (GPS) capability of the tablet to record the location of the household. Officials use near real-time maps and reports to quickly identify hot-spots and transmission patterns. Mapping of cases was done using Quantum-GISTM software version 2.12.0 and cluster analysis was performed using Bernoulli spatial scan statistic through the SaTScan® software. All 10 districts had malaria although incidence was lower in Pemba than Unguja. Unguja had an annual incidence increased from 6.3 per 1000 population in 2013 to 14.8 per 1000 population in 2015, of which 60% of cases in Unguja occurred between weeks 18 and 30 of each year. Among 9,352 cases identified during the surveillance, which were matched to 32,355 controls, we identified significant spatial clusters of malaria cases localized in Urban and West districts in Unguja, Wete and Micheweni districts in Pemba. Our results confirm the existence of a spatial heterogeneity and the seasonality of malaria transmission in Zanzibar, providing evidence for implementation of targeted interventions.

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IMPLICATION OF FLUCTUATING COMMODITY AVAILABILITY ON SUSTENANCE OF GAINS OF POPULATION BASED INTERVENTIONS

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Preventing malaria in pregnancy remains a daunting challenge in ensuring healthy mothers and newborns in malaria endemic communities. This study examines the effect of fluctuating antimalarial commodity support for the administration of intermittent preventive therapy for malaria in pregnancy (IPTp) using sulphadoxine pyrimethamine (SP), on the sustenance of gains of improved antenatal clinic visits and consequent reduction in the incidences of malaria among pregnant women accessing care in public health facilities across Enugu State, Nigeria. This study assessed clinical records of antenatal visits, administration of sulphadoxine pyrimethamine (SP), and confirmed cases of malaria among pregnant women during the high transmission period, across 255 health facilities. Trend analyses of percentage uptake of IPTp, were compared with and pattern of antenatal visits, and reported incidence of malaria over the study period. Findings: Using the first year as a baseline, observed 42% cumulative increase in antenatal visits in the second year appears to correspond significantly with the introduction of IPTp with an over 300% increase in uptake, and over 3% reduction in incidence of malaria in pregnancy (7.5% to 4.2%). With reduction in availability, IPTp uptake dropped in the third year to 5.6%, a proportionate reduction in antenatal visit of 6%, and malaria in pregnancy reduction was 1.6% (4.2% to 2.6%). As the availability dropped further in year 4 (-31.3%), the antenatal visits and malaria in pregnancy recorded relative marginal increases at 14.7% and 2.9% respectively. Further percentage reductions in antenatal visit at 22.4%, marginal increase in malaria in pregnancy incidence of 4.8% recorded in year 5 corresponds to the percentage reductions in IPTp uptake 23.6% occasioned by non-availability of commodities. In conclusion, it is imperative that interventions of which commodity supply is a critical component, has the tendency to impact positively on uptake and invariably produce and sustain desired results if emphasis is on ensuring unhindered commodity availability and improved access.

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HUMAN POLYMORPHISM ASSESSMENT AGAINST THE SAFETY AND EFFICACY USAGE OF PRIMAQUINE

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In all malaria-endemic countries outside sub-Saharan Africa, *Plasmodium vivax* infections are becoming the major challenge for control programs. The relapsing nature of *P. vivax* makes elimination difficult without radical treatment with 8-aminoquinoline drugs such as primaquine or tafenoquine against the long-lasting liver stage, hypnozoites. The safety and efficacy of 8-aminoquinoline drugs are however crucially dependent on each individual's glucose-6-phosphate dehydrogenase (G6PD) and cytochrome P450 2D6 (CYP2D6) genotype, respectively. Assessment of the impact of G6PD and CYP2D6 genotypes on the usage of 8-aminoquinoline drugs is hence important for designing optimal treatment schedules and drug-based public health inventions. A sensitive next-generation sequencing-based method to genetically characterise G6PD and CYP2D6 was established. This assay is suitable for large-scale screening strategy to evaluate the risk of primaquine therapy in different populations. Validation of assay performance will be conducted in a cohort of children (6 months to 12 years old) from Solomon Islands (2013-2014), which were tested for point-of-care RDT G6PD deficiency and treated with primaquine if clinical vivax malaria were observed. The ability to rapidly assess the genetic background for safe and efficient usage of primaquine in a given population has important implications for the planning of potential mass drug administration intervention programs. This study thus provides guidance for a better informed *P. vivax* elimination strategy.

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CHARACTERIZATION OF POPULATIONS CROSSING FORMAL AND INFORMAL BORDERS ON THE CAMBODIA-LAOS BORDER, INCLUDING IDENTIFICATION OF MALARIA INFECTION AND ARTEMISININ RESISTANCE INFECTION

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Recent evidence identified malaria prevalence among cross-borders to be as high as 11.5% in Cambodia-Laos border. As information is available for international border post which is not available at along the porous borders. The aim of this study is to estimate the potential contribution of crosser border to the spread of malaria and artemisinin resistance. A cross sectional study is being conducted at 7 informal and 1 international Cambodia-Laos border posts, in Stung Treng province. A total of 4500 individuals are expected to be tested between April 2015 and February 2016: sample collection started in September 2015. Participants are diagnosed malaria by using RDT and RT-PCR analysis. A structured questionnaire is capturing: demographic characteristics, history of travel, occupation. All crossers borders are eligible to participate and a written consent is required. Positive cases by RDT treat at the spot. Double entry questionnaire in EpiData 3.1 and PCR data is entered in Ms Excel database and then merged by unique identifiers in to STATA 12. A descriptive analysis of the primary data was conducted: demographic characteristics, RDT and RT-PCR results disaggregated by species. RT-PCR positive cases were also disaggregated and classified according to their fever status. A total of 291 samples were tested; 66.3% were male and the majority had

between 15-40 years old (63.2%); agricultural work was main occupation 68.7% but in Srei Champa Post, 20% of tested were security personnel. RT-PCR results: 16.2% of tested were deemed positive: informal border posts registered higher malaria prevalence than international border post (19.8% and 17.1% vs 11.4%). Among the positive, 87.2% were Cambodian and 12.8% Laos. RDT results indicated 2.7% were positive: international border post was also higher than informal posts (5.7% vs 0.9% and 2.3%). RT-PCR results: 65.1% of positive cases were asymptomatic: 16.3% were *P.f*, 69.8% *P.v*, and 13.9% *P.f/P.v.* Despite sample size limitations, differences in malaria prevalence among different types of border seem to exist.

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AN ASSESSMENT OF NATIONAL MALARIA SURVEILLANCE SYSTEMS FOR MALARIA ELIMINATION IN THE ASIA PACIFIC

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Heads of Government from Asia and the Pacific have committed to a malaria-free region by 2030. The Asia Pacific Leaders Malaria Alliance (APLMA) recognises that for malaria elimination to succeed, it is essential to have an accurate picture of malaria incidence over time and space. Having a closer look at each country's disease reporting system is a crucial component of this endeavour. It helps to recognise gaps in surveillance and develop evidence to support health system strengthening. This study describes the sources of malaria incidence data collected by national malaria control programmes (NMCPs) in 22 countries in the Asia Pacific targeting elimination. From April 2015, a short survey was sent to each NMCP. It collected country-specific information on existing sources of malaria incidence data, the system for collecting and collating these data and the role of the private sector in malaria treatment. Follow-up with key persons was done to ensure quality of survey responses, which were then stored in a secure database. Summary tables and thematic maps were generated to facilitate effective communication of findings to policymakers. Twenty-one countries completed the survey. Most of the malaria incidence data collected by NMCPs originate from government facilities, while many do not collect comprehensive malaria incidence data from mobile and migrant populations, the private sector or the military. All data from village health workers was included by 9/19 countries and some by 4/19. Other sources of data included police, plantations and other government ministries. Malaria is treated in private health facilities in 17/18 countries, while antimalarials are available in pharmacies in 15/18 and shops in 6/18. Countries should be supported to improve the completeness of malaria surveillance data collected from existing sources. In addition, a regional effort is warranted to include additional sources of malaria case data in the national surveillance database for each country, in particular from village health workers, mobile and migrant populations, the private sector, non-governmental organisations and the military.

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DECREASED ENDEMIC MALARIA IN SURINAME: MOVING TOWARDS ELIMINATION

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Suriname has moved from being the country with the highest annual parasite index in the Americas to one on the threshold of elimination. The progress toward elimination in the stable populations of Suriname between 2000 and 2015 is reviewed. Data were obtained from the Medical Mission and the Ministry of Health Malaria Program case-reporting systems, and analyzed with a focus on disease burden and differentiation of the disease geographically, by malaria species, age, ethnicity and trophocite and gametocyte infection rates. Between 2000 and 2015 there were 57.811 locally acquired cases of malaria in the stable populations of Suriname. A significant reduction in authochthonous malaria cases was observed from 2006 to 2015. The number of imported malaria cases is higher than the number of locally acquired cases since 2014, with a total of 10 imported cases vs 5 authochthonous cases in 2015. The overall decline in malaria case incidence can be attributed to active case detection in high risk areas, free distribution of impregnated bed nets in all transmission areas, public awareness campaigns and improved accessibility of diagnosis and treatment. The results from Suriname show how the local availability of good quality diagnosis and treatment are essential for the success of a malaria control program.

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RAPID REPORTING AND A SPATIAL DECISION SUPPORT SYSTEM TO STRENGTHEN MALARIA ELIMINATION INTERVENTIONS AND RESEARCH IN ZAMBEZI REGION, NAMIBIA

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As countries transition from control to elimination, surveillance systems must shift from periodic and aggregated case reporting to real-time reporting on individually geo-located cases. In Zambezi region Namibia, completed case forms often remained at health facilities (HFs), with delayed entry into databases that would permit finer scale analyses. To address these challenges a national rapid reporting system was piloted in western Zambezi (pop.~35381) and linked to a geographical reconnaissance (GR) of 8026 households conducted 2014-15. The GR mapped and collected baseline data on households and included a QR code affixed to each doorframe and resident's health passport. The rapid reporting system involves HF entry of minimum essential data on confirmed cases via tablet, sent by 3G network to a secure cloud database. A spatial decision support system (SDSS) was developed, incorporating a geographical information system (GIS)-based framework, a surveillance database, graphical maps, and expert knowledge. The SDSS includes GR data, retrospective 2012-14 incidence data from HF registries (including 3152 rapid diagnostic test-confirmed cases from 2013-15), and ongoing incidence data collected (2015-) by rapid reporting system. The SDSS enables users to plot cases to the household level within 24 hours of case detection, track progress by household during community-level investigations, and generate transmission risk maps. Challenges have included ensuring patients carry health passports and building capacity for nurses to complete the rapid report despite heavy workload. The Zambezi region SDSS has 3 roles: (i) to support Ministry of Health and Social Services (MoHSS) targeting of human and financial resources towards most at-risk areas; (ii) to support the implementation of a randomized controlled study exploring innovative case response strategies; and (iii) to coordinate the region's MoHSS and research activities. This represents one of the first reports of an SDSS being used to help a country move towards elimination, support research, and contribute to making that research more accessible and acceptable to local actors.

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DOCUMENTATION AND REACTIVE DETECTION OF MALARIA CASES IN A ZONE OF PRE-ELIMINATION FROM JULY TO SEPTEMBER 2015

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The ongoing decrease of the cases of malaria in Senegal has led the National Malaria Control Program (NMCP) to set a target of pre-elimination in the low transmission north and acceleration of control in the higher transmission south. In the north, malaria cases are investigated and reactive active case detection is conducted. Implementation started in 2011 in one northern district, and in 2015, the NMCP decided to progressively extend investigations in a northern region, adding three additional districts of the same region (St-Louis). Following extensive informational meetings and consultations with administrative, health and local authorities, health district personnel and community stakeholders were trained. Of the 580 cases diagnosed, 20% (115) were traveling through, and had no local address and 88% (508/580) were investigated. Of these, 59% of cases had traveled outside the district during in the previous 15 days. Among the 580 diagnosed cases, the 80% (465/580) who had addresses in the district were eligible for reactive active case detection within 7 days, with 82% (380) of these conducted within the correct time frame. Active case detection carried out in the household of the index case revealed 1.3% (57/4,466) test positivity, and in high risk members of five households around the index case, 0.6% (13/2,279) test positivity. Of the 70 additional cases detected through active case detection, 63 were in the household of the index case. The investigation of the cases of malaria in areas of pre elimination initiated by the NMCP and the responsibility of the district health management teams gave very satisfactory results. The NMCP plans to extend the strategy to other low transmission districts, integrating lessons learned to improve results and ensure success, namely involvement of all administrative and community actors, overseeing close and regular at the beginning and throughout the intervention, good coordination between the district teams and hospitals, and the need to consider focusing the investigation only at the household of the index case.

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COLLABORATIVE EFFORTS TO IMPROVE PREVENTION OF MALARIA IN PREGNANCY IN BURKINA FASO THROUGH USE OF IPTP-SP

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Malaria remains the first cause of consultation (47%), hospitalization (62%) and death (31%) in health facilities in Burkina Faso (2014 Statistical Yearbook). Pregnant women are among the most vulnerable to malaria. Intermittent preventive treatment in pregnancy (IPTp) is a priority intervention in the Burkina Faso 2011-2015 National Malaria Strategic Plan. In 2012, IPTp2 was low across the country at 53%. The President's Malaria Initiative (PMI) supported the National Malaria Control Program (NMCP) in implementing the national malaria control strategic plans. IPTp was promoted through 3 strategies: advocacy and policy updates, capacity building, and behavior change communication. Malaria prevention and management guidelines and job aids updated stressed IPTp in line with WHO recommendations. 185 trainers were trained who in turn organized one-day briefings for over 1,300 healthcare providers from 1081 health facilities (61.3% of health facilities nationally) on the revised guidelines, which were distributed along with job aids. Health information system tools now reflect new IPTp guidance, and 190 district and regional level data managers were trained in their use. 208 community health workers were trained in sensitization and community mobilization around early ANC attendance. Over 3000 radio and TV spots were aired on 28 stations on the importance of IPTp. In 21 project districts in 2013, IPTp2 and IPTp3 coverage rates based on ANC registration were 54% and 0%. Following the interventions, rates in these districts increased to 72% (IPTp2) and 23% (IPTp3) in 2014 compared to 63% and 8% in the other 42 districts. These efforts have resulted in improvements in IPTp service delivery and reporting, Based on successes, training and guideline dissemination continued in 2015 across the country so that all health facilities received copies of the new guidelines and 82% of districts received training.

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FINDING THE LAST FEW CASES: MOST EXPOSED INDIVIDUALS LESS LIKELY TO PARTICIPATE IN MALARIA SCREENING IN A PRE-ELIMINATION SETTING IN VIETNAM

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In a one-year cohort study comprising five malariometric screening surveys (n=456) in a remote, forested and recently endemic region of Central Vietnam, only 6 asymptomatic malaria infections (3 *Plasmodium falciparum* and 3 *P. vivax*) were identified, leading to speculation that local transmission may be fading out to elimination. However, people who spend overnights in forest fields, the most significant local risk factor for malaria, may be less likely to participate in screening due to regular absences from the villages where the surveys were conducted, leading to challenges in the identification of the remaining infection reservoir. Ancillary to the cohort study, an exploratory mixed methods study, comprising ethnographic fieldwork and a cross-sectional survey (n=160) was conducted to assess variation in malaria exposure-related behaviours and participation in screening. Full participation in all five screening surveys (40%) was not associated with spending overnights at forest fields (n=72), but individuals spending periods of one week to more than one month at their fields (n=16) were significantly less likely to participate in all screenings (12.5%). There was no difference in bed net availability by duration of stay, though 57% of bed nets at the fields had tears. In addition, people staying for longer durations at the fields were less likely to consistently sleep under a bed net, stayed in larger family groups, with fewer bed nets per person, and were twice as likely to have new unused LLINs stored in the village home, which suggests bed nets in use were older. Finally, those staying for longer durations at their fields had lower malaria knowledge. In conclusion, this study identified a sub-population with multiple malaria risk factors including extended stays in forest fields, lower effective bed net use, and lower malaria knowledge, but who are less likely to participate in screening surveys, which challenges elimination efforts. Mixed methods studies can improve the robustness of findings from small samples and highlight small populations in which the last few malaria cases are more likely to occur.

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IMPROVING IVERMECTIN´S EFFICACY AS A VECTOR CONTROL TOOL: REDUCING ITS METABOLISM AND EXCRETION TO PROLONG THE MOSQUITO-KILLING WINDOW

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The potential use of ivermectin mass drug administration to kill mosquitoes and reduce malaria transmission is gaining momentum. This endectocide has the potential to target mosquitoes feeding during the temporal and spatial gaps left by core vector control tools (insecticide treated nets and indoor residual spraying) and reduce residual malaria transmission, possibly easing the path to elimination. With a plasma half-life of 12-18 hours, however, either multiple daily doses or slow-release formulations are required to sustain effective mosquitocidal plasma concentrations. An additional alternative is the pharmacological inhibition of the metabolism and excretion of the drug. This strategy forms the basis of several drug combinations used effectively and safely in oncology and HIV treatment. Ivermectin is a substrate of both the CYP 450 (CYP) 3A4 and the P-glycoprotein (P-gp). We conducted an animal model experiment showing the effect of ketoconazole, a CYP3A4/P-gp inhibitor, in the oral pharmacokinetics (PK) of ivermectin. Six adult hybrid minipigs (37-60 kg) received a single oral dose of 800 mcg/kg of ivermectin. Blood samples for plasma-HPLC drug quantification were taken hourly for the first 8 hours and then at regular intervals for two weeks. The main PK parameters were calculated including the area under the curve (AUC) and the time above 6 ng/ml (a concentration known to kill 50% of biting *Anopheles gambiae*). The experiment was repeated after a wash-out period of one month, the animals were randomized to receive premedication with ketoconazole or nothing as a control before ivermectin. In animals pre-treated with the CYP3A4/P-gp inhibitor ketoconazole, we saw a statistically significant 6-fold increase in the time above mosquito-killing concentration and a significant increase in the AUC (1750 vs 464 ng/ml·hr). No sign of neurological toxicity was seen. Modifying the metabolism and excretion of ivermectin by means of CYP3A4/P-gp inhibitors appears to be an alternative strategy for prolonging its mosquito-killing effect and possibly increase its efficacy in reducing malaria transmission.

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CONTROL AND PRE-ELIMINATION OF MALARIA IN THE YUNNAN PROVINCE OF CHINA FROM 1983 TO 2013

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Yunnan faces an increasing risk of imported malaria cases from border countries, which Myanmar, Laos and Vietnam. With imported malaria thus becoming a major challenge in the context of malaria elimination in the province. The aim of this study is to understand the past and present malaria situation, and the challenges involved in its control, in Yunnan Province. A retrospective study of the past 30 years' of surveillance data and relevant sources on malaria in Yunnan was conducted. Data on malaria cases from 1983 to 2013 were collected from the China Information System for Disease Control and Prevention, as well as from case investigation reports.Results: From 1983 to 2013, a total of 375,602 malaria cases were reported in Yunnan Province; among these 739 resulted in death. Of the total number of malaria cases, 72.7% were infected with *Plasmodium vivax*, 21.2% with *P. falciparum*, 0.02% with *P. malariae*, 1.4% were mixed infection cases, and 4.7% were untyped cases. Out of the total number of reported cases, 207,956 were reported from the 25 border counties, accounting for 55.4% of the total malaria cases, and 44.6% (167,646) were reported from the mainland counties (the other 104 counties) of the province. The annual malaria incidence rate decreased from 64.8 per 100,000 in 1983 to 0.9 per 100, 000 in 2013. Among the 25 border counties, the malaria incidence rate decreased from 179.8 per 100,000 in 1983 to 4.5 per 100,000 in 2013, and the mainland counties malaria incidence rate decreased from 45.4 per 100,000 in 1983 to 0.3 per 100,000 in 2013. In 1983, malaria was prevalent in the northwest of Yunnan, Zhaotong city (northeast of Yunnan), and Yuanjiang-Honghe River Valley and border areas, while it was prevalent in the western and southern border areas of Yunnan in 2013. The population segment at high risk of contracting malaria consists of young male farmers and migrant workers. Conclusion: From 1983 to 2013, malaria control has been effective in Yunnan Province. Malaria has almost been eliminated in the mainland areas, and future control interventions should focus on the border areas.

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EFFECTIVENESS OF MALARIACONNECT FOR REAL-TIME CASE NOTIFICATION TO STRENGTHEN SURVEILLANCE IN SOUTH AFRICA

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South Africa is targeting malaria elimination by 2018. Strengthening the surveillance system is key to the country achieving its elimination goal. South Africa's historical paper-based notification system led to reporting delays before data was entered into the national malaria information system (MIS). To enable real-time case notification, South Africa developed MalariaConnect, an Unstructured Supplementary Service Data (USSD) tool for health care workers to notify malaria cases immediately from any mobile phone. MalariaConnect was deployed in November 2015 across 297 public health care facilities. These facilities are located in South Africa's five endemic districts (Ehlanzeni, Mopani, Umkhanyakude, Uthungulu, and Vhembe) and had previously reported at least 1 malaria case from 2012-2014. MalariaConnect notifications were compared against the paper-based data to assess consistency of reporting, and timeliness through a paired t-test. User acceptability has been assessed using an unstructured interviewer-administered questionnaire. From November 2015 through March 2016 there were 1234 cases reported through the paper-based system from facilities enrolled on the MalariaConnect project. On average, cases were notified through MalariaConnect within 1 day of patient diagnosis, compared to 5.1 days in the paper-based system (95% Confidence Interval 4.09-5.17: p 0.001). Of these, 70% (n=865/1234) were reported through MalariaConnect. Of the 108 healthcare workers that were interviewed and had used MalariaConnect, 96% (n=104/108) were willing to continue using the system. MalariaConnect has significantly improved timeliness of case reporting in South Africa and achieved high user acceptability. In 2016 South Africa will focus on increasing reporting rates through continued follow up visits and by strengthening systems for data-driven response.

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MALARIA ELIMINATION BY REACTIVE CASE DETECTION: CHALLENGES AND OPPORTUNITIES

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As several regions with historically substantial vectorial capacity approach eliminating malaria, there is a need to understand which features of a malaria surveillance and response system are most crucial for achieving local elimination and stemming resurgence. To investigate how local hotspots and spatial biases in surveillance quality interact to sustain local transmission, an individual-based model of malaria transmission, including vector life cycle dynamics and within-host immunity, was adapted to explicitly simulate transmission at the spatially-connected household level. A community of a few hundred households in Gwembe District, Southern Province, Zambia, where elimination operations are currently underway, was used as a model system. Vector bionomics, treatment-seeking, bednet usage, and mass drug campaign schedules and coverages were modeled according to household-level survey data from the area. Simulations predict that case management rate is the strongest determinant of success of reactive approaches, and high coverage and magnitude of response cannot completely compensate when a portion of symptomatic infections are left untreated. Rather than devoting resources to coordinating extensive reactive campaigns, we propose that a more effective strategy would be to improve access to treatment through strategic placement of village health workers and active community engagement on the importance of promptly treating cases.

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RWANDA TOWARDS MALARIA PRE-ELIMINATION: ACTIVE CASE INVESTIGATION IN A LOW ENDEMIC DISTRICT

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Rwanda has seen an increase in malaria cases recently with an increase from 514,173 cases in 2012 to 1,957,402 cases in 2015. This change can be attributed to an increase in temperature, rainfall, and resistance to insecticides. Despite this setback, Rwanda is aiming to reach the pre-elimination phase by 2018. In January 2015, 11 health facilities in Rubavu, a low endemic district, started implementing reactive active case detection after training 55 health care providers and 11 lab technicians on the topic. This strategy involves screening and treating individuals living in close proximity to passively detected cases, also known as index cases. Index cases can be used to identify population groups that are sources of infection. From January 2015 to December 2015, 16,434 cases of Malaria were detected and treated at 11 health facilities in Rubavu District. Among these cases, 2,917(17.8%) index cases were investigated and 4,943 individuals (between 1 and 2 contacts for each index case) living in proximity of index cases were tested using rapid diagnostic tests by health care providers. Of these, 508 (10.3%) tested positive for malaria and were treated according to national guidelines. This data shows that the number of investigated cases is still lower than the national guidelines of screening 5 individuals residing between 100 to 500 meters of every confirmed case. This low rate could be due to the increase of malaria cases in Rwanda which has placed a burden on health care providers and health facilities in areas like Rubavu which used to be low endemic malaria areas. Additionally, data gathered through supervision activities has indicated a need for additional training on screening investigations in order to adhere to national guidelines and conduct the investigations more efficiently. Active case investigation could be improved by training and involving more health care providers such as community health workers who could reduce the burden on health center staff. The additional support for case investigation activities and improved training can help to achieve higher coverage of individuals located near index cases.

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THE UTILITY OF MALARIA RAPID DIAGNOSTIC TESTS AS A TOOL IN ENHANCED SURVEILLANCE FOR MALARIA ELIMINATION IN VANUATU

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As part of efforts to eliminate malaria, Vanuatu has piloted the implementation of enhanced malaria surveillance and response strategies since 2011. This involves passive case detection (PCD) in health facilities, proactive case detection (Pro-ACD) and reactive case detection (Re-ACD) in communities using malaria rapid diagnostic tests (RDTs). While RDTs improve case management, their utility for detection of malaria infections in ACDs in this setting is unclear. We evaluated the utility of malaria RDTs as diagnostic tools in PCD, in five rounds of Pro-ACDs and five rounds of Re-ACDs conducted in Tafea and Torba Provinces, Vanuatu between 2011 and 2014. In PCD conducted in Tafea Province in 2013, a RDT-positive rate of 0.21% (2/939) and a PCR-positive rate of 0.44% (2/453) was observed in fever patients, demonstrating less than 1% of suspected malaria cases in this province were due to malaria. In Pro-ACDs conducted in Tafea and Torba Provinces, RDT-positive rates in 2013 and 2014 were 0.14% (3/2145) and 0% (0/2823), respectively, while the corresponding PCR-positive rates were 0.72% (9/1242) and 0.79% (9/1141). PCR identified villages in both provinces appearing to be transmission foci with a small number of low-density infections, mainly *P. falciparum* infections. In five rounds of Re-ACD, RDTs did not identify any additional infections while PCR detected only one among 173 subjects screened. These results demonstrate that both Tafea and Torba Provinces in Vanuatu has achieved very low malaria prevalence. In these low-transmission areas, conducting Pro-ACD and Re-ACDs using RDTs appears not cost-effective and may have limited impact on interrupting malaria transmission due to the small number of infections identified by RDTs and considerable operational resources invested. More sensitive, field deployable and affordable diagnostic tools will improve malaria surveillance in malaria elimination settings.

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BACK TO THE FUTURE: REVISITING THE ROLE OF CHLOROQUINE FOR MALARIA ELIMINATION IN MOZAMBIQUE

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Chloroquine (CQ) was used for many decades as the mainstay of antimalarial treatment, but was progressively abandoned due to increasing parasite resistance throughout the majority of malaria-endemic areas, particularly in Sub-Saharan Africa. Recent evidence from Malawi suggest that CQ sensitivity may be returning in places where discontinuation has reduced the drug pressure to the parasite populations. While this does not support the reintroduction of CQ as first line therapy, it suggests that, if proven sensitive in a given area, it could play a prophylactic role in malaria

elimination strategies when used in combination with other drugs or tools. Additionally, due to its high safety profile, CQ could also be considered an alternative for prophylaxis in first-term pregnancies and young infants.

A randomized, single-blind, placebo-controlled trial in asymptomatic Mozambican adults was conducted in the district of Manhiça, Southern Mozambique. Participants were followed up at days 0, 1, 2, 3, 7, 14, 21 and 28. The primary study endpoint was the rate of adequate and parasitological response (ACPR) to therapy on day 28 (PCR-corrected). Blood-slides and filter papers were collected at every study visit to measure parasite density and differentiate recrudescenses from new infections. A total of 52 and 27 participants were included in the CQ and Placebo group respectively. Mean parasite density at study entry was 517p/µL and there were 7 lost-to-follow-up participants in each arm. A PCR-corrected ACPR was 89% (95%CI 80%-98%) in the CQ arm and 36% (95%CI 17%-59%) among those in the placebo group (p< 0.001). In conclusion, this exploratory study suggests the return of CQ sensitivity in the South of Mozambique, implying its potential role as a prophylactic drug to be used in malaria elimination efforts such as mass drug administration, in combination with other effective anti-malarials. To further explore this question in Mozambique, additional studies will be performed on adults with clinical malaria and special populations.

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HETEROGENEITY OF *PLASMODIUM FALCIPARUM* MALARIA CASES IN MATABELELAND SOUTH: ANALYSIS OF CASES REPORTING TRAVEL HISTORY IN THE EARLY STAGES OF AN ELIMINATION PROGRAM

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As malaria prevalence decreases, imported malaria cases pose a challenge to malaria programs due to their potential to instigate local transmission. Travel history of RDT-confirmed cases, available through routine passive surveillance, was used to estimate potential for malaria importation in Zimbabwe's Matabeleland South province, where an elimination agenda was launched in 2013. Individual travel history records from 1,190 cases reported between September 2014 and June 2015 were analysed to quantify the number of cases travelling within Matabeleland South, to other parts of Zimbabwe and outside Zimbabwe. Reported travel locations of cases were geo-located. 185 (15.5%) of cases were reported to have travelled outside their home in the past 4 weeks, of which 99 (53.5%) were domestic travellers and 86 (46.5%) were international travellers. Beitbridge (63%) and Gwanda (21%) reported greatest proportion of cases that travelled. 44.3% of cases with travel outside Matabeleland South were identified in low transmission risk settings (<1 case per 1,000) and 20% were in high and medium transmission risk areas (>10 cases per 1,000) of the province. Environmental Health Practitioners classified 80 (6.7%) of all 1,190 confirmed cases as imported (transmission occurred outside Matabeleland South), 11 (0.9%) as intraported (transmission occurred outside case's home district but within Matabeleland South), and 744 (62.5%) as local (transmission occurred in home district of case). As locations reported in travel history data were described by coarse spatial resolutions, accurate classification was a challenge. Characteristics such as age, gender, and occupation will be explored to determine the demographic differences between travellers and non-travellers. Improvements in data collection, validation of travel history and its link to infections (including clustering of local cases) are needed for successful national and regional elimination of malaria.

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QUANTIFYING MALARIA-ATTRIBUTABLE FEVER IN AFRICA, AND THE DIFFERENCE IN TREATMENT-SEEKING RATES FOR MALARIA-COINCIDENT FEVERS AND NON-MALARIAL FEBRILE ILLNESS

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Malaria is typically assumed to be the causal parasite of febrile illness in Africa. While it is increasingly common for individuals to receive a diagnostic test for malaria before receiving treatment, it remains possible that the individual could have a co-infection with another disease that is in reality the underlying cause of their fever. Using paired observations of two-week fever history and *Plasmodium falciparum* malaria positivity from household survey datasets, we use model-based geostatistics to estimate prevalence of malaria-attributable fever, malaria-coincident fever, and non-malarial febrile illness across Africa. We show that febrile illness that is directly attributable to malaria accounts for a decreasing proportion of malaria-coincident febrile illness with increasing malaria prevalence, and that non-malarial febrile illness is widespread across the continent. Additionally, using household survey data on treatment-seeking rates for febrile illness, we show the difference in treatment-seeking rates between individuals with a malaria-coincident fever and non-malaria fevers. We show that if a substantial number of symptomatic malaria infections are not seeking care for their symptoms in low-transmission areas, then small-scale outbreaks may remain undetected, causing a problem for malaria elimination.

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SPATIAL DISTRIBUTION OF MALARIA CASES AND VECTORS IN A HYPO-ENDEMIC AREA OF WESTERN KENYA HIGHLANDS

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Malaria transmission in hypo endemic areas of Western Kenya highlands have been significantly reduced by extensive use of insecticide treated bed nets. However, residual transmission continues to maintain the disease in the community. Ecological studies suggest that there are foci of transmission. This study was undertaken to determine whether the distribution of malaria cases is random or clustered. Forty four microscopically confirmed index malaria cases from a local hospital were identified and homesteads of these patients geo-referenced. Household members in the index case population were screened for malaria. Houses located at a distance of 500 - 1000 m radius from the index case houses were identified and blood samples obtained for malaria diagnoses. This group served as the control. Mosquitoes were collected in the index case houses and control houses using pyrethrum spray catches. The number of malaria cases and vector density was compared between index case households and control population. In the index case household, the prevalence of malaria cases was 8 % compared to 4 % in the control houses. In the index case houses, vector density was 0.11 for *Anopheles gambiae* and 0.38 for *An. funestus*. In contrast, vector density in the control houses was 0.04 for *An. gambiae* and 0.27 for *An. funestus* .The malaria prevalence in the index case household was 2 fold greater than the control household. The indoor house density for *Anopheles gambiae* and *Anopheles funestus* were respectively 2.8 and 1.4 fold, greater in the

index houses compared to the control. Differences in the indoor density of the mosquitoes species were significant (P = 0.04). The data indicates higher transmission and malaria prevalence in the index case houses compared to the control, hence an indication of non-random vector and case distribution. Analysis of spatial distribution will be undertaken to show relationship between malaria cases and potential breeding habitats.

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SEVERE FLOODING AND MALARIA TRANSMISSION: IMPLICATIONS FOR DISEASE CONTROL IN AN ERA OF GLOBAL CLIMATE CHANGE

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There are several mechanisms by which global climate change may impact malaria transmission, including those that relate to changes in the frequency of extreme weather events including heat, drought, and floods. We sought to assess how the increased frequency of extreme precipitation events associated with global climate change will impact malaria transmission in highland areas of East Africa. We used a differences-in-differences, quasi-experimental design to examine spatial variability in the incidence rate of laboratory-confirmed malaria cases and malaria-related hospitalizations comparing villages at (1) high vs. low elevations, (2) with and without rivers, and (3) upstream vs. downstream before and after severe flooding that occurred May 1, 2013 in the Kasese District of Western Uganda. Findings: During the study period 7,596 diagnostic test were performed and 1,285 patients were admitted with a diagnosis of malaria. We observed that extreme flooding resulted in an increase of approximately 30% in the risk of an individual having a positive malaria diagnostic test in the post-flood period in villages bordering a flood-affected river compared with villages further from a river with a larger relative impact on upstream vs. downstream villages (adjusted RR 1.91 vs. 1.33). In conclusion, extreme precipitation such as the flooding described here may pose significant challenges to malaria control and elimination programs, and will demand timely and sustained responses to prevent and mitigate deleterious impacts on human health.

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MALARIA IN THE FIRST TRIMESTER OF PREGNANCY: INCIDENCE AND ASSOCIATED RISK FACTORS IN BENIN, SUB-SAHARAN AFRICA

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In Africa, preventive drug strategies against malaria in pregnancy are recommended from the 2nd trimester and bed nets are rarely used in early pregnancy. Therefore, women remain insufficiently, or not, protected during the first trimester, when malaria may be particularly deleterious for the mother and the child. The incidence of and risk factors associated with malaria in the first trimester have been poorly explored so far. A subsample of 200 pregnant women recruited before conception, were followed up monthly until delivery. Malaria was detected during the 1st, 2nd and 3rd month of pregnancy. A multivariate mixed model was used to assess factors associated with malaria during the first trimester. The cumulative incidence of malaria during the first trimester of pregnancy was 17.8% (11.2% during the first month). Early gestational age (≤ 6 weeks' gestation) (aOR: 2.69 [1.35-5.37]) and living in a lakeside area (aOR: 0.17 [0.04-0.85]) were the main factors significantly associated with malaria in the first trimester. In conclusion, malaria was highly incident during the first trimester of pregnancy, particularly during the first month and in women living far from the lake. The consequences of these infections for the mother and her child need to be assessed.

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FIRST PILOT PROJECT FOR ACTIVE SURVEILLANCE OF ASYMPTOMATIC MALARIA CASES IN HISTORICALLY ENDEMIC REGIONS IN PARAGUAY BY TWO METHODS MICROSCOPY AND MULTIPLEX SEMINESTED PCR

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In 2014, Paraguay was placed among the 16 countries that reported zero indigenous cases of Malaria. The implementation of active surveillance to detect potential asymptomatic cases in historically endemic regions in Paraguay is needed considering the indigenous parasite is *Plasmodium vivax* that contrary to *P. falciparum*, can remain latent as hypnozoites in the host. In the present study we applied the microscopy and the molecular diagnosis to search for sub-patent parasitemias in asymptomatic cases. Adults from a total of 15 localities from three departments, Alto Paraná, Caaguazú and Canindeyú were selected, based on the records of the SENEPA considered as the last localities that reported at least one case in the period of 2007-2011. The sample size obtained for 95% confidence was 361 but 332 samples were collected and analyzed up to now and informed consent of each of them was obtained. Thick smears were analyzed by microscopy. DNA samples were extracted from blood drop dried on filter paper and analyzed by the Seminested Multiplex PCR using the primers that amplify the 18 S rDNA for the four species that cause Malaria (*P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*). Human 18S rDNA was amplified as internal control. Sensitive essays allowed to detect until 0,01 ng of genomic *P. falciparum* DNA. From the 332 samples, 11.4% were from the department of Canindeyú, 30.1% from Alto Parana and 58,4% from Caaguazú. The distribution per locality was: 11,4% from Pira Vera, 10.5% from Maracamoa, 7.8% from Nueva Esperanza, 11.7% from Mision Verbo Divino, 2.4% from San Juan, 1.8% from Mbarigui Indígena, 17.2% from Mil Palo, 0.9% from Nueva Brasilia, 4.2% from Nueva Esperanza, 11.1% from Nueva Toledo, 1.8 from Ñu Jhovy, 3.6% from Pindoí, 2.4% from Santa Clara, 7.5% from Santa Teresa and 5.4 from Yby Moroti. Fifty seven percent were female and 43% were male. We could not detect any sub-patent parasitemia that can reveal the presence of asymptomatic cases. The results obtained in this study are very promising for our country, at this stage where all the efforts are done towards the eradication of the Malaria.

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MALARIA IN AN INTERNALLY DISPLACED PERSONS CAMP IN THE DEMOCRATIC REPUBLIC OF CONGO

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Malaria remains a leading cause of death in children under 5. Malaria prevention and treatment efforts in the Democratic Republic of the Congo (DRC) are hindered by armed conflict, which has resulted in the displacement of approximately 2.7 million individuals. This study aimed to describe malaria cases treated at a health centre serving an internally displaced persons (IDP) camp in DRC. The study took place in Luchebere IDP camp in the DRC which housed 2,580 individuals (318 children <5) at the time of the study in 2014. The camp consisted of two waves of IDPs: (1) ~1,400 individuals from Masisi and Walikale in Sept 2013; and (2) ~1,500 individuals from Northwest Masisi in Jan-Feb 2014. Febrile patients

presenting to the only health clinic in the area, which provides free medical services to IDPs, were tested for malaria using a rapid diagnostic test (Paracheck®). Demographic and clinical data were abstracted from clinic records. Uncomplicated malaria cases were treated with artemisinin-based combination therapy, according to WHO recommendations, and severe malaria with intravenous quinine. Between January and July 2014, 751 patients presenting to the clinic with fever were tested for malaria. 323/751 (43%) tested positive, including 169/279 (61%) children <5. The incidence of malaria requiring treatment in the IDP camp was estimated to be at least 210 per 1,000 at risk per year overall and 910 per 1,000 at risk per year in children under 5 (>3-fold higher than the WHO Africa region). Of the participants with malaria, 292/323 (91%) had uncomplicated disease and 28/323 (8.8%) had severe malaria. There were 4 deaths, 2 in children <5. 452/751 (60%) of patients had a bednet in their shelter and of these, 259/452 (59%) reported using the bednet. Malaria accounted for a higher proportion of febrile illness among patients from the second wave of IDPs who had lived in the camp for <6 months (72% vs 12%, p<0.0001), and among those who reported that they did not use a bednet (62% vs 8.9%, p<0.0001). The findings suggest that control measures targeting this high risk population may reduce the burden of malaria, particularly children under 5 and recent arrivals to the IDP camp.

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PREVALENCE OF SUBMICROSCOPIC MALARIA IN PREGNANCY IN COLOMBIA

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For countries progressing towards malaria elimination, highly sensitive diagnostic methods are required to target reservoirs of transmission. Currently, microscopy is the gold standard for malaria diagnosis however, more sensitive tests are needed to identify low-density submicroscopic infections. Pregnant women are an important risk group as they may harbour submicroscopic infections which could impact clinical outcomes for the mother and fetus. Here, a reverse transcription real-time PCR (RT-qPCR) was developed for detection of the 18S rRNA gene for *Plasmodium falciparum* and *P. vivax*. The assay was validated with 42 blood samples and demonstrated a specificity and sensitivity of 100% when compared to qPCR. To investigate the prevalence of submicroscopic malaria in pregnancy, a cohort of 200 women from Colombia were recruited and followed longitudinally via antenatal visits until delivery. Of the 38 women followed up so far, 19 were positive for submicroscopic malaria during pregnancy. 228 samples were screened by RT-qPCR, of which 27 samples tested positive. 22%, 59%, and 19% of the infections were caused by *P. falciparum*, *P. vivax*, or mixed, respectively. Additionally, samples from asymptomatic subjects and from febrile patients were collected from Colombia and tested via microscopy and RT-qPCR to investigate the frequency of submicroscopic malaria outside of pregnancy. Of the 84 asymptomatic samples screened by RT-qPCR, 6 and 10 were positive for *P. falciparum* and *P. vivax*, respectively. Further, of the 322 febrile samples screened by RT-qPCR, 36 and 66 were positive for *P. falciparum* and *P. vivax*, respectively. These results reveal a high frequency of submicroscopic malaria in this region. We report that 50% of pregnant women followed up in this study were positive for a submicroscopic infection at some point during pregnancy. 15% of the asymptomatic population and 27% of the febrile population were also positive for submicroscopic malaria. We propose that the use of more sensitive tests and active surveillance during pregnancy will become a necessity for the end goal of malaria elimination in this part of Latin America.

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CORRELATION BETWEEN MALARIA INFECTION DURING PREGNANCY AND ADVERSE BIRTH OUTCOMES IN VARIED MALARIA TRANSMISSION SETTINGS IN UGANDA AND BURKINA FASO RESPECTIVELY

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Preterm delivery and low birth weight are the leading causes of neonatal mortality and morbidity in children below the age of five years. The mechanisms under which malaria leads to low birth weight, still birth and preterm labor are poorly understood. Using data from a prospective cohort in which; 990 pregnant women were enrolled, 853 (85.2%) followed through delivery and resulting in 838 live births, we assessed the association between malaria infection during pregnancy and adverse birth outcome that included low birth weight, still birth, abortions and preterm delivery in two African clinical settings (Uganda, hyperendemic, and Burkina Faso, seasonal transmission). Women were categorised according to their malaria infection history during the study: Baseline, high parasite group and low parasite group. A total of 338 (40.8%) were in the baseline group, 310 (37.4%) were in the high-level infection group, 120 (14.5%) were in the low-level infection group, and 60 (7.2%) had occult or early infections. Overall there were 230 adverse birth outcomes across the two study sites; this represented 27.0% of all births: Uganda (83) and Burkina Faso (147). Premature births represented 74% of adverse outcomes. The proportion of preterm births was highest in low parasite group (0.215) in Burkina Faso and (0.296) in Uganda. Infection group was not found to be a significant predictor of an adverse outcome (P>0.05) using logistic regression. Although proportion of infants weighing <2.5kg was highest in the occult infection group (0.169), the proportion of infants with low birth weight was not significantly associated with infection group (P=0.257) even after adjusting for potential confounders (age, gravidity, country and weeks of amenorrhea at delivery); infection group did not significantly influence the odds of having a low birth weight infant (P=0.603). In the cohort of women enrolled in this study, malaria infection during pregnancy was not statistically associated with adverse birth outcomes.

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MAPPING CLIMATIC, GEOGRAPHIC AND SOCIO-ECONOMIC DETERMINANTS OF MALARIA IN MALAWI FOR MALARIA RISK ASSESSMENT

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The 2016-2030 WHO global technical strategy for malaria aims to reduce malaria case incidence by 90% in 2030. Achieving this will not only depend on new interventions but require optimal use of novel technologies and methods. More accurate profiling of geographical variation in malaria over time, aimed at identifying transmission hotspots, will enable more targeted control. Modelling interactions between various potential drivers and their effect on malaria risk can also lead to a better understanding of transmission dynamics. Modern spatio-temporal statistical models and Bayesian predictive inference are particularly well suited to mapping health outcomes in low resource settings for (at least) three reasons. Firstly, they enable more precise prediction in data-sparse

regions by exploiting spatio-temporal dependence in the health outcome of interest in addition to associations with spatio-temporally dense environmental covariates. Secondly, they can simultaneously capture spatial variation at large and small scales. Finally, they deliver honest assessments of predictive uncertainty. We use spatio-temporal statistical models to investigate the contribution of climatic, environmental and socio-economic factors to district-level variation in malaria risk in Malawi. Outcome data (malaria cases) are taken from an age stratified health management information system data covering all 28 districts in Malawi between July 2004 and December 2015 while socio-economic data are obtained from national surveys. Remotely sensed climate data averaged over the districts are used to capture the impact of climatic variations on transmission. We first assessed covariate effects in a non-spatial model to identify the most important significant drivers of malaria, which we then used to build a spatio-temporal model and generate Bayesian predictive maps of spatial variation in disease risk. These predictive maps serve two purposes: to highlight areas of unusually high and low risk that could inform sub-district surveillance and control strategies; and to target augmented sampling designs on areas where current predictions are imprecise.

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RURAL-URBAN DIFFERENCES IN THE UTILIZATION OF MALARIA PREVENTIVE AND TREATMENT SERVICES BY WOMEN OF REPRODUCTIVE AGE GROUP IN NIGERIA

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Nigeria witnessed a dramatic improvement in the provision of universal access to malaria prevention, diagnosis and treatment tools. The objective of this analysis is to describe rural urban differences in the utilization of malaria preventive and treatment services in Nigeria by women of reproductive age group to inform malaria control programming and planning. A cross sectional survey involving 6,344 women aged 15-49 years selected through multi staged cluster sampling procedure. Descriptive analysis was done to obtain weighted prevalence and proportions of women background characteristics and selected outcome variables. Univariate and multivariate logistic regression analyses were then conducted to obtain crude and adjusted odds ratios for the hypothesized association. Statistical testing was done using the adjusted Wald test. An estimated 30% of the women reported the use of net the night before the survey. 30% of them claimed their children had experienced fever in the preceding 2 weeks before the survey but only 14% had a malaria confirmatory test for their children. An estimated 65% percent of the women who sought treatment for their children used the private sector. Rural women have about twice the odds of using a net than their urban counterparts (2.12(1.47; 3.06), p<0.001). The odds of using nets decrease with any form of formal education, with increasing wealth index and among older women. Further multivariate analysis showed that younger, rural women are more likely to use net OR (1.77(1.12; 2.80) p=0.013) while the inverse relationship between level of education and net use persisted. There is no evidence of an association between place of residence and malaria diagnostic testing. Utilization of net is generally low with more tendencies to use net among rural, un-educated and lower socio economic status. Treatment of malaria based on clinical suspicion is widely prevalent and majority of the women patronize the private sector. Recommendations were made for public health strategies to encourage use of nets; improve infrastructures for malaria diagnosis and strengthening of the health system.

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DESIGN OF A CLUSTER SURVEY STUDY TO DESCRIBE THE EPIDEMIOLOGY OF MALARIA GAMETOCYTE CARRIAGE AND TRANSMISSION DYNAMICS IN A HOLOENDEMIC TRANSMISSION SETTING IN KISUMU COUNTY, WESTERN KENYA

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To better target malaria control interventions, more information is needed about the human, mosquito and environmental factors that affect who is carrying gametocytes and who among these carriers is transmitting malaria. While human and parasite factors, including the intensity and duration of gametocytemia, affect infectiousness at the individual level, entomological factors such as mosquito exposure dictate the transmission potential of each individual and the infectious reservoir as a whole. Most malaria transmission studies have not measured these factors simultaneously and longitudinally. Here, we describe the design of a pilot cluster design survey study that is simultaneously and longitudinally assessing human, parasite and entomologic factors that influence malaria transmission in Kisumu, Kenya. The study area is a 369km2 region located on the northeastern shores of Lake Victoria that has been mapped using Global Positioning System (GPS) technology and divided into clusters that measure 1km by 1km. Thirty clusters were randomly selected, ensuring at least a 1 km buffer zone around a selected cluster and that each selected cluster had at least 4 households with each household having at least 1 person in all of the following age categories: above 25 years, below 5 years and between 6 and 25 years. Field workers with GPS devices were sent to the homesteads to obtain consent from the heads of the households. The clusters cover 18% of the total study area. Each cluster is visited weekly and each homestead monthly to collect resting mosquitoes as well as epidemiological data and blood samples from the study participants. Testing for the presence of asexual and sexual stages by microscopy and molecular methods is performed on the samples collected from the study participants. Female mosquitoes are dissected and tested for the presence of *Plasmodium* sp. An analysis of blood meal is done for fed female mosquitoes. Individuals identified to harbor gametocytes undergo mosquito feeding assays; a subset of adults undergo both direct and membrane feeding assays for direct assay comparison. Preliminary findings will be presented.

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INCREASE IN MALARIA AWARENESS AND REDUCTION IN MALARIA PREVALENCE IN ENDEMIC DISTRICTS OF BANGLADESH: EVIDENCE FROM FOLLOW UP MALARIA PREVALENCE SURVEY 2013

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Malaria is endemic in 13 districts of Bangladesh. A baseline malaria prevalence survey across the endemic districts of Bangladesh in 2007 was conducted by our group, when the point prevalence was reported around 39.7 per 1,000 population. Followed by the two rounds of Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) funded intervention by

National Malaria Control Programme and BRAC led NGO consortium we had conducted a follow up survey during August to November 2013 in 70 upazilas (sub districts) of 13 malaria endemic districts of Bangladesh to measure the reduction following GFATM interventions.. We used a multi-stage cluster sampling technique to collect 9750 blood samples from same number of households. We used "FalciVax" rapid diagnostic tests (RDT) to diagnose malaria from blood from randomly selected individual from a household and the test result was recorded. The same RDT was used during the baseline survey. As in the baseline survey, the household head or available eldest person was interviewed using a pre-coded structured questionnaire to collect data on the knowledge and awareness to malaria of the household. Based on weighted calculation, overall malaria prevalence was found 1.4 per 1,000 population. The proportion of *Plasmodium falciparum* mono infection was 78% while *P. vivax* and mixed infection of these two species were 11% in both cases which was 90.2% for *P. falciparum* mono infection 5.3% and 4.5% for *P. vivax* and mixed infection during 2007. Bandarban was the highest malaria prevalent district (6.7 per 1,000 population) in the follow up survey. Knowledge on malaria sign and symptoms and mode of transmission were found better in follow up survey (97.3%) than the baseline survey (34.0%). Use of insecticide treated bed nets for prevention of malaria was found high (90.2%) at the respondents level during follow up survey. Overall, people in Chittagong hill tracts areas had slightly better knowledge than those in other areas. In a nutshell, a reduced point prevalence of malaria and increased level of knowledge were reported in the follow up malaria prevalence survey in Bangladesh.

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SEASONAL CHANGES IN THE ANTIBODY RESPONSES AGAINST *PLASMODIUM FALCIPARUM* ANTIGENS ON ISLANDS IN LAKE VICTORIA, KENYA

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Anti-malaria antibody responses can help characterize heterogeneous in malaria transmission. In the present study antibody responses to *Plasmodium falciparum* AMA-1, MSP-119 and CSP were measured to assess the transmission intensity in meso-endemic settings in Lake Victoria. Two cross-sectional surveys were conducted in dry and wet seasons in 2012 comprised of five settings: Ungoye (mainland), Mfangano (large island) and small islands (Takawiri, Kibuogi, Ngodhe). Individuals provide a finger-blood sample to assess malaria infection by rapid diagnostic test (RDT). Exposure to malaria antibodies were detected by ELISA using eluted dried blood form filter paper. Of 5044 participants, RDT tests were done in 4852 (96.2%) and 4112 (81.5%) were tested for serology. The overall seroprevalence was 64.0% for AMA-1, 39.5% for MSP-119, and 12.9% for CSP. Within settings, seroprevalences for merozoite antigens were higher in Ungoye and Mfangano than in small islands, showed different patterns between seasons and consistently high in the wet season for CSP ($p<0.01$). The overall seroprevalence and antibody titers generally increase with age group ($p<0.001$). Seroconversion rates (SCR) demonstrated different patterns between seasons where AMA-1 seroconversion rates constantly high and similar in Ungoye, decreased in Mfangano and Takawiri but increased in Kibuogi and Ngodhe from dry to wet seasons. Increasing age was strongly associated with increased odd of seropositivity in all settings. We observed heterogeneity of parasite prevalence and serological indices acroos study sites and temporal changes in the force of infection islands in Lake Victoria. These data suggest that AMA-1 sero-epidemiological analysis may have a role in assessing short-term changes in exposure especially in high or seasonal transmission settings and appeared to best reflect transmission intensity.

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IMPACT OF ANTIMALARIAL INTERVENTIONS ON MALARIA MORBIDLY AND MORTALITY IN HOSPITALS FROM 2001-2015, SENEGAL

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The Senegal National Malaria Control Program and partners accelerated malaria control in the last decade, with nationwide roll-out of artemisinin-based combination therapy (ACT) starting in 2006, and rapid diagnostic tests (RDTs) starting in October 2007. Since 2003, insecticide-treated nets (ITNs) have been distributed through health facilities to pregnant women and children under five years, and since 2008, mass campaigns of ITN distribution have targeted children under five years, with universal coverage starting in 2010, and 2014. Since 2007, indoor residual spraying has been implemented in seven districts. We assessed the trends of malaria cases, hospitalizations and deaths at 35 hospitals and in all 76 districts during the period of the scale-up of malaria control interventions. Data collected from all hospitals and districts from 2001-2015 were used to assess the impact of accelerated malaria control. Numbers of outpatient and inpatient cases and deaths were compared between the 2001-2007 period and the accelerated - intervention period of 2008-2015. From 2001 to 2007, the proportion of suspect cases confirmed increased slightly from 2.2% to 4.0%. With the introduction of RDTs, this increased to 99.3% in 2015, with a mean test positivity rate of 42% during the 15 year period. The proportion of all consultations due to malaria decreased from 39.7% in 2001 to 26.9% in 2007 (pre-RDT). After the introduction of RDTs and change in definition from clinical to laboratory-confirmed, the proportion of all consultations due to malaria decreased from 9.1% in 2008 to 5.0% in 2015. The proportion of patients hospitalized for malaria accounted for 11.5% of all hospitalizations in 2001, and decreased to 7.5% in 2015. From 2001 to 2007, the proportion of hospitalized deaths attributable to malaria decreased from 24.5% in 2001 to 8.1% in 2007. From 2008 to 2015, the proportion of hospitalized deaths attributable to malaria decreased from 7.1% to 3.5%. We conclude that malaria morbidity and mortality has decreased substantially during the scale up of malaria control interventions.

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ASSOCIATION BETWEEN HOUSE QUALITY AND MALARIA INFECTION IN SUB-SAHARAN AFRICA: A MULTI-COUNTRY ANALYSIS

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Improvements to housing may contribute to malaria control and elimination by reducing house entry by malaria vectors and thus exposure to biting. We tested the hypothesis that malaria infection risk is lower in modern, improved housing compared to traditional housing in sub-Saharan Africa (SSA). All Demographic and Heath Surveys (DHS) and Malaria Indicator Surveys (MIS) that measured malaria infection by rapid diagnostic test or microscopy in SSA were analysed. Houses built using non-rudimentary wall, roof and floor materials were classified as modern and all other houses were classified as traditional. Conditional logistic regression was used to determine the association between house quality and prevalence of malaria infection in children aged 0-5 years, adjusting for age, gender, intervention coverage, household wealth and geographic cluster. We will present the association between house quality and the odds of malaria infection in children and discuss the potential of improved

housing as an intervention against malaria in a range of transmission settings across SSA. Improved housing may be an important strategy to prevent the re-introduction of malaria in areas where malaria has been eliminated.

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NOVEL APPLICATION OF A SPATIAL ANALYSIS METHOD IDENTIFYING FINE-SCALE SPATIAL CLUSTERING OF MALARIA DISEASE AND MOSQUITO VECTORS AMONG MATCHED CASES AND CONTROLS IN BLANTYRE, MALAWI

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Matching cases and controls in epidemiological studies usually produces greater statistical power in analyses, but precludes direct analysis of matched variables on the outcome. We analyzed clinical cases of malaria in under-five year olds in Blantyre Malawi (n=187) with age-, and residence-matched controls (n=286) who were *Plasmodium*-negative from the same clinic. To explore spatial patterns of household environmental risks among malaria cases and vectors, we used nonhomogeneous Poisson point process (NPPP), an analytic method employed in landscape ecology, which treats the cases as a 'presence-only' data set and omits controls from analysis, to determine environmental variables associated with malaria case or anopheline-positive households. Using this method, non-random spatial distributions of case ($p<0.01$) and anopheline-positive households ($p<0.01$) were detected. In a 'pseudo-absence' logistic regression model informed by NPPP results, environmental variables associated with both malaria case- and anopheline-positive households included natural roofs ($p<0.01$), unfinished walls ($p=0.02$), open eaves ($p=0.02$), and proximity to open water ($p=0.02$). NPPP is a method that can expand the use of matched case-control data that has been matched on location, although there is a cost in power related to diminished sample size. These results demonstrate that there are environmental factors associated with increased risk of malaria in urban and peri-urban Blantyre.

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SCALING-UP AND USING ROUTINE MALARIA SURVEILLANCE DATA TO IDENTIFY MALARIA HOTSPOTS AND TARGET MALARIA CONTROL INTERVENTIONS DURING AND AFTER THE EBOLA EPIDEMIC IN GUINEA

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Malaria is the principal cause of morbidity and mortality in Guinea; its burden was exacerbated by the recent Ebola outbreak. Due to historical limitations in the routine health management information system, the Guinea National Malaria Control Program (NMCP) developed a monthly reporting system for malaria epidemiologic and commodity data prior to the outbreak. With the expansion of access to rapid diagnostic tests for malaria, the reporting system has facilitated accurate tracking of malaria burden through widespread diagnostic confirmation of cases. The scale-up of this system also coincided with the Ebola outbreak and the related interruptions in health care service delivery in Ebola-affected areas. The malaria reporting system is based on standardized forms completed at health facilities. Data are entered into the electronic system at the district level and analyzed by the NMCP. The NMCP issues a monthly malaria bulletin that is disseminated nationally and regionally. The bulletin reports malaria incidence, testing and treatment rates, and commodity stock levels for Guinea's 38 health districts. Each bulletin also highlights the 10 health facilities reporting the highest incidence of malaria cases. Completeness of data entered at the district level increased from 66% for the first issue in November 2014, to 82% by March 2015, to 97% in January 2016. As data completeness improved, the bulletin allowed the NMCP to identify a high malaria transmission hotspot in Boffa district, where annualized malaria incidence in certain areas was as high as 425 cases per 1,000 population. Follow-up investigations based on surveillance data allowed NMCP and district health officers to target behavior change communications to hotspot areas. Despite staffing and logistics challenges related to the Ebola outbreak, the NMCP was able to successfully scale up a new malaria-specific reporting system. This system allowed the NMCP to improve data reporting from remote areas and to begin using routine surveillance data to identify malaria hotspots and target interventions.

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AN OUTBREAK OF AUTOCHTHONOUS MALARIA IN THE ATLANTIC FOREST, STATE OF RIO DE JANEIRO, BRAZIL

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In the state of Rio de Janeiro (RJ) malaria transmission was considered eliminated in 1968 but since 1993 some sporadic autochthonous cases with history of having visited native locations of the Atlantic Forest (AF) are been described with no identifiable source of infection. From 2006 to 2014, 51 autochthonous malaria cases were reported, an average of 5.6 cases/year. We describe an outbreak of autochthonous malaria that occurred in 2015 in the AF of RJ state diagnosed in Fiocruz. A FOUR-fold increase over the average of autochthonous malaria cases from RJ, with 25 individuals without history of travelling outside the State being diagnosed with malaria infection. Most male (23/25) aged 7-59 years (median 41y), with clinical presentation characterized by sudden onset of fever, chills, headache, and myalgia. The time from onset of symptoms and diagnosis varied from 3 to 21 days (median 13 days). The fever pattern was daily progressing to a tertian pattern after a median of 10 days. Microscopic examination (thick blood smear) showed low parasitaemia (48 p/mm3 to 1200 p/mm3) with unusual morphological forms of *Plasmodium vivax*. All cases were positive for *P. vivax* by PCR, and this is currently been investigated by genomic sequencing. No patient presented clinical or laboratory complications and all were treated with chloroquine and primaquine, as recommended by Brazilian guidelines. Most (22/25) were inhabitants of RJ capital city, who visited AF areas for leisure or work-related activities in vegetation-dense areas (median 8 days). The median time between probable exposure and onset of symptoms was 24.5 days (range: 17 to 41). No individual had visited well-established malaria endemic areas or had any other risk factor for infection. The presumed areas where the infections occurred are widespread throughout the AF with mountainous topography and very dense native vegetation coverage, a natural habitat for malaria vectors *Anopheles* (Kerteszia), and with circulation of non human primates. This indicates that the transmission can occur, and may reflect the existence of a zoonotic transmission cycle.

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BREAKDOWN IN MALARIA CONTROL IN VENEZUELA - 440% INCREASE IN MALARIA CASES

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Venezuela's health care system is in deep crisis at all levels. Patients in urban and rural areas do not have access to essential drugs and prevention activities have been severely affected. The country is experiencing a breakdown in malaria control and national health authorities stopped reporting malaria epidemiological information in November 2015. The routine weekly malaria reporting system started in the 1960s and plays a key role in the identification of malaria epidemics and it is a useful tool for decision-making at the national malaria program. The objective of this study was to describe the changes in the total number of confirmed malaria cases (officially reported) between 2000-2015 and compared them with the adjusted malaria estimations for the same period. Malaria morbidity data was updated incorporating information from the Venezuelan health system including annual national epidemiological bulletin, the world malaria report 2015, and data on relapses, recrudescences, under reporting and self-treatment rates. Between 2000-2015, Venezuela officially reported 794,531 cases of malaria contrasting with the estimated 1,172,285 malaria cases for the same period. Compared to the baseline in 2000, in 2015 Venezuela had a 350% and 440% increased of reported and estimated malaria cases respectively. This represent approximately 25% of the malaria cases in the Americas. 80% of Venezuelan's malaria morbidity is concentrated in Sifontes Municipality, Bolivar State a complex operating environment with high mobilization, crime rates, illegal mining and lack of security. Malaria interventions are limited in the country: there is not mass/routine distribution of bednets, stock out of antimalarials are common, no malaria rapid test are routinely used and logistical/administrative limitations impedes the implementation of the activities. It is unjustifiable that in a country that has a trillion dollars from oil revenues in the last decade has derailed its health's investment to protect its population from malaria. There is a need to act now to prevent further spread of this malaria epidemic in Venezuela and neighboring countries.

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EFFECTS OF SEASONALITY AND HOST FACTORS ON LONGITUDINAL *PLASMODIUM FALCIPARUM* GAMETOCYTE PREVALENCE IN KENIEROBA, MALI

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In order to build upon recent gains in global malaria control and elimination efforts, it is necessary to enhance our current understanding of the spatiotemporal dynamics of parasite carriage and the human risk factors affecting them in areas with varying transmission patterns and intensities. To achieve this objective, sensitive molecular diagnostic tools have been recently developed and applied in epidemiological surveys of both *Plasmodium falciparum* blood-stage parasites and sexual-stage gametocytes in endemic areas. However, many of these studies are cross-sectional by design and fail to effectively address the longitudinal dynamics of gametocytes that are critical for transmission. In Kenieroba, Mali, where malaria transmission follows a seasonal pattern, we conducted a one-year longitudinal cohort study and used molecular methods to assess the dynamics of both total parasite and gametocyte prevalence among village residents. From June 2013 to May 2014, we followed a cohort of 500 individuals aged 1-65 years that represented the age structure of the village population, and measured both *P. falciparum* parasitemia (PCR) and gametocyte prevalence (stage-specific RT-PCR) in peripheral blood every two weeks. Approximately 80% of *P. falciparum* DNA-positive individuals were found to be gametocyte-positive, regardless of the time of year. In addition to asexual parasite prevalence, host age (peak at 9-16 years) and gender (higher in males) were also significantly associated with longitudinal gametocyte prevalence. Among *P. falciparum*-positive individuals, longitudinal gametocyte prevalence over the one-year period was found to be consistently high (median range: 72-82%) among children aged 16 years or less, and then declined with increasing age. Other host factors (i.e., G6PD genotype, ABO blood type, and sickle-cell trait) showed no significant association with longitudinal gametocyte prevalence. Our findings show that asexual parasite prevalence, and host age and gender are important determinants of longitudinal gametocyte prevalence in a seasonal, high-transmission area of Mali.

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THE EFFECT OF HOLES IN LONG-LASTING INSECTICIDAL NETS ON MALARIA: A CASE-CONTROL STUDY IN MALAWI, 2013

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Long-lasting insecticidal nets (LLINs) are a cornerstone of malaria prevention. Holes develop in LLINs over time, which compromise their physical integrity; however, the effect of holes on the risk of malaria infection is not well known. After a mass LLIN distribution in southern Malawi in 2012 that led to >95% LLIN coverage among children, we conducted one of the first in-depth studies to assess the relationship between LLIN holes and malaria. From March–September 2013, we enrolled febrile children ages 6–59 months who consistently slept under LLINs (every night for two weeks before illness onset) in a case-control study at a clinic. Cases were positive for *Plasmodium* by microscopy, and controls were negative. Digital photographs of participants' LLINs were taken and analyzed using image processing software to measure holes. Total hole area was divided into quartiles and the World Health Organization's (WHO) proportionate Hole Index (pHI) cut-offs: <79 cm^2 (good), 80–789 cm^2 (damaged), and >790 cm^2 (too torn). We compared hole characteristics between case and control LLINs using non-parametric and logistic regression analyses. Of 248 LLINs analyzed, 97 (39%) were from cases. Overall, 86% of LLINs had at least one hole. The median number of holes per net was 9 for case and control LLINs (p=.82). Hole location was divided into roof, upper halves, and lower halves. For both case and control LLINs, the median number of holes in the roof was 0 and in the upper halves was 2. The median number of holes in the lower halves of LLINs was 6 for cases and 7 for controls (p=.63). Median total hole area was 10 cm^2 for controls and 8 cm^2 for cases (p=.10). Multivariate modeling showed no association between pHI or total hole area quartiles and malaria, controlling for child age, caregiver education, socioeconomic status, windows, closed versus open housing eaves, and iron versus thatched roof. LLINs in this study were in relatively good condition one year after an LLIN campaign, which may be why holes were not associated with increased odds of malaria. Future studies should examine associations between LLIN holes and malaria risk in other populations and with more damaged nets.

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INTER-PROVINCIAL DIFFERENCES IN MALARIA CASE MANAGEMENT PRACTICES IN ANGOLA: A CROSS-SECTIONAL HEALTH FACILITY SURVEY

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Malaria accounts for the largest portion of healthcare demand in Angola. A pillar of malaria control in Angola is the appropriate management of malaria illness, including testing of suspect cases with rapid diagnostic tests (RDTs) and treatment of confirmed cases with artemisinin-based combination therapies (ACTs). Periodic systematic evaluations of malaria case management are recommended to measure health facility (HF) readiness and adherence to national case management guidelines. Cross-sectional HF surveys were performed in low-transmission Huambo and high-transmission Uíge Provinces in early 2016. In each province, 45 HFs were randomly selected from among all public HFs. Survey teams performed inventories of malaria commodities and conducted exit interviews and re-examinations, including RDT testing, of a random selection of all patients completing outpatient consultations. Key HF readiness and case management indicators were calculated adjusting for the cluster sampling design. Availability of RDTs on the day of the survey was 71% (54-83) in Huambo and 85% (67-94) in Uíge. At least one formulation of an ACT was available in 83% (66-92) of HFs in Huambo and 79% (61-90) of HFs in Uíge. A total of 590 patients in Huambo and 634 in Uíge were re-examined. Among re-examined patients in Huambo, 8.9% (95% CI: 5-15) were true malaria cases, compared to 32% (26-39) in Uíge. Testing rates of suspect malaria cases in Huambo were 31% (23-39) versus 70% (55-82) in Uíge. Overall, 28% (13-50) of patients with uncomplicated malaria, as determined during the re-examination, were appropriately treated with an ACT with the correct dose in Huambo, compared to 62% (44-77) in Uíge. The results reveal important differences between provinces. Despite similar availability of RDTs and ACTs, testing and treatment rates were significantly lower in Huambo compared to Uíge. A majority of true malaria cases seeking care in HFs in Huambo were not appropriately treated with antimalarials, highlighting the importance of continued training and supervision of healthcare workers in malaria case management, particularly in areas with decreased malaria transmission.

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CLINICAL MALARIA INCIDENCE RATE COLLECTED DURING MALARIA TRANSMISSION BLOCKING VACCINE STUDY IN ADULTS VOLUNTEERS IN BANCOUMANA, MALI

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Malaria control strategies have focused on children under the age of 5 years and pregnant women. However, more studies in West Africa showed that clinical malaria attacks also occur in adults living in areas of high endemicity. The present study was undertaken to investigate the clinical malaria incidence in adults living in malaria endemic area. A total of 120 and 200 volunteers aged from 18 years to 50 years old were enrolled respectively in malaria vaccine study of Pfs25-EPA/Alhydrogel® and of combined Pfs230D1M-EPA/Alhydrogel® and Pfs25-EPA/Alhydrogel® in Bancoumana, Mali. Clinical malaria data were collected from May 2013 to December 2014 and from May 2015 to December 2015 respectively in study cohort of Pfs25-EPA/Alhydrogel® and of combined Pfs230D1M-EPA/Alhydrogel® and Pfs25-EPA/Alhydrogel®. Malaria smear and or rapid diagnostic test (RDT) was performed in case of clinical symptoms to confirm clinical malaria before the treatment initiation. In 2013, from July to December, the incidence rate of clinical malaria during that transmission season period was 0.96 (101/110) episode of malaria per person per season. In 2014, during the same malaria transmission season period, the incidence rate of clinical malaria was 0.87 episode of malaria per person per season (94/107), during that year, the overall incidence rate of clinical malaria was 0.99 (106/107) episode per person per year. In 2015, from July to December, the incidence rate of clinical malaria during that transmission season period was 0.91 (174/191) episode of malaria per person per season. During that year, the overall incidence rate of clinical malaria was 0.96 (185/191) episode per person per year. The incidence of malaria continues to be high and seasonal in Bancoumana in adults population. This malaria burden in adult population needs to be taken into account in malaria control strategies.

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GENETIC POLYMORPHISM OF MEROZOITE SURFACE PROTEIN2 (MSP2) IN *PLASMODIUM FALCIPARUM* ISOLATES FROM PAWE DISTRICT, NORTHWEST ETHIOPIA

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In malaria-endemic regions, *Plasmodium falciparum* infection is characterized by extensive genetic diversity. Describing this diversity provides important information about the local malaria situation. This study was conducted to evaluate the extent of genetic diversity of *P. falciparum* in Pawe, in the northwest Ethiopia. A total of 92 isolates from patients with uncomplicated *P. falciparum* attending Pawe Health Centre was collected from September to December, 2013.Genomic DNA was extracted using Chelex® method and anlysed by length polymorphism following gel electrophoresis of DNA products from nested-PCR of msp2 (block 3) targeting allelic families of FC27 and 3D7/IC. There were 22 different *MSP2* alleles, 11 corresponding to the3D7/ IC/ and 11 to the FC27 allelic family. However, isolates of the 3D7/IC allelic family showed

higher frequency (87%) compared to FC27 (85%). The overall multiplicity of infection was 2.8 (CI 95% 2.55-3.03), seventy-six percent of isolates contained multiple infections. The heterozygosity index was 0.75 for msp2 .There was no statically significant difference in the multiplicity of infection by either age or parasite density. In conclusion, this study showed that the genetic diversity in *P. falciparum* isolates from northwest Ethiopia was high and mainly of multiple infections.

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ROLE OF ALPHA-THALASSEMIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN *PLASMODIUM FALCIPARUM* TRANSMISSION FROM HUMAN TO MOSQUITO

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Large evidence is available showing that human genetic variation affects susceptibility to infectious diseases, but it is unknown whether it also affects the host efficiency to transmit pathogens. We have previously shown that haemoglobin S and C, known to protect from clinical *Plasmodium falciparum* malaria, increase the transmission of the parasite from the human host to the mosquito vector. In this study we evaluated the role of 3.7 alpha deletional thalassemia and glucose-6-phosphate dehydrogenase deficiency, in the ability to infect mosquitoes.To assess the impact of these genetic factors on the ability to infect mosquitoes, we conducted Standard Membrane Feeding Assays on blood samples from 69 children aged 3-15 years from the village of Soumousso, Burkina Faso with known alpha thalassemia and G6PDA- genotypes. A total of 15515 *Anopheles* were dissected on day seven after membrane feeding and oocysts were detected by microscopy in mosquito guts.We found that alpha thalassemia increases both the prevalence and density of *P. falciparum* infection in mosquitoes while G6PDA- increases the prevalence but not the density of infection.These results confirm that human genetics variation affects *P. falciparum* transmission from human to mosquitoes.

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GENETIC DIVERSITY AND COMPLEXITY OF *PLASMODIUM FALCIPARUM* ISOLATES IN NORTH-CENTRAL NIGERIA

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Malaria is a parasitic disease of significant public health importance in Nigeria. Population-specific investigation of the genetic diversity of the parasites is important for effective vaccinological and chemotherapeutic intervention. This study determine the genetic diversity of *Plasmodium falciparum* isolates using two antigenic markers in individuals attending health facilities in Idah and Ibaji Local Government Areas of Kogi State, North-Central, Nigeria. DNA was extracted from finger-prick blood samples collected from *P. falciparum* positive individuals, followed by polymerase chain reaction genotyping which targeted the MSP-1 and MSP-2 allelic families. All the three families of MSP-1 (K1, MAD20 and RO33) and two of MSP-2 (FC27 and 3D7) were observed among the isolates. Prevalence of (MSP-1) K1, MAD20 and RO33 were 70%, 20% and 40% respectively for Idah while prevalence of 40%, 10% and 20% was recorded for MSP-1 (K1, MAD20 and RO33) respectively. Analysis of MSP-2 allelic families revealed prevalence of FC27 family was 40% in Idah and 30% in Ibaji while 3D7 had a prevalence of 20% in both Idah and Ibaji. The frequency of FC27 genotypes was higher than 3D7 in both populations. Multiplicity of Infections (MoI) for MSP-1 was higher in Ibaji (1.30) than Idah (1.05) while MoI with MSP-2 families was lower in Ibaji (2.00) than Idah (2.13). There was no significant difference between the MoI values in Idah and Ibaji ($P > 0.05$). The expected heterozygosity (HE) value was 0.56 for MSP-1 and 0.84 for MSP-2 showing higher diversity in MSP-2. The findings showed a high genetic diversity of *P. falciparum* in the study populations providing population-specific genetic information on the malaria parasites circulating in North-Central Nigeria.

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INFLUENCE OF GENETIC AND EPIGENETIC VARIATIONS ON MALARIA SUSCEPTIBILITY

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Erythrocyte phase of *Plasmodium falciparum* malaria infection results from complex membrane sorting and signaling. Proteins in the erythrocyte membrane lipid rafts regulate membrane sorting and signaling processes in erythrocytes, and hence some of these proteins (Gαs and ß2AR) and their interacting proteins (ADORA2A and GRK5) were expected to influence pathogen entry to erythrocytes. It is also known that entry of malaria parasite in patients induces the synthesis of inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-10. Altered gene expression of cytokines regulated by mutations or epigenetic mechanisms, and cytokines by themselves inducing epigenetic changes may influence malaria pathogenesis. Epigenetic modifications of drug transporters like P-glycoprotein may also influence the chemotherapy for malaria. We hypothesize, that genetic and epigenetic variants in GNAS, ADRB2, ADORA2A, GRK5 and ABCB1 genes may influence the malaria susceptibility. To test the hypothesis, a case - control study of individuals affected by *P. falciparum* malaria versus healthy controls, was performed. Genetic and/or epigenetic variations of the genes were analyzed after PCR-RFLP and direct DNA sequencing. Western blotting was used to access the levels of ABCB1 protein. Genetic association was observed at allele, genotype and haplotypes of SNPs in the genes, and significant DNA methylation differences among cases and controls. Our study provides evidence for the proposed role of studied genes mediated mechanisms in the etiology of malaria susceptibility.

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K13 POLYMORPHISMS IN *PLASMODIUM FALCIPARUM* FROM LOW AND HIGH TRANSMISSION AREAS IN KENYA

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The development of artemisinin resistance in Southeast Asia (SEA) threaten malaria control globally. The recent genetic marker Kelch 13 propeller has proven useful in identifying resistance but only in SEA. Sub-Saharan African (SSA) countries show mutations in this marker but none of the haplotypes are those reported in SEA. Further, none of the observed mutations in SSA exhibit delayed parasite clearance, defined as longer than 3 days following treatment with artemisinin. On the Kenya's coast, a study showed a decelerated responsiveness to ACTs but it was not clear whether it was due to declining immunity or changes in parasite sensitivity. With this realization it is important for SSA countries to identify single nucleotide polymorphisms (SNPs) that would best describe resistance in this part of the world. A total of 380 *Plasmodium falciparum* clinical isolates, collected before 2003 and after the introduction of artemisinin combination therapy, were screened for Kelch 13 propeller mutations. These were collected from regions with different malaria transmission intensities in Kenya which include Kisumu, Kombewa, Marigat, Kericho, Kisii and Malindi. Twenty six pre-ACT isolates screened showed mutation at position 493 (7.7%) and 539 (7.7%). Mutations in both these positions

have been reported in SEA and have shown delayed parasite clearance 3 days post treatment. Mutation at position A578S accounted for 3.8% of the mutations. This mutation has been reported in Bangladesh and SSA and is said to alter protein interactions although not reported to have any delayed parasite clearance following treatment with ACTs. Unique SNPs were also found with D464N accounting for 15% of the mutations. There is an urgent need for continued identification of ACT resistance markers for SSA parasites, in part to ease traditional laborious surveillance efforts and to aid in prompt action in the event of identified ACT resistance.

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GAMETOCYTE CARRIAGE IN A LOW MALARIA TRANSMISSION AREA OF GHANA

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Gametocytes (the sexual stage parasites of *Plasmodium falciparum*) play a major role in malaria transmission in different endemic settings and their identification is important for transmission-blocking interventions. Asymptomatic individuals in malaria endemic areas tend to harbor both microscopic and submicroscopic gametocytes of *P. falciparum* which can infect mosquitoes and contribute to malaria transmission. Knowledge of the population at risk of harboring gametocytes is important in determining the infectious reservoir for transmission-blocking interventions. This cross-sectional study therefore aimed to determine the prevalence of *Plasmodium falciparum* gametocytaemia among a cohort of children and its association with the transmission pattern in a low malaria transmission area of Ghana. A total of 181children within age groups 2-5, 6-10 and 11-15 years took part in this study. Finger prick and venous blood samples were taken from the children and screened for the prevalence of *Plasmodium falciparum* parasites by microscopy, Nested PCR targeting Plasmodium DNA and Real-Time PCR targeting both 18SrRNA gene and transcripts using the gametocyte marker pfs25. Data collected were analyzed using R regression and GraphPad Prism 5. Out of 181 children, 3.9% (7/181) had microscopically confirmed asexual parasites, with no gametocytes found (0/180). Asexual parasite prevalence was 4.4% (2/48), 2.5% (2/80) and 5.8% (3/53) for 2-5, 6-10 and 11-15 years respectively. nPCR gave an overall parasite prevalence of 7.2% (13/181) with prevalences of 1.1% (2/181), 2.2% (4/181) and 3.9% (7/181) for ages 2-5, 6-10 and 11-15 years respectively. qPCR showed 6 out of 13 nPCR positive samples had gametocytes with one sample missing. Within the age groups, individuals carrying gametocytes were 1, 3 and 3 for ages 2-5, 6-10 and 11-15 years respectively. The study has therefore determined the presence of submicroscopic gametocytaemia in the cohort and this is likely to contribute to malaria transmission in the study area.

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GENETIC DIVERSITY AND NATURAL SELECTION AT DOMAIN I OF *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1 NIGERIAN ISOLATES: RELATIONSHIP WITH T-CELL RESPONSES OF MALARIA PATIENTS FROM LAGOS NIGERIA

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The *Plasmodium falciparum* apical membrane antigen I (AMA1) is a leading malaria vaccine candidate antigen. Population genetic analyses of vaccine candidate antigens provide insights into the status and natural dynamics of diversity and evolution in these antigens. T-cell responses mediate immunity against malaria. This study describes the extent of genetic polymorphisms and selection at the hyper-variable domain I of AMA1 among *Plasmodium falciparum* isolates circulating in the Nigerian population and the consequence on T-cell responses of malaria patients. The Domain I of AMA1 gene was amplified in a nested-PCR and sequenced in both directions from 195 P.falciparum isolates collected from microscopically confirmed P.falciparum dry blood spots of patients from the three senatorial districts in Lagos, Nigeria. Pro-inflammatory cytokines were determined by capture ELISA. A total of 74 AMA1 haplotypes were observed among 195 isolates sequenced. Forty-eight of these 74 haplotypes are new and here reported for the first time. The nucleotide diversity (ϖ) was in the order Ajeromi > Ijede > Lekki while the number of haplotypes (H) was highest in Ajeromi with relatively higher transmission. Analysis of the inter-population genetic differentiation showed moderate gene flow and genetic differentiation (Fst range = 0.007-0.037) between two populations. Analysis of the non-synonymous and synonymous mutations, Tajima's D and recombination rates showed evidence of positive selection in the AMA1 antigen with high rates of recombination events while Phylogenetic analysis showed no population-wise clustering. The relationship between genetic diversity in AMA1 and innate immune responses revealed no statistically significant association. In Nigeria, PfAMA1 is under positive natural selection with 48 new haplotypes reported. Genetic diversity level and selection were highest in Ajeromi, the gene flow among these populations were moderate. T-cell components of immunity against malaria are independent of genetic polymorphisms in AMA1. Data reported here will update information needed for the development of effective malaria vaccine.

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GENETIC STRUCTURE OF *PLASMODIUM FALCIPARUM* ISOLATES IN PRE-ARTEMISININ THERAPY ERA OF INDIA

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Population studies conducted on *Plasmodium falciparum* have revealed its success to perpetuate high genetic diversity and this diversity has hampered all attempts to control malaria. Limited information is available about genetic sub-structuring in Indian falciparum populations. We want to evaluate the genetic diversity and population structure prevailed in high and low falciparum prevalent malaria endemic areas, and to predict the geographic origin of Indian falciparum malaria before artemisinin regimen. In this study, samples were collected from six study sites from both low and high falciparum prevalent areas across India. The samples were collected in year 2002-2006, when artemisinin was not introduced as antimalarial treatment in India. Twelve polymorphic microsatellite markers were used to understand the genetic structures of falciparum populations. All the parasite populations were analyzed for genetic diversity, linkage disequilibrium and genetic structure. The measures of genetic diversity revealed all microsatellite loci to be polymorphic and the number of alleles per locus varied from 4 to 14. The mean expected heterozygosity (He) were from 0.376 ± 0.036 and 0.864 ± 0.039, revealing a moderate to high level of genetic diversity at these loci. Evaluation of geographic population structure within and among populations using F-statistic and STRUCTURE analysis revealed genetically distinct groups in accordance with transmission intensity of different geography. Results of this study will provide an insight towards the population structure of falciparum prevailed before artemisinin regimen in India and also provide a basis to study how artemisinin could affect the population structure in Indian falciparum population.

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DOES A SINGLE PERIPHERAL BLOOD SAMPLE FROM A MALARIA-INFECTED INDIVIDUAL CAPTURE ALL PARASITE GENOTYPES PRESENT IN AN INFECTION?

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There is contradicting data about whether or not a single peripheral blood sample accurately captures all parasite genotypes present in a malaria infection. Previous studies have demonstrated a rapid turnover of parasite genotypes during the course of an asymptomatic infection with some genotypes appearing while others disappearing from the peripheral blood. This rapid turnover of genotypes suggests that a peripheral blood sample taken at a single time-point contains only a subset of parasite genotypes present in the entire malaria infection. Parasite genotypes not detected in the peripheral blood are thought sequester in deep tissues precluding their detection in the peripheral blood. However, recent studies have shown that parasites sequestered in deep tissues are genetically identical to those circulating in the peripheral blood. This suggests that a single peripheral blood sample effectively captures all the parasite diversity present in the infection. These discrepant findings may have resulted from the poor resolution of msp1/2 genotyping methods used to determine the genetic composition of infections. To resolve problems associated with msp1/2 measures of infection complexity, we have employed a more-sensitive and less ambiguous 24-SNP Taqman assay to obtain the DNA fingerprint of malaria parasites sampled from adults with asymptomatic malaria over the course of seven consecutive days. We have used this approach to examine whether or not the within-host parasite genetic diversity in asymptomatic individuals remains constant or changes over seven consecutive days. Results from the first set of asymptomatic infections will be presented and discussed at the meeting.

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COMPARISON OF TWO GENOTYPING METHODS FOR DISTINGUISHING RECRUDESCENCE FROM RE-INFECTION IN ANTIMALARIAL DRUG EFFICACY/EFFECTIVENESS TRIALS

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In areas of intense malaria transmission, individuals treated for malaria may encounter new episodes of malaria parasitaemia during the period of follow-up. Without comparing the genetic identity of pre-treatment and post-treatment parasites, it is difficult to resolve whether the recurrence is as a result of treatment failure (recrudescence) or a new infection. Genotyping of the merozoite surface proteins 1 and 2 (msp 1 and 2) is the current gold standard for genotyping infections to correct drug efficacy/effectiveness data. However interpretation of msp1 and msp2 data is often ambiguous and subjective. Therefore, new and better methods for distinguishing recrudescence from re-infection are urgently needed. We compared the performance of the msp1 and msp2 genotyping with a high sensitivity and high resolution 24 single nucleotide polymorphism (SNP) Taqman assay in a cluster-randomized effectiveness trial in an area of high malaria transmission in Malawi. Filter paper samples were collected on day 0 and day 42 of follow-up from children with malaria aged 4-11 (n=106) treated with either artemether-lumefantrine or dihydroartemisinin-piperaquine. Parasite DNA was extracted from pre-treatment (day 0) and post-treatment (day 42) and genotyped using msp1 and msp2 genotyping method and 24-SNP Taqman assay as previously described.The agreement between the two methods was 86%. Discordant results were often due to false positive msp1/2 results. We found a high rate of re-infection with 33% of new episodes of parasitemia detected on day 42 of follow up. Rate of treatment failure based on SNP barcoding of day 0 and d42 filter paper blood samples was 3%. A full comparison of the two genotyping methods for distinguishing re-infections and treatment failure will be presented during the meeting.

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MICROSATELLITE ANALYSIS REVEALS DIFFERENT TRANSMISSION PATTERNS IN THE PERUVIAN AMAZON

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Population genetics studies provides critical information about where and when the infection took place, so imported cases as result of migration can be separated from endogenous cases that indicate the efficacy of the control programs. To address the hypothesis that endogenous transmission is the main mechanism that maintain malaria in rural communities in Peruvian Amazon, 1031 *Plasmodium vivax* positive samples were identified from a population-based cohort by PCR performed every three months from March 2013 to March 2014 in two communities: Cahuide (CAH), community with high mobility by road; and Lupuna (LUP), riverine and isolated community. 390 samples were chosen for microsatellite (MS) genotyping with 9 previously reported MS and 7 new MS. High genetic diversity was observed among communities (He 0.622 ± 0.045) but it was low (0.38 ± 0.043) in LUP in December 2013. Higher proportion of polyclonal infections was found in CAH (19%-36%) in comparison to LUP (5%-24%). AMOVA analysis showed that most of the variance occurs within population (58%) and among communities (37%), suggesting strong population structure within and between communities. Genetic differentiation was high between communities, but low between months in each community (Pairwise FST 0.039), except for LUP in March 2013 which showed moderate differentiation with respect to other months in LUP (0.25), but low differentiation in comparison to CAH (0.09). Analysis of population structure revealed the presence of 4 clusters or subpopulation within these communities (Cluster A, B1, B2 and C).Cluster A was mainly present in March and June 2013 in CAH and only in March 2013 in LUP. Cluster C was predominantly along the follow-up in LUP, except in March 2013. Neighbor joining and burst analysis showed a clonal expansion of these two clusters. Regarding clusters B1 and B2, they are polyphyletic groups that did not maintain fixed over time. In conclusion, results are consistent with an outbreak in Cahuide caused by a clonal expansion of cluster A. In contrast, Lupuna showed endogenous stationary transmission pattern caused by Cluster C that prevails along the seasons.

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POPULATION GENETICS OF *PLASMODIUM VIVAX* IN MICROSCOPIC AND SUB-MICROSCOPIC INFECTIONS IN RIVERINE AND ROAD COMMUNITIES

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Plasmodium vivax population diversity has been reported along time in different settings in the Peruvian Amazon. The aim of this study was to explore the genetic diversity and parasite population structure of *P. vivax* parasites from microscopic and submicroscopic infections in two communities: Cahuide (Km 56 at Iquitos-Nauta road) and Lupuna (accessible only by river), in Loreto. 473 *P. vivax* positive samples were selected from a cohort of 1031 *P. vivax* positive samples based on DNA quantity that will allow Microsatellite (MS) analysis. These samples were collected from March 2013 to March 2014 (168 samples from Cahuide and 305 samples from Lupuna) by monthly active case detection surveys. All samples were confirmed as positive to *P. vivax* by microscopy and/or PCR, DNA was quantified by qPCR. Only 437 samples were used for genotyping using 16 microsatellites. Genotyping was performed and subsequently analyzed by capillary electrophoresis using an ABI PRISM 3100 avant genetic analyzer. Based on microscopy and/or PCR, from the total number of samples, 177 (35%) were classified as submicroscopic and 296 (65%) as microscopic. From genotyped samples, 339 (77%) were classified as monoclonal infections and 98 (29%) were polyclonal infections. Around 361 had an almost-complete allele profile (75% or higher). Our results showed an average genetic diversity (He) of 0.565 for Cahuide and 0.52 for Lupuna, meaning high genetic variability for both communities. A total of 51 haplotypes were found, 21 haplotypes were present in Cahuide and 30 in Lupuna. 2 haplotypes with the highest frequencies where found one in each community. In Cahuide, "haplotype-20" is present only during March and June 2013 in 23 samples; whereas in Lupuna "haplotype-42" is present along year 2013. Interestingly 4 population clusters were found in both communities with no differentiation between microscopic and submicroscopic infections. This data suggests that there is no genetic differences between parasites from microscopic and submicroscopic infections and are stable along time. Population structure of *P. vivax* might be explained by other factors rather than type of infection.

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VALIDATING A SNP-BASED BARCODING TOOL FOR *PLASMODIUM VIVAX* IN PAPUA NEW GUINEA

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Plasmodium vivax is one of the four species of Plasmodium that cause malaria in Papua New Guinea (PNG) and is the dominant species in some areas of PNG. Efforts to reduce and eventually eliminate malaria in PNG will require a combination of different strategies and tools to effectively monitor the parasite. We have validated a field deployable SNP-based barcoding tool for genotyping *P. vivax* parasites in PNG. We selected *P. vivax* field samples with infections containing single parasite clones for validating this new tool. The selection of single clone infections was based on genotyping length polymorphic molecular markers, Pvmsp1F3 and microsatellite MS16. We then performed High Resolution Melting (HRM) analysis on amplicons from established polymerase chain reaction assays. Genotyping was performed on 24 SNPs, which were previously identified amongst a global panel of isolates. These SNPs are spread across all 14 chromosomes of *P. vivax* and are located on putatively neutral sites. Eight of the 24 SNPs had a minor allele frequency of ≥0.15 across three different geographic areas of PNG. These were identified as suitable candidates for genotyping *P. vivax* parasites in PNG. A complete 8-SNP-haplotype was obtained for 42 (44%) of the 96 samples tested. Amongst these there were 33 unique haplotypes identified. The discriminatory power of SNP-based barcoding will be compared with that of the fragment length polymorphic markers which are currently being used to genotype *P. vivax* in PNG. SNP-based barcoding is a field based tool that can be adapted to platforms that perform HRM and with further validation may be useful for studying *P. vivax* population genetics in PNG.

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LONGITUDINAL POOLED DEEP SEQUENCING OF *PLASMODIUM VIVAX* KELCH PROPELLER DOMAIN IN CAMBODIA REVEALS A LACK OF DIRECTIONAL SELECTION

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The emergence of artemisinin resistance among *Plasmodium falciparum* in the Greater Mekong region threatens malaria treatment and control interventions. Mutations in K13 (PF3D7_1343700) provide potential molecular markers of artemisinin resistance in *P. falciparum*. The aim of this study was to survey the sympatric species, *P. vivax*, in Cambodia, for mutations in the orthologous gene (K12, PVX_083080) that might similarly confer artemisinin resistance. 359 clinical isolates collected in western and northern Cambodia from 2009-2013 were organized into eleven pools by province and year. The propeller domain of PVX_083080, 2139 bp in length, was amplified using PCR. Amplification products were submitted to the Ion Torrent Personal Genome Machine®. Single nucleotide polymorphisms (SNPs) were identified using a C++ module and a pileup approach with filters for mapping quality, base quality, read length, and strand bias. In total, 3,898,057 reads from *P. vivax* pools and 1,005,303 reads from sequencing controls were obtained and analyzed for SNPs. Control sequences demonstrated no false-positive SNPs at the quality cut-offs used. In addition, simulations validated the C++ module for SNP detection to 0.5% frequency within each pool. Among the eleven pools, we found 23 SNPs across twelve codons in the kelch propeller region. Twelve of the SNPs produced nonsynonymous mutations, none of which were maintained year-to-year. Two synonymous mutations persisted over multiple time-points. Five mutations were shared between parasite populations, of which, one mutation (V552I) has been previously reported in northeastern Cambodia. However, none of our detected SNPs produced orthologous artemisinin-resistance conferring *P. vivax* mutations and did not persist within the populations, which suggests a lack of directional selection on the K12 propeller region attributable to artemisinin drug pressures. Next-generation sequencing described several SNPs that were not described previously by traditional surveillance techniques, providing a timely way to conduct in-depth molecular surveillance for early artemisinin resistance detection.

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EVOLUTION OF SOLUBLE HLA-G LEVELS DURING PREGNANCY AND INFANCY IN A BENINESE POPULATION EXPOSED TO MALARIA INFECTION

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Human Leucocyte Antigen-G is a non-classical HLA class I molecule firstly described on the surface of extravillous cytotrophoblast cells at foeto-maternal interface. HLA-G binds its inhibitory receptors present on the surface of immune cells (monocytes, NK, T,B and dendritic cells) modulating host's immune response.These immunosuppressive properties of HLA-G are crucial and benefic during pregnancy where HLA-G plays a crucial role in maternal-fetal tolerance. There are known associations between high levels of circulating soluble HLA-G (sHLA-G) and either parasite or viral infections (HIV,cytomegalovirus)and it has been suggested that the induction of sHLA-G expression could be a mechanism via which infectious agents subvert host immune defence. To explore more precisely interactions between soluble HLA-G and malaria,latent class analysis was used to test whether distinct sub-populations of children, each with distinctive soluble HLA-G evolutions may suggest the existence of groups presenting variable malaria susceptibility. This study was conducted in Benin from 2010 to 2013 and 165 children were followed from birth to12 months and soluble HLA-G was quantified by Elisa method.Three groups of children were identified: one with consistently low levels of soluble HLA-G during follow-up, a second with very high levels and a last intermediate group. In all groups, low birth weight, malaria infection and high exposure to malaria transmission were associated with high level of soluble HLA-G.Placental malaria was not. Presence of soluble HLA-G in cord blood increased the probability of belonging to the highest trajectory. These results, together with previous ones,confirm the importante role of HLA-G in the individual susceptibility to malaria. Assaying soluble HLA-G at birth could be a good indicator of newborns more fragile and at risk of infections during childhood.

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MEMORY T CELLS METABOLISM DURING CHRONIC MALARIA INFECTION

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Malaria infection kills up to 0.85 million people each year. The first generation of vaccine does not generate long-lived protection. We have shown, in *Plasmodium chabaudi* infection that CD4 effector memory T cells generated protect from parasitemia and pathology. However, the mechanisms underlying development and maintenance of this long-lived protective memory T cells (Tmem) are not well understood. Recent findings have highlighted the importance of cellular metabolism in Tmem generation. Specifically, fatty acid oxidation (FAO) has been associated with CD8 Tmem development in acute infection. Substrates for FAO in CD8 Tmem are generated through the fatty acid synthesis (FAS) pathway. However, it's not clear whether FAS pathway controls Tmem differentiation or survival. Using transcriptomic analysis, we found upregulation of FAS genes in Tmem compared to effector (Teff) in malaria-specific CD4 T cells. Interestingly, blockade of FAS pathway *in vivo* using TOFA (*Acc1-specific*), impairs Tmem development. To determine when FAS is required for Tmem differentiation, *P. chabaudi*- infected mice were treated with TOFA at the priming or contraction phase of the immune response. Preventing fatty acid synthesis during priming inhibits memory formation and reduces parasitemia. Using stable isotope tracer, memory T cells show high phospholipids synthesis. Together these data suggest that an early shift to FAS is important for CD4 Tmem differentiation and may prove crucial to development of malaria vaccine.

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IL-15 COMPLEX-STIMULATED NK CELLS PROTECT MICE FROM CEREBRAL MALARIA

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To date, no effective adjunctive therapies exist for severe malaria. *Plasmodium falciparum* is the main cause of severe malaria in humans and accounts for about 600,000 deaths per year, mainly in children in sub-Saharan Africa. Cerebral malaria (CM) is one of the most lethal complications of severe malaria. Infection of susceptible mouse strains such as C57BL/6 with *Plasmodium berghei* ANKA (PbA) induces a fatal neurological syndrome from 6-10 days post-infection (dpi). We found that prophylactic or therapeutic treatment of C57BL/6 mice with interleukin (IL)-15 complexes (IL-15C; IL-15 bound to an IL-15Rα-Fc fusion protein) prevented the development of PbA-induced CM. IL-15C treatment stimulates Natural Killer (NK) and CD8 T cells. Interestingly, adoptive transfer of IL-15C-stimulated NK cells, not CD8 T cells, prevented CM. Similar complexes formed with IL-2 (IL-2C; IL-2 bound to the anti-IL-2 S4B6 antibody) also causes robust expansion and activation of NK cells, but NK cells from mice treated with IL-2C failed to protect against CM. Comparative RNAseq analysis of IL-15C and IL-2C-treated NK cells identified novel gene expression patterns, demonstrating previously unappreciated differences between these cytokine complex signaling cascades in NK cells. Interestingly, IL-15C treatment resulted in reduced CD8 T cell activation in the brain at 6 dpi and reduced blood brain barrier breakdown, suggesting that IL-15C-stimulated NK cells limit the CD8 T cell-mediated pathology in CM. Indeed, a large subset of NK cells in the spleen, blood, and brain of IL-15C-treated, but not IL-2C-treated, mice produced the immunoregulatory cytokine IL-10 on day 3 pi. These data indicate that NK cells - which are typically involved in promoting inflammatory responses - can restrain damaging immune responses. A mechanistic understanding of CM pathogenesis and the process of cytokine complex perturbation will provide an important foundation for the identification of new therapeutic targets and aid in the development of adjunctive therapies for treating severe malaria.

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THE ROLE OF INFLAMMATION AND MICROVASCULAR DAMAGE/REPAIR IN THE PATHOGENESIS OF CEREBRAL MALARIA

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Cerebral Malaria (CM), a severe form of malaria, caused by *Plasmodium falciparum* remains a major cause of morbidity and mortality. Currently, there is no available test to predict potential CM patients, as well as mortality or recovery from the syndrome. The disease results from a combination of vascular and inflammatory immune system dysfunction. Triggering receptor expressed on myeloid cells 1 (TREM-1) has been shown to potentiate inflammatory response. A recent preliminary study has

shown that there is an increase in soluble TREM-1 production in CM as compared to uncomplicated malaria (UM). Another study in our lab has shown that there is lower levels of endothelial progenitor cells (EPC) in CM children as compared to UM and Healthy controls (HC). Based on this result, it could be suggested that there could be an association between inflammation and microvascular damage/repair in the pathogenesis of cerebral malaria. To study this hypothesis, children between the ages of 2-12 years who are either CM, UM or HC have been recruited into the study. Samples were taken at three or four time points i.e. Day 0, (Recovery-for only CM), Day 7 and Day14. TREM-1 data and EPC data would be correlated to give a better insight into cerebral malaria pathogenesis. Findings from this study could be employed in the diagnosis of CM.

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CLINICAL DEVELOPMENT OF A VAR2CSA-BASED PLACENTAL MALARIA VACCINE PLACMALVAC: QUANTIFYING VACCINE ANTIGEN-SPECIFIC MEMORY B & T CELL ACTIVITY IN BENINESE PRIMIGRAVIDAE

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Pregnancy associated malaria (PAM) is a major public health problem associated with poor pregnancy outcomes that commonly include maternal anemia and fetal growth alterations, whilst neonatal and infant health can also be affected. A malaria vaccine that targets the pre-erythrocytic stages of the parasite will not prevent the consequences of PAM. The identification of the parasite antigen VAR2CSA that is implicated in the pathophysiology of PAM has led to the development of a candidate vaccine by an EU-funded consortium (PlacMalVac project: German, Danish, French and Beninese Partners). The vaccine is currently under Phase I trial in Germany and Benin. As part of the PlacMalVac project, we quantified B and T cell memory responses to the VAR2CSA sub-unit vaccine candidate in a cohort of pregnant primigravid Beninese who were followed up throughout pregnancy. Clinical and parasitological data were collected every month from 37 primigravid women recruited at the beginning of their pregnancies and followed through to delivery. Mononuclear cells from peripheral blood collected on 4 occasions (first and fifth month of pregnancy, at delivery and 6 months post-delivery) were isolated and cryopreserved under liquid nitrogen. The concentrations of the cytokines IL-5, IL-6, IL-10, IL-13, IFN-γ and TNF-α produced in response to the vaccine antigen, to PPD and to PHA were quantified in supernatants of stimulated cells using cytometric bead array. The frequencies of vaccine antigen-specific antibody-secreting memory B cells were evaluated in the same cell samples using standard ELISPOT assays. Preliminary analysis shows that the profile of vaccine-specific B cell populations increased as a function of women's *Plasmodium falciparum* infection histories, whilst tetanus toxoid-specific B cell frequencies increased following tetanus vaccine boosts administered according to national guidelines. Multivariate analyses are under way to illustrate in detail the effects of *P. falciparum* infections during first pregnancies on the establishment of cellular immunological responses to the vaccine antigen.

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THE INFLUENCE OF INHIBITORY MOLECULES ON TREG CELLS DURING *PLASMODIUM VIVAX* MALARIA

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Malaria is still considered a major helath problem worldwide, and the *Plasmodium vivax* is the most spread causative agent, with 80% of incidence in Brazil. The balance between pro- and anti-inflammatory responses is essential to limit immune response-mediated pathology and regulatory T cells (Treg) probably play an important role in this process. Recently our group demonstrated that the expression of inhibitory receptors on T cells regulates cytokines production by *P. vivax*-specific cells. The expression of one of these inhibitory receptors, the programmed death- 1 (PD-1), negatively regulates Treg function in patients chronically infected with HCV. Since the function of *P. vivax*-specific T cells is impaired due to inhibitory receptors expression, our goal is to assess the expression of these receptors on Treg upon malaria infection and to evaluate their function. Our hypothesis is that the increased expression of inhibitory receptors on Treg during *P. vivax* infection, affects the functions of these cells in regulating inflammatory responses. Peripheral blood mononuclear cells were collected from *P. vivax*–infected patients and from the same individuals after treatment, in Porto Velho-RO. Leukocytes were analyzed by flow cytometry. Our data show that *P. vivax* infection triggers an increase in the frequency of Treg cells and in the frequency of cytotoxic T lymphocyte attenuator (CTLA-4) and PD-1 expressing Treg. The expression of CTLA-4 and PD-1 on Treg was correlated with the bilirubins serum levels. Importantly, PD-1$^+$ Treg express lower levels of Forkhead Box 3 (FoxP3) than PD-1$^-$ Treg when analyzed *ex vivo* or after culture. Moreover, PD-1$^+$ Treg become able to produce IFN-γ. All together, our results indicate that malaria infection triggers the expression of PD-1 and decreases the expression of FoxP3 in Treg, phenomenum that could affect its regulatory functions.

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ANTIBODIES TO *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN-1 AND CIRCUMSPOROZOITE PROTEIN ARE ASSOCIATED WITH PROTECTION FROM HOSPITALIZATION AFTER SEVERE MALARIA DISEASE

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Severe malaria is a leading cause of morbidity and mortality in children. We hypothesize malaria-specific antibodies are markers of exposure and immunity; higher antibody levels will protect children against subsequent hospital sick visits and admission. A prospective cohort study was conducted at Mulago Hospital in Kampala, Uganda. Children between 18 months and 12 years with severe malaria were enrolled: cerebral malaria (CM, n=221), severe malarial anemia (SMA, n=198); and age-matched community controls (CC, n=170) and followed for one year. At enrollment, serum samples were collected and assessed for IgG antibody levels to apical membrane antigen-1 (AMA-1), circumsporozoite protein (CSP), glutamate rich protein (GLURP) and merozoite surface proteins-1 (MSP-1) using a multiplex assay. Children with SMA were the youngest, 33.5 months 41.0 months (CM), and 46.3 months (CC). Children with CM and SMA had significantly higher antibody levels for all antigens. Children with CM had higher antibody levels against malaria-specific antigens than children with SMA or CC, $p<0.05$ for all comparisons. The rate of returning sick visits for clinical malaria in the year following enrollment was 19.5% (n=43) for children with CM, 23.2% (n=46) for children with SMA, and 15.3% n=26 for CC. Higher antibody levels to AMA-1 were associated with protection from clinical malaria in CM and CC, (odds ratio

(OR), 95% confidence interval (CI): CM, 0.97(0.95, 1.00), p=0.05; CC, 0.97(0.95, 1.00), p=0.05). Following adjustment for age, the antibody levels against AMA-1, CSP and MSP-1were not protective against clinical malaria for all comparisons, (OR) 95% (CI) 0.99, (0.92, 1.06), P=0.71; 1.04, (0.98, 1.00), p, 0.17; 0.94, (0.88, 1.00), P=0.06), respectively. Despite having higher exposure to malaria, children with severe malaria were not protected from clinical malaria in the year following admission. These data suggest that acquisition of IgG antibodies against the antigens we measured may not be sufficient to protect against clinical malaria. Further studies are needed to look at IgG subclass responses in this cohort.

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ANTIBODY DEPENDENT CELLULAR INHIBITION IS ASSOCIATED WITH PROTECTION AGAINST FEBRILE MALARIA IN A LONGITUDINAL COHORT STUDY INVOLVING GHANAIAN CHILDREN

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The antibody dependent respiratory burst and opsonic phagocytosis assays have been associated with protection against malaria; however, other mechanisms may also be involved. The antibody dependent cellular inhibition (ADCI) assay is yet to be correlated with protection in longitudinal cohort studies (LCS). We investigated the relationship between ADCI activity of immunoglobulin G prior to malaria season and risk of malaria in a LCS involving 98 Ghanaian children. Purified IgG was tested in ADCI assay and in schizont extract Enzyme-Linked Immunosorbent Assay. Antibody-dependent cellular inhibition activity increases with age and high ADCI (75% SGI) activity was significantly associated with protection against malaria. The importance of IgG3 in the ADCI mechanism was also substantiated. In conclusion, findings here suggest a potential usefulness of the ADCI assay as a correlate of protection to guide malaria vaccine studies.

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THE PRESENCE, PERSISTENCE AND FUNCTIONAL PROPERTIES OF DUFFY BINDING PROTEIN II ANTIBODIES ARE INFLUENCED BY HLA CLASS II ALLELIC VARIATIONS

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Plasmodium vivax infects human reticulocytes through the interaction between the Duffy binding protein (region II, DBPII) and its cognate receptor on reticulocytes, the Duffy antigen/receptor for chemokines (DARC). A high proportion of individuals naturally exposed to P.vivax fails to develop antibodies that inhibit the DBPII-DARC interaction, and genetic factors that modulate humoral immune response are poorly characterized. Here, we investigate if DBPII responsiveness could be HLA class II-linked. A community-based open cohort study was carried-out in a community of the Brazilian Amazon region, in which 336 non-related volunteers were genotyped for HLA class II (DRB1, DQA1 and DQB1 loci), and their DBPII immune responses were followed-up (baseline, 6 and 12 months) by conventional serology (DBPII-IgG antibodies) and functional assays (DBPII-Binding Inhibition Antibodies, BIAbs). In silico analyzes evaluated the relative binding affinities of DBPII peptides for class II molecules associated with distinct immune outcomes. After 12-month follow-up, the results demonstrated an increased susceptibility of DRB1*13:01 carries to develop and sustain their DBPII-IgG antibody response, and strengthen as persistent non-responder individuals harboring the haplotype DRB1*14:02-DQA1*05:03-DQB1*03:01. The HLA class II polymorphisms also influenced the functional proprieties of DBPII antibodies, with three alleles (DRB1*07:01 DQA1*02:01 DQB1*02:02) that comprise a single haplotype linked with the presence and persistence of the BIAbs response. Finally, quantitative prediction of DBPII-HLA class II binding affinity demonstrated that the nonresponse to DBPII was not due to failure of immune epitopes to bind HLA class II molecules. In conclusion, the current study confirms the heritability of DBPII antibody response, with genetic variation on HLA class II influencing in both the development and persistence of IgG antibody responses. Further studies on knowledge of the binding affinities of DBPII peptides for class II molecules linked with antibody responses might be useful for vaccine development.

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UNCOMPLICATED MALARIA CHILDREN AND ADULTS WITH IN MALARIA HYPERENDEMIC AREA OF BURKINA FASO TREATMENT AND ANTIBODIES PRODUCTION

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Artemisinin-based Combination Therapies (ACTs) are the first line drug for the treatment of uncomplicated malaria in most endemic countries. They quickly clear the parasitaemia and reduce fever. In animal model, it has been found that artemisinin derivatives have an immunosuppressive effect. In the present study we assessed the effect of ACT on malaria antigens specific antibodies in population living in malaria hyperendemic area and repeatedly having uncomplicated malaria.In 2013, patients presenting with uncomplicated malaria were recruited and allocated to receive ACTs and follow up to 2 years. Antibodies titer against three P.falciparum blood stage antigens (MSP3, GLURP R0, GLURP R2) were measured by ELISA before and twenty eight days after treatment and during subsequent uncomplicated malaria episodes.In total 478 volunteers were recruited for antibody measurement. Antibody levels were always high Twenty eight days after the initiation of the treatment for all tested antigens but not significative. IgG titer measurement for MSP3 antigen show a trend between D0 and D28 with respectively 7.52 [1.5 13.6] AU and 7.85[2.5 13.1] AU. Subsequent malaria episodes also appear to have a boosting effect on antibody responses.Concomitant parasitaemia initiate and boost immunological responses in population naturally exposed to malaria and Artemisinin-based Combination Therapies seem not to have immunosuppressive effect.

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CELLULAR IMMUNE RESPONSES FOLLOWING CONTROLLED HUMAN MALARIA INFECTIONS BY DIRECT VENOUS INOCULATION OF CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES IN MALARIA-NAÏVE, MALARIA-IMMUNIZED AND SEMI-IMMUNE AFRICAN INDIVIDUALS

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Controlled human malaria infection (CHMI) trials represent an important tool to test vaccine and drug efficacy against *Plasmodium falciparum* malaria, as well as to study the immune response of the human host under controlled conditions. We studied B and T cell responses in volunteers with three different malaria-immunology backgrounds from CHMI trials performed in Europe and Africa. All volunteers received a direct venous inoculation (DVI) of 3,200 cryopreserved *P. falciparum* sporozoites (PfSPZ, Sanaria® PfSPZ Challenge), with cellular studies performed at baseline (prior to challenge), and at different time points following challenge. Trials: 1. Malaria-naïve individuals: Barcelona, Spain; 2. Malaria-immunized individuals: Tübingen, Germany, volunteers were challenged 10 weeks after being immunized with PfSPZ Challenge by DVI or placebo both under chloroquine treatment (Sanaria® PfSPZ-CVac); 3. Lambaréné, Gabon, semi-immune Gabonese volunteers, including both sickle cell trait (AS) and no sickle cell trait (AA) individuals. The study included volunteers who developed patent parasitemia and also those who remained protected following challenge. B cell phenotyping was performed in all samples *ex vivo* by flow cytometry (FACS) with a panel of 11 markers aimed at analysing different B cells subsets including classical, atypical and marginal zone-like memory B cells. T cellular studies were performed after *in vitro* stimulation with NF54 *P. falciparum* infected red blood cells (all samples) and PfSPZ (only a subset of samples), with a cellular panel of 11 markers aimed at analysing T regulatory cells, γδT cells, cytotoxicity markers (CD107a) and cytokine production (IL-1, IL-10, INFγ). These studies provide relevant information on/about the immune response at B and T cell level in individuals with different malaria-exposure and immunology backgrounds that can/may/should guide the study of correlates of protection in malaria-vaccine trials involving naïve and semi-immune subjects.

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HIGH TOTAL IGG LEVELS AND IGG1 SUBCLASS AGAINST MSP10 PROTEIN ARE ASSOCIATED TO PROTECTION IN ASYMPTOMATIC SERA FROM *PLASMODIUM FALCIPARUM* INFECTED PATIENTS FROM THE PERUVIAN AMAZON REGION

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A previous study conducted by our group demonstrated that recombinant protein MSP10 was highly reactive in sera from symptomatic and asymptomatic patients infected with *Plasmodium falciparum* from a low transmission setting in the Peruvian Amazon region. The aim of this study was to evaluated the humoral response in sera from 122 symptomatic (Sym), 21 asymptomatic (Asym) patients infected with *P. falciparum* and 20 controls negative (Ctr) against recombinant MSP10 (rMSP10) and to compare total IgG and subclass profiles (IgG1, IgG2 and IgG3) in naturally exposed individuals living in this region by ELISA. Total IgG responses, calculated as optical density (OD), were significantly higher in Asym vs. Sym and both groups had a higher OD levels compared to Ctrl. Likewise, IgG1 subclass showed a significant difference between the 3 groups with higher OD mean level in Asym followed by Sym. IgG2 showed significant differences between Asym vs. Control and Sym vs. Control. Nevertheless, IgG3 only had basal levels and a significant difference between Asym vs. Ctrl and showed the lowest OD levels. No negative correlation was found between parasitemia and humoral response in any of the groups.The results here demonstrated that rMSP10 was able to induce a differential response in IgG1 levels between Sym and Asym being higher in the last group showing association to clinical protection against this antigen. It was also found that total IgG levels were high in both Asym and Sym patients in comparison to Ctrl confirming our previous results. Interestingly, this preliminary study shows rMSP10 as a potential antigen for the identification of asymptomatic individuals which are known to represent an infectious reservoir in malaria endemic areas. Definitely a larger sample number would be needed to ascertain this.

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IS TIMING OF *IN UTERO* EXPOSURE KEY IN PREVENTING FETAL PRIMING?

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In malaria endemic regions, pregnant women are at increased risk for malaria. Prenatal exposure to malaria parasites or their soluble antigens modifies subsequent immune responses and susceptibility to malaria infection. Timing of *in utero* exposure may be the key in whether immune imprinting occurs. In this study we examined whether the timing of exposure and early detection and treatment of malaria in mothers can prevent or limit fetal priming and the resultant phenotypes. 300 pregnant Kenyan women were enrolled in the study. Mothers received malaria prophylaxis during the antenatal care (ANC) visits and were treated if found positive for malaria during pregnancy. Malaria was diagnosed by blood smear and PCR. We examined T cell immunity to *Plasmodium falciparum* merozoite surface protein-1 (MSP1-42) and the peptides to MSP1-42 in cord blood lymphocytes (CBL) and assayed for lymphocyte proliferation and used Bioplex to detect IL-2, IFN-γ, IL-13, IL-5, IL-10 and TNF-α. Newborns were categorized as: i) malaria +ve at ANC visits and delivery (n=21) ii) malaria +ve at ANC visits and -ve at delivery (n=195) iii) malaria -ve at ANC visits and delivery (n=84). Preliminary analysis was done

using one way ANOVA with Dunnetts multiple comparisons to compare the 3 groups. Data, though not statistically significant, shows a reduced proliferation to malaria-antigens in offspring of women positive for malaria at delivery as compared to the malaria negative. Th2 response and IL-10 was greater in offspring of mothers with malaria at ANC and delivery (mean IL-13 273.9 pg/ml vs 2.86 pg/ml; IL-5 20.56 pg/ml vs 3.09 pg/ml and IL-10 27.5 pg/ml vs 1.03 pg/ml); though data was not statistically significant. These preliminary results show a high cellular immune response to malarial Ags in CBL isolated from neonates whose mothers were exposed to malaria later in pregnancy as compared to those exposed early into pregnancy. Further testing and analysis is ongoing to determine the implications of early detection and treatment of antenatal malaria on neonates' immunity to malaria.

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PHAGOCYTIC FUNCTION OF MONOCYTE SUBSETS DURING ACUTE UNCOMPLICATED MALARIA IN KENYAN CHILDREN

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Monocytes play an important role in innate and adaptive immunity to malaria. Human blood monocytes are classified into 3 subsets according to levels of CD14 and CD16 expression (classical $CD14^{hi}CD16^{-}$, intermediate $CD14^{hi}CD16^{+}$, and nonclassical $CD14^{lo}CD16^{+}$). The functional roles of the subsets during malaria infection are still being described. Cryopreserved peripheral blood mononuclear cells (PBMC) were obtained from 9 children in western Kenya at presentation with acute uncomplicated malaria and 6 weeks following treatment. Phagocytic activity was determined using assays in which CFSE-labeled Pf-infected erythrocytes (IE) were opsonized with heat-inactivated plasma (pooled Kenyan adult (KA) plasma as a positive control, or malaria-naïve North American (NAM) plasma as a negative control). After incubation of IE with PBMC, the cells were stained with fluorescently-labeled anti-CD14 and anti-CD16 antibodies and subjected to flow cytometry. The percentage of monocytes within a population that had phagocytosed IE was calculated according the the CFSE signal. Opsonic phagocytic activity of classical monocytes and all monocyte subsets combined was decreased during acute malaria compared to 6 week recovery (classical subset median values 20.8% vs. 28.9%, $p < 0.01$; all monocytes 25.5% vs. 43.8%, $p < 0.01$). Phagocytic activity of the monocyte subsets in the presence of NAM-opsonized IE did not differ between acute and recovery. Intermediate and nonclassical monocytes displayed greater phagocytic activity compared to classical monocytes in the presence of NAM-opsonized IE (9.2% and 7.3% vs. 4.5%, respectively, p values < 0.01) as well as KA-opsonized IE (52.4% and 62.6% vs. 27.8%, respectively, p values < 0.01). These data indicate that opsonic phagocytic function of monocytes is decreased during acute malaria compared to 6 weeks following treatment. Compared to the classical subset, intermediate and nonclassical monocytes were more efficient at phagocytosing IE.

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CD68 REGULATES PARASITE DENSITY OF *PLASMODIUM YOELII* 17XNL MURINE MALARIA

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CD68 is a highly glycosylated transmembrane protein found on macrophages and other immune cells. Although CD68 is a well-known marker for macrophages, its function is poorly understood. Recently, this molecule has been identified as a candidate receptor for *Plasmodium* sporozoite invasion of Kupffer cells– the specialized macrophages that line the sinusoids in the liver. In this study, we measured the effect of CD68 on the erythrocytic stage of *Plasmodium* murine malarias using mice deficient for the *CD68* gene. Loss of CD68 had no effect on susceptibility to experimental cerebral malaria or on parasite burden during lethal *Plasmodium berghei* ANKA infection. However, absence of CD68 resulted in a two-fold increase in parasite density at peak parasitemia (day 10 post-infection) during non-lethal *P. yoelii* 17XNL (*Py* 17XNL) infection. To better understand the immune mechanism of CD68-mediated control of *Py* 17XNL parasitemia, we compared the major immune cell subsets by flow cytometry and measured serum cytokine and antibody levels in wildtype verses mice lacking the *CD68* gene over the course of *Py* 17XNL infection. Significant differences were observed in the number of macrophages, natural killer cells, and B cells expanded in the spleen. In addition, production of the IL-12p70, IL-10, MCP1, MIP1β, G-CSF, and RANTES cytokines were significantly altered in the absence of CD68. Lastly, there was a dramatic difference in the quantity of IgG in the serum of WT verses CD68 knockout mice, suggesting that CD68 may alter the B cell response during *Py* 17XNL malaria. Studies are underway to determine the role of CD68 in the differentiation of macrophages into the M1 and M2 subsets, and to dissect the mechanism of CD68-mediated regulation of B cell immunity by *in vivo* depletion experiments. The results of these studies will be useful in discerning the function of CD68 and in understanding the requirements for immune protection to malaria.

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TACI CONTRIBUTES TO *PLASMODIUM YOELII* HOST RESISTANCE BY CONTROLLING THE KINETICS OF TFH AND GC FORMATION AND ANTIBODY DEVELOPMENT

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The transmembrane activator and calcium-modulator and cyclophilin ligand receptor (TACI) is involved in B-cell survival, antibody isotype switching and plasma cell generation. TACI expression is severely impaired in murine and human newborns as compared to adults. We challenged TACI knock-out (KO) mice with *P. yoelii* (Py) NL in order to evaluate the role of TACI in response to malaria infection. We found that the parasitemia levels were significantly elevated in TACI KO-mice (61.8% at day 18) compared to C57BL-6 WT-mice (11.1% at day 11), and that parasite clearance was substantially delayed in the TACI KO (27 days) mice relative to WT controls (18 days). We also determined that TACI KO-mice have a delay in anti-Py NL IgG-antibody production when compared with the WT-mice. Since the interaction of T follicular helper (Tfh) cells with antigen specific B cells in the germinal center (GC) is essential for the generation of antibody responses against T cell-dependent antigens, we measured the formation of Tfh and GC in the spleens of Py NL infected mice. Interestingly, we determined that while Tfh cell numbers were elevated on day 10 in both the strains, WT mice Tfh cell numbers sharply declined by day 15 as the number of Tfh cells in TACI KO-mice remained high. Coinciding with parasite clearance kinetics, TACI KO Tfh cells declined to baseline values on day 25. Similar to the Tfh cells, GC B cells remained high in TACI KO-mice after their decline on day 15 in WT mice. Finally, we detected elevated serum levels of the TACI ligand B cell-activating factor belonging to the TNF family (BAFF) in infected TACI KO mice as compared to the WT mice. Conclusion: Susceptibility of TACI deficient mice to Py NL infection appears to be based on altered Tfh and GC kinetics, which in turn results in delayed antibody development.

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IMMUNOLOGICAL EFFECT OF SEASONAL MALARIA CHEMOPREVENTION (SMC) WITH SULFADOXINE-PYRIMETHAMINE (SP) AND AMODIAQUINE (AQ) IN CHILDREN UNDER 10 YEARS IN THE SOUTHEASTERN PART OF SENEGAL

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In developing countries, malaria is still a major public health problem and children are the most affected individuals. In order to strengthen malaria control, Seasonal Malaria Chemoprevention (SMC) has been developed. This strategy is very effective in preventing malaria clinical episodes but its effect on children's immunity is not well documented. This study aimed to assess the immunological effects of SMC among children under 10 years living in the southern part of Senegal (Velingara). The study was nested in a cluster randomized trial assessing the impact of SMC with a single dose of Sulphadoxine-Pyrimethamine (SP) and 3 doses of Amodiaquine (AQ). Two cross-sectional surveys were carried out at baseline (October 2010) and a year after intervention (September 2011). Thick and thin blood smears were used to assess malaria prevalence. Blood was collected on filter paper for serological measurement by ELISA to measure IgG anti-MSP1_42 and anti-AMA1. Logistic regression analysis was performed to assess factors associated with the production of antibodies. A total number of 1611 children under 10 were included (866 children in 2010 and 745 children in 2011). Malaria prevalence was 10.39% in 2010 and 5.03% in 2011. The seroprevalence of anti-MSP1_42 anti-AMA1 antibodies was higher in 2010 compared to 2011 providing a significant reduction of IgG production at 11.4 AU (95%CI [8.3-14.4]) for MSP1_42 and 7.2 AU (95%CI [4.5-9.9]) for AMA1. Seroprevalence increased with age and *Plasmodium falciparum* carriage while it decreased according to the area and the study period. In conclusion, SMC is an effective strategy for malaria prevention in children under 10 years. The strategy can as well induce a decrease of IgG anti-AMA1 and anti-MSP1_42 which are associated with the protectiion against malaria. Consequently this strategy needs to be renewed each year in areas where malaria is highly seasonal to avoid a resurgence of malaria, while promoting the use of other antimalarial interventions.

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MALARIA INTERVENTION SCALE-UP IN AFRICA: STATISTICAL EFFECTIVENESS PREDICTIONS FOR HEALTH PROGRAM PLANNING TOOLS, BASED ON DYNAMIC TRANSMISSION MODELLING

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Malaria prevention and treatment needs to expand, and national strategies and budget allocations be evidence-based. We statistically summarized dynamically simulated relations between intervention coverage scale-up and impact, to inform a malaria module in the Spectrum program planning tool. The dynamic transmission model OpenMalaria was used to simulate health impacts of insecticide-treated net usage (ITNs), indoor residual spraying (IRS), management of uncomplicated malaria cases (CM) and seasonal malaria chemoprophylaxis (SMC) over a 10-year horizon, for a range of African settings with stable endemic falciparum malaria. ITN effectiveness was parameterized by fitting to estimates from Cochrane review of ITN trials. Generalized linear regression models (GLMs) were used to summarize impact patterns in the simulations. GLMs explained 94-97% of variation in simulated post-intervention parasite prevalence (three age groups, three 3-year horizons); 86-97% for case incidence and 74-95% for malaria mortality, which was most stochastic. For a given effective population coverages, CM and ITNs were predicted to avert most infections, cases and deaths, with lower impacts for IRS. Impact of SMC was limited to young children reached. Proportional impacts were similar across ages, larger at lower endemicity, and (except for SMC) largest in low-endemic settings with low seasonality. Vector control and CM, by reducing endemicity and immunity, entailed a partial rebound in malaria mortality among over 5 year olds from around 7 years after scale-up in low-endemic settings. SMC did not reduce endemicity, but slightly shifted malaria to older ages by reducing immunity in children reached. Incremental health impacts for single-intervention scale-up started to diminish noticeably above 40% coverage, while in high-endemic settings CM and ITNs acted synergistically by lowering endemicity. Statistical models can emulate epidemiological dynamics and inform strategic planning and target setting for malaria control.

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UNDERSTANDING THE MECHANISM OF *PLASMODIUM VIVAX* HYPNOZOITES REACTIVATION

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Plasmodium vivax hypnozoites serve as the major source of maintaining *P. vivax* transmission in endemic countries. Controlling this parasite requires a thorough understanding of the factors determining the timing of *P. vivax* hypnozoite reactivation, which is not completely understood. In this study, we analyze previously published data from infected patients who were treated for blood stage infection and subsequently followed-up until detection of *P. vivax* blood stage parasites. We develop a mathematical model of the frequency of hypnozoite reactivation from the liver, and apply this to published treatment-to-infection studies to investigate the frequency of hypnozoite reactivation over time. We first investigate whether the timing hypnoziote reactivation is constant with time or whether there is evidence that reactivation is induced by previous infection, and subsequently slows with time. We fitted our model to the dynamics of *P. vivax* reactivation of military personnel returning from endemic region, and found that reactivation dynamics were consistent with being induced by previous episodes of infection, and then the reactivation rate slowing over time with a half-life of around 60 days (CI: 45- 89). We also analysed time-to-*P. vivax* infection in patients under continual exposure in an endemic area of PNG, and found that although the initial rate of infection was highest in patients infected with *P. falciparum* at baseline, follow by those infected with *P vivax* and those uninfected at baseline, there was evidence for slowing of *P. vivax* reactivation rate with time. Mathematical modeling of the reactivation of *P. vivax* hypnozoites after anti-malarial treatment provides insights into the timing of reactivation, and has important implications for understanding treatment trials and planning eradication strategies.

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USING CELL PHONE DATA TO IMPROVE MALARIA TARGETING AND MITIGATE THE NEGATIVE EXTERNALITY OF INTERNAL POPULATION MOVEMENT

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The effect of population movement on malaria prevalence has been studied through small case control studies or using modeled parasite prevalence maps linked to human movement data. Yet, case control studies only cover a small geographic area and are not representative of a whole region or country, and using parasite prevalence becomes more difficult in areas that are at a pre-elimination stage. In those areas of low transmission, population movement is critical because most new cases

are imported from abroad or other parts of the country, and contribute to secondary cases. This study utilizes a unique dataset of cell phone usage for 9 million people in Senegal to measure population movement, and combines it with monthly malaria case data available at the health post level for the North of the country. This type of analysis, linking detailed movement data from cell phone records to case data on malaria makes it possible to study the effect of population movement in low transmission settings. In addition, movement is broken down into residents returning after travel away from home and visitors coming in from outside areas, in order to better understand which travelers are at the highest risk for spreading the disease. An important effect of population movement on malaria cases is found, especially for the very low malaria transmission districts that receive many travelers. The majority of the effect comes from residents travelling and returning, with each expected infected case coming into an area in the north leading to 2.5 new cases of malaria in that area, and very little impact from visitors. In addition, simulations demonstrate which are the districts in Senegal that are the leading exporters of the disease to low malaria areas of the country, and how targeting these districts could lead to larger decreases in prevalence. This paper also contributes to the research field of malaria more generally, demonstrating how as areas approach elimination, environmental factors like rainfall decrease in importance and human factors, such as travel, play a more prominent role in driving transmission.

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CLUSTER-LEVEL DETERMINANTS OF REACTIVE CASE DETECTION PERFORMANCE IN MALARIA ENDEMIC SETTINGS: A MONTE-CARLO SIMULATION AND MODEL EMULATION STUDY

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In recent years, many countries and regions within countries have achieved unprecedentedly low levels of malaria and are now looking towards elimination. Paradoxically, many current surveillance systems face the challenge of identifying and responding to increasingly rare and often hidden infections. Health officials within these settings are exploring novel approaches to malaria surveillance, such as reactive case detection (RCD), to combat this challenge. Little research has been done to evaluate if such an approach improves the sensitivity of malaria surveillance systems. The aim of this study was to develop a model emulator which predicted the cluster-level sensitivity of RCD, given a set of cluster-level attributes. RCD sensitivity, defined as the proportion of the malaria reservoir detected, was simulated within clusters of geo-coded census data from southern Zambia using a previously described monte-carlo algorithm. Eight rounds of parasite census data were used to maximize variability in the cluster-level attributes, consequently increasing the performance of the model emulator. Cluster-level attributes such as prevalence, treatment-seeking behavior, population density, rainfall, forestation, and others were taken both from the Zambia data and from remote sensing data sources. Program modifiable attributes, such as CHW coverage, were also included in the model emulation. The model emulator was formed by regressing monte-carlo-simulated RCD sensitivity on a function of the cluster-level attributes, including complex interactions between them. Preliminary results suggest that local treatment-seeking behavior and prevalence will be major determinants of RCD sensitivity. The results of this study supplement the limited empirical research on malaria RCD and provide new information for decision making around RCD implementation.

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WITHIN-HOST DYNAMICS AND SPREAD OF DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA

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In the malaria parasite *Plasmodium falciparum*, drug resistance emerges more readily in low-transmission areas than in high-transmission settings. One possible explanation for this phenomenon is that within-host dynamics of *P. falciparum* infections make it harder for drug-resistant parasites to spread in high—transmission settings. With intense transmission, multi-strain infections are common; therefore, a drug-resistant mutant will likely have to compete against several drug-sensitive strains in any host it infects, reducing its chances of survival and onward transmission. To examine the effects of within-host competition on the emergence of resistance, we constructed a nested mathematical model which describes the within-host dynamics of individual infections as well as the dynamics of transmission in the host population. We used the model to examine the effect of transmission intensity on the evolutionary emergence of drug resistance; we also investigated how the impact of transmission intensity depended on host immunity and antimalarial drug use.

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IS THE USE OF HRP2-DETECTING RAPID DIAGNOSTIC TESTS SUFFICIENT TO SELECT FOR HRP2-NEGATIVE *PLASMODIUM FALCIPARUM* PARASITES?

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Successful control and elimination of malaria relies on rapid and accurate diagnosis of clinical cases and surveillance. The use of malaria rapid diagnostic tests (RDTs) has significantly expanded over the past decade with the majority of tests detecting *histidine-rich protein 2* (HRP2), expressed only by *Plasmodium falciparum* (Pf). However, HRP2-negative parasites have been reported in several regions, most notably the South America Amazon, but more recently countries such as India, Myanmar, Ghana, Mali and Senegal. A high prevalence of HRP2-negative parasites can undermine the utility of HRP2-detecting RDTs. While the selection forces on these parasites are unclear it has been hypothesized that the use of HRP2-detecting RDTs could be the primary mechanism for selection of HRP2-negative parasites. In this study we test this hypothesis using an individual-based mathematical simulation model of *P. falciparum* transmission. The purpose was to determine whether diagnosis using HRP2-detecting RDTs alone provides a sufficient selective force to allow newly introduced HRP2-negative parasites to become established within a community, and the probability of this occurring. Results indicate that the probability of successful introduction of HRP2-negative parasites is influenced by transmission intensity, half-life of the ACT companion drug and type of RDT used for diagnosis (HRP2 only vs HRP2/pan-pLDH combination test). In certain circumstances there is also a considerable lag between introduction and clinical evidence of HRP2-negative parasites. Since wide-spread emergence of HRP2-negative parasites has the potential to impact on timely and accurate diagnosis of malaria, improved understanding of the factors facilitating the establishment of non-HRP2 expressing parasites allows for targeted surveillance and monitoring, and subsequently the adaptation of case management strategies in regions with or susceptible to HRP2-negative parasites.

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PLASMODIUM VIVAX AND *P. FALCIPARUM* INFECTION DYNAMICS IN CO-ENDEMIC SETTINGS: RELAPSES, RECRUDESCENCES, RE-INFECTIONS AND THE ROLE OF CO-INFECTION

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Plasmodium vivax infected populations are often co-endemic with *P. falciparum* malaria, with co-infection frequently observed in individuals. *P. vivax* infection dynamics are qualitatively different from *P. falciparum* due to liver-stage infection with hypnozoites which activate to cause relapses. Intensely sampled longitudinal data on genotyped malaria infections is assembled from cohorts spanning the *P. vivax* and *P. falciparum* endemic world, including Papua New Guinea, Solomon Islands, Thailand and Brazil. Individual and population-level infection dynamics are analysed using a mathematical model with statistical inference implemented in a Bayesian framework. Examples are presented where *P. vivax* recurrences are probabilistically classified into relapses, recrudescences and re-infections, depending on primaquine treatment, transmission intensity, and genotype detectability. *P. vivax* infections have shorter durations than *P. falciparum* infections, with the duration of both decreasing in higher transmission settings. We also present multi-site analyses of the association between *P. falciparum* induced fevers and *P. vivax* relapses, and of the impact of co-infection on the duration of blood-stage infections. The combination of longitudinal data, genotyping of samples and statistical analysis presented here allows for probabilistic identification of *P. vivax* relapses and estimation of their contribution to onwards transmission.

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OPTIMIZING THE GLOBAL ALLOCATION OF MALARIA FUNDS

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The burden of *Plasmodium falciparum* malaria remains high and efforts at control are resource-constrained. Optimal allocation of both internal and global financing is therefore paramount. In light of this, and coinciding with the Global Fund's fifth replenishment call we undertook work to inform the allocation and spending of domestic, bi-lateral and multi-lateral funding for malaria control. We used a well-established mathematical model of malaria to describe the cost and impact of varying coverage levels for 4 key interventions (LLINs, IRS, SMC and treatment) across a wide range of epidemiological strata. We used these simulations to estimate the impact of intervention packages on malaria transmission in Global Fund supported countries. We optimised, at the first administrative unit, the spending of domestic financing within country and the distribution and spending of external and Global Fund financing across countries to maximise the number of cases or deaths averted. Targeting interventions can potentially lead to improved impact, with substantial benefits from countries optimising internally. We demonstrate the trade-off between maximising burden reductions and targeting countries for elimination. The optimal allocation is driven by four key factors: baseline transmission intensity, population at risk, current transmission and domestic financing capability.

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DEVELOPMENT OF A NEW SOFTWARE TOOL AND ANALYSIS METHOD TO IMPROVE DETERMINATION OF GLUCOSE-6-PHOSPHATE-DEHYDROGENASE (G6PD) STATUS

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For the radical cure of *Plasmodium vivax* infection, safe administration of 8-aminoquinoline drugs is critical, but these drugs can be administered only after the determination of G6PD status. Cytochemical staining allows for the determination of G6PD activity in an individual red blood cell. It is possible to estimate the relative proportions of G6PD-deficient cells to those with normal G6PD activity. This research describes a software tool to standardize the analysis of flow cytometry in screening for G6PD-heterozygous females. Its primary function is to provide standardized ratios of "normal" to "G6PD-deficient" cells in an individual blood sample. The goal is to determine zygosity of individuals spanning the G6PD polymorphisms found in African and Southeast Asian populations referenced to DNA sequencing methods. Methods: A reference quantitative assay and cytofluorometry method were used to determine G6PD status. A software tool for standardization and interpretation of cytochemical staining for G6PD status has been developed. Criteria for calling zygosity in females was determined using DNA sequencing as the reference assay. The tool was validated with 472 females and males spanning the G6PD polymorphisms found in African and Southeast Asian populations referenced to DNA sequencing methods. Validation of the analysis tool showed 100% sensitivity and 100% specificity for both normal and G6PD-deficient males when referenced to DNA sequencing. For females with varying levels of G6PD activity, the specificity and sensitivity were 91% and 98% respectively for homozygous deficient, 97% and 91% for heterozygous, and 91% and 100% for homozygous normal. In onclusion, a methodology for standardizing the analysis of cytofluorometry in screening for G6PD-heterozygous females has been developed and packaged into a free, open-source software tool that is available online. The software tool is able to correctly call zygosity with a high level of sensitivity and specificity, even across polymorphisms found in Southeast Asian and African populations, making it a useful tool in the identification of G6PD-heterozygous females.

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SPATIAL MODELING AND HETEROGENEOUS ANALYSIS OF THE EFFICACY OF LONG-LASTING MICROBIAL LARVICIDING ON MALARIA OUTDOOR TRANSMISSION IN WESTERN KENYA HIGHLANDS

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The rising insecticide resistance and increase in outdoor transmission have greatly hampered the effectiveness of currently available first line malaria prevention tools such as long lasting insecticidal nets (LLIN) and indoor residual spray methods. Among the alternative intervention methods are being developed and tested in the field, microbial larvicides represent a promising supplemental intervention tool that may tackle outdoor transmission and pyrethroid insecticide resistance. Long-lasting microbial larvicides (LLML) are particularly attractive because reduce the number of reapplications and thus costs associated with insecticide application, and subsequently they may be potentially more cost-effective. To determine the efficacy of LLML in reducing malaria transmission and optimal field application strategies, the Epidemiological Modeling (EMOD) model for Malaria Transmission developed by Institute for Disease Modeling (IDM) was employed to simulate sites with different malaria prevalence and

landscape scenarios through its Computation Modeling Platform Services. Three sites in western Kenya highland and lowland with well characterized vector ecology and malaria prevalence were simulated using the multi-node spatial model with vector migration method of EMOD. The results show that the beneficial killing efficacy of nets might gradually be reduced due to increased insecticide resistance and outdoor transmission in the absence of other supplemental interventions. The potential reduction of population infected rate after LLML application can reach up to 24%. Re-treatment of aquatic habitats every 4 months would lead to consistent reduction in malaria transmission. In summary, the modeling analyses suggest that LLML has the potential to provide significant added benefits to LLIN for malaria control in a range of sites with different transmission intensities and landscape characteristics.

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USING A BAYESIAN GEOSTATISTICAL MODEL TO UNDERSTAND LOCAL-SCALE HETEROGENEITY IN MALARIA RISK: THE EXAMPLE OF BUNKPURUGU-YUNYOO DISTRICT IN NORTHERN GHANA

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Bayesian methods have been used to generate country-level and global maps of malaria risk on a large geographical scale. However, these maps may lack the ability to identify smaller scale heterogeneity and may not be ideal for operational malaria activities. The aim of this study is to apply Bayesian geostatistical models to high-resolution malaria data in order to construct a predictive model of local-scale spatial heterogeneity. We used existing malaria parasitemia survey data from a 30x40km study area in the Bunkpurugu-Yunyoo district of northern Ghana, consisting of 10,366 children from 438 geo-coded communities sampled between November 2010 and November 2013 bi-annually. A Bayesian hierarchical model using a Gibbs sampler and Metropolis Hasting algorithm estimated parameter values for geostatistical predictions and accounted for spatial dependency at individual and community level. To permit generalizability of the model to other districts, we selected only remote-sensed variables, including environmental factors - such as elevation, temperature, rainfall - and GIS-derived demographic factors such as distance to health facility, urban centres, roads and water bodies. Overall, malaria prevalence in the district varied between 19% and 90%, showing a north-east to south-west gradient of predicted risk with the highest prevalence rates found at lower elevations. Model selection revealed elevation and distance to urban centre to be important covariates. The general distribution is heavily weighted between the two modest urban centres, showing lower risk in urban centres compared to rural areas, with some indication that a threshold distance-to-urban-centre exists for malaria risk. Model predictions revealed high variability in malaria prevalence in areas previously assumed to be homogenous, indicating important shortcomings of country level spatial modelling from a programmatic perspective. Our modelling approach could potentially be applied to infer fine-scale malaria risk in other areas of northern Ghana and beyond, using readily available remote-sensed data.

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GEOGRAPHIC TARGETING OF MALARIA INTERVENTIONS IN MYANMAR USING A DYNAMIC ECONOMIC EPIDEMIOLOGICAL MODEL

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Two interventions receive the majority of malaria control funding in Myanmar i) insecticide treated bed nets and ii) early diagnosis and treatment through malaria community health workers. While malaria funding has increased markedly in recent years, universal coverage of both interventions is not currently affordable nor likely to be financially sustainable. This study focuses on 52 priority townships and aims to provide practical recommendations for targeted geographic allocation of these interventions such that impact on malaria is maximised from the investment. Malaria surveillance in Myanmar is undergoing substantial improvement but does not currently capture detailed incidence data from non-governmental organizations. A data repository was established to collect and process historical incidence data from governmental and non-governmental sources. This information was used within a dynamic economic epidemiological model to estimate intervention costs and effects in terms of Disability Adjusted Life Years averted within each township. Township specific intervention recommendations given a fixed total budget are obtained via a resource allocation algorithm. Scenario analysis was employed to illustrate alternative allocation results under variations of certain model or parameter assumptions, such as cost sharing with other disease funds. Finally, uncertainty analysis presents the consistency of intervention allocation result separately for each townships, given the total prior parameter uncertainty. Final results will be completed prior to the conference.

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SIMULATING WITHIN-VECTOR GENERATION OF MALARIA PARASITE DIVERSITY

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Plasmodium falciparum, the most virulent malaria parasite causing disease in humans, undergoes asexual reproduction within the human host, and sexual reproduction within the vector host, *Anopheles* mosquitoes. Consequently, the mosquito stage of the parasite life cycle provides an opportunity to create novel parasite genotypes in superinfected mosquitoes, a likely contributor to the observed high degree of parasite diversity in both high and low transmission settings. This diversity has important implications for disease transmission and malaria control, however the mechanisms driving this diversity within the vector remain under investigation. To understand the role that vector biology plays in modulating the generation of parasite diversity, we developed a two-stage model framework that estimates the genetic diversity across a population of mosquitoes as a consequence of different bottlenecks and expansion events occurring during the vector-stage of the parasite life cycle. In the first stage of this framework, we developed the first stochastic model of within-vector *P. falciparum* parasite dynamics and simulate the dynamics of two competing parasite genotypes, emulating superinfection. Coupled to this model of parasite dynamics is the second stage of our framework: a model of sequence diversity generation through recombination between genotypes within a mosquito. Our model framework demonstrates that bottlenecks from the ookinete to oocyst stage decrease diversity from the initial gametocyte population in a mosquito's blood meal, and increases with the development of sporozoites. Furthermore, the bottlenecks in the transition from the human host to a mosquito population results in a pool of parasites that has increased in diversity at the population level.

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REMOTELY SENSED ENVIRONMENTAL CONDITIONS AND MALARIA MORTALITY IN THREE MALARIA ENDEMIC REGIONS IN WESTERN KENYA

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Malaria is an important cause of morbidity and mortality in endemic countries. The malaria mosquito vectors depend on environmental conditions, such as temperature and rainfall, for reproduction and survival. To investigate the potential for weather driven early warning systems to prevent disease occurrence, the disease relationship to weather conditions needs to be carefully investigated. Where meteorological observations are scarce, satellite derived products provide new opportunities to study the disease patterns depending on remotely sensed variables. In this study, we explored the lagged association of Normalized Difference Vegetation Index (NVDI), day Land Surface Temperature (LST) and precipitation on malaria mortality in three areas in Western Kenya. The lagged effect of each environmental variable on weekly malaria mortality was modeled using a Distributed Lag Non Linear Modeling approach. For each variable we constructed a natural spline basis with 3 degrees of freedom for both the lag dimension and the variable. Lag periods up to 12 weeks were considered. The effect of day LST varied between the areas with longer lags. In all three areas, malaria mortality was associated with precipitation. The risk increased with increasing weekly total precipitation above 20 mm and peaking at 80 mm. The NDVI threshold for increased mortality risk was between 0.3 and 0.4 at shorter lags. This study identified lag patterns and association of remote-sensing environmental factors and malaria mortality in three malaria endemic areas in Western Kenya. Our results show that rainfall has the most consistent predictive pattern to malaria transmission in the study area. Results highlight the potential for developing locally based early warning forecasts that could reduce the disease burden by enabling timely control actions.

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EFFECT OF IMMEDIATE VS. DELAYED IRON THERAPY ON NEUROCOGNITIVE OUTCOMES IN CHILDREN WITH SEVERE MALARIA

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Malaria and iron deficiency are associated with neuropsychological deficits, but iron supplementation given concurrently with antimalarial treatment per the standard of care may not be well absorbed due to malaria-induced inflammation. We compared neuropsychological functioning after 6 and 12-months between children with cerebral malaria (CM) or severe malarial anemia (SMA) who received ferrous sulphate on admission concurrently with antimalarial treatment (immediate group) or four weeks after admission (delayed group). We hypothesized that children with delayed iron treatment would have better iron uptake, therefore better neurocognitive outcomes at 12-months follow-up. We recruited 239 Ugandan children aged 18 months - 4.9 years for 12 months: 79 with CM, 77 with SMA, and 83 healthy community children (CC). All CM, SMA and 35 CC were iron-deficient (zinc protoporphyrin (ZPP)≥80 mmol/mol heme) and randomly assigned to start a 3-month course of ferrous sulphate immediately or after four weeks. Children were assessed for overall cognitive ability, attention, and associative memory 1 week and 6 and 12 months after admission. Administration of iron immediately vs. delayed did not lead to significantly different neurocognitive outcomes in cognitive ability, attention or associative memory in children with CM or SMA at 12-months follow-up. However, a mixed effects model analysis that included outcomes at all three time points showed that children with CM in the immediate treatment group exhibited better attention scores than the delayed group. Regardless of treatment arm, CM had significantly worse cognitive ability and associative memory scores, and SMA had significantly worse cognitive ability scores compared to CCs at 12-months. Delay of iron may not result in improved long-term neurocognitive outcomes in children with severe malaria as compared to immediate iron treatment. In children with CM, it was associated with worsened long-term attention. Administration of iron therapy with either timing may not prevent worsened neurocognitive outcomes in children with severe malaria when compared to community children in the area.

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IMPROVING REPORTING OF WEEKLY MALARIA DATA THROUGH THE ELECTRONIC INTEGRATED DISEASES SURVEILLANCE AND RESPONSE (E-IDSR) IN TEN REGIONS OF TANZANIA

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To ensure that malaria epidemics are detected and addressed within two weeks of onset, the Tanzania National Malaria Control Program monitors malaria cases reported weekly via the electronic Integrated Disease Surveillance and Response System (e-IDSR). Implementation of the e-IDSR started with 67 health facilities (HF) in November 2013 and progressively scaled-up to reach 2,967 HFs by January, 2016, covering a total of 10 out of 25 regions. This study reports trends in reporting rate, gaps, and the ongoing initiative to improve low reporting rates. At the HF a health worker compiles data for each epidemiological week (Monday to Sunday) and submits the previous week's data via mobile phone by dialing number *152* 05#. Reports are submitted every Monday by 3:30pm, later submission is considered late reporting. Malaria data reported are number tested with RDT/microscope, positive and clinical malaria case. The submitted report goes directly into the DHIS2 information management system and can be accessed by officials at district, regional, and national levels. Overall, in 2013 the reporting rate was 49%, 63.3% in 2014, and 38.3% in 2015. Reports received on time (by Monday 3:30 pm) were 35.6% in 2013, 36.4% in 2014 and 10.8% in 2015. There was considerable variation in reporting rate between districts. In 2015, only 3(5%) out of 60 districts had reporting rates which met the national target of 80% (range 80%-84%). 13(22%) out of 60 districts had a reporting rate ranging from 50%-74%. Overall malaria data reported in 2013 showed that a total of 54,985 people tested of which 15, 654 (28%) had malaria, in 2014 and 2015 a total of 1,211,789(87%) and 2, 458,807 (89%) people were tested of which 474,556(39%) and 817,494(33%) tested positive for malaria respectively. Complete and timely reporting of malaria cases is crucial to prevent and mitigate malaria outbreaks. The e-IDSR reporting rate remains far below target and has faltered after three years. To improve reporting a series of 3 day workshops incorporating practices and lesson learned from the best performing districts is being conducted and attended by regional and district authorities in all 10 regions.

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"I FEEL SO BAD BUT HAVE NOTHING TO DO." A QUALITATIVE STUDY OF UGANDAN CAREGIVERS' EXPERIENCES OF PARENTING WITH CHILDREN WITH SEVERE MALARIA AND SUBSEQUENT REPEATED EPISODES OF UNCOMPLICATED MALARIA

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Severe malaria (SM) and repeated malaria attacks (RMA) are major public health concern. In the endemic regions, children experience 2-5 malaria attacks annually. The experience of caregivers with children having repeated malaria attacks has not been documented. The purpose of this study was to explore caregivers' experiences in parenting of a child with a history of severe malaria followed by repeated episodes of uncomplicated malaria. This was a qualitative study that used phenomenological approaches. Twenty five caregivers of children previously exposed to severe malaria and who had experienced repeated subsequent episodes of uncomplicated malaria were purposively selected. Data was collected using in-depth interviews conducted in Luganda, a native dialect. Interviews were audio recorded, transcribed and translated into English language. Data was manually analyzed using content analysis. The main themes and subthemes exploring caregivers' experiences of parenting a child with a history of severe malaria and repeated malaria episodes were generated. From the interviews, the 4 main themes were identified. These included; societal burden where children are left with community members when their caregivers have to inevitably work; disagreements in seeking healthcare from traditional, spiritual or modern medicine among caregivers, family and the community; family life disruptions involving breakdown of relationships and inadequate male-spouses involvement in the child care; and a sense of helplessness in management of a child with severe malaria and repeated malaria episodes. Severe malaria and repeated malaria episodes affect not only the children who have these illnesses but also their caregivers' parenting experiences. There is need for caregiver-parenting sessions towards the management of children who have had severe malaria and repeated subsequent episodes of malaria.

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PREVALENCE OF SEVERE VIVAX MALARIA: A SYSTEMATIC REVIEW AND META-ANALYSIS SINCE 1900

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Malaria caused by *Plasmodium vivax* was long considered to have a low mortality, but recent reports suggest that severe and complicated vivax malaria may be more common than previously thought. The primary objective of this systematic review and meta-analysis was to describe the reported clinical characteristics and the geographical variation in prevalence of reported severe vivax malaria and its change over time derived from English-language articles published since 1900. Medline and Scopus databases were searched for original papers on severe vivax malaria. A total of 77 studies with reported severe vivax malaria and 63 studies with no reported severe vivax malaria (totaling 46,411 and 6,753 vivax malaria patients, respectively) were included. The 77 studies with reported severe vivax malaria were mainly from India (n = 33), USA (n = 8), Indonesia (n = 6), and Pakistan (n = 6). Among the 77 studies reporting severe vivax malaria, severe thrombocytopenia (<50,000/ mm3) was the most common "severe" manifestation (888/45,775 with pooled prevalence of 8.6%). The case fatality was 0.3% (353/46,411). In conclusion, *P. vivax* can cause severe and even fatal disease. More detailed epidemiological studies are needed which dissociate causation from association to refine the definition and estimate the prevalence of severe vivax malaria.

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COMPARISON OF METHEMOGLOBIN LEVEL BETWEEN CHILDREN AND ADULT AFTER TREATMENT WITH PRIMAQUINE

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Primaquine prevents relapse and sterilizes infectious sexual plasmodia, but confusion surrounds its use. It is able to convert hemoglobin to methemoglobin, producing cyanosis. Limited data support the influence of primaquine on level of methemoglobin in children. A prospective study part of a clinical trial was done in an endemic area of malaria, North Sumatera, Indonesia. Patients diagnosed with *Plasmodium vivax* were given ACTs and low dose primaquine for 14 days. Methemoglobin level was measured on day 0, 7 and 14. A descriptive analysis and un-paired ttest were carried out. Among 3168 patients that were screened, 331 (10.45%) was enrolled. We found 65.86% children and 34.14% adult. Mean level of Methemoglobin in children on day 0 was 1.75% compared to 1.53% in adult, p=0.03. On day 14, Mean methemoglobin level in children was 5.46% and adult 4.76%, p=0.03. 12/218 (5.5%) of children had methemoglobin level > 10% compared to adult (5.3%) 6/113. The highest methemoglobin level (19.6%) was found in a child on day 14, fortunately without any symptoms. An increase level of methemoglobin found in malaria patients in North Sumatera, Indonesia after receiving treatment of primaquine. Children were more prone to methemoglobinemia compared to adult.

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HEALTH SYSTEM STRENGTHENING

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Kisumu is one of the top 15 counties with poor maternal and neonatal health indicators. Malaria in pregnancy (MIP) is one of the conditions associated with poor pregnancy outcomes including maternal anaemia, miscarriages, low birth weight and neonatal deaths. However, the uptake of the effective interventions like intermittent preventive treatment of malaria in pregnancy using sulfadoxine pyrimethamine (IPTp-SP) has remained belowthe national targets at 50% for 2 or more doses and 33% for 3 or more doses in areas providing IPTp.To enable the country move towards achieving the national targets,ministry of health has developed simplified guidelines on how to prevent and treat malaria in pregnancy and how to create demand for health services by communities. To build the capacity of Muhoroni subcounty to scale up the effective MIP interventions a) health care workers (HCWs) in public, faith-based organizations, community-based organizations and private sector providing antenatal care services (ANC) services were trained on provision of the interventions b) community health volunteers (CHVs)were trained on MIP messaging at community levelto sensitize pregnant women and create demand for health services. A total of 264 out of 312 (84.6 %) HCWs in 30 out of 32 (93.8 %) facilities providing ANC were trained. A total of 318 out 340 (93.5 %) targeted CHVs in all 34 community units were trained on sensitization of pregnant women on importance of adhering to scheduled ANC visits. To reduce the poor maternal and neonatal health indicators associated with effects of malaria in pregnancy, it is important to scale up the coverage rates of MIP interventions to the set national targets. The subcounty in an effort to increase coverage towards the national targets built the capacity of HCWs on prevention and treatment of malaria in pregnancy and CHVs on sensitization of pregnant women to attend scheduled ANC visits.

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REDUCTIONS IN MALARIA IN PREGNANCY AND ADVERSE BIRTH OUTCOMES FOLLOWING INDOOR RESIDUAL SPRAYING OF INSECTICIDE IN UGANDA

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Indoor residual spraying of insecticide (IRS) is a key intervention for reducing the burden of malaria in Africa. However, data on the impact of IRS on malaria in pregnancy and birth outcomes is limited. We conducted an observational study nested within a trial of intermittent preventive therapy during pregnancy in Tororo, Uganda. Women were enrolled at 12-20 weeks of gestation between June-Oct 2014, provided with insecticide treated bednets, and followed through delivery. From Dec 2014-Feb 2015, carbamate-containing IRS was implemented in Tororo district for the first time. Exact spray dates were collected for each household. The exposure of interest was the proportion of time during a woman's pregnancy under protection of IRS. Of 289 women followed, 134 had no IRS protection during pregnancy, 90 had >0-20% IRS protection, and 65 had >20-43% protection. During pregnancy, malaria incidence (0.49 vs 0.10 episodes ppy, P=0.02) and parasite prevalence (20.0% vs 8.9%, P<0.001) were both significantly lower after IRS. At the time of delivery, the prevalence of placental parasitemia was significantly higher in women with no IRS protection (16.8%) compared to women with 0-20% (1.1%, P=0.001) or >20-43% IRS protection (1.6%, P=0.006). Compared to women with no IRS protection, those with >20-43% IRS protection had a lower risk of LBW (20.9% vs 3.1%, P=0.002), preterm birth (17.2% vs 1.5%, P=0.006), and fetal/neonatal deaths (7.5% vs 0%, P=0.03). In cocnlusion, in this setting, IRS was temporally associated with lower malaria parasite prevalence during pregnancy and at delivery, and improved birth outcomes.

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HIGH-THROUGHPUT METABOLOMICS TO IDENTIFY BIOMARKERS OF RELAPSES IN *PLASMODIUM VIVAX*-INFECTED PATIENTS

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Plasmodium vivax is the most widely distributed cause of human malaria worldwide, and presently more than 40% of the global population is at risk of infection with this parasite. *P. vivax* develops latent stages in the liver, where the hypnozoites can generate new acute malaria episode months or years after the initial infection. Recently, systems biology and mathematical modeling studies have highlighted the role of relapses in *P. vivax* infection in geographical areas where the parasite is transmitted. Therefore, the new agenda of malaria eradication includes controlling relapses in *P. vivax* infected patients. Although this is an important challenge towards eradication, a differential diagnosis for relapse in patients is currently unavailable. Thus, the aim of this study was to identify human metabolites that are differentially expressed in relapse compared to the primary infection in the same individuals. To reach this goal, high throughput untargeted metabolomics was performed on serum samples from individuals with clinical cases of initial infection and relapses. More than 2,800 metabolites features were detected using this platform, and 86 metabolites showed significant differences between the two groups (primary x relapse). Significant metabolites included inosine and taurine, and the former increased in relapses and the latter decreased in relapses. These metabolites can serve as potential biomarkers of relapse in *P. vivax* infection, and confirmation of their identities will determine their full potential.

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NEW EFFORTS AIMED AT REPLACING ARTEMISININS FOR MALARIA TREATMENT: IDENTIFICATION OF NOVEL, DRUG-LIKE AND FAST-ACTING COMPOUNDS WITH TRANSMISSION BLOCKING POTENTIAL FROM WITH PHENOTYPIC SCREENING

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Malaria is a mosquito-borne protozoal infection caused by parasites of the genus Plasmodium. Despite efforts to eradicate malaria in the past century, the disease remains a major global health problem. Nowdays, according to the 2014 World Health Organization (WHO) global malaria report, 3.3 billion people are at risk of being infected with malaria. From the 198 million cases reported in 2013 the disease led to ca. 500,000 deaths.[1] Plasmodium has been able to adapt to the different treatments developed by humans along history. Part of it as a consequence of its complicated life-cycle which involves resistance to the current first line treatments Artemisins Combination Therapies (ACT) is also arising. The wide knowledge of the illness and the awareness of governments/health systems/funding agencies, makes the current time a unique opportunity to change the course of this disease and achieve eradication. GSK efforts are focused on identifying quality novel chemotypes suitable for oral administration and activity versus strains resistant to current antimalarials in the clinic. Ideally that new molecule should present activity versus the parasite forms involve in the transmission of the disease. [2,3] Therefore, we are seeking the next generation of antimalarials. In this communication we will discuss our latest advances in the field. References [1] World Health Organization. World malaria Report 2014; WHO Press: Geneva, Switzerland, 2014. http://www.who.int/malaria/ publications/world_malaria_report_2014/report/en/ (accessed August, 4, 2015). [2] F. J. Gamo, et. al., Nature 2010, 465, 305-312.; [3] J. Lelièvre et al, PLoS ONE 2012, 7(4), e35019 The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents. All animal studies were ethically reviewed and carried out in accordance with European Directive 86/609/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

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TRENDS IN U.S. PRESIDENT'S MALARIA INITIATIVE-FUNDED INDOOR RESIDUAL SPRAY COVERAGE AND INSECTICIDE CHOICE IN SUB-SAHARAN AFRICA (2008-2015): URGENT NEED FOR AFFORDABLE, LONG-LASTING INSECTICIDES

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The changing pattern of US President's Malaria Initiative-funded IRS in sub-Saharan Africa between 2008-2015 was studied. IRS coverage in sub-Saharan Africa increased from <2% of the at-risk population in 2005, to 11% or 78 million people in 2010, mainly as a result of increased funding from PMI. The scaling up of IRS in sub-Saharan Africa has been successful in several epidemiological settings. However, the spread and intensification of pyrethroid resistance in malaria vectors led many control programmes to spray alternative insecticides. Between 2009-2013, pyrethroid spraying decreased from 87% (13/15) of PMI-funded countries conducting IRS to 44% (7/16), while bendiocarb use increased from 7% (1/15) to 56% (9/16). Long-lasting pirimiphos-methyl CS received WHOPES recommendation in 2013 and was scheduled to be sprayed in 85% (11/13) of PMI-funded countries conducting IRS in 2015. The gradual replacement of relatively inexpensive pyrethroids firstly with bendiocarb (carbamate) and subsequently with pirimiphos methyl CS (organophosphate) has contributed to downscaling of most PMI-funded IRS programmes. Overall, there was a 53% decrease in the number of structures sprayed between years of peak coverage and 2015, down from 9.04 million to 4.26 million structures. Sizeable reductions in the number of structures sprayed were reported in Madagascar (56%, 576,320

to 254,986), Senegal (64%, 306,916 to 111,201), Tanzania (68%, 1,224,095 to 389,714) and Zambia (63%, 1,300,000 to 482,077), while in Angola, Liberia and Malawi PMI-funded spraying was suspended. The most commonly cited reason was increased cost of pesticides, as vector resistance necessitated switching from pyrethroids to organophosphates. There are worrying preliminary reports of malaria resurgence following IRS withdrawal in parts of Benin, Tanzania and Uganda. At present, there are several countries reliant on organophosphates and carbamates for IRS and increasing resistance is a serious threat that could result in IRS no longer being viable. A portfolio of new cost-effective insecticides with different modes of action is urgently needed.

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SENTINEL-SITE SURVEILLANCE FOR MALARIA MORTALITY AND ITS POTENTIAL FOR EBOLA VIRUS DISEASE OUTBREAK DETECTION IN GUÉCKÉDOU, GUINEA

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Community-based surveillance systems are used to document program impact on mortality from diseases such as malaria. They could also act as surveillance systems for outbreak detection. We conducted a retrospective analysis of data from a prospective malaria mortality surveillance system in Guéckédou, Guinea through which data was reported monthly from July 2011-July 2014. In 46 sentinel sites, 40 with a malaria control program (program) and 6 without (comparison), cause of death as reported by the deceased's family was recorded by key informants. Deaths were classified as due to malaria or another cause. Suspect Ebola virus disease (EVD) deaths were those reported as due to symptoms compatible with the EVD case definition. Deaths were aggregated by area and analyzed by 6 month surveillance period (1-6) corresponding to the dry (January-June) and rainy (July-December)seasons. Logistic regression models were used to investigate temporal trends in malaria-attributable mortality and suspect EVD mortality. From July 2011-June 2014 approximately 45,000 individuals were monitored by the surveillance system, 1,242 deaths were reported. The majority (55.2%, n=686) were reported as due to malaria. Proportional mortality attributable to malaria decreased from 66.8% to 40.3% ($p<0.001$) in the program area and from 65.8% to 59.2% ($p=0.782$) in the comparison area. 75.2% (n=934) of all deaths occurred at home, 17.8% (n=221) occurred in a health facility. In total, 68 deaths [range 6-17, by period] were classified as EVD suspect and increased over time ($p=0.021$). Seventeen suspect EVD deaths were reported during period 6, January-June 2014, when EVD was detected; these included the first two (11.7%, 2/17) laboratory confirmed EVD deaths in the region. Community surveillance can be used to document program impact on mortality and complement health facility surveillance particularly in areas where the majority of deaths occur in the community. Such systems could also be used for outbreak detection. Had community surveillance for outbreak detection been in place, the EVD outbreak in Guéckédou might have been identified earlier.

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BUILDING CAPACITY TO ACCELERATE IPTP UPTAKE THROUGH THE ADOPTION OF 2012 WHO IPTP GUIDANCE IN MALAWI

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Malawi adopted the World Health Organization's updated guidance on intermittent preventive treatment in pregnancy (IPTp) in 2013. Support from the US President's Malaria Initiative through USAID funded health projects, enabled collaboration between the National Malaria Control Program (NMCP) and the Reproductive Health Directorate (RHD) of the Ministry of Health, to build capacity from national to district to frontline health facility levels to implement the updated IPTp policy. These partners updated IPTp policy in the National Malaria Treatment Guidelines, and developed appropriate training manuals. All 5708 health workers from the 304 facilities in the 15 project districts were trained on the IPTp policy and guidelines. Post-training test scores of health staff increased over pre-test by an average of 40 percentage points. The community action cycle approach engages community volunteers and local community based organizations to identify and solve local problems and was used to encourage pregnant women to attend antenatal care (ANC) and receive IPTp and long lasting insecticide-treated nets. Health information system data from the 15 Districts were used to compare ANC and IPTp coverage for 2012 and 2015 fiscal years (Oct.-Sept.). ANC registration in the project area rose from 113,683 to 394,116. IPTp1 as a proportion of ANC registration rose from 52% to 87%, and IPTp2 increased from 17% to 62%. While IPTp3 doses were recorded in the ANC registers, reporting forms in 2015 still did not include space to enter this IPTp3. Observations at clinics showed IPTp3 and 4 were provided. Malawi's experience shows that collaboration between NMCP and RHD as well as between clinics and communities not only disseminated knowledge of the new policy, but resulted in increased uptake of services and protection of pregnant women from malaria.

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ACCURATE MALARIA MICROSCOPY: IS IT THE READER OR QUALITY OF PREPARED SMEARS? A QUANTITATIVE COMPARISON OF FALSE POSITIVES/NEGATIVES VS SMEAR QUALITY IN TANZANIAN MILITARY HEALTH FACILITIES

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Quality malaria microscopy remains the gold standard for malaria diagnosis. For the past 5 year WRAIR/TPDF has implemented innovative strategies to increase access to quality malaria case management through monitoring and improving quality of malaria microscopy. The accuracy and reliability of results are critically dependent on the quality of the blood film preparation, the staining procedure and the microscopist's expertise in reading the smears. Studies have demonstrated poor malaria microscopy performance in Africa, including Tanzania. Walter Reed and Tanzania Peoples Defence Forces (TPDF) have established a Quality Monitoring and Improvement Program. The key objective is to perform crosschecking of malaria slides prepared and examined in current and future research sites. All testing sites are provided with high quality equipment, reagents and supplies to perform malaria microscopy. All blood films prepared for routine testing are stored and a monthly QC sample is crosschecked by the Walter Reed Malaria Program. The QC samples are selected in situ from the laboratory register on a quarterly basis. 10 negative and all positive slides for each month are selected using a random representative sampling protocol. The quality of blood films are quantitatively assessed macroscopically for blood film preparation quality and microscopically for staining quality and reading accuracy. The quantitative assessment of preparation, staining and reading shows correlation between the quality of the smear and accuracy of the results reported. In this study we will

present the data on the trend of false positive rates as compared to increase or decrease on the total quality index of preparation and staining of blood smears over the past 12 months.

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A CROSS-SECTIONAL SURVEY OF KNOWLEDGE AND PERCEPTIONS RELATED TO PREVENTION AND TREATMENT OF MALARIA IN PREGNANCY AMONG HEALTH CARE PROVIDERS AT HEALTH FACILITIES IN TANZANIA

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A cross-sectional survey was conducted among 131 health-care providers in rural Tanzania to examine the knowledge and availability of malaria preventive and curative treatment for pregnant women recommended by the World Health Organization and contained in Tanzanian national guidelines. Perceptions of harm attributable to malaria infection and, separately, to malaria treatment were recorded, as well as malaria-specific training received in the last three years. Thirty-nine percent of providers correctly stated that malaria during the first trimester should be prevented by sleeping under insecticide treated nets only. In contrast, 80% of providers correctly reported that malaria is to be prevented using insecticide treated nets and intermittent preventive treatment of malaria with sulfadoxine-pyrimethamine during the second and third trimesters. Knowledge of appropriate first-trimester case management was correctly reported by 50% of providers, increasing to 60% when providers were asked to describe curative care for pregnant women in their second and third trimesters. Approximately 30% of the providers stated having received malaria training in the last three years. Untreated malaria infection was viewed as extremely harmful by over 93% of providers to pregnant women and their unborn babies in any trimester. Malaria treatment was considered harmful to pregnant women in the first trimester and unborn babies in the second and third trimesters by 44% and 45% of providers, respectively. Only 48%, 44% and 69% of providers reported having sulfadoxine-pyrimethamine, quinine, and artemisinin-combination treatment available at the health facility, respectively, for preventive and curative care. These results suggest that the scale up of interventions to reduce the burden of malaria in pregnancy will, in part, require substantial improvements in supply chain management as well as pre-service training and in-service retraining of health-care providers in appropriate preventive and curative care with an emphasis on the safety of treatments by trimester.

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DEVELOPMENT OF *PLASMODIUM FALCIPARUM* EXFLAGELLATION ASSAY

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The standard membrane-feeding assay (SMFA) is considered a gold standard functional assay for transmission-blocking vaccine (TBV) development against *Plasmodium falciparum* malaria. However, it has been shown that SMFA reproducibility is poor when % inhibition of oocysts is low. In addition, the assay needs an insectary which can provide *Anopheles* mosquitoes and fulfill all regulatory requirements to maintain infectious mosquitoes. Therefore, we evaluated an exflagellation assay (EXA) for assessment of functional activity of transmission-blocking antibodies. Since EXA does not involve parasite development process in mosquitoes, it is possible that EXA could show better precision than SMFA. Two monoclonal antibodies (mAbs), 1B3 (anti-Pfs230) and IIC5B10 (anti-Pfs48/45), both of which have shown strong functional activities in SMFA, were utilized for the assay development. We first optimized the protocol of EXA, e.g., ratio of test antibody and gametocyte culture, incubation time. In the optimized EXA, clear enhancement of inhibition was observed in the presence of complement for both mAbs, while IIC5B10 mAb does not require complement to work in SMFA. We confirmed the specificity of inhibition using 4B7 mAb, which recognizes Pfs25, one of the post-fertilization TBV candidates. Both 1B3 and IIC5B10 mAbs showed dose-dependent inhibition in the EXA, and the inter-assay precision of IIC5B10 mAb was much better in EXA than in SMFA. The results indicate that EXA could be one option to evaluate functional activity of transmission-blocking antibodies which target pre-fertilization antigens.

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SAFETY AND IMMUNOGENICITY OF WITH NOVEL MALARIA VACCINE CANDIDATE, R21 ADJUVANTED WITH MATRIX M1

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Falciparum malaria remains one of the leading infectious causes of morbidity and mortality worldwide. Development of a durable efficacious vaccine is a priority, and there remains an urgent need to improve efficacy to achieve the World Health Organisation goal of a deployable vaccine with at least 75% durable efficacy against clinical malaria by 2030. R21 has been developed at the Jenner Institute, University of Oxford. This is an improved RTS,S construct, an antigen derived from the pre-erythrocytic circumsporozoite protein, which is an abundant coat protein involved in sporozoite development and hepatocyte invasion. R21 comprises recombinant particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP) fused to HBsAg, but without the excess of unfused HBsAg protein found in RTS,S. It was GMP manufactured in Pichia pastoris at the Clinical Biomanufacturing Facility at Oxford University. We undertook a Phase I, open-label clinical trial to assess the safety and immunogenicity of R21 administered alone (Group 2; n=4) and with the novel saponin-based adjuvant, Matrix M1 (Group 1 and 3; n=10 per group). Safety was assessed by active and passive collection of local and systemic adverse events. The immunoassay of most interest was the antibody response to NANP because this correlates with vaccine efficacy after RTS,S/AS01 administration, and induction of antibody levels comparable to or greater than RTS,S/AS01 would suggest likely vaccine efficacy. Preliminary analysis shows that the vaccine is safe and well tolerated with the majority of adverse events being mild in nature and short-lived. Initial assessment of antibody responses indicate antibody levels elicited at least as high as reported for RTS,S/AS01 in previous studies. This trial was the first administration of R21 in humans and safety and immunogenicity profiles observed support further testing in Phase IIa studies using a controlled human malaria infection model.

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PHASE I VACCINE TRIAL FOR EBA-175 RII INDUCES HIGH LEVELS OF BINDING INHIBITORY ANTIBODIES THAT TARGET KEY FUNCTIONAL EPITOPES

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Naturally acquired antibody responses against blood-stage parasites are associated with protective immunity. Erythrocyte Binding Antigen-175 (EBA-175) is a leading vaccine candidate of *Plasmodium falciparum* that plays a key role in merozoite invasion by binding to glycophorin A on the erythrocyte surface. We have previously shown that antibodies to EBA-175 are associated with protection and can inhibit EBA-175 binding to glycophorin A. This binding is mediated through Duffy-binding like (DBL) domains in Region II of the protein. EBA175 Region II was recently assessed in a dose escalation Phase I trial adjuvanted with Alum and individuals (n=71) were shown to develop high titres of IgG but relatively low growth inhibitory responses. Our study aimed to further assess the properties and functional activity of these vaccine-induced antibodies and relate the magnitude of these responses with antibodies acquired following natural infection in a cohort of children from Papua New Guinea (n=206). There were no statistically significant differences in total IgG levels between the vaccine-induced and naturally acquired responses (p=0.883). However, vaccine-induced responses were significantly lower compared to naturally-induced responses for IgG1 (p=0.003) and IgG3 (p<0.001); both key subclasses associated with protection from symptomatic disease in naturally immune populations. Vaccine induced antibodies were able to inhibit EBA-175 binding, especially for the 20µg per dose or greater. Furthermore, the vaccine was able to elicit antibodies that targeted epitopes shared with the functional monoclonals, R217 and R218. Importantly, there was a high correlation between binding inhibitory activity and antibodies to the R217 epitope (0.8860; p<0.0001). This study demonstrates that a recombinant EBA-175 vaccine can induce high level binding inhibitory antibodies that target key epitopes on EBA-175 Region II however the differences in subclass responses requires further optimisation. The exploration of adjuvants is required to ensure that the vaccine-induced immunity is optimised further and exceeds natural immunity.

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PROTEOMIC ANTIBODY PROFILING OF U.S. AND AFRICAN VOLUNTEERS IN MULTIPLE CLINICAL TRIALS OF PFSPZ VACCINE

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A vaccine against malaria would benefit the millions affected worldwide and accelerate efforts to eliminate malaria. Immunization with PfSPZ Vaccine, a metabolically active, non-replicating malaria sporozoite vaccine, induced high-level protective efficacy against *Plasmodium falciparum* (Pf) malaria infection in multiple clinical trials. An antibody assay that predicted protection would facilitate vaccine development and deployment. To identify the target proteins for such an assay, we developed whole Pf proteome microarrays representing 4,805 unique genes, or approximately 91% of the Pf proteome, to screen sera from volunteers in clinical trials of PfSPZ Vaccine who underwent controlled human malaria infection (CHMI) or followed for risk of malaria. Sera have been assessed for proteomic antibody profiles in three clinical trials of U.S. volunteers and clinical trials of African volunteers from Mali and Tanzania. We have detected over 1,500 responses to Pf proteins in PfSPZ Vaccine recipients. Over 50 Pf proteins have been identified with an immunogenic profile and association with sterile protection after CHMI. A combination of 6 proteins (PfCSP, PfMSP5, PfGSK3, PfLRR9, PfDOC2 and PF3D7_1030200) that predicted protection was identified in one clinical trial with 32 U.S. volunteers, which had a cross-validated AUC of 0.89 and sensitivity and specificity of 92% and 89%, respectively. This signature of protection has been confirmed in an independent clinical trial. These 6 Pf proteins were selected for further development as companion assays to PfSPZ Vaccine for travelers and potential components of subunit malaria vaccines. Unique antibody profiles were observed between trials of PfSPZ Vaccine in U.S. and African volunteers. A signature of protection is being investigated for African PfSPZ Vaccine recipients for development of a companion assay to predict when subjects will be protected during malaria elimination.

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A HIGHLY INFECTIOUS *PLASMODIUM YOELII* PARASITE, BEARING PLASMODIUM CIRCUMSPOROZOITE PROTEIN

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Plasmodium circumsporozoite protein (CSP) is a major surface antigen present in the sporozoite (Spz) stage of a malaria parasite. RTS,S vaccine, the most clinically advanced malaria vaccine, consists of a large portion of *Plasmodium falciparum* CSP (PfCSP). A highly infectious, recombinant rodent malaria, *Plasmodium yoelii* parasite bearing a full-length PfCSP (PfCSP/Py) was generated by double cross-over homologous recombination. This PfCSP/Py parasite produced up to 30,000 Spz in mosquito salivary glands, which is equal or even higher than the number of Spz produced by wild-type *P. yoelii* parasites. Five bites of PfCSP/Py-infected mosquitoes could induce blood infection in BALB/c mice. Our new transgenic parasite that expresses a full-length PfCSP may become a useful tool for researchers to investigate immunity against PfCSP in a mouse model.

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VECTORED PFCSP VACCINES BASED ON BACULOVIRUS DUAL EXPRESSION SYSTEM AND ADHU5 INDUCE STRONG PROTECTIVE EFFICACY AGAINST TRANSGENIC *PLASMODIUM BERGHEI*

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The baculovirus-vectored vaccine based on the "baculovirus dual expression system (BDES)" has been exploited as a novel vaccine platform for malaria. The gene encoding the human decay-accelerating factor was incorporated into the BDES malaria vaccine expressing the *Plasmodium falciparum* circumsporozoite protein (PfCSP). The newly developed BDES vaccine "BDES-sPfCSP2-Spider" resulted in complement resistance both *in vitro* and *in vivo*. To improve the vaccine efficacy, baculovirus expressing mouse interleukin-12 (mIL-12) and the adenoviral vaccine expressing PfCSP "AdHu5-sPfCSP2" were generated. Large-scale immunization studies were conducted in mice, and the protective efficacy was examined by using biting of mosquitoes infected with transgenic *P. berghei* sporozoites expressing PfCSP. After the priming immunization with AdHu5-sPfCSP2, booster immunization with BDES-sPfCSP2-Spider together with the mIL-12 vector conferred strong protective efficacy as compared to the controls (29 mice out of 44 were protected; 65%), following the high level of anti-PfCSP IgG titer. Thus, we propose that the prime-boost regimen using adenovirus and BDES offer great potential as a new malaria vaccine platform.

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MALARIA TRANSMISSION-BLOCKING VACCINE ANTIGEN DISCOVERY USING NATURALLY ACQUIRED FUNCTIONAL ANTIBODY

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Transmission-blocking vaccines (TBV) target the *Plasmodium falciparum* parasite's sexual stages to interrupt its life cycle and would be useful for elimination efforts. We previously identified human sera from Malian adults whose purified IgG conveyed high transmission-blocking activity (TBA) by standard membrane feeding assay (SMFA) against laboratory cultured gametocytes fed to A. stephensi mosquitoes. Here, we describe the results from an iterative subtractive screening of a gametocyte stage cDNA phage display library using naturally acquired IgG with versus without TBA. A set of novel TBV candidate antigens was identified including 3 proteins with hits in four independent differential screens. The top 9 candidates are being evaluated in an animal model using DNA immunization via gold particle bombardment, and the top 3 mentioned above using protein immunization as well. Briefly, synthetic genes were cloned into pCI-SF mammalian expression vector and pET-24b(+) for *E. coli* protein expression, respectively. Mammalian cell transient transfection was used to assess expression from pCI-SF clone's prior to animal immunizations. Independently, cobalt affinity column was used for protein purification from pET-24b(+) bacterial expression. Protein and DNA immunogens are being used to immunize small animals, and functional immune responses evaluated by SMFA will be reported.

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ASSOCIATION OF SPECIFIC VAR2CSA HAPLOTYPES WITH WORSENED BIRTH OUTCOMES IN WOMEN WITH *PLASMODIUM FALCIPARUM* PLACENTAL MALARIA IN MALAWI AND BENIN

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Pregnancy associated malaria (PAM) causes adverse pregnancy and birth outcomes including low birth weight (LBW) and small for gestational age (SGA). Placental accumulation of *Plasmodium falciparum* is mediated by VAR2CSA. This protein's ID1-DBL2x region is considered a minimal binding epitope and is a promising vaccine candidate against PAM. We hypothesized that variation in the ID1-DBL2x region would be associated with differential prevalence of poor birth outcomes. Using two clinical cohorts of women with placental malaria at delivery, we deep-sequenced the 1.6kb ID1-DBL2x region in 101 placental samples in Malawi and Benin to characterize genetic diversity and identify pathogenic clades. In Malawi, we identified two genetic clades which resembled the sequences of the current vaccine candidate antigens, 3D7 & FCR3. In Benin, along with 3D7-like and FCR3-like clades, three other unique clades were detected.

We estimated the association of specific clades with birth weight, LBW, and SGA, controlling for confounders using inverse probability weights. In our study population, the mean (SD) infant birth weight in Malawi was 2677g (540g) and 2840g (380g) in Benin. Prevalence of LBW was 19.6% (n=11) in Malawi and 13.3% (n=6) in Benin; prevalence of SGA was 16.1% (n=9) in Malawi and 24.4% (n=11) in Benin. In phylogenetic analyses, the variants present in the placentae of women delivering LBW or SGA infants clustered more readily in the 3D7-like clade in Malawi but were more evenly distributed in Benin. Compared to women infected with FCR3-like only variants, women infected with 3D7-like only variants delivered infants with lower birth weight (-267.99g; 95% CI: -466.43g - -69.55g) and higher odds of LBW (OR: 8.19; 95% CI: 1.65 - 40.57) and SGA (OR: 3.65; 95% CI: 1.00 - 13.38). These associations were attenuated in Benin, but were overall supported by country-level analyses. The results from our study provide evidence that 3D7-like genetic variants of VAR2CSA in parasites infecting the placenta are associated with worse birth outcomes including LBW and SGA. This supports the development of polyvalent vaccines that target multiple clades of VAR2CSA to combat PAM.

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EFFICACY OF PFSPZ VACCINE AGAINST HETEROLOGOUS MALARIA CHALLENGE IN MALARIA-NAÏVE ADULTS

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Sterile protection lasting 14 months against *Plasmodium falciparum* (Pf) malaria has been achieved in humans after intravenous (IV) injection of a non-replicating, cryopreserved Pf sporozoite (SPZ) vaccine (PfSPZ Vaccine). Further development is focused on identification of a dose-sparing immunization regimen that induces durable protection against heterologous Pf strains. We conducted an open-label trial of PfSPZ Vaccine, composed of attenuated, aseptic, purified cryopreserved PfSPZ, at a dose of 9.0 x105 PfSPZ administered IV 3 times at 8-week intervals to 15 healthy, malaria-naïve adults. Vaccinated and non-vaccinated control volunteers underwent controlled human malaria infection (CHMI) by exposure to mosquitoes carrying infectious PfSPZ of homologous 3D7 and heterologous 7G8 Pf strains at 19 and 33 weeks, respectively, after final immunization. Antibody and cellular immune responses were assessed. PfSPZ Vaccine was well tolerated. After CHMI with homologous PfSPZ at 19 weeks, 9/14 volunteers (64%) remained without parasitemia compared to 0/6 controls (P=0.012, Fisher's exact test, one-sided). Six non-parasitemic volunteers underwent repeat CHMI with heterologous PfSPZ at 33 weeks, and 5/6 vaccinees remained without parasitemia compared to 0/6 controls (P=0.0076). Pf-specific antibody, CD8, CD4, and γδ T cell responses were detected in all vaccinees. A 3-dose regimen of PfSPZ Vaccine conferred sterile protection for at least 33 weeks against CHMI with heterologous Pf and induced broad-based PfSPZ-specific immune responses. Ongoing studies using higher doses are evaluating protective efficacy in travelers, military personnel, and infants and adults living in endemic areas.

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IMMUNIZATION BY MOSQUITO BITE WITH RADIATION ATTENUATED SPOROZOITES (IMRAS): A PHASE 1 CLINICAL TRIAL

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Human clinical trials have demonstrated that immunization with radiation-attenuated *Plasmodium falciparum* sporozoites (PfRAS) by mosquito bite is an excellent model for malaria vaccine development conferring the highest levels of sterile protection. In the study Immunization by mosquito bite with radiation-attenuated *P. falciparum* sporozoites, or IMRAS, we have applied a systems biology approach to this model to improve current understanding of immune mechanisms of protection by comparing sterilely protected with non-protected study subjects. The clinical study was designed such that approximately 50% of immunized human subjects would be protected against homologous controlled human malaria infection (CHMI) to facilitate the analysis of biomarkers and correlates of protection. Earlier studies suggested that immunization with a total of 960 bites from mosquitoes infected with PfRAS would yield 50% protective efficacy. The study was conducted with two sequential cohorts, each consisting of twelve true-immunized and four mock-immunized human subjects. Subjects in both cohorts underwent five immunization sessions every four weeks receiving approximately 200 infectious bites per immunization session. Immunization procedures were well-tolerated, and there were no vaccine-related serious adverse events. All twelve infectivity controls (six per cohort) became parasitemic and none of the mock-immunized subjects were protected. Surprisingly, despite the similar number of total infectious bites in each cohort, the percentage of subjects protected in the two cohorts was quite different. Six of the 11 (55%) true-immunized subjects in the first cohort were sterilely protected against parasitemia after primary challenge at 23-25 days post-last immunization. In the second cohort, 9 of 10 (90%) true-immunized subjects were protected after primary challenge. We will present a detailed analysis of all factors which may have impacted the discordant levels of protective efficacy in the two cohorts; such data may provide key information for the development of a highly protective malaria vaccine.

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TOLERABILITY, SAFETY AND EFFICACY (ADULTS) OF ESCALATING DOSES OF PFSPZ VACCINE ADMINISTERED BY DIRECT VENOUS INOCULATION TO TANZANIAN INFANTS, CHILDREN, ADOLESCENTS AND ADULTS

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PfSPZ Vaccine administered by direct venous inoculation (DVI) has been shown to be extremely well tolerated and safe and induce durable, sterile protection against homologous and heterologous *Plasmodium falciparum* (Pf) parasites in controlled human malaria infection (CHMI)(U.S. and Tanzania) and field (Mali) studies in doses up to $2.7x10^5$ Pf sporozoites (SPZ). However, while good, the protective efficacy has not yet been shown to achieve our target of at least 80% sterile protection for at least 6 months, and PfSPZ Vaccine has not yet been studied in adolescents, children, or infants. All current data indicate that increasing the numbers of PfSPZ per dose will increase protective efficacy. To address both deficiencies, we are conducting a double blind, normal saline placebo-controlled trial at the Bagamoyo Clinical Trials Unit of the Ifakara Health Institute in Tanzania. The trial is aimed at determining if 3 doses of up to $9.0x10^5$ PfSPZ (young children and infants) and $1.8x10^6$ PfSPZ (adults, adolescents, older children) are well tolerated, safe and immunogenic and protect adults against CHMI. 93 female and male subjects were enrolled in an approximately 2:1 ratio (vaccinee to control) in the five age groups including 18 volunteers in each of the adult, 11-17, 6-10, and 1-5 year age groups, and 21 in the 6-11 months group, plus an additional 6 adults to serve as infectivity controls for CHMI. Two of the three planned doses have been administered to adults, adolescents, and older children and 50% of the younger children and infants. The study is still blinded. However, there have been no serious adverse events, nor any indication of differences in adverse events among the age groups, or of a safety signal. Adults will undergo CHMI by DVI of PfSPZ Challenge (aseptic, purified, infectious PfSPZ) in May and June 2016, and the study will be completed in September 2016. The complete un-blinded results of tolerability, safety, immunogenicity, and protective efficacy will be presented, as will plans for the next Tanzanian study designed to support licensure for the mass vaccine administration indication.

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CLINICAL MANIFESTATIONS OF *PLASMODIUM FALCIPARUM* INFECTION IN TANZANIAN ADULTS AFTER CONTROLLED HUMAN MALARIA INFECTION BY INJECTION OF PFSPZ CHALLENGE

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The clinical manifestations of Plasmodium falciparum (Pf) malaria in volunteers in the United States and Europe after controlled human malaria infection (CHMI) by exposure to mosquitoes carrying Pf sporozoites (SPZ) and by injection of aseptic, purified, cryopreserved, infectious Pf sporozoites (SPZ), a product called Sanaria® PfSPZ Challenge, have been well described. We conducted the first CHMI study in Africans using intradermal injection of PfSPZ Challenge; 21 subjects developed malaria. Since then we have conducted three different CHMIs by direct venous inoculation (DVI) of PfSPZ Challenge; 56 subjects developed malaria. In May and June 2016 we will conduct two more CHMIs by DVI of PfSPZ Challenge in 26 subjects. In general with the same dose of PfSPZ Challenge the pre-patent periods are longer and the clinical manifestations less in previously exposed Tanzanians as compared to subjects in the U.S. and Europe with no previous exposure to malaria. We will present a detailed analysis of the parasitological findings and the clinical manifestations in the Tanzanian subjects, and contrast them with those reported for non-immune subjects. These data will provide a foundation for groups in Africa to design, justify, and execute CHMIs to assess the effects of antimalarial drugs and vaccines, naturally acquired immunity, and genetic background on malaria.

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CHEMOPROPHYLAXIS VACCINATION (CVAC) WITH SANARIA® PFSPZ CHALLENGE AND PYRIMETHAMINE: PHASE 1 TRIAL TO DETERMINE SAFETY AND PROTECTIVE EFFICACY AFTER EXPOSURE TO ONLY *PLASMODIUM FALCIPARUM* LIVER STAGES

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Direct venous inoculation (DVI) of aseptic, purified, infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) (Sanaria® PfSPZ Challenge) under chloroquine (CQ) prophylaxis (Sanaria® PfSPZ-CVac approach) protected 100% (9/9) of malaria naive vaccinees against homologous controlled human malaria infection (CHMI), 9 weeks after the third and last dose. CVac-CQ exposes vaccinees to both liver and blood stage antigens, thus the contribution of each parasite stage to immunity is unclear. We are investigating the safety and protective efficacy of CVac using CQ and pyrimethamine chemoprophylaxis (liver stage only exposure) versus CVac-CQ. Upon determination in a pilot study with 2 subjects that pyrimethamine dosing at days 2 and 3 post PfSPZ Challenge inoculation was sufficient, the main study enrolled 12 subjects to receive CVac using CQ and pyrimethamine and 6 subjects to receive CVac using CQ only. Subjects completed 3 rounds of PfSPZ-CVac at 4 weeks intervals. Thus far, PfSPZ-CVac has been well tolerated with the majority of adverse events (AEs) being transient Grade 1 AEs, with one serious AE reported. No subjects developed parasitemia, detected by thick blood smear, or clinical malaria. Subpatent parasitemia detected by polymerase chain reaction (PCR), was seen on days 7 and 8 post PfSPZ Challenge inoculation in during CVac-CQ, but not in any subject receiving CVac with CQ and pyrimethamine. All eligible vaccinated subjects and 5 subjects in an infectivity control group will undergo CHMI with 3,200 PfSPZ Challenge in June 2016. Unblinded tolerability, safety, immunogenicity, and protective efficacy results will be presented. This is the first trial to evaluate whether exposure limited to live replicating liver stage parasites in humans results in the development of immunity against homologous CHMI.

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DEVELOPMENT OF A PFSPZ VACCINE REGIMEN TO PROTECT MILITARY PERSONNEL AGAINST *PLASMODIUM FALCIPARUM* INFECTION

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Plasmodium falciparum (Pf) sporozoites (SPZ) are the only immunogens ever shown to induce >90%, sterile protective immunity against malaria.

Sanaria® PfSPZ Vaccine, an aseptic, purified, cryopreserved radiation attenuated Pf sporozoite (SPZ) malaria vaccine administered by direct venous inoculation has been shown to be safe, well-tolerated, easy to administer, immunogenic and highly protective. In order to be utilized as a vaccine to protect deployed military personnel, the vaccine must have an efficacy of >80% against infection with heterologous strains of Pf for at least 6 months. A series of clinical trials conducted by the Naval Medical Research Center, Walter Reed Army Institute of Research and other members of the International PfSPZ Consortium have demonstrated a distinct dose response for protection induced by PfSPZ Vaccine. Gradually increasing the total amount of vaccine (administered in as few as 3 immunizations) has generated increasing levels of protection and anti-PfCSP antibody titers. After reaching >90% protective efficacy against short-term homologous controlled human malaria infection (CHMI) at 3 weeks post final dose, we set the next benchmarks to include protection against 1) long-term homologous CHMI (6 months post final dose), 2) short-term heterologous CHMI, 3) long-term heterologous CHMI and finally, 4) protection against *P. vivax*. In our most recently completed clinical trial, we had unprecedented success in reaching the first and second of these benchmarks. We expect to meet the third benchmark (protection against long-term heterologous CHMI) with one of the regimens being assessed in our newly initiated clinical trial. These include a 3 dose regimen with up to 4 times as many PfSPZ per dose (1.8x10^6) as in our previous study and a regimen with the highest dose given in humans thus far (2.7x10^6), followed by two doses of 9x10^5. We will present a detailed analysis of the dose response to PfSPZ Vaccine, preliminary results from our ongoing trial, plans to assess protection against *P. vivax* CHMI and plans for Phase 3 testing to support submission for FDA licensure.

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SAFETY, TOLERABILITY AND EFFICACY OF DOSE ESCALATING DIRECT VENOUS INOCULATION WITH RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* NF54 SPOROZOITES (PFSPZ VACCINE) IN HEALTHY MALIAN ADULTS

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A double blind, randomized Phase 1/2 clinical trial is being conducted in Mali, West Africa to assess the safety, immunogenicity and protective efficacy of increased doses of Sanaria® PfSPZ Vaccine administered via direct venous inoculation (DVI) against natural malaria exposure and controlled human malaria infection (CHMI) in healthy 18-50 year old adults. The initial dose escalation pilot study (part A) started in January 2016. Five volunteers received 1 dose of PfSPZ Vaccine ($4.5x10^5$ PfSPZ) and five volunteers received 1 dose of PfSPZ Vaccine ($9.0x10^5$ PfSPZ) in a staggered manner. Following no identified safety concerns, 30 additional volunteers have received three doses of PfSPZ Vaccine ($18.0x10^5$ PfSPZ) at eight week intervals in the dry season. These 30 subjects will also undergo further evaluation, including examination of protective efficacy against homologous CHMI via PfSPZ Challenge in May/June 2016. Fifteen additional subjects will be enrolled as infectivity controls during CHMI. The targeted dose ($18.0x10^5$ PfSPZ Vaccine), having been shown to be safe and tolerable, is now being administered to a larger main cohort in a double blind, randomized, placebo controlled trial (part B) to examine the protective efficacy of the vaccine against naturally occurring infection. During the malaria transmission season (August-December), volunteers will be examined and blood smears obtained every 2 weeks for 20 weeks in total, with the primary efficacy endpoint being observation of a positive blood smear following receipt of third vaccination. Safety, tolerability, immunogenicity, and protective efficacy from the pilot phase (part A) and safety and tolerability from the main phase (part B) will be presented.

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ANTIBODY PROFILING BY PROTEIN MICROARRAY OF HUMAN VOLUNTEERS PROTECTED BY IMMUNIZATION WITH RADIATION-ATTENUATED *PLASMODIUM VIVAX* SPOROZOITES

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A randomized, single-blinded clinical trial was conducted with Duffy positive (Fy+; Pv susceptible) individuals (n=21) assigned to either radiation attenuated sporozoites (RAS) or control groups; 14 received bites from irradiated (150 ± 10 cGy) Pv-infected *Anopheles* mosquitoes (RAS) and 7 from non-irradiated non-infected mosquitoes (control). Fy- (Pv refractory) volunteers (n=7) were immunized with non-irradiated Pv-infected mosquitoes. Eight weeks after the last immunization, 19 Fy+ and Fy- volunteers received a controlled human malaria infection (CHMI) with non-irradiated Pv–infected mosquitoes. Nineteen volunteers completed 7 immunizations (12 RAS, 2 Ctl and 5 Fy-) and received a CHMI. Five of 12 (42%) RAS volunteers were protected compared with 0/2 controls. None of the Fy- volunteers developed infection by the 7th immunization or after CHMI. To test whether protection might also be associated with IgG profiles, serum samples from day (0), after each immunization dose and 60 days post-CHMI were probed on a custom protein microarray displaying 500 *P. vivax* sero-reactive antigens. We observed that, while Fy- volunteers respond vigorously to non-irradiated vaccine, the RAS vaccine appeared to be only weakly antigenic in Fy+ volunteers (despite being protective in 5/12 RAS volunteers). Nevertheless, we observed significant changes in antibody profiles between protected and unprotected RAS volunteers post-CHMI, consistent with similar studies in *P. falciparum* vaccinees. We conclude that, protection from *P vivax* challenge can be achieved with relatively low doses of RAS vaccine.

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CHANGING THE PARADIGM OF VACCINE DEVELOPMENT: TURNING THE TARGET PRODUCT PROFILE (TPP) ON ITS HEAD

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Vaccines are lacking for many complex pathogens such as parasites and fungi, may be only partially protective, or may not be approved for key populations like children and pregnant women. There is an exigent need to enhance the efficiency of vaccine development, to generate new and better products, and to protect vulnerable populations. The International PfSPZ Consortium is developing a vaccine against a "refractory" pathogen - the malaria parasite - on an efficient and accelerated time line, with plans to submit a Biologics License Application in 2018 for a sporozoite-based vaccine providing > 80% sterile protection against malaria infection for > 6 months. The principles we have followed may be of value to the field. 1) We have avoided a detailed target product profile (TTP), even though this is often considered the first step in development. We believe TTPs force thinking "inside the box" and suppress innovation. Rather, we have ignored conventional restrictions such as adherence to traditional manufacturing methods, storage conditions, and routes of administration, contending that vaccines have just 3 required attributes (our universal TPP):

safety, tolerability and efficacy. 2) We selected a vaccine platform based on established proof-of-concept for high-level protection in humans. While a prototype vaccine may stray from other practical norms, high-level protection trumps everything else as the cornerstone of success. 3) The logistics of vaccine manufacturing, storage, delivery and administration have been addressed head-on as bioengineering and clinical challenges that need to be overcome. 4) Optimizing the immunization regimen has required classic vaccinology studies to adjust dose size, number and interval, and to select the best route of administration. 5) Speed of development has been enhanced by flexible trial design, receptivity to new approaches, intense transparent collaboration, immediate data communication, and frequent consultation with regulatory authorities, all driving toward licensure. These concepts will be discussed with the goal of promulgating a new, highly effective paradigm for vaccine development.

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DETERMINANTS OF INSECTICIDE TREATED NET USE AMONG UNDER-FIVE CHILDREN IN NIGERIA

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The use of Insecticide-treated nets (ITN) is the most cost effective malaria preventive measure. However, malaria remains a big killer of children in Nigeria accounting for about 30% of the under-fives (U5) deaths. Despite massive distribution campaigns nationwide, ITN use among (U5s) is low. This study assessed factors influencing ITN use among U5s across households in Nigeria.We analyzed the 2013 Nigerian Demographic and Health Survey dataset with specific focus on ITN use in children. A total of 17,664 U5s who slept in households with at least one ITN the night prior to the survey were studied. We conducted binary logistic regression analysis to identify factors influencing ITN use by U5s in (1) households that have at least one ITN and (2) households with at least one ITN for every two people (universal coverage). Data analysis was conducted using STATA (version 14.0) and results were considered significant at $p < 0.05$. In households with ITN ownership, only 28.5% of U5s slept under the ITN the night before the survey. Significantly, children less than three years old, those who reside in urban areas, those from other regions of the country except the North East had higher odds of using ITN the night before the survey. Also, children from households with less than 7 members and those from the second and third wealth quintiles had significantly higher odds of using ITN. On the second regression model among households with universal coverage, U5s who reside in urban areas, those from other regions of the country except the North East and those from the second and third wealth quintiles still had significantly higher odds of sleeping under ITN the night before the survey.Our findings have programmatic implications for malaria control among U5s in Nigeria. Alongside sustained ITN distribution to improve ITN coverage, health promotion/education efforts targeting parents/guardians on the relevance of ITN use in malaria prevention among U5s should be emphasized. Interventions to improve ITN use should be intensified among rural dwellers, larger households, and residents of North-Eastern Nigeria.

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TRANS STAGE DYNAMICS OF MIDGUT MICROBIOTA OF *ANOPHELES ALBIMANUS* FROM THE PACIFIC COAST OF COLOMBIA

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Colombia is the second country in Latin America in number of malaria cases reported, with an average in the last decades of over 100,000 cases per year, but underreporting is presumed. Some bacteria of the *Anopheles* mosquitoes gut microbiota seem to be involved in blocking development of the *Plasmodium* parasites and are being studied as alternative paratransgensis approach. Little is known about the autochthonous microbiota of the Latin-American anopheline mosquitoes; therefore, in this study, the midgut microbiota of fourth-instar larvae and adults of the main Colombian malaria vector *An. albimanus* was characterized. The midgut microbiota of specimens collected in the locality of the Colombian Pacific Coast was analyzed by both, culture-dependent and Mi-Seq Illumina high-throughput technology. Higher bacterial species richness was detected in immature stages while various bacterial species were detected in both stages, larval and adult females. Gram positive bacteria predominated in larvae and similar number of Gram positive and Gram negative bacteria were detected in adults. Current metagenomics analysis will complement the information on the bacterial community composition and their dynamics within the different stages of this important *Anopheles* vector species. This knowledge provides the basis for the design of new and effective vector interventions to control malaria transmission.

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FACTORS ASSOCIATED WITH OWNERSHIP OF INSECTICIDE TREATED NETS IN HOUSEHOLDS IN UGANDA

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Malaria is still a global public health challenge and in Uganda, it accounts for 40% of hospital outpatient visits, 20% of hospital admissions, and 14% of hospital deaths. Use of Insecticide treated nets (ITNs) is effective for malaria prevention and control in households. Universal coverage of ITNs (defined as one ITN for two people regardless of age or gender) is recommended for all households to achieve impact. Mass ITNs distribution campaigns remain a cornerstone in efforts to achieve universal coverage. As such, Uganda recently conducted a mass ITN distribution campaign, distributing over 33 million nets country wide. We examine factors associated with universal ownership of ITNs among households in Uganda to guide programmatic planning. We analyzed the Uganda Malaria Indicator Survey data collected in 2013/14. Factors associated with universal ownership of ITNs in households were examined using logistic regression. Independent variables assessed included area of residence, age and sex of household head, household socio-economic class, number of rooms in the household, and whether the household had odd or even number household size. Households in the lowest socio-economic class (OR = 0.641, p = 0.00) were less likely to have universal ownership of ITNs compared to those in the highest socio-economic class. Households with household size as an odd number (OR = 0.600, p = 0.00) were also less likely to have universal ownership of ITNs. Allocation strategies for future mass ITN distribution campaigns need to incorporate households in low socio-economic classes as a first step. More consideration should also be accorded to households with odd number household size.

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LABORATORY AND SEMI-FIELD EVALUATION OF A LONG-LASTING MICROBIAL LARVICIDE FOR MALARIA VECTOR CONTROL

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Bio-larvicides can target both insecticide resistant and outdoor biting mosquitoes however, currently available formulations have a short duration of efficacy and the need for frequent applications hence more costs involved. The study was designed to evaluate the efficacy and duration of activity of a new slow release briquet formulation of Bacillus thuringiensis israelenis (Bti) and Bacillus sphaericus (Bs) under laboratory and semi-field conditions. One and Two larvicide briquets were dissolved in separate plastic tanks to make two different Bti/Bs solutions while a non-Bti/Bs briquet was dissolved in another plastic container to make the control solution. After 24 hours, 2 liters of each solution and twenty 2nd - 3rd instar of field collected *Anopheles* larvae were added to a set up of 10 small plastic basins and placed in the insectary. The same was done for the semi-field experiment and left in the open outside the insectary. The surviving larvae and pupae in each plastic basin in both treatment and control set-ups were counted daily. The experiment was repeated on day 3, 7, 2 weeks and thereafter monthly. Results in the lab show both larvicide solutions gave high larval mortality of about 90% for the 5 months period with no larval pupation observed during the first two months. About 5%, 8% and 11% of the larvae pupated within 3, 4 and 5 months respectively for the one briquet larvicide. For the two briquet solution, about 2.5%, 4.5% and 7.5% of the larvae pupated within 3, 4 and 5 months respectively. More than 75% larval pupation was observed in all the 5 months in the control set up. In the semi-field, both the larvicide solutions gave high larval mortality for 3 months and there was no larval pupation observed in the two larvicide solutions during the first two months. In the control set up larval pupation was more than 80% for all the 4 months. This study showed that this new Bti/Bs briquet formulation is effective in killing larval mosquitoes for more than 4 months.

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DEVELOPMENT AND EVALUATION OF A NOVEL PIPE TRAP FOR OUTDOOR HOST-SEEKING MALARIA VECTOR SURVEILLANCE IN WESTERN KENYA

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Monitoring outdoor biting malaria vectors is essential to monitor residual malaria transmission and evaluate the likely success of vector control measures. Human landing catch (HLC) has been considered standard method for monitoring host-seeking vectors. However, its use is facing increasing ethical concern. CDC light trap has been used as an alternative, but it does not provide a direct estimate of human biting rate. In this study, we evaluated a novel pipe trap for surveillance of outdoor host-seeking malaria vectors. The pipe trap was made of pipe that pumps human odor from sleeping room to outdoor station and CDC light trap which was connected to the outer end of the pipe. Fan was fitted in to the pipe to enhance outflow of the odor. The sampling efficiency of the pipe trap was compared with outdoor CDC light trap (LT-outdoor) in cross-over experimental design in lowland (Ahero) and highland (Iguhu) settings of western Kenya from November 2015 to February 2016. A total of sixty trap-nights were done for each trap per site. Overall, 2,924 *Anopheles* mosquitoes were collected by pipe trap and LT-outdoor. Of these *An. arabiensis, An. funestus, An. gambiae, An. pharoensis* and *An. coustani* accounted for 18.4%, 6.2%, 0.3%, 39.8%, and 35.3%, respectively. In Ahero, pipe trap yielded significantly (Mann-Whitney p = 0.003) higher numbers of An. arabiensis (mean = 5.53, 95% CI: 3.61-7.45) than LT-outdoor (mean = 2.48, 95% CI: 1.36-3.60). Similarly, the pipe trap caught significantly (p = 0.002) higher numbers of *An. funestus* (mean = 1.65, 95% CI: 1.15-2.15) than LT-outdoor (mean = 0.78, 95% CI: 0.43-1.13). The mean numbers of other species collected did not vary significantly between the two traps. In the highland, mean numbers of An. gambiae and An. funestus was 0.1 and 0.15 in the pipe trap, and 0.05 and 0.1 in the LT-outdoor, respectively. Both traps collected mostly unfed mosquitoes, with higher proportion recorded in the pipe trap. The pipe trap attracted higher numbers of human seeking malaria vectors, suggesting its potential to replace HLC. Validation of the pipe trap for collecting outdoor human biting mosquitoes will be done by comparing against Malaise trap.

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ESTIMATING BEDNET DEMAND IN TANZANIA USING A DISCRETE CHOICE EXPERIMENT

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During the last 10 years, widespread evidence confirms that insecticide-treated bednets (ITNs) are an effective tool for decreasing malaria incidence. While this evidence has supported mass distribution of free ITNs in various sub-Saharan African countries, no current information exists regarding private household demand for ITNs. This study estimates private demand for bednets in Tanzania using an experimental approach, namely a non-hypothetical Discrete Choice Experiment (DCE). The results should prove useful to policymakers, donors, and bednet manufacturers as they explore the extent that upper and middle income households might share the cost of ITN distribution and identify preferences among potential users for various bednet attributes (texture, shape and size). Tanzania provides a suitable locale to explore these questions because the government recently implemented several nationwide distribution campaigns. The study gauges the impact of these campaigns on private demand. Moreover, the DCE provides an improved estimate of demand elasticity and willingness-to-pay for specific attributes, including the pre-treated insecticide. Existing estimates of bednet demand in Tanzania were conducted prior to the introduction of pre-treated long lasting nets (LLINs) and before the mass distribution campaigns. Consequently, the bednet market has experienced potentially significant demand and supply side shocks. The DCE method involves displaying two different nets to each participant and assigning prices to the nets. Participants then choose the net they would prefer to buy, or neither one. The DCE replicates a market environment since participants receive a small stipend, with the cost of the chosen net deducted from the stipend. DCEs are routinely used for market studies in high-income countries but this study marks their debut application to bednets. Data collection will occur during May 2016, with 800 participants spread across two rural and two urban locations.

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KNOWLEDGE, ACCEPTANCE AND WILLINGNESS TO PAY FOR INDOOR RESIDUAL SPRAY IN RURAL AND URBAN COMMUNITIES IN NIGER STATE, NIGERIA

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Malaria is the leading cause of U5 mortality (20%) and responsible for 11% maternal mortality in Nigeria. The Nigeria National Malaria Elimination Programme (NMEP) adopted In-door Residual Spray (IRS) in 2007 as one of her key strategies for reducing malaria burden in

the country. A quantitative study was conducted among households in Niger state to assess the knowledge, acceptance, and willingness to pay for IRS by households to inform nationwide IRS implementation. Respondents were selected through a multistage sampling technique. Data were collected by trained research assistants using semi-structured questionnaire, administered to 400 consenting households across six communities in two local government areas. Ethical approval for the study was granted by the Niger State Ministry of Health. Association between Willingness to pay and socio-demographic characteristics such as age, sex, education, religion and socio economic status was explored using the Chi Square Test. Result showed that only 8.4% of the respondents have ever heard of IRS therefore information on IRS was provided to all households that had never heard of it before the study. Although about 70% of the households were using LLIN to prevent malaria, 88.4% were willing to have their houses sprayed while 71% where willing to pay for IRS. Majority of the respondents were willing to pay at most N1000 (about $5) per spray while about 1.2% wanted IRS provided at no cost. Place of residence (urban, rural), status of respondent (whether household head or not), ownership of dwelling place and level of education were observed to be significantly associated with willingness to pay for IRS. The study revealed low knowledge of IRS in households in Niger state but a high level of acceptance and willingness to pay once the benefits of IRS have been provided to respondents. To facilitate acceptance of IRS, implementers should obtain consent from head of households and sensitization on the importance of IRS should be carried out. Findings from this study will guide implementation of state-wide implementation of IRS in Niger state.

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EVALUATION OF THE IMPACT OF IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION ON MORBIDITY AND MORTALITY IN YOUNG CHILDREN: A QUALITATIVE STUDY IN NORTHERN GHANA

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Seasonal Malaria Chemoprevention (SMC) intervention was introduced in the Upper West Region of Ghana in July 2015. This was by the recommendation of WHO as an additional tool which has been shown to be effective, safe and feasible in preventing malaria among children under five years in areas with high seasonal malaria transmission. Community involvement is critical in controlling malaria. This may determine the effectiveness of the intervention. A study was undertaken to assess the community acceptability of this intervention in addition to other malaria control measures in reducing the burden of malaria among children under five years in the Upper West Region of Ghana. Fifty interviews and eight focus group discussions were conducted in the Lawra district where SMC intervention was introduced for the first time. Participants consisted of parents and guardians of children who received the intervention and community health workers and health volunteers. The intervention was generally acceptable to the local population who opined that it had led to a reduction in the prevalence of malaria in the district compared to previous years. An overwhelming majority of the respondents said the drug was very good because it prevented malaria in children who took it.Drug adherence was very good and there was no serious adverse event associated with drug intake. The various adverse events that occurred did not result in interruption of the treatment course. Community members and other stakeholders believe that the SMC intervention played a role in reducing the burden of malaria in the study area and wish that the program would continue so that their children would continue to benefit. Some participants called for the intervention to be introduced into the EPI and made compulsory for all children under five years. Acceptability of the intervention was very high among parents and other stakeholders in the study area. They however wanted to be counselled on the potential side effects and their management. Community acceptability of SMC is very high and can contribute to control malaria in areas where malaria transmission is seasonal.

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THE IMPACT OF INDOOR RESIDUAL SPRAYING ON THE DENSITY AND PARITY RATE OF *ANOPHELES GAMBIAE* S.L. IN OROMIA REGION - ETHIOPIA

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Entomological monitoring is an integral part of the US President's Malaria Initiative's (PMI's) vector control efforts. Data on key entomological indicators is regularly collected and used to guide vector control programs and track their impact. A non-randomized, interrupted controlled time series design was used to assess the impact of IRS on vector density and parity rate in Ethiopia. Pre-spray data was collected from one control and two intervention districts in monthly intervals from March to August 2015, while post-spray data was collected monthly from September to November 2015 from the same sites. Pyrethrum spray and human landing catch mosquito sampling methods were used to determine indoor resting density and parity rate, respectively. Before spray, the mean indoor resting density of the main vector, *Anopheles gambiae* s.l., was 4.1 (n=496 mosquitoes) and 1.03 (n=124) per house per day in the intervention and control sites, respectively. Post spray, the mean vector density dropped to 0.49 (n=59) in the intervention and to 0.73 (n=88) in the control districts. The decline in vector density was four-fold in the intervention sites as opposed to only a 30% decline in the control site. The reduction was statistically significant in the intervention (p=0.025) but not in the control (p=0.738) site. The difference noted between the two treatments might be due to the impact of IRS.At the same time, data on parity rate for *An. gambiae* s.l. was collected pre- and post-spray from the intervention and control sites. Before the spray campaign, the parity rate was 79.75% (n=553) in the intervention and 96.94% (n=193) in the control sites. After spraying, the parity rate dropped to 54.57% (n=405) in the intervention sites but increased to 100% (n=148) in the control site. The decline and increase in parity rate observed after spray as compared to before spray in the intervention and control sites, respectively, were statistically significant. (p<0.001). The change detected in parity rate might partially or totally be attributed to the impact of IRS. Further study is needed to understand if the reduction in density and parity rate has led to reduced malaria transmission.

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STRATIFICATION OF INDOOR RESIDUAL SPRAYING (IRS) IN BIOKO ISLAND: METHODOLOGY AND IMPACT

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The Bioko Island Malaria Control Project (BIMCP) uses a GIS-based Campaign Information Management System (CIMS) that uniquely identifies each household based on geographical location. 2014 spray data revealed Island wide coverage of just 57% with high refusal rates, well below the WHO recommended coverage for effectiveness. Due to this, as well as budget constraints that did not support long term-sustainability of Island-wide IRS, the BIMCP stratified households and communities targeting spraying at the most vulnerable populations only, relying on LLINs in other areas. To stratify households by vulnerability, we utilized community-level parasite prevalence and risk of importation derived from the 2014 MIS, quality of housing and 2014 IRS coverage from the CIMS. As the 2014 MIS sampling frame was based on sentinel sites, MIS values for prevalence and importation in communities not

included in the MIS sample were imputed. Based on this information, a risk score (R) and a risk-acceptability score (Ra) were derived for each community. Communities with the highest R and Ra values were selected for IRS- targeting, given the available budget, 30% of all households. Spray coverage in targeted communities was high, with an overall coverage of 80%. As such, in 2016, the BIMCP re-ran the stratification but removed previous IRS coverage as a criteria utilizing only prevalence, importation and housing quality - our measures of vulnerability. In addition, the 2015 MIS sampling frame was all communities in the Island with 20 households or more, eliminating the need to impute data. MIS data from 2014, 2015, and 2016 will be analyzed in communities that were stratified for IRS and in those that were not, to determine if the stratification of IRS and replacement of LLINs as the main control strategy in all communities resulted in a differential change in prevalence of infection across communities. An initial analysis of 2014 and 2015 data revealed prevalence of infection decreased both in communities stratified for IRS and those where LLINs alone were deployed, indicating that the withdrawal of IRS from less vulnerable communities did not lead to adverse consequences.

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COST-EFFECTIVENESS OF MALARIA CONTROL MEASURES: A CLUSTER-RANDOMIZED CONTROL TRIAL OF IRS AND ITNS IN MOZAMBIQUE

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Malaria endemic countries face challenging decisions regarding choice and financing of interventions to prevent malaria. To maintain the gains in malaria control and move toward elimination, malaria endemic countries need information on the cost effectiveness of interventions and combinations of interventions. Vector control programs, through insecticide treated nets (ITN) and indoor residual spraying (IRS), are a critical component of national malaria strategies. Yet, widespread insecticide resistance - particularly to pyrethroids, is a serious threat to program effectiveness. Additionally, important questions remain regarding the benefits and costs of IRS in the context of expanding ITN coverage. In order to inform national vector control strategies, this study will compare the cost-effectiveness of different interventions in a malaria endemic region of Mozambique by determining the cost per case averted of ITNs alone, as compared to ITNs plus IRS. Clusters will be randomized in mid-2016 to receive either IRS with the organophosphate, Actellic CS (intervention), within a context of high coverage of pyrethroid impregnated ITNs or ITN coverage only (control). Data collection is from 2016 to 2018 with baseline data available by October 2016. Changes in malaria incidence from strengthened health facility data, community parasite prevalence and net use from community surveys, entomological inoculation rates and resistance levels from entomological surveillance, and intervention costs will provide evidence on the impact of the intervention. Routine case burden data, cost data, cross-sectional prevalence surveys and entomological monitoring will be triangulated to determine overall and incremental impact and cost-effectiveness of IRS plus ITNs compared with ITNs alone. These findings will be critical to support informed-decision making to control and eliminate malaria by malaria programs in Mozambique and other high-burden countries.

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DETERMINING THE PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF MIXED FUNCTION OXIDASES IN *ANOPHELES GAMBIAE* S.L. WITH PYRETHROID RESISTANCE IN RELATION TO SITE SELECTION FOR A TRIAL OF COMBINATION LLINS IN MALI

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The only long-lasting insecticidal nets (LLINs) currently under consideration for use in areas of pyrethroid resistance are LLINs treated with pyrethroids plus piperonyl-butoxide (PBO), a synergist that inhibits the activity of mixed function oxidases (MFOs) in resistant mosquitoes. This study sought to document the prevalence of MFO mechanisms in pyrethroid-resistant An. gambiae s.l. mosquitoes in different geographic areas of Mali. This information was used to identify sites for an intervention trial involving PBO-containing LLINs. *Anopheles gambiae* s.l. larvae were sampled in 12 sites in central and southern Mali from August-December 2015. Local vector populations were screened with the CDC bottle bioassay tests for permethrin and deltamethrin resistance. Bottle bioassays with PBO as a synergist were used to determine the association between reduced survival and elevated levels of MFOs. Taxonomic identification of species as well as presence of the L1014F and L1014S kdr (knock down resistance) mutations were determined by polymerase chain reaction. Based on WHO's 2013 resistance classification guidelines (mortality rate < 90%), evidence of high-frequency pyrethroid resistance was observed in all 12 test sites. Mortality increased (P97%). This suggests evidence of MFO-based metabolic resistance mechanisms involved in pyrethroid resistance. High variability was observed in resistance and the effect of PBO on mortality among sites. L1014F kdr allelic frequencies were >60% in *Anopheles coluzzii*, >40% in *An. gambiae* s.s. L1014S kdr allelic frequencies were <15% in An. coluzzii, <10% in An. gambiae s.s. This study demonstrated high variability of MFO mechanisms in wild-caught mosquitoes with pyrethroid resistance in central and southern Mali. Even though use of PBO did not restore full susceptibility, several sites were identified as potentially suitable for further evaluation of combination LLINs with PBO.

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WHY NO NETS? AN IN-DEPTH INVESTIGATION INTO THE DECREASE IN NET ACCESS ON BIOKO ISLAND AFTER BED-NET DISTRIBUTION

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From December 2014 to June 2015, the Bioko Island Malaria Control Project (BIMCP) conducted a mass-top up LLIN distribution campaign on Bioko Island, Equatorial Guinea. Despite good reported coverage (88% of households received at least 1 LLIN), results from the BIMCP's 2015 Malaria Indicator Survey (MIS) reported low levels of net ownership (69%)

and low net access (32%) approximately 7 months following distribution. While the distribution campaign ensured that one net was distributed per sleeping area, achieving an average of one net per 2 residents in 88% of households, a better understanding of the reasons behind the reduction of nets is needed to allow BIMCP to implement strategies against a decrease in net access in future campaigns. An initial analysis of MIS questions regarding net ownership indicated that a high proportion of households reported a dislike of the net (16.2%), while other households reported giving them to relatives on the mainland (12.7%). A mixed quantitative and qualitative study will be performed to determine the reasons behind the reduction in bed net access. A random sample of 150 households on Bioko Island will be selected from BIMCP's Campaign Information Management System (CIMS) and MIS databases. These databases use a GIS-based household mapping system, used to easily locate and track every household location to be sampled. A semi-structured interview will be employed to determine participant household's current net access relative to the number of nets distributed during the campaign and the number of nets reported in the subsequent MIS, what happened to the nets that were lost and the reasons why, current net usage by household member type (e.g. under-5's, pregnant women, etc.), their knowledge and attitudes acquired from the distribution teams and/or other media about LLINs, and their perceptions about the distribution process. The analysis will seek to discern what the primary reasons are for the unexpected decrease in net access. Findings will inform the BIMCP on how to reduce the LLIN loss rates, in planning for the upcoming 2017 LLIN distribution and for future continuous distribution.

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DETERMINANTS OF BED NET USE CONDITIONAL ON ACCESS IN POPULATION SURVEYS IN GHANA

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Out of 23 African countries surveyed with a Demographic and Health Survey (DHS) in the 2012-2015 period, Ghana ranks 21st in net use conditional on access (NUCA) (with 57.5%), and only Namibia and Nigeria have a larger proportion of people not using nets despite having access to a space under one. In literature, self-reported reasons for not using nets include discomfort (due to heat), disruption of sleeping arrangements, and low mosquito density. We explored if 'geographical region', 'residence' (urban or rural), 'wealth quintile', 'spraying of the dwelling with residual insecticides' (IRS), the 'humidex' in the month of survey, 'connection to electricity', the 'mean age' of the nets in the household, the 'nets : people ratio' in the household and the 'respondent heard a message on the use of nets' could explain NUCA with beta-binomial regression models that corrected for spatially correlated random effects in the Ghana 2014 DHS. Similarly, this was done with these variables (except the not collected one on messaging) for the Ghana 2011 Multiple Indicator Cluster Survey (MICS), with as additional variables 'ethnicity', 'religion' and an estimate of 'vector density in the month of survey'. In the DHS, 'wealth quintile', 'electricity', 'residence', 'messaging', 'humidex' and 'IRS' (in order of decreasing importance) individually improved model fit, as assessed by the deviance information criterion (DIC), compared to a null model without covariates, and also all these variables combined in one model improved the DIC over simpler models. In the MICS, the variables 'region', 'net age', and 'IRS' improved the fit when individually added to the null model. 'Net age' improved the fit when included into a model with 'region' as covariate. Differences in results between 2011 and 2014 are possibly due to the 2011 MICS being held in the midst of a mass distribution campaign, with 4 regions (partly) completed and 6 not yet begun. Surprisingly, the 'nets : people ratio' (the higher the ratio, the less need for sharing nets and possibly disrupting sleeping arrangements) did not explain NUCA and the humidex (a measure of comfort due to heat) only did so in the 2014 DHS.

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JOINT DISTRIBUTION OF ACUTE RESPIRATORY INFECTION, DIARRHEA AND STUNTING AMONG CHILDREN UNDER THE AGE OF FIVE YEARS IN SOMALIA

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The aim of this study was to assess the spatial co-occurrence of acute respiratory infections (ARI), diarrhoea and stunting among children the age of five years in Somalia. Data were obtained from routine bi-annually nutritional surveys conducted by the Food and Agriculture Organization (FAO) from 2007 - 2010. A Bayesian hierarchical shared component model was fitted to the spatial components of the three health conditions concurrently. Risk maps of the common spatial effects at 1 x 1 km resolution were derived. The empirical correlations of the proportion were 0.37, 0.63 and 0.66 for ARI and stunting; diarrhoea and stunting and ARI and diarrhoea respectively. Spatially, the posterior residual effects ranged from 0.04 to 18, 0.19 to 5.39 and 0.07 to 8.16 for shared component between ARI and stunting; diarrhoea and stunting; and ARI and diarrhoea respectively. This analysis shows clear spatial shared component between ARI, diarrhoea and stunting in Somalia with the southern part of the country experiencing a higher than usual rate of these conditions. Intervention aimed at reducing the rates of these three health conditions should focus on the common risk factors particularly in the South in Somalia.

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SHANCHOL, THE ORAL CHOLERA VACCINE IS SAFE AND IMMUNOGENIC WHEN STORED AT ELEVATED TEMPERATURES IN BANGLADESHI PARTICIPANTS

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Immunization against cholera is recognized as a major intervention for control of the disease, however the requirement for a cold chain has limited its use in resource poor settings.The aim was to determine the safety and immunogenicity of Shanchol after stored at temperature upto 42°C. The study conducted in adult participants in Dhaka, Bangladesh. The vaccine kept under standard (Group A; 2-8°C) and at three elevated temperatures (Group B; 25°C, Group C; 37°C, Group D; 42°C) for 14 days. Vibriocidal and LPS antibody responses were determined at baseline and 7 days after each dose. 580 participants; 145 in each group (A=2-8 °C ; B=25 °C; C=37 °C and D= 42°C) were vaccinated. Only 17 mild adverse events, which did not differ between groups. Increases of vibriocidal responses observed at day 7 and 21 ($P<0.001$) compared to day 0 in all groups. Over four-fold increases to *V. cholerae* O1 Ogawa were observed at day 7 and/or day 21 after vaccination in at all groups, with responder rates of; 76%, 80% , 69% and 74% in Groups A, B, C, and D ($p=0.240$). Significant vibriocidal antibodies to *V. cholerae* O1 Inaba and O139, and LPS- Ogawa and Inaba antibody responses also observed after each dose ($p<0.001$); with comparable seroconversion rates between Group A and Groups B, C, and D. Interpretation: This is the first report of the testing

the temperature stability of Shanchol in people. Shanchol is safe and immunogenic in endemic settings even when kept outside the cold chain. Further study should conduct to assess the impact in younger age group.

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HOST AND ENVIRONMENTAL CORRELATES OF MULTI-DRUG RESISTANCE IN KENYAN CHILDREN WITH ACUTE BACTERIAL DIARRHEA

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Bacterial diarrhea results in significant morbidity and mortality in children in sub-Saharan Africa. Antibiotic treatment may be important in this population but is limited by concerns about antibiotic resistance. Stool/rectal swab samples of children aged 6 mos - 15 yrs presenting with acute diarrhea in western Kenya were cultured for bacterial pathogens. HIV uninfected children with identified *Shigella* or *Salmonella* species, or enteropathogenic [EPEC], enterotoxigenic [ETEC], enteroaggregative [EAEC], or enteroinvasive *Escherichia coli* [EIEC] were included in this substudy. Resistance to ampicillin, ceftriaxone, ciprofloxacin, cotrimoxazole, and tetracycline was determined using MicroScan Walkaway40 Plus. To evaluate correlates of multi-drug resistance (MDR [resistance to ≥ 3 classes of antibiotics]), we used multivariable log-binomial regression to estimate prevalence ratios (PRs) and 95% confidence intervals (CIs). Of 336 children in the analysis, median age was 19 mos (interquartile range: 10-39 mos), 38% used unimproved sanitation facilities and 10% were HIV-exposed. Resistance to cotrimoxazole (96%) was most common among all pathogens, followed by ampicillin (81%) and tetracycline (77%). Phenotypic MDR was identified in 11% of children; and in 38% of *Shigella*, 40% of *Salmonella*, 76% of EPEC, 54% of ETEC, 78% of EAEC, and 77% of EIEC isolates. Children 6-24 mos were more likely to have MDR infections identified than those 24-59 mos (aPR = 1.56 [95% CI: 1.25, 1.92]). Children whose caregivers used a shared pit latrine or who openly defecated were more likely to have MDR (aPR = 1.71 [95% CI: 1.02, 2.86]) than those with flush or unshared toilets. Duration of exclusive breastfeeding, malnutrition, maternal HIV, water source, and hand washing were not associated with MDR infections in this study. Young age and unimproved sanitation may be associated with MDR as a result of greater exposure to fecal contamination. Community antibiotic use may select for resistant enteric bacteria in settings with poor sanitation by eliminating susceptible bacteria before excretion and increasing inter-species transfer of resistant plasmids.

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GENOTYPIC IDENTIFICATION OF AMPC β-LACTAMASES PRODUCTION IN DIARRHOEAGENIC E.COLI FROM CHILDREN UNDER FIVE AND MOLECULAR DOCKING OF THEIR PROTEINS

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AmpC beta-lactamases are bacterial enzymes that hydrolyse 3rd generation extended spectrum cephalosporins and cephamycins engendering resistance to these categories of antibiotic. AmpC β-lactamase expression can increase in the nosocomial pathogens, triggered by exposure to antibiotics and β-lactamase inhibitors with the β-lactam function. Therefore, AmpC β-lactamase is an important target for developing novel effective antibacterial therapies. The objective of this study was to evaluate the Real Time PCR as a rapid diagnostic tool for simultaneous detection of AmpC beta-lactamase producing E.coli and in silico determination of docking sites of AmpC proteins.During one year period from July 2012 to July 2013, 120 stool samples were collected, including 80 diarrheagenic E.coli and 40 controls from children in University College of Medical Sciences and Guru Teg Bahadur Hospital, East Delhi. E.coli was diagnosed for AmpC beta lactamase production using conventional phenotypic tests. DNA extraction was done, and extracted DNA was used as a template for Real Time PCR. Bioinformatics tools were used for molecular docking. Real time PCR detected target genes of AmpC beta lactamase in 18.75% and 22.5% in cases and controls respectively. Chi square test and Fisher's exact test were used to determine statistical significance of data. Real time PCR assay will save time and help investigators to explore the role of multidrug resistant *E. coli*. Active binding sites will be useful in synthesis of new drugs. Modeling and docking studies may provide useful insights for developing new antibiotic drugs to minimize multidrug resistance.

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CAMPYLOBACTER SPECIES, CAPSULE TYPES AND VIRULENCE FACTORS CIRCULATING AMONG CHILDREN IN EGYPT

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Campylobacter is one of the most frequently isolated bacterial pathogens in children with diarrhea. There are 47 recognized Penner serotypes of *Campylobacter jejuni*, represented by 35 capsule types. The capsule serves as a target for vaccine development. *Campylobacter* can also harbor the type-6 secretion system (T6SS), which is a known virulence factor among Gram negative pathogens. Data are lacking on the distribution of *C. jejuni* capsule types in most developing countries. The prevalence of the T6SS within *Campylobacter* species and capsule types has not previously been reported. The US Naval Medical Research Unit #3, based in Cairo, Egypt conducted three prospective diarrheal studies in Abu Homos, Egypt from 1995-2000. In total, 531 *Campylobacter* spp. isolates were obtained from 397 children. Each isolate was re-grown and DNA was extracted for speciation, capsule typing and hcp gene presence, a marker for T6SS. Of the isolates, only 522 and 501 were respectively available for capsule and T6SS analysis; 60.9% (318) were *C. jejuni*, 37.9% (198) were *C. coli* and 1.1% (6) were unable to be speciated. Capsule types were identified in 88.4% (281) of the *C. jejuni* isolates; HS-2 and HS-3 were the most prevalent (both 11.9%). The hcp gene was found in 57.5% (172) of *C. jejuni* isolates compared to 19.4% (38) of *C. coli* isolates ($p<0.001$) and 83.3% (5) of the unspeciated isolates ($p<0.578$). The presence of the hcp gene among *C. jejuni* capsule types varied significantly (0-100%). This study provides epidemiological data regarding the distribution of circulating *C. jejuni* capsule types. Within this cohort, eight capsule types together accounted for 72% of *C. jejuni* infections. The heterogeneity of *C. jejuni* serotypes is higher than previously reported, although prior studies of serotype distribution within Africa are limited and have not focused on pediatric infections. Our data suggests that the T6SS is more commonly found among *C. jejuni* isolates compared to *C. coli*. We also highlight significant gaps that persist. Further studies are needed to determine whether specific capsule types or presence of the T6SS are associated with more severe clinical illness.

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ENTEROPATHOGENS DISTRIBUTION AND BURDEN WITHIN ORAL CHOLERA VACCINE RECIPIENTS IN SOUTH SUDAN

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The efficacy of oral cholera vaccine (OCV) is poorer in younger children than adults and there is great heterogeneity in immunogenicity within and between different sub-populations. Multiple oral vaccines have shown decreased efficacy in the setting of concurrent enteric infections. The objective of this study was to explore the distribution of enteropathogens within stool of recipients of OCV and the relationship between pathogen carriage and immunogenicity. As part of a mass vaccination campaign in an internally displaced person's camp in South Sudan, we obtained paired stool and serum samples from a subset of recipients who received 2 doses of the OCV Shanchol 14 days apart. We used the vibriocidal assay to determine titers just before the first dose (day 0) then 14 and 28 days after. We extracted nucleic acid from stool samples and performed multiplex real-time PCR on 18 bacterial, viral and protozoal targets. Stool from a total of 89 subjects were examined, 13 (15%) of which reported diarrhea the week prior to vaccination. Overall, 39 of 89 (44%) subjects had at least one pathogen identified in stool, with 19 (21%) having two or more identified. Among those aged 1-5 years (young children, n=8), 6-17 years (older children, n=26) and 18-60 years (adults, n=55), we found stools to be positive for at least one pathogen in 50%, 50%, and 38% of subjects, respectively. The most commonly identified pathogens were Shigella spp. (13% of subjects), D fragilis (12%), norovirus (12%), and STEC (9%). 32 of 46 (70%) subjects with vibriocidal data available seroconverted. We did not find any significant differences in the presence of co-infection, type of co-infection or extent of pathogen burden between those who did and did not seroconvert. We demonstrate that a large proportion of OCV recipients in an internally displaced person camp in South Sudan had asymptomatic carriage of enteropathogens. While these preliminary analyses suggest that these co-infections may not be associated with immune responses to OCV, we are in the process of completing analysis of additional pathogens, including helminthic targets, and final data will be available by time of presentation.

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EFFECT OF *HELICOBACTER PYLORI* INFECTION ON GROWTH TRAJECTORIES IN YOUNG ETHIOPIAN CHILDREN: A LONGITUDINAL STUDY

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Helicobacter pylori infection has been associated with early childhood growth impairment in high and middle-income countries; however, few studies have examined this relationship within low-income countries or used a longitudinal design. We examined the possible effects of *H. pylori* infection on growth trajectories in a cohort of young Ethiopian children. In 2011/12, 856 children (85.1% of the 1006 original singletons in a population-based birth cohort) were followed up at age six-and-a-half. An interviewer-led questionnaire administered to mothers provided information on demographic and lifestyle variables. Height and weight were measured twice, and the average of the two measurements was used. Exposure to *H. pylori* infection was assessed using a rapid *H. pylori* stool antigen test. The independent associations of positive *H. pylori* infection status (measured at ages 3 and 6.5 years) with baseline height and weight (age 3) and height and weight growth trajectory (from age 3 to 6.5 years) were modeled using Hierarchical Linear Models. At baseline (age 3), children's mean height was 85.7 cm and their mean weight was 11.9 kg. They gained height at a mean rate of 8.7 cm/year, and weight at a mean rate of 1.76 kg/year. *H. pylori* infection was associated with lower baseline measurements and linear height trajectory (β=-0.74cm, and -0.79cm/year, respectively), after controlling for demographics and markers of socio-economic status. However, the positive coefficient was associated with quadratic growth in height among *H. pylori* infected children (β=0.28, 95% CI, 0.07 to 0.49, p<0.01), and indicated increase in height trajectory as the child increased in age. In conclusion, our findings add to the growing body of evidence supporting that *H. pylori* infection is inversely associated with childhood growth trajectory, after controlling for a range of factors associated with reduced growth and *H. pylori* status.

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ANTIMICROBIAL RESISTANCE PATTERNS AMONG INTERMEDIATE - TO LONG-TERM TRAVELERS TO CUSCO, PERU, WITH DIARRHEA

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In order to characterize antimicrobial resistance among travelers with diarrhea in Cusco, Peru, we prospectively collected stool from students older than 18 years of age presenting consecutively to the Amanta Spanish School's physician with diarrhea between June 2003 and July 2010. We performed antibiotic susceptibility testing using the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2011). Reduced susceptibility to ampicillin and first-generation cephalosporins was common among all isolates, except Shigella sonnei. Non-*Campylobacter* isolates were nearly uniformly susceptible to ceftriaxone. *Campylobacter coli* and *C. jejuni* isolates had reduced susceptibility to erythromycin and both quinolones, naldixic acid and ciprofloxacin, whereas susceptibility to azithromycin was 100% for both species and susceptibility to sulfamethoxazole-trimethoprim 100% and 94%, respectively, for both species. For the *E. coli* enteropathogens, ETEC was the most common isolate and was highly susceptible to amoxicillin-clavulanic acid (89%), ceftriaxone (100%), azithromycin (79%), and ciprofloxacin (93%), but showed greatly reduced susceptibility to cefazolin (64%), erythromycin (4%), sulfamethoxazole-trimethoprim (61%), and tetracycline (61%). Susceptibility trends for EAEC were similar to ETEC. Of the Shigella sp., S. sonnei strains were more common than S. flexneri and notable differences in susceptibility between the two species were found. Shigella sonnei was more susceptible to ampicillin (100%), cefazolin (100%), and sulfamethoxazole-trimethoprim (100%) than S. flexneri, but less susceptible to azithromycin (56%). Ciprofloxacin, tetracycline, and ceftriaxone susceptibility was 100% for both S. sonnei and S. flexneri. These results suggest that azithromycin is the most appropriate empiric treatment for travelers' diarrhea in this region, as it will effectively treat ETEC, Shigella, and fluoroquinolone-resistant *Campylobacter*-associated diarrhea, whereas ciprofloxacin would only effectively treat ETEC and Shigella.

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LETHALITY OF FIRST CONTACT DYSENTERY EPIDEMICS ON PACIFIC ISLANDS

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Infectious diseases de-populated many isolated Pacific islands when they were first exposed to global pathogen circulation from the 18th century. Although the mortality was great, the lack of medical observers makes determination of what happened during these historical epidemics largely speculative. Bacillary dysentery caused by Shigella is the most likely infection causing some of the most lethal island epidemics. The fragmentary historical record is reviewed in order to gain insight into the possible causes of the extreme lethality that was observed during first contact epidemics in the Pacific. Subacute dysentery occurred following first contact measles epidemics but not in subsequent smaller measles outbreaks. Immune aspects of the early dysentery epidemics and post-measles infection resulting in subacute inflammatory enteric disease suggest that epidemiologic isolation was the major lethality risk factor on Pacific islands in the 19th century. Other possible risk factors include HLA homogeneity from a founder effect and pathogen-induced derangement of immune tolerance to gut flora. If this analysis is correct, then Pacific Islands are currently at no greater risk of emerging disease epidemics than other developing countries despite their dark history.

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PLASMA AND MEMORY B CELL RESPONSES TARGETING O-SPECIFIC POLYSACCHARIDE (OSP) ARE ASSOCIATED WITH PROTECTION AGAINST *VIBRIO CHOLERAE* O1 INFECTION AMONG HOUSEHOLD CONTACTS OF CHOLERA PATIENTS IN BANGLADESH

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Vibrio cholerae is a noninvasive intestinal pathogen, and the cause of cholera, a severe dehydrating illness in humans. The mediators of protection against cholera are currently unknown. However, we have previously shown that IgG memory B cell responses targeting lipopolysaccharide (LPS) of *V. cholerae* O1 correlate with protection against *V. cholerae* O1 infection among household contacts of cholera patients. Protection against cholera is serogroup specific, and serogroup specificity is defined by the O-specific polysaccharide (OSP) component of LPS. Therefore, we prospectively followed household contacts of cholera patients to determine whether OSP-specific immune responses present at the time of enrollment are associated with protection against *V. cholerae* infection over a 30 day period. Two hundred forty two household contacts of 150 index patients with cholera were enrolled. The presence of OSP-specific IgG memory B cell responses in peripheral blood on study entry was associated with a decrease in the risk of infection in household contacts (43% reduction; $p = 0.048$). No protection was associated with cholera toxin B subunit (CtxB)-specific memory B cell responses. In addition, the presence of OSP-specific plasma IgA, IgM, and IgG antibody responses on study entry was also associated with protection among household contacts. These results suggest that immune responses that target OSP, both in plasma and memory responses, may be important in mediating protection against infection with *V. cholerae* O1.

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ROTAVIRUS VACCINE TAKES SEASONAL SIGNATURE OF CHILDHOOD DIARRHEA BACK TO PRE-SANITATION ERA IN BRAZIL (AND THAT IS A GOOD THING!)

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Diarrhea mortality and morbidity have not only declined substantially in developing nations, but also switched from a summer-bacterial to a predominantly winter-viral seasonal pattern. However, little is known about the effect of the rotavirus vaccine introduction on seasonal patterns of diarrheal disease. Here we examined the long-term evolution of diarrheal morbidity and mortality risks across age and geography in Brazil, including the effect of rotavirus immunization (introduced in 2006) and other development indicators. Nationwide mortality (1979 - 2013) and hospitalization (1998 - 2013) data were obtained from the Brazilian Ministry of Health. Secular trends and seasonality were inspected for each Brazilian state and age group using the software Epipoi. For most states, the primary peak in mortality risk among children under 5 years occurred from December to April (summer/early autumn) from 1979 - 1988, then switched to the period between June and October (winter) by 2000 - 2005 (just prior to the 2006 implementation of the rotavirus vaccine for children under 1), and next shifted back to summer/early autumn by 2007 - 2013. A similar pattern was observed for hospitalizations. In contrast, the risk of diarrhea-related death among older children (5 to 19 years) and adults (≥ 20 years) did not have a well-defined seasonality or spatial pattern until 2007, when a trend towards summer/autumn peaks of mortality was observable. Hospitalizations for these age groups tended to peak in summer/early autumn throughout the period. Rotavirus vaccination policies were not only associated with a change in the risk of death, but also with a shift in the timing of peaks in risk in children under 5 years, which is reminiscent of the summer-diarrhea period. Additionally, young children were shown to have distinct annual disease patterns compared to other age groups, suggesting a different etiology of disease.

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REACTIVE VACCINATION IN NSANJE, MALAWI USING AN ORAL CHOLERA VACCINE

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Malawi experiences regular cholera outbreaks, particularly in the southernmost district of Nsanje (population: 292,083 people) where Lake Malawi drains, causing frequent inundations. Flooding occurred in January 2015 and the President of the Republic of Malawi declared a state of disaster. One month later the first cholera case was reported, marking the beginning of an outbreak, during which Nsanje was one of the most heavily affected districts. At that time, the International Vaccine Institute (IVI) had planned to conduct a preemptive oral cholera vaccination (OCV) campaign in Nsanje in collaboration with the Malawi Ministry of Health (MOH), targeting 50,000 people with a two-dose regimen of Shancol® (Shanta Biotechnics, Hyderabad, India). As the outbreak began to spread, the Malawian MOH addressed the International Coordinating Group (ICG) and requested additional cholera vaccine doses from the WHO emergency OCV global stockpile to equip a public health response. IVI-procured vaccines were repurposed for this activity. A multi-organizational task force was put in place to plan and deliver cholera vaccination. 160,482 people including 70,000 internally displaced people were targeted for vaccination; overall 156,592 (97.6%) received the first dose, of which 108,440 (67.6%) received both scheduled doses. Here we report on the experiences in planning the activities, the outcome and lessons learnt

of the vaccination campaign as well as a comparison of this outbreak response to other reactive oral cholera vaccine (OCV) campaigns in sub-Saharan Africa conducted to date.

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HUMAN IMMUNE RESPONSES AGAINST ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) YGHJ MUCINASE, A PROMISING NEW ETEC VACCINE ANTIGEN

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The human enterotoxigenic *Escherichia coli* (ETEC) vaccine development effort has mainly been focused on inducing protective immunity by immunization with the ETEC colonization factor (CF) and heat-labile toxin antigens. But the main contributors to childhood ETEC diarrhea are heat-stable toxin-producing ETEC strains, of which about one third lack any known CFs. Identifying additional broad-coverage ETEC vaccine antigens is therefore important. YghJ, a chromosomally encoded mucinase common to most *E. coli*, and present in close to 100% of all ETEC, is a promising alternative. YghJ helps ETEC reach the small intestinal cell wall by breaking down the protective mucus barrier, and ETEC seem to secrete substantially more YghJ than other *E. coli*. We aim to describe immune responses to YghJ in human volunteers who have been experimentally infected with wild-type ETEC strains. Saliva, serum and intestinal lavage were obtained from adult human volunteers before (d0) and 10 days after (d10) experimental infection with wild-type strains TW10598 (O6:H16, CS2, CS3, CS21, STh, LT; 30 volunteers) and TW10722 (O11 5:H5, CS5, CS6, STh; 9 volunteers). T-cell responses were measured by flow cytometry gating for activated CD4 T cells expressing CD25 and CD134 after 2 days culture with YghJ. Antibody responses were measured by immunoassays where beads had been coated with purified recombinant YghJ. In preliminary analyses we found serum IgA antibody responses towards YghJ increasing significantly from d0 to d10; mean fluorescence 2625 to 14187, p=0.043, while IgG responses did not increase significantly. T cell responses increased significantly from 0.73 to 1.37 percent activated YghJ-specific CD4 T cells out of all CD4 T cells, p=0.008. In general there was higher background to YghJ than to more distinct ETEC proteins like the CS5 colonization factor, suggesting previous exposures to this protein. We have found that human volunteers experimentally infected with ETEC induce an anti-YghJ IgA antibody response, as well as T-cell responses. Further investigation into YghJ responses in saliva and intestinal lavage fluids are ongoing and results will be presented.

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OBSERVATIONS ON THE NATURAL HISTORY OF CYSTIC ECHINOCOCCOSIS IN UNTREATED AND ALBENDAZOLE-TREATED PATIENTS

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The natural history of cystic echinococcosis (CE), a complex, neglected, and arcane zoonotic disease with a global distribution, is still being elucidated. This study examined the natural history and albendazole-induced changes of CE, and whether the standardized WHO ultrasound (US) classification reflects these changes. Patient data were collected during mass US screening surveys in endemic regions amongst transhumant populations, the Turkana of Kenya, and the Berber of the Mid-Atlas Mountain region of Morocco. Cysts were categorized using the WHO US classification (CL, and CE1 to CE5). Patient records occurring prior to the receipt of treatment, and after the administration of albendazole, were selected. 852 paired before/after observations of 360 cysts (10 CL, 140 CE1, 7 CE2A, 14 CE2B, 5 CE3A, 144 CE3B, 38 CE4, 2 CE5) from 257 patients (241 Turkana, 16 Berber) who went untreated for up to 9789 days (mean 600 days, median 215 days) were analyzed using a McNemar-Bowker $\chi 2$ test for symmetry, which achieved significance ($p < .0005$). Next expected observations of cyst type exhibited no instances of regression to earlier types, only progression. A McNemar-Bowker $\chi 2$ test of 1414 paired before/after observations of 288 cysts (93 CE1, 6 CE2A, 6 CE2B, 5 CE3A, 158 CE3B, 19 CE4, 1 CE5) from 157 Turkana patients treated with albendazole also achieved significance ($p < .0005$). Regression from the CE4 to CE3B type occurred in a small percentage (2.05%) of these observations. The significant finding of asymmetry for the McNemar-Bowker test and the absence of instances of cyst regression for the natural history group re-affirms that the standardized WHO US classification is reflective of the natural history of CE, and more reflective of the natural history of CE than earlier classifications. Similarly, the significant McNemar-Bowker test for the albendazole-treated group demonstrates that the WHO classification also reflects the albendazole-induced changes in cysts. The regression of CE4 to CE3B in a few of the albendazole-treated cysts may reflect the stability of the CE3B cyst type.

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G1 STRAIN OF *ECHINOCOCCUS GRANULOSUS* IN SUDANESE PATIENTS: A THREATENING ALARM

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Cystic echinococcosis is a zoonosis caused by the cestodes *Echinococcus* spp. Its life cycle involves dogs and other canids as definitive hosts for the intestinal tapeworm, as well as domestic and wild ungulates including human as intermediate hosts for the tissue -invading metacestode (larval) stage. It was previously suggested that few data are available on the frequency of CE in humans in Sudan compared with published data on animal cystic echinococcosis. Few recent reports mentioned a prevalence of about 0.33% in central Sudan and sporadic cases in different areas in the Sudan. Recently, cystic echinococcosis is having an increased attention in Sudan as it is now more frequent having patients suffering from either respiratory or abdominal symptoms found to be associated with the presence of cyst or cysts in a vital organ associated with either of these systems. This emerging situation necessities studying the factors which have an impact on the increasing frequency of such an important disease in a country like Sudan suffering from tribal un restless and poverty. One of these factors was thought to be that 100% of cyst material obtained from human were genetically identified as *Echinococcus* canadensis G6. The infectivity of this taxon for humans was in doubt for many years, and even today the number of confirmed human cases is small and scattered all over the world and that was considered as the main reason of having a rather rare disease in Sudan despite all epidemiological conditions for autochthonous transmission of CE. From the other hand, *E. granulosus* G1 which is highly prevalent in regions known to be endemic for the disease was thought to be extremely rare in most of Sudan, as there was only one record from a dog in the central part of the country. However characterization of genetic materials recently obtained from human patients showed that this strain is present in human. A situation alarming the need of further studies to investigate to what extent is it present, population at risk and preventive measure. Hence this study represents the first study confirming the presence of G1 in patients in Sudan, knocking an alarm and discussing a preventive policy.

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CDNA LIBRARY FROM *TAENIA SOLIUM* RACEMOSE CYST: A NOVEL TOOL FOR TRANSCRIPTOMICS IN NEUROCYSTICERCOSIS

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Human neurocysticercosis (NCC), caused by the larval stage or cysts of the flatworm *Taenia solium*, is the most frequent parasitic disease of the brain in humans and is endemic in many developing countries. The murine model of NCC using *T. crassiceps* has advanced our understanding of immunopathology. However most molecular mechanisms involved in the host-pathogen interaction remain unknown. The best studied form of NCC is parenchymal disease in which cysts are lodged in the parenchymal tissue. Cysts that develop in subarachnoid spaces can grow into very large multivesicular structures commonly named "racemose cysts", which appear to differ in many characteristics from parenchymal cysts, with an aggressive course of infection that is difficult to treat, and has a high mortality rate. Investigation of *T. solium* at the molecular level is limited to detection and identification; a characterization of genes involved in regulation of growth, proliferation, and virulence has not been undertaken to date. The identification of the genes involved in survival and proliferation of racemose parasites would advance our understanding of this aberrant and malignant form of the disease. To this end, we isolated total RNA from a human *T. solium* racemose cyst, obtained at surgery, synthesized cDNA, generated double strand cDNA (ds cDNA) by long distance PCR, purified the ds cDNA using CHROMA SPIN columns, and cloned it into pSMART2IFD (In-Fusion, Clontech). Electrocompetent *E. coli* TG1 cells were transformed with these plasmids and a cDNA library was generated successfully. This library, the first generated from a *T. solium* racemose cyst, will allow systematic studies of the development and biology of the parasite and will aid in the search for novel targets for diagnostic and anthelmintic drugs. Our immediate aim is amplification and sequencing of full-length cDNA fragments by long distance PCR for cloning and identification of genes with significant roles in proliferation and cyst growth, and to determine their role in the development of the racemose parasite. This cDNA library promises to serve as a tool for detailed studies of the biology of *T. solium*.

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RISK FACTORS FOR REFRACTORY EPILEPSY DEVELOPMENT IN NEUROCYSTICERCOSIS

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Epilepsy is a major public health problem linked to increased mortality. The helminth infection Neurocysticercosis (NCC) is the number one cause of adult epilepsy worldwide; 70-80% of people with NCC experience seizures that can develop into treatment-refractory epilepsy (TRE). Early intervention before recurrent seizures establish abnormal electrical circuits in the brain could impact disease progression, but in order to do so predictors of TRE in NCC patients need to be identified. To identify such predictors, we conducted a retrospective review of 39 randomly selected subjects from a large clinical trial of patients with NCC and seizures. These subjects presented a mean of 14.9 months following their first seizure (SD, 64.3 months), most commonly had partial seizures (29/39 subjects, 74%), and had a mean of 3.3 viable or degenerating lesions due to NCC (range 1-21, SD 6.6). Eleven subjects went on to develop TRE (defined as seizures > 2 months after treatment for NCC despite anticonvulsant therapy) and 28 did not. We examined a number of clinical factors in these patients in order to identify variables associated with TRE. The primary risk factor identified was a strong and sustained inflammatory response to the parasite. This was demonstrated by a significantly increased number of bands seen on the initial NCC western blot in subjects who developed TRE (GM 5.8 bands) compared to those who did not (GM 3.8 bands, $p<.05$). As further evidence that ongoing brain inflammation is associated with epilepsy risk there was an increased (though not statistically significant) percentage of subjects with continued edema on follow-up imaging 6-12 months following treatment in those with TRE (2/6, 33%) compared to those without (2/18, 11%, $p=.6$); the total volume of edema at follow-up was also increased in those who developed epilepsy (22.7 cm^3) compared to those who did not (1.8 cm^3, $p=.2$). These findings provide a compelling rationale for further exploring signs and symptoms of ongoing inflammation as predictors of epilepsy development in NCC patients; this knowledge could aid in the development of targeted antiepileptogenic treatments.

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A CROSS-SECTIONAL ABATTOIR STUDY ON *TAENIA HYDATIGENA* INFECTIONS IN PIGS IN BURKINA FASO

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Taenia hydatigena is a non-zoonotic tapeworm transmitted between dogs, and ruminants or pigs. While *T. hydatigena* does not cause clinical signs in pigs, its presence impacts the validity of serological tests used for the diagnosis of current porcine *T. solium* infection through cross-reactions. Estimating *T. hydatigena* prevalence is crucial to validly interpreting *T. solium* serological results. The only five studies published in Africa, including 888 pigs, reported prevalences from 1.4 to 6.6%. We performed a cross-sectional study in Koudougou, Burkina Faso, to estimate the prevalence of *T. hydatigena* in slaughtered pigs. Pigs came from nearby villages practicing traditional breeding with free roaming. Over 35 days, 452 pigs were carefully inspected post-mortem for the presence of abdominal *T. hydatigena* cysticerci including the liver. In addition, meat inspectors routinely inspected the carcasses for *T. solium* cysticerci and blood samples were taken for B158/B60 Ag-ELISA examination. Cysticerci were stored in ethanol for molecular analysis by PCR-RFLP. *T. hydatigena* cysticerci were found in liver, abdomen and both in 27, 12 and one pigs, respectively. Meat inspectors found seven carcasses infected with *T. solium* cysticerci; molecular analysis confirmed six of these, one was identified as *T. hydatigena*. Seven cysts found in the liver were molecularly identified as *T. solium*. Overall, 41 carcasses (9.1%) were found infected with *T. hydatigena* and 14 (3.1%) with *T. solium*; two pigs were infected with both *Taenia* spp. 219 pigs (48.5%) were positive to the Ag-ELISA of which 23/41 (56%) and 9/14 (64%) were of pigs with *T. hydatigena* and *T. solium* cysts, respectively. The relatively high prevalence of *T. hydatigena* in this study suggests that estimates of current *T. solium* infection prevalence should be adjusted for the presence of *T. hydatigena*. Adjustment values can be estimated through post-mortem examination. *T. solium* prevalence derived from meat inspection is invalid as routine meat inspection has a low sensitivity, and pigs with tongue cysts are mostly diverted to the unofficial slaughter circuit.

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EFFICACY OF A SINGLE-DOSE OF OXFENDAZOLE AT THREE DIFFERENT FORMULATIONS AGAINST THE LARVAL STAGE OF *TAENIA SOLIUM* IN NATURALLY INFECTED PIGS

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Oxfendazole (OFZ) is the drug of choice against porcine cysticercosis. This drug is commercialized as a broad-spectrum antiparasitic for farm animals. However, its high cost limits its use by many health programs in low-income countries, so local formulations of OFZ could be an alternative for its implementation This study evaluated the efficacy of three different formulations of OFZ against porcine cysticercosis in a randomized controlled trial. Twenty-eight pigs naturally infected pigs with *Taenia solium* cysts were distributed in the following treatments: OFZ in a commercial formulation (Synanthic 9.06% Fort Dodge®, Mexico), OFZ in two experimental formulations (22.5% and 10.9%) and an untreated group. Experimental formulations were prepared in the Laboratory of Pharmacology at the National University of San Marcos University in Lima, Peru using OFZ p.a (Spectrum Laboratory Products Inc, Gardena, CA). OFZ was given to pigs at a single oral dose of 30 mg/kg under experimental conditions. Necropsies were performed at day 60 post-treatment and the carcasses were examined for the presence of cysts in muscle samples and brains. OFZ efficacy against cysts in muscle was 100% for the three treatment groups (viable cysts were not observed) compared to the untreated group ($p<0.001$). OFZ efficacy in brain was variable and non-statistical differences in the proportion of pigs with viable cysts in brains among treatments were observed. Although some differences in the plasmatic maximum concentration and the time to peak concentration in plasma after oral administration of OFZ at 30mg/kg in pigs were reported previously using the same formulations, we found no differences in the efficacy among the tested OFZ formulations. Since pigs are is a vital component in the transmission chain of *T. solium*, a cost-effective treatment in endemic areas should greatly help to eliminate the source of infection in humans. From a practical point of view, our results provide evidence that local formulation of OFZ at high concentrations (thus requiring lower volumes to be given to pigs) can be cheaper and efficient alternatives for use in the control of *T. solium*/cysticercosis.

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NEUROCYSTICERCOSIS AND EPILEPSY AMONG PEOPLE LIVING NEARBY PIGS HEAVILY-INFECTED WITH CYSTICERCOSIS IN RURAL ENDEMIC PERU

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Neurocysticercosis (NCC) causes substantial neurologic morbidity in endemic regions around the world. Ring strategy for control involves focusing treatment resources for taeniasis and porcine cysticercosis within clusters (rings) of households surrounding a heavily-infected pig. This strategy is based on the assumption that risk of parasite transmission is greatest within the rings. At the end of a community trial of ring strategy in northern Peru, we offered neurologic screening to all participants who resided within an identified ring. Our objective was to describe the prevalence of NCC and epilepsy in these rings. We offered screening to 576 ring residents by applying a 9 question seizure survey. Our study physician evaluated positive respondents to confirm the diagnosis using International League Against Epilepsy criteria. Those with confirmed epilepsy were offered a non-contrast CT of the head. We also tested sera using EITB LLGP to detect anti-cysticercus antibodies and ELISA B60/B158 to detect cysticercosis antigens. Those with strongly positive ELISA (ODR>=3) were offered a non-contrast MRI of the brain. 527 people completed seizure screening and 114 (21.6%) were positive. The physician evaluated 108, of which 35 had confirmed seizures and 16 had epilepsy (lifetime prevalence 30 per 1000). 12 with epilepsy accepted CT scan and 5 (41.7%) had parenchymal calcifications. None had viable cysts. Of the 514 that provided a blood sample, 241 (46.9%) were seropositive by EITB, 39 (7.6%) were positive by ELISA, and 12 (2.9%) were strongly positive by ELISA. 11 accepted MRI and 8 (72.3%) had NCC, including 5 with subarachnoid cysts, 5 with parenchymal vesicular cysts and 2 with parenchymal granulomas. Epilepsy and NCC are common among ring residents although it is unclear whether the risk is greater than in the general population as no controls were evaluated in this study. The high positive predictive value of ELISA in this population suggests a potential role for antigen screening of blood or urine to allow early detection and intervention of people with NCC, particularly those with subarachnoid cysts, the most malignant presentation.

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TAENIA SOLIUM AND NEUROCYSTICERCOSIS BURDEN AND DECREASED ACADEMIC PERFORMANCE ASSOCIATED WITH BRAIN INFECTION IN SCHOOL AGED CHILDREN, SOUTHWEST CHINA

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Neurocysticercosis (NCC) is caused by larval forms of the pig tapeworm, *Taenia solium*, invading the brain and has been shown to cause cognitive deficits in adults. We characterized *T. solium* exposure as well as NCC burden and resulting cognitive deficits in school-aged children living in

southwest China. We surveyed 3,038 students aged 10- 15 years in poor areas of southwest China, characterizing exposure history, collecting blood for *T. solium* serologic testing by ELISA, and administering a standardized math test. We characterized children as probable NCC cases if they had positive serologic testing and reported serious neurologic symptoms (seizures or recurrent headaches or weakness). We randomly selected a subset of students representing all possible combinations of serologic results and presence or absence of neurologic symptoms for in- depth testing that included brain MRIs and additional cognitive testing. 6% of children (175/2867) had positive serologies demonstrating exposure to *T. solium*, with some counties having prevalences higher than 15%. 15% (459/2953) of children reported consumption of undercooked pork in the month prior to the survey and 25% (761/3027) reported seeing encysted parasites in their meat in the past year. 14% (408/2781) reported being treated with a deworming medication within the past year. Children reported seizures (93 cases, 3%), severe headaches (>6 headaches per month, 241 cases, 8%), and recurrent extremity weakness (>6 episodes per month, 59 cases, 2%). 2% of children (65/2867) met criteria as probable NCC cases. Children classified as probable NCC cases had significantly lower math scores compared to their uninfected classmates (average score difference of 0.65 standard deviations, p=0.02). MRI scans on a subset of students revealed evidence of *T. solium* brain involvement in 9% (6/63). Our results suggest that *T. solium* infection and NCC are wide spread in school-aged children in southwest China. Children with NCC underperform academically and this may contribute to a cycle of poverty. Our results suggest the need for *T. solium* eradication efforts to decrease disease burden in school-aged children.

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EFFICACY AND ADVERSE EVENTS OF NICLOSAMIDE IN A LARGE SCALE CYSTICERCOSIS ELIMINATION DEMONSTRATION PROGRAM ON THE NORTH COAST OF PERU

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Taeniasis is an important parasitic infection as the immediate cause of neurocysticercosis (NCC). Mass drug administration with niclosamide (NSM) is one strategy to control the disease. NSM is reported to be 90-95% efficacious against taeniasis and is considered safe for use in endemic regions given that it is minimally absorbed and therefore poses no risk to individuals with NCC. However, there is little published information regarding treatment efficacy and adverse events of NSM when used in endemic community settings. We evaluated the efficacy and adverse events of NSM during a large-scale cysticercosis elimination demonstration program in Tumbes, Peru, in which we offered three rounds of mass treatment with oral NSM, at 4 month intervals, to 79,191 rural residents older than 2 years of age. We collected post-treatment stools after the first round of NSM to diagnose taeniasis using coproantigen ELISA, and collected an additional stool sample at 30 days for those with taeniasis to evaluate treatment efficacy. We visited all participants in their homes to collect information about adverse treatment events. At total of 158,201 doses were administered across all 3 rounds with 68,751 (86.8%) people receiving at least one dose. Of the 45,391 people who provided a post-treatment stool sample, 235 had taeniasis. 210 provided a follow-up stool sample and 59 had evidence of persistent infection after 30 days, representing a treatment efficacy of 71.90% (151/210). The prevalence of adverse events related to ingestion of NSM was 0.90% (1418/158,201), of which 98.73% (1400/1418) were of mild intensity. There were no severe adverse events. Abdominal pain was the most frequently reported adverse event (571/1418; 40.27%). Adverse events were more common among females [PR=2.33; 95% CI 2.06-2.65] and among those that received NSM in more than one treatment round [2 doses PR 1.18; 95% CI 0.97-1.44; 3 doses PR 1.46; 95% CI 1.27-1.67). NSM is a safe drug for use in mass drug administration although the treatment efficacy is substantially less than previously reported which may reduce control effect.

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VISUALIZING NEUROCYSTICERCOSIS AND THE IMPACT OF CYSTS ON EPILEPTOGENESIS USING INTERACTIVE 3D MODELS

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Neurocysticercosis (NCC) is a preventable, disabling disease that affects approximately 50 million people worldwide; it is in critical need of research, medical education and increased global awareness and has been classified as a neglected tropical disease by the World Health Organization. A person contracts NCC by ingesting eggs from *Taenia solium*, a parasitic tapeworm. The eggs migrate through the body tissues into the brain where they develop into cysts. Eventually, these cysts can trigger a massive inflammatory response in the host and debilitating symptoms emerge such as seizures and the development of epilepsy. Seizures are the most common symptom associated with NCC, occurring in 70-80% of infected individuals, and NCC is the most common cause of adult acquired epilepsy in the world. This research investigates whether 3D modeling and interactive visualization aid researchers in comparing the neural cysts of patients with NCC to better understand why some infected individuals develop chronic epilepsy while others suffer from isolated seizures or do not seize at all. Patient data was segmented out to create interactive 3D models, which were embedded in a user interface. Emphasis was placed on the location and stage of cysts and surrounding edema. Patient EEG data was represented in the 3D models to visualize which parts of the brain are involved in seizure activity. The final application was embedded in a globally accessible web based user interface. The user can rotate the models in 3 dimensions, control the transparency and visibility of the EEG data, and view patient lesions on a generalized brain to account for the high variability of neuroanatomy between individuals. This project helps researchers explore the relationship between cyst location, stage, and number and the development of seizures or chronic epilepsy. This project uses biomedical visualization to enable research on NCC and epilepsy through the creation of a multimodal interactive. It demonstrates the critical role of biocommunciation in shaping medical research, clinical decisions, and patient outcomes through visual translation of clinical data.

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EPIDEMIOLOGIC CHARACTERIZATION OF *HYMENOLEPIS NANA* INFECTION IN CHILDREN AGED 2 TO 15 YEARS OLD ON THE NORTH COAST OF PERU

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Hymenolepis nana is among the most common intestinal parasites and an important public health disease in impoverished areas given it's morbidity in children and ease of transmission. Understanding the risk factors for transmission in endemic regions may guide strategies to reduce the burden of infection. We conducted a cross-sectional secondary analysis of data collected during a large community-based study of 107 villages (73,161 people) in Tumbes, Peru, to evaluate risk factors for *H. nana* infection among children. Of the 20,249 eligible residents based on age (2-15 years old), 14,671 provided a single stool sample that was analyzed by sedimentation and microscopy for the presence of *H. nana* eggs. We used binomial family generalized linear models with log link to calculate crude and adjusted prevalence ratios of sociodemographic factors that may influence infection risk. The crude overall prevalence of *H. nana* infection was 7.61% (1124/14,761), and was slightly higher among males (8.59%; 95% CI 7.95-9.22) than among females (6.65%; 95% CI 6.08-7.21). In the multivariable model, the prevalence of *H. nana* infection increased 8% (adjusted prevalence ratio, aPR 1.08, 95% CI 1.06-1.09) with each additional year of age, was 30% higher among females than males (aPR 1.30, 95% CI 1.16-1.46), and 27% lower among rural dwellers compared to urban dwellers (aPR 0.73, 95% CI 0.64-0.84), after controlling for household clustering and other variables. Water source, particularly in-ground storage tanks filled by water truck, also increased the risk compared to piped water (PR 2.70, 95% CI 2.34-3.11), as did the lack of latrine (PR 2.00 95% CI 1.73-2.33). *H. nana* infection is a relatively common intestinal infection in northern Peru. Improvements to water and sanitation infrastructure may help reduce the burden of this and other intestinal infection in the region.

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EFFICACY OF SINGLE DOSES OF PRAZIQUANTEL 5-10 MG/KG FOR TAENIASIS UNDER CONTROLLED CONDITIONS IN RURAL COMMUNITIES OF THE NORTHERN COAST OF PERU

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Taenia solium has two stages, the adult tapeworm in the human intestine (Taeniasis) and the larval cyst in humans and pigs (cysticercosis). Praziquantel (PZQ) is considered the first line treatment for taeniasis, although there are concerns for potential adverse effects in people with neurocysticercosis (NCC) with viable cysts. Niclosamide is a safe alternative although its availability is limited. The objective of this study was to assess the efficacy of PZQ for intestinal taeniasis in an area endemic for *Taenia solium* at the usual single oral doses of 5 mg/kg or 10mg/kg (maximum dose 900 mg) under controlled conditions to ensure the safety of participants. Taeniasis was prospectively identified from the endemic region of Piura, Peru, in a series of mass stool screening interventions between 2011-2013. People with taeniasis 12-65 years old were eligible for participation and underwent a battery of screening tests to rule out NCC before treatment, including a) non-contrast brain CT scan to identify viable cysts, b) EITB for cysticercosis antibodies and c) cysticercosis antigen detection. Participants with viable cysts on CT or those with positive circulating antigen received niclosamide as an alternative to PZQ. The remaining cases received a single oral dose of PZQ at either 5 mg/kg or 10 mg/kg, with stool samples collected 30 days post treatment to evaluate cure. Of the 67 age-eligible cases, 10 refused treatment, 14 (21%) were given NSM because of positive antigen results. CT scan demonstrated 30 patients (44.7%) with parenchymal calcifications and apparently none with viable cysts. Of the remaining 43 participants with taeniasis, 33 (76.7%) received 5 mg/kg PZQ and 10 received 10 mg/kg of PZQ. Rates of cure as assessed in those who provided a 30-day follow-up stool sample were 26/29 (89.6%) for the 5 mg group and 10/10 (100%) for the 10 mg group. In conclusion, PZQ at 5 or 10 mg/kg was highly effective to treat *T. solium* taeniasis with few side effects in this selected subgroup of patients with no viable cysts on CT and negative circulating antigen.

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GEOSPATIAL ANALYSIS OF CYST BURDEN IN PIGS AS AN INDICATOR FOR LOCAL TRANSMISSION OF *TAENIA SOLIUM*

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Taenia solium is a parasite that is the leading cause of preventable epilepsy in the developing world. Taenia eggs are released into the environment through the stool of humans infected with an adult intestinal tapeworm (taeniasis), and cause cysticercosis when ingested by pigs or other humans. Ring strategies for the control of *T. solium* operate by screening and/or treating human for taeniasis if they live near pigs heavily infected with *T. solium* cysts. Traditionally, these heavily infected pigs have been diagnosed by examining the tongues of pigs for cysts, but higher sensitivity methods, such as improved serologic testing or ultrasonographic imaging, are needed in order to advance current control efforts. These tests require that we define thresholds of heavy infection that will perform best in the context of ring strategies. For this study, we performed necroscopic examination to determine the total body burden of viable *T. solium* cysts in pigs purchased from eight villages as part of a concurrent prospective ring intervention. Human stool was collected and processed for the presence of *Taenia* sp. coproantigen indicative of active taeniasis, and the geographic coordinates for each household were recorded. We assessed the prevalence of taeniasis inside rings of 50 and 100 meters around pigs of different cyst burdens in order to determine the rings that best indicated high-risk geographic foci for taeniasis. Of 152 seropositive pigs necropsied, 12 (8%) had ≥ 100 viable cysts, 9 (6%) had 10-99 cysts, and 27 (18%) had 1-9 cysts. There were 33 cases of taeniasis among all 1,323 people tested for a prevalence of 2.4%. Pigs with ≥100 cysts were significant indicators of local taeniasis when assessed at a distance of 50 meters (PR=2.74; 95% CI: 1.09, 6.91), but did not show an effect at greater distances. Pigs with fewer than 100 cysts did not significantly predict for local increases in the prevalence of taeniasis. The results of this study indicate that tests intended to diagnose heavily infected pigs should aim to detect pigs with at least 100 cysts. Lower detection limits are unlikely to yield additional cases of taeniasis in the context of ring interventions.

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PATHOGENESIS OF SEIZURES IN NEUROCYSTICERCOSIS: FROM CYSTICERCOTIC LESIONS TO SEIZURE SEMIOLOGY

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Neurocysticercosis (NCC) is the most common parasitic infection of the central nervous system and constitutes a major health problem. The aim of this study is to determine the concordance between semiologic manifestations and lesion topography in patients with NCC and seizures. In this retrospective cohort analysis patients with one or two NCC lesions in any stage were selected from participants in three randomized clinical trials of antiparasitic treatment. Out of 220 randomized patients, 58 (26.4%) had one or two lesions and 8 (13.8%) of them apparently had only primarily generalized tonic clonic seizures and were excluded, leaving 50 patients for analysis. Forty-two (84%) had the symptomatogenic zone, solely determined by seizure semiology, and at least one NCC lesion in the same sublobar brain region. Twelve (24%) patients had seizures semiologically arising from the peri-operculum region (perisylvian part of the frontal, parietal and temporal lobes), characterized by speech arrest, face motor or somatosensory seizures and epileptic vertigo or dizziness. Fifteen (30%) patients showed concordance between semiologic manifestations with the superior sublobar central region (parts of the frontal and parietal lobes), characterized by clonic motor or somatosensory seizures of extremities. In the 10 (20%) patients who had a calcified lesion and a viable cyst, the calcified lesions had not relation with the semiology of seizures. Out of 23 (46%) patients who had seizures after antiparasitic treatment was given in the randomized clinical trials, two (8.7%) had a new symptomatogenic zone and both showed a suitable semiological-topographical concordance for another viable lesion. In conclusion, roughly 85% patients with one or two NCC lesions and seizure history exhibit semiologic-topographic concordance suggesting a direct influence of the parasitic lesions in the very near surrounding brain tissue such the main sustainable cause of seizures. Follow-up studies including modern neuroimaging techniques and ictal-EEG to appropriate establishing the ictal onset zone should help us to better understand epileptogenesis in human NCC.

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A POTENTIAL CANDIDATE ENOLASE FROM *TAENIA SOLIUM* EXPRESSED IN BACULOVIRUS SYSTEM FOR INMUNODIAGNOSIS OF SWINE CYSTICERCOSIS

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Taenia solium, is a zoonotic parasite that infects both humans (as a final host) and swine (as an intermediate host). The pig acquires cysticercosis when it eats contaminated food with human feces with viable *T. solium* eggs. In endemic countries it is considered a serious public health problem. In the search for new antigens for immunodiagnostic of swine cysticercosis, we have annotated a glycolytic enzyme called enolase, whose function in Taenia species is not yet well understood. We have expressed the enolase of *T. solium* with their respective posttranslational modifications in the baculovirus-insect cell expression system (BES). We cloned the coding sequences in pFastBac HTA and the gene rearrangement was made in the baculovirus genome (bacmid) in *E. coli* DH10 Bac. We used bacmids for viral particles production and we obtained 3x108 viral particles in the infected sf9 insect cells. These were used to express 740 µg/mL of recombinant enolase (52 kDa), also it was identified by Western blot with polyclonal rabbit antibodies (1/5000) specific for enolase. The BES is efficient to express recombinant enolase with posttranslational modifications. Low titers of antibodies to recognize enolase suggest that they are highly antigenic. Finally, we evaluated the usefulness of the recombinant protein for the diagnosis of porcine cysticercosis through the detection of antibodies in sera samples of infected and non-infected pigs using ELISA test. We obtained 98,7 % of sensibility and 66,7 % of specificity.

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USEFULNESS OF TOURNIQUET TEST FOR DIAGNOSING DENGUE INFECTION IN ADULT

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Dengue infection becomes an important cause of fever among adult patients in the tropics. Tourniquet test (TT) is a classical bedside diagnostic tool which may aid clinician in the rural area where specific laboratory tests are not available. This study aims to determine the accuracy of TT in adult patients presenting with fever in the Bangkok Hospital for Tropical Diseases. A retrospective study was conducted in adult patients (age >18 years) presenting with acute febrile illness during 2012-2013. A TT performed using the standard technique once at presentation. Dengue infection was diagnosed by any of the following criteria: positive dengue specific NS-1 Ag. Total 1,157 adult patients with a median age of 30 years (18-96 years) were enrolled in this study. The male to female ratio was 1:1.4 and the mean duration of fever was 3.5 days. 259 cases (22.4%) had dengue infection. TT positive was found in 27.3% of patients. In our study, TT showed sensitivity 51.4%, specificity 79.5%, positive predictive value 41.9%, negative predictive value 85.0% and accuracy 73.2%. These parameters are varied by the day of fever on which TT was performed. The sensitivity of TT increased but the specificity decreased among patients with a longer history of fever. Patients with dengue infection were more likely to have positive TT than other febrile illness (OR 4.09, 95% CI 3.06-5.49). Among patients with dengue infection, the TT had no predictive value on the severity of dengue, risk of bleeding and length of stay in the hospital. Tourniquet test might be a useful predictor of the dengue infection in adult patient with acute febrile illness. However, among patients with confirmed dengue infection, tourniquet test had very little predictive value in the clinical course and outcome.

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SPIDR-WEB: AN NGS BIOTECHNOLOGY PLATFORM FOR DIAGNOSTIC, BIOSURVEILLANCE AND TRANSCRIPTOMIC APPLICATIONS

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We are transforming the field of infectious disease diagnostics with the development of the Sample Prep for Infectious Disease Recognition With EDGE Bioinformatics (SPIDR-WEB). SPIDR-WEB is a sample-to-result biotechnology platform that enables efficient use of next generation sequencing (NGS) for pathogen detection in clinical samples. NGS has become a powerful tool for detection and characterization of both known and emerging pathogens. The main advantage of NGS is its non-biased approach that identifies all organisms in a sample. This is in contrast to traditional molecular assays that force us to look for a set of specific pathogens. In most clinical samples, the relative abundance of pathogen nucleic acids (DNA or RNA) is vanishingly small. Therefore, vast amounts of sequence data must be generated and analyzed to identify rare pathogen sequences. SPIDR-WEB is a sample-to-result process that relies on efficient laboratory and in silico steps. Clinical samples mostly comprise non-informative host RNAs or abundant housekeeping gene transcripts. SPIDR-WEB incorporates removal of non-informative RNAs (RNR), thereby enriching all other RNAs, including those from pathogens. This step enables either higher sensitivity and specificity, or less expensive and faster sequencing. Our custom EDGE bioinformatics data analysis platform provides rapid read classification at all taxonomic levels, and reliably detects all organisms present in a sample. EDGE is an efficient process, as it uses databases with pre-computed signatures, instead of aligning sequencing reads to the entire Genbank. In addition to RNR and EDGE, SPIDR-WEB includes robust, inexpensive and rapid sample lysis, RNA extraction, and library preparation steps. We want to implement SPIDR-WEB in both research and clinical settings to support a multitude of applications, such as discovery of novel mechanisms and biomarkers, study host-pathogen interactions, improve vaccines and therapeutics, and complement current diagnostic tools and help improve their utility.

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EPIDEMIOLOGY OF LEPTOSPIROSIS AMONG PATIENTS PRESENTING WITH ACUTE FEBRILE ILLNESS TO LAKESIDE HEALTH CENTERS IN RURAL RWANDA

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Leptospirosis is an infectious disease caused by leptospires, which are transmitted directly or indirectly from animals to humans. Minimal data are available on leptospirosis epidemiology in Africa, including Rwanda. This study aimed to determine the frequency of leptospirosis infection as cause of acute febrile illness in patients presenting to three lakeside health centers in rural Rwanda. Included patients were 21 years old and above, with axillary temperature 37.50C or more, and a negative test for malaria. Serum samples were collected and tested with a Rapid Diagnostic Test {Standard Diagnostic Bioline) for anti-*Leptospira* IgG/IgM antibodies. Among 421 acute febrile patients, the study found a seroprevalence of 22.1% (93/421) for leptospira IgG positivity and an incidence of 5.5% (23/421) for IgM positivity; all IgM positive patients also had IgG antibodies and 85/93 cases with positive leptospira serology came from a single health center. Both IgG and IgM antibodies were strongly associated with lake swimming, livestock exposure and fish farming. Conjunctival suffusion was the physical examination finding most strongly linked with seropositivity. This study demonstrates the presence and localized endemicity of human leptospirosis infection in Rwanda. National mapping of prevalence, and enhanced clinician awareness supported by greater diagnostic resources, are needed to combat this under-recognized disease.

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PREVALENCE OF *TRYPANOSOMA CRUZI* AMONG NON-ISCHEMIC CARDIOMYOPATHY PATIENTS PRESENTING FOR CLINICAL MANAGEMENT AT THREE MEDICAL FACILITIES IN SOUTHEASTERN TEXAS, USA

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Infection with the parasite *Trypanosoma cruzi* (Chagas disease) causes a progressive dilating cardiomyopathy in approximately 30% of affected patients. Anecdotally referred to as the "silent killer", most patients are unaware of their disease status due to an asymptomatic or mild, non-specific acute infection period. Sudden cardiac disease is the first presenting symptom in 35% of patients with cardiac manifestations, and is the most common cause of mortality. Disease burden estimates suggest 7 million patients are living with chronic Chagas in Latin America. In the United States, we have a substantial burden of Chagas disease among immigrant populations, and we have recently started to recognize a growing number of locally acquired cases. Our current study aimed to understand the prevalence of *T. cruzi* infection in patients presenting for clinical management of known non-ischemic idiopathic cardiomyopathy

at one of three medical facilities. All housed in Houston, Texas, the three medical facilities serve distinct and unique populations: 1) Latin American immigrants living in Texas, 2) impoverished Texas residents with increased vector exposures, and 3) Texas residents with a high socioeconomic status. From this large cohort of cardiac patients, we identified a considerable proportion of previously undetected *T. cruzi* infection. We will discuss the clinical presentations and transmission risk factors of those patients who tested positive for Chagas disease compared to idiopathic cardiomyopathy patients with a negative serology. Our findings have important clinical implications for cardiologists practicing throughout the United States, as well as clinicians in other non-endemic countries providing healthcare to Latin American immigrants.

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OUTCOMES OF PATIENTS WITH SEVERE INFECTION IN UGANDA ACCORDING TO ADHERENCE TO WHO INTEGRATED MANAGEMENT OF ADOLESCENT AND ADULT ILLNESS FLUID RESUSCITATION GUIDELINES

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The World Health Organization's Integrated Management of Adolescent and Adult Illness (IMAI) recommendations for fluid resuscitation of patients with septic shock (IMAI-shock) and severe respiratory distress without shock (IMAI-SRDS) have not been validated. Therefore, we sought to describe outcomes in hospitalized patients in Uganda meeting these clinical criteria based on whether or not they received IMAI recommended fluid resuscitation. We performed a secondary analysis of data from a prospective cohort of severely septic adult patients admitted to Mbarara Regional Referral Hospital in Uganda that included the volume of intravenous fluids patients received during the first 6 hours of resuscitation. We selected patients meeting criteria for IMAI-shock or IMAI-SRDS and used logistic regression to determine predictors of outcomes. We evaluated 136 patients with IMAI-shock and 41 patients with IMAI-SRDS. During the first 6 hours of resuscitation, for patients with IMAI-shock, those that received IMAI recommended fluid volume (N=28) received more than those that did not (3L vs 1.5L, p<0.001) and there was no difference in mortality (30% vs 36%, p=0.788). Receipt of IMAI recommended fluid volume was associated with admission O2 saturation <90% (aOR 2.9, 95%CI 1.1-7.1, p=0.025) and in-hospital mortality was associated with wasting (aOR 3.9, 95%CI 1.4-11.0, p=0.026) and ambulation (aOR 0.2, 95%CI 0.09-0.6, p=0.005). For patients with IMAI-SRDS, those that received IMAI recommended fluid volume (N=9) received less than those that did not (0.5L vs 1L, p<0.001) and there was no difference in mortality (22% vs 57%, p=0.08). Receipt of IMAI recommended fluid volume and in-hospital mortality were both associated with a Glasgow Coma Scale score <15 (aOR 0.07, 95%CI 0.007-0.7, p=0.021; aOR 6.9, 1.04-45.7, p=0.045). In conclusion, IMAI recommended fluid resuscitation did not improve outcomes for patients with IMAI-shock or IMAI-SRDS. Further studies are needed to better understand the optimal resuscitation strategy for patients with severe infection in resource-limited settings such as Uganda.

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A RETROSPECTIVE STUDY OF MALARIA-RELATED DEATHS IN CHILDREN THAT DIED ON ADMISSION WITH SYMPTOMS OF FEVER MANIFESTATION IN A SECONDARY HEALTH CARE INSTITUTION IN WESTERN REGION OF GHANA

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Malaria contributes significantly to deaths in children. Children are vulnerable to severe consequences of malaria due to lack of anti-parasite and clinical immunity. Malaria causes death either directly being the underlying cause of death as in the case of cerebral malaria and severe malaria anemia (SMA) or indirectly by contributing to the cause as in the case of malaria in a child with pneumonia or hypoglycemia. This is a retrospective study which evaluated the malaria-related deaths among children who died on admission over a 3 year period. The study was done in the children's ward of Effia nkwanta hospital. A total of 223 dead children medical records were reviewed. Percentage mortality was 15.7% and Case fatality rate for malaria was 13.7%. All-cause mortality and malaria-specific deaths decreased from 21.5% and 24.3% in 2010 to 11.1% and 4.4% in 2012 respectively. 17.9% (40/223) tested positive for malaria with Cerebral malaria 37.5% (15/40), Severe malaria anemia 30 %(12/40), and Severe malaria 25% (10/40). A continuous unrelenting implementation of child survival and malaria control programs is very necessary to protect children from malaria and other childhood killer diseases.

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INFECTIOUS ETIOLOGIES OF FEBRILE ILLNESSES IN CAMEROON

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Fever is a common cause of patients seeking treatment in healthcare facilities in most tropical countries and poses a diagnostic and therapeutic challenge to healthcare workers in resource limited areas. Diagnosis of febrile illnesses in most malaria endemic countries mostly focuses on confirming or ruling out malaria. Thus, healthcare workers are often faced with the challenge on the course of action to take in treating febrile patients negative for malaria. The lack of information on the specific etiologic agents of non-malaria febrile illnesses prevents effective management of otherwise often treatable diseases. Despite their importance, there is no published data on the epidemiology of non-malaria febrile illnesses in Cameroon and their true burden remains unknown. In this study, we sought to identify pathogens that cause febrile illnesses in Cameroon. We recruited 551 febrile patients (6 months and older) in three different geographical regions of Cameroon. Blood and stool specimens were collected to perform rapid diagnostic test, ELISA, microsphere immunoassay, microscopy, culture and PCR to identify various etiologic agents of febrile illnesses. Of the 551 participants, 50% had malaria, 41.5% had one or more acute respiratory viral infections (influenza A (9%), influenza B (9%), adenovirus (10%), respiratory syncytia virus (17.5%), and parainfluenza virus (8%)), 7% had typhoid, 1.8% had acute toxoplasmosis, 18% had gastroenteric infections, 2% had dengue, 6% had West Nile virus infection, 0.5% had leptospirosis, 1% had acute

chikungunya virus infection, while 13% of the participants were co-infected with malaria and one or more non malarial pathogens. Moreover, 91% participants were presumed to have malaria based on fever, of which 41% were negative for malaria by PCR. Our results show evidence of non-malaria febrile illnesses in a malaria endemic region, which should be considered by clinicians in the differential diagnosis of febrile illnesses. But, lack of access to diagnostic tests impedes precise clinical management of febrle illnesses.

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EVALUATION OF SAFETY TOOL FOR AMBULATORY LEPROSY PATIENTS AT RISK OF ADVERSE OUTCOME

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Leprosy treatment requires a complex management approach consisting of ongoing laboratory monitoring, screening for factors that will adversely affect response to corticosteroids, engagement of allied health services, and prolonged follow-up. Given that leprosy is complicated to manage, a novel safety tool was developed and implemented in our practice in March 2015. Our objective was to evaluate its utility through retrospective chart review. We reviewed charts of patients with leprosy treated over a 3.5-year period: 3 years pre-implementation, and the 6-months following implementation. Outcomes included: loss to follow-up; monitoring of key laboratory parameters; allied health services engagement; baseline ophthalmologic assessment; and risk mitigation interventions such as prescription of GI and bone protection if on steroids. Seventeen patients with leprosy were treated during the enrolment period: 10 males (58.8%), and 7 females (41.2%). Of 17 patients enrolled, 8 were treated pre-implementation, and 9 post-implementation. Seven patients (41.2%) were classified as paucibacillary, and 10 (58.8%) as multibacillary. Five patients (29.4%) were lost to follow-up, all of whom were lost prior to implementation of the safety tool. One (12.5%) pre-implementation patient was sent for baseline ophthalmologic assessment vs. 8 (88.9%) post-implementation, (p=0.01). Only post-implementation patients received referrals for occupational therapy and social work. Seven (77.8%) patients were referred for in-home occupational therapy assessments and 33.3% (n=3) were referred for social work assessments. Laboratory parameters were routinely monitored for both groups. GI and bone protection were provided for all patients on steroids. Implementation of the safety tool has established a user-friendly method for systemizing all elements of care that are critical to appropriate management of leprosy. The tool has been particularly useful in ensuring the involvement of all consulting and allied health services necessary for optimizing outcomes of leprosy patients.

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EVALUATION OF A CLINIC-BASED QUALITY STRUCTURE FOR MEDICINES TO TREAT PARASITIC INFECTIONS

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Frequently used drugs in the Tropical Disease Unit (TDU) of Toronto General Hospital, including albendazole and ivermectin, are only available through the Special Access Programme (SAP) of Health Canada. As there are multiple points at which errors can occur in this process, a tracking system was implemented to ensure quality control of SAP-supplied medications and to evaluate the turnaround time (TAT) and success rate of SAP applications for drugs to treat common illnesses evaluated in the TDU. We aimed to evaluate the utility of this tracking system. A retrospective review of the TDU SAP logs for 2013-2015 was undertaken, and data including the TAT for each drug, incomplete notification rate (i.e., request by SAP for additional details before approval), and final approval rates over time were analyzed by one-way ANOVA or Yates' correction Chi square analysis, respectively. The mean TAT decreased progressively from 2013 to 2015, from 9.27 ± 10.3 days to 7.38 ± 8.03 days to 5.15 ± 1.93 days (p=0.04). The rates of incomplete notifications for albendzole in each of 2013 to 2015 were 25%, 54%, and 31%, respectively. For ivermectin, the incomplete notification rates in each of 2013 to 2015 were 4%, 16%, and 17%, respectively. Overall final approval rate for albendazole or ivermectin was 100% in all years. First time success and incomplete notifications differed between ivermectin and albendazole, with 87% of applications for ivermectin approved immediately compared to 64% for albendazole (p=0.0033). For the indication of Strongyloides hyperinfection (n=6), specifically, the mean TAT for ivermectin was 5.11 ± 0.694 days. It is critical that patients receive the necessary medications for their helminthiases , as rejections or delays in drug approval increase the likelihood of patient adverse outcomes including death. This is especially true for entities such as disseminated strongyloidiasis or ruptured hydatid cyst. Prior studies have demonstrated increased patient morbidity and mortality resulting from delays associated with the SAP drug approval process. Our clinic-based system may have contributed to the improved TAT noted over time.

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IMMUNIZATION AGAINST TETANUS DURING PREGNANCY: SEROLOGICAL INVESTIGATION FOR MATERNAL AND NEONATAL ANTIBODIES IN WEST REGION OF BURKINA FASO, BOBO DIOULASSO

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In Burkina Faso, neonatal tetanus often occurs often during aseptic childbirth and is related to infection of the umbilical cord in a baby born of an unimmunized mother. The Centers for Disease Control and Prevention (CDC) estimate that over 270,000 deaths occur annually worldwide as a result of neonatal tetanus. It accounts for about one-half of tetanus deaths in Burkina Faso. This study aims to assess risk factors linked to inadequate immunization against tetanus and immunity status of the newborn. One hundred venous blood plasmas were collected from pregnant women during eutocic childbirth and were compared with blood sample umbilical cordon from their newborns. The tetanus antibody titers were determined by enzyme-linked immunosorbent assay (ELISA kit; IBL International GMBH, Germany) in accordance with the manufacturer's recommendation. The results were reported in IU/mL, and they were standardized by comparing them to calibrated World Health Organization (WHO) reference sera. Overall, 100 women were assessed, 74% are illiterate, 57% are in age group 18-25 years old and 26% are on their second pregnancy. Most of these women, 93% have tetanus antibody titers upper than 1.00 IU/mL. Geometric mean of tetanus antibody titers was 3.66 (95%CI 3.13-4.28) in mothers and 3.87 (95%CI 3.28-4.58) in newborns. There is a significant correlation between antibody titer in the two groups (Spearman's coefficient=0.86, p<0.05). But there is no obvious link between tetanus antibody titers, the number of doses of tetanus vaccine given during pregnancy on the one hand (coeff=0.3, p<0.5) and the interval since the last dose on the other hand (coeff=0.3, p=0.85). Pregnant women and their newborns are well immunized against tetanus in Bobo Dioulasso. There is no reliable evidence related with gravidity, number of tetanus vaccine doses given during pregnancy and the tetanus antibody titer.

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EVALUATING TREATMENT OUTCOMES OF AMBULATORY LEPROSY PATIENTS RECEIVING OFLOXACIN-CONTAINING MULTIDRUG THERAPY REGIMENS

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There is an ongoing need to evaluate the efficacy of treatment options for leprosy, as the standard dapsone and clofazimine-containing multidrug therapy (MDT) continues to face barriers such as safety and tolerability. The fluoroquinolone ofloxacin has shown promise as an effective component of MDT, however, few data on the efficacy of ofloxacin-containing MDT for the treatment of leprosy in non-endemic areas are available. We evaluated the treatment outcomes of leprosy patients receiving ofloxacin-containing MDT regimens in our practice. We performed a retrospective chart review of all patients treated for leprosy in our practice over a 3.5-year period. Primary outcomes evaluated were cure rate; frequency of occurrence of leprosy reactions; frequency of dapsone-associated adverse effects; and treatment adherence. Analysis was descriptive, but data were also stratified by age, sex, spectrum of disease (paucibacillary vs. multibacillary), region of origin, and treatment regimen, and compared by odds ratios (ORs). During the enrolment period, 17 patients were treated with ofloxacin-containing MDT regimens: 10 males (58.8%) and 7 females (41.2%). Ten patients were classified as multibacillary, and 7 as paucibacillary. At the time of analysis, 5 patients (29.4%) had fully completed MDT, and all were deemed cured clinically, with a median duration of follow-up of 35 months (range 27 to 66 months). Five patients (29.4%) were lost to follow-up after completion of treatment, and 7 (41.2%) are still in active treatment. Ten patients (58.8%) experienced either ENL (n=3, 17.6%) or reversal reactions (n=5, 29.4%) or both (n=2, 11.8%). Of those receiving a dapsone-containing regimen (n=12), 5 (41.7%) developed clinically significant methemoglobinemia, and 2 (16.7%) developed hemolysis. Patients from the Philippines (n=5) were more likely to undergo reversal reactions than those from the Indian subcontinent or Africa (n=12) (p=0.028). Although limited by sample size, our results demonstrate high cure rates and positive treatment outcomes with ofloxacin-containing MDT, with reaction rates comparable to those documented in other studies.

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INTUSSUSCEPTION SURVEILLANCE AMONG CHILDREN BEFORE ROTAVIRUS VACCINE INTRODUCTION IN BAMAKO, MALI

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Intussusception (IS) is a major cause of bowel obstruction in infants that has been linked to infective agents and more recently to rotavirus vaccination. As rotavirus vaccine introduction proceeds in GAVI-eligible countries, where few studies of IS have been performed to-date, there is keen interest in documenting the epidemiology of IS. This retrospective study provides baseline data among children from 0 to 15 years with IS admitted to the major pediatric surgery unit in Bamako, Mali at the Hôpital Gabriel Touré (HGT) from 2009 to 2014 (when rotavirus vaccine was introduced). We reviewed operating room and hospital registers to identify cases who met the definition of IS at Level 1 of Diagnostic Certainty from the IS Brighton Collaboration Working Group (2004). During the 6 year study period, IS was diagnosed in 458 (6.1%) of 7,429 children hospitalized on the pediatric surgery unit of HGT; 66.8% were male and 61.6 % were younger than 1 year of age. The mean period incidence was 84.6 per 100,000 infants (range 29-81). The most common symptoms of IS were abdominal mass (78.4%), rectal bleeding (54.4%), vomiting (70.3%), crying/colic (54.8%) and abdominal distention (34.9%). IS was confirmed pre-operatively by sonogram in 381 (83.2%) and /or by X-ray in 7 (5.0%). Surgery was the sole therapeutic modality used, and was performed in 441 patients (96.3%), while 5 were lost to follow-up, 9 improved, and 3 died before surgery. Among 433 with surgically-confirmed IS, there were 23 spontaneous reductions, 309 manual reductions, 28 intestinal resections, and 35 ileostomies; 61 required a second surgical procedure. After surgery, 378 (82.5 %) of cases were discharged in good condition, 11 (2.4%) had complications and 64 (13.9%) died. Children who died were younger, had a longer duration of symptoms, and were more systemically ill. Intussusception is a relatively frequent occurrence in children under 1 year of age in Bamako, Mali and is associated with a high mortality. The ability to monitor changes in incidence related to vaccine is complicated by natural annual variation. Efforts are needed to improve outcome of IS and to detect possible vaccine-related cases.

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FIELD CHALLENGES IN CONDUCTING RESEARCH IN MULTIDRUG RESISTANT MALARIA AND PRE-ELIMINATION SETTINGS

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As efforts to eliminate malaria scale up, a range of practical challenges in conducting research in these settings arise which makes quality clinical research increasingly difficult. This analysis sought to gain a more comprehensive understanding of the difficulties encountered by research and field staff based in the field in these settings to improve the success of future research projects in malaria elimination settings. Reports from field-based studies were reviewed using content analysis to identify the challenges that were reported while conducting the research. In depth interviews were conducted with Village Malaria Workers (VMWs) involved in the research to identify challenges that may not have been reported in official reports. Informal interviews were conducted to provide more insight into the common themes identified from the report content analysis. As malaria elimination progresses, the smaller numbers of study participants available presents a range of practical field challenges that can impact upon the study outcomes. This is further affected by the selection of exclusion criteria. We found that in Western Cambodia migrant and mobile populations (MMPs) were frequently excluded from studies because of potential difficulties in study follow-up. This led to significantly reduced numbers for study enrolment and the exclusion of a critical high-risk group. We also also found that implementation challenges are reported sporadically and are often not formally reported. Conclusions This is the first study reporting the implementation challenges faced in conducting clinical trials in malaria elimination and drug resistance settings. The findings of the analysis will assist in informing the design of future projects to increase the likely success of these studies. The inclusion of MMPs as participants in future studies, although presenting some challenges will also reduce potential biases and makes the research more relevant for elimination in these settings.

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PARASITE AND *MYCOBACTERIUM TUBERCULOSIS* CO-INFECTION IN IMMIGRANTS

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Tuberculosis (TB) is the leading infectious cause of mortality worldwide, and parasitic infections are endemic in 21 of the 22 countries with the highest TB burden. Although data suggest that parasitic infections alter susceptibility to infection with *Mycobacterium tuberculosis* (Mtb), it remains unclear whether this association varies by parasite species. We aim to assess differences in susceptibility to Mtb infection and immune response to Mtb based on parasite species. Immigrants receiving routine clinical care at Boston Medical Center are enrolled (anticipated sample size of 400). Information on the social and medical history of participants is collected from medical records. Participants are tested for TB infection with the Quantiferon Gold-in-Tube (QGIT) assay and for parasites with stool microscopy and antigen testing and with serology for those with eosinophilia or relevant symptoms. QGIT supernatants are analyzed to quantify Th1, Th2, and Treg responses and compare these between individuals with protozoal infections, helminths, and no parasites. Thirty-nine participants have been enrolled to date: median age is 28 (range 10-76) and 25 (64.1%) are male. Of 36 with QGIT results, 8 (22.2%) are positive and, among 23 with stool testing results, 8 (34.8%) have at least one parasite. Among those with parasites, 7 (87.5%) have at least one protozoal infection (5 (71.4%) Blastocystis hominis, 1 (14.3%) Endolimax nana and Blastocystis hominis, 1 (14.3%) Endolimax nana, Dientamoeba fragilis and Blastocystis hominis), and 1 (12.5%) with schistosomiasis (based on serology). Comparing those with protozoa (n=7) to those with no parasites (n=15), 4 (57.1%) and 3 (20.0%) are QGIT positive (p=0.08). Those with protozoa are of similar age (median age 28 vs. 29) and more likely to be female (4 (57.1%) vs. 4 (26.7%), p=0.17) than those with no parasites. Immunologic analyses are pending. This ongoing study of newly arrived immigrant patients at Boston Medical Center will provide data to delineate the association between protozoa and helminth response to latent tuberculosis infection. Participant enrollment and data analyses are ongoing.

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Lauren A. Saag[1], Rachel L. Epstein[2], Jackson B. Mesick[1], Sondra S. Crosby[2], Sarah L. Kimball[2], Elizabeth D. Barnett[2], Natasha S. Hochberg[2]

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THE IMPACT OF SYSTEMATIC POINT-OF-CARE ULTRASOUND ON MANAGEMENT OF PATIENTS IN A RESOURCE-LIMITED SETTING

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Although target point-of-care (POC) ultrasonography has been shown to benefit patients in resource-limited settings, it is not clear whether a systematic POC ultrasound assessment in these settings can also lead to similar changes in patient management. A pre-defined systematic set of POC ultrasound scans were performed on inpatients at a tertiary referral hospital in Tanzania to see if this resulted in changes to patient management. Of the 55 patients scanned, an abnormality was detected in 75% (n=41), and a change in patient management was recommended or implemented on the basis of POC ultrasound findings in 53% (n=29). The main impact was earlier initiation of treatment due to more rapid and accurate diagnosis. Further research is warranted to determine whether systematic POC ultrasonography would result in improved patient outcomes in resource-limited settings.

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INTRODUCTION OF THE MISGAV-LADACH CAESAREAN SECTION TECHNIQUE TO A NIGERIAN TEACHING HOSPITAL

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When compared to the traditional Caesarean section technique, the Misgav-Ladach method for Caesarean section has been shown to be more efficient, leading to decreased operating time, peri-operative bleeding, and need for post-operative painkillers . We compared the two techniques sequentially after the introduction of the Misgav-Ladach method to a teaching hospital in Nigeria via a teaching DVD provided by the World Health Organization. 144 patients who underwent Caesarean-sections between March 2007 and November 2009 were compared. The first 72 received a traditional Caesarean section before the WHO training had been completed. The second group of 72 after the training received the Misgav-Ladach method. The two groups were analyzed with a multivariate analysis of covariance controlling for patient age and the acuity of the indication for surgery (emergent vs. urgent). Overall, mean surgical time was lower in the Misgav-Ladach group (55.8 minutes) compared to the traditional method group (72.3 minutes; $p < 0.001$). There were no significant differences in blood loss, number of transfusions, or pain medication doses

between the two groups. To examine differences in resident and attending use of the techniques; 25 resident cases were compared to 119 attending cases. The residents had longer surgical times (69.2 minutes) than attendings (58.9 minutes; p <0.034) there were no technique interactions. As such, the Misgav-Ladach group had significantly lower surgical times regardless of resident vs. attending physician. The Misgav-Ladach method appears to be faster than the traditional technique. In general, there were trends indicating less blood loss and pain medications in the Migav-Ladach group though only surgical time was significant. These differences could have systems implications , especially in low-to-middle income countries, where resources are less available. The fact that the training took place through a DVD and that the benefit was seen in both attendings and resident physicians shows promise for an effective means of disseminating the skills needed to incorporate this technique in areas of high need.

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NEW STRATEGIES FOR THE DEVELOPMENT OF ANTIVENOM THERAPIES TO TREAT SNAKEBITE

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Snakebite is a neglected tropical disease that affects ~1.8 million people each year. Of these cases, upwards of 94,000 deaths occur, primarily in the rural regions of south Asia and sub-Saharan Africa. The mainstay of snakebite therapy is antivenom - polyclonal antibodies from hyperimmune animal serum derived from horses or sheep immunised with small amounts of snake venom. Different antivenoms are produced to treat bites by different medically important snakes, as the venom toxins that cause pathology vary from species to species. This process of generating commercial antivenoms has remained largely unchanged for the past century and, consequently, these therapeutics have many limitations: (i) limited paraspecifity at treating bites by different snake species, (ii) low specificity in terms of the number of antibodies that are specific to venom proteins and (iii) high incidences of adverse reactions due high volumes of foreign protein being delivered to patients. Here we describe new experimental strategies that we have developed to produce 'next-generation' antivenoms that display increased paraspecific cross-reactivity and specificity to toxic snake venom proteins. Using 'omic' technologies to characterise toxin gene expression in the venom gland and toxin proteins in secreted venom, we have elucidated the variation in venom composition observed in many snakes of medical importance. This facilitates the identification of conserved regions of venom-encoding genes that can be used as antigens to stimulate the production of antibodies with paraspecific activities. We show that experimental antivenoms developed using few epitope-string DNA immunogens are capable of stimulating antibody repertoires with comparable cross-reactivity to traditional antivenom, and which are capable of neutralising venom-induced pathology *in vitro* and *in vivo*. Such approaches provide new tools amenable for the development of 'pathology-specific' or 'continent-specific' antivenoms that are based on mixtures of monoclonal antibodies and will be more specific and efficacious at lower doses than existing treatments for snakebite.

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MONITORING OF PATIENTS OPERATED FOR TRACHOMATOUS TRICHIASIS IN THE KAYES REGION OF MALI

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The Kayes region of Mali has historically had a large trachoma burden. Implementation of the World Health Organization's SAFE strategy (Surgery, Antibiotics, Facial cleanliness and Environmental improvements) has led to significant reductions in trachoma prevalence. The Mali national program has a long standing surgery program to treat the trachomatous trichiasis (TT) cases and reduce the estimated national TT backlog. High quality data regarding surgical outcomes are essential to timely and accurate evaluation of trachoma programs. The aim of this study was to assess the quality of TT surgery by following up patients who underwent TT surgery in Kayes region, Mali, three to six months following the operation. A cross-sectional study was conducted in 8 health districts in Kayes region from June 2014 to February 2016. To be eligible patients must have been operated by mobile surgery teams within three to six months prior to the field trip and on the national surgery record. Fifteen to 20 patients were randomly selected from the national program's operated patient registry for each field visit. In total, 206 patients were enrolled and completed a structured interview and ophthalmological assessment. Concordance of collected data with that sent to the PNSO was verified for each patient interviewed. 143 of the 206 enrolled patients (69.4%) were interviewed, with a median age of 62.5 (range 9-94) and a ratio of 2.6:1 for females to males. Among the 143 patients interviewed, 27 (18.9%) patients have not been operated. Of the 116 patients operated for TT, in 24 (20.7%) cases, the reported operated eyelid did not match the actual operated eyelid. Suture removal did not occur in the recommended timeframe in 16 cases. Post-operative TT was found in 33/116 (28.4%) patients, and 28/116 (24.1%) had continued tearing in the operated eye after surgery. This study underscores the importance of post-operative follow-up to verify data completeness and quality, as well as surgical outcomes of patients. Refresher training for operators and TT surgery supervision must be strengthened to ensure high quality TT surgery to achieve trachoma elimination in Mali.

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PARASITIC DISEASES IN CAMBODIA: A NATIONAL SEROSURVEY OF WOMEN 15 TO 39 YEARS OF AGE BY MULTIPLEX BEAD ASSAY

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A national survey of Cambodian women of childbearing age was conducted in 2012 to assess immunity to vaccine preventable diseases such as polio, measles, and rubella. The availability of a nationally representative serum panel offered an opportunity to measure IgG

antibody levels to a number of parasitic diseases using a multiplex bead assay previously developed in our lab. Recombinant antigens from *Plasmodium falciparum*, *P. vivax*, *Toxoplasma gondii*, *Taenia solium*, *Strongyloides stercoralis*, and lymphatic filariasis were included in the multiplex assay panel, and national and regional prevalence values were estimated from the IgG antibody data. Few women were positive for cysticercosis or toxoplasmosis (<6% seroprevalence) and no geographic clustering was obvious. Malaria and lymphatic filariasis were mainly confined to the North region of the country with several distinct hotspots. Infection with S. stercoralis was widespread in the population (45.9% prevalence): urban residents (32% seroprevalence) had lower levels of infection than rural residents (50% seroprevalence). Integration of multiplex bead assays into nationally representative population serosurveys can provide valuable information on the presence, prevalence, and distribution of parasitic disease and can be used to evaluate the impact of public health interventions.

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A PROSPECTIVE STUDY OF SCABIES OUTBREAKS IN TEN RESIDENTIAL CARE FACILITIES FOR THE ELDERLY

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Scabies is a global health problem and common in closed communities. It represents a significant problem in residential care facilities (RCF) for the elderly in the UK and other countries. Clinical presentations in the elderly can differ from those in younger individuals. In RCFs where a scabies outbreak was suspected and prior to treatment, two physicians including a dermatologist performed examinations of residents, including dermoscopy and skin scrapings. . Scabies diagnosis was classified as definite/probable/possible. Outbreaks were defined as ≥2 suspected cases. Residents were treated with a topical scabicide twice. A second visit after treatment was performed. 230 residents were examined across 10 RCFs between February 2014 and February 2015. The median age was 86.9 years and 175 (76.1%) were female. 157 individuals (68.3%) had dementia. 61 (26.5%) were diagnosed with scabies. Of these 3 had crusted scabies. The median number of cases/RCF was 6 (2-11). The number of cases of scabies categorised as definite was 8 (13.1%). The mite was visualised using dermoscopy in 7 cases (11.5%) and identified on skin scraping in 3. Burrows were detected in 41.0% of cases. 31 (50.8%) individuals were asymptomatic. Dementia was significantly associated with having scabies (OR=2.37 95% CI 1.37-4.09). At the second visit (median interval of 44 days, range 37-81 days) there were no new cases of scabies. 10 individuals diagnosed with scabies at the first visit were identified as still having possible (8) or probable (2) scabies. Scabies is a difficult diagnosis to make in this population with more than half of affected individuals being asymptomatic often with subtle clinical signs. Dermoscopy and skin scrapings were of limited value in identifying affected individuals. This is the first study to confirm that dementia is a risk factor for scabies in this group. Our findings are of potential relevance to the understanding and management of scabies in highly endemic communities, RCFs in tropical settings and other closed communities. Elderly members of communities should be carefully examined as they may have no symptoms or typical signs.

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BACTERIAL ETIOLOGY AND OUTCOME OF CHILDHOOD LIFE THREATENING INFECTIONS IN THE GAMBIA: EUCLIDS IN WEST AFRICA

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Despite the importance of severe bacterial infections as a major cause of morbidity, disability and mortality in African children, data on the factors contributing to poor outcome of severe childhood bacterial infection in West African children is limited. We aimed to prospectively identify factors related to poor outcome. Patients aged 1 month to 16 years presenting with suspected sepsis and/or severe focal infections to two urban hospitals in The Gambia recruited for the European Union Life-threatening Infectious Disease study (www.euclids-project.eu) were part of this analysis. Basic demographic data, clinical features, outcome and pathogen identified using standard bacterial culture and molecular diagnostics were documented. From January 2013 to September 2015 we recruited 411 children, 96(23.3%) developed poor outcome; 54(13.1%) died and 42(10.2%) developed sequelae. The common bacterial pathogens identified were *Staphlococcus aureus* (37,24.7%), *S. pneumoniae* (31,20.7 %), *Neisseria meningitidis* (25,16.7%), and *Haemophilis influenzae* (20,13.3 %). The factors that were associated with poor outcomes were young age, presence of a cor-moridity, prior stay at another health facility and duration of symptoms prior to presentation. More than half of the mortality cases were less than 24 months with median age (IQR) of 16.3(5.9-60.0) months and sepsis was present in 39/54(72.2%) of those who died (p=0.004). 29/54 (69%) died within 48 hours of admission and gram negative organisms were the commonest(12/42(22.2%) associated with mortality. Abnormalities in the musculoskeletal system and central nervous system at presentation were more often associated with development of sequelae. Poor outcome is common (23.3%) in childhood life-threatening infections. A potential vaccine preventable disease was found in 27/96 (28.1%) of those with poor outcome. The attainment of MDG 4 of reduction of childhood mortality requires a review and improvement of vaccine policies as well as research into the production of novel effective vaccine against S. aureus.

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MALARIA PREVENTIVE MEASURES DURING ROUTINE CARE AMONG CHILDREN WITH SICKLE CELL DISEASE IN MALAWI

Graham Ellis[1], Godwin Chipoka[2], Pilirani Mafunga[2], Christopher Stanley[2], Tisungane Mvalo[2], Portia Kamthunzi[2], Isobel Kambalami[2], Peter Wasswa[3], Kate Westmoreland[4], Seyed Nouraie[5], Nigel Key[4], Kenneth Ataga[4], Satish Gopal[2]

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The contribution of malaria to sickle cell disease (SCD) morbidity in sub-Saharan Africa is unclear, particularly for children receiving chronic care including malaria preventive measures. In a Malawian pediatric SCD cohort, we describe use of preventive measures and prevalence for malaria, and explore associations with levels of hemoglobin (Hb) and lactate dehydrogenase (LDH). Weekly sulfadoxine/pyrimethamine (SP) was the local standard-of-care for malaria chemoprophylaxis. SP adherence, bed net use, and malaria history were assessed using a standardized survey. Overall, 94 children with SCD were evaluated with mean age 8.2 (SD 4.5) and median Hb 7.2 g/dL (IQR 6.6-7.7). 92 (98%) were receiving SP at evaluation. Of these, 53 (58%) reported perfect adherence to SP and 88 (96%) reported adherence to ≥75% of doses. The most common reasons for missed doses were running out of SP and guardian traveling. 66 (70%) reported regular bed net usage, with costs reported as the

major barrier to nonuse. 6 (6%) reported a history of malaria, and of 72 cross-sectional blood smears collected over three months including the transmission season, no malaria parasites were seen. Neither self-reported history of malaria, Hb, nor LDH correlated with SP adherence or bed net use. In conclusion, we observed high rates of adherence to SP and bed net use in a pediatric SCD cohort in Lilongwe. Self-reported malaria was very infrequent and laboratory-confirmed malaria was not detected, suggesting good effectiveness of malaria preventive measures in this population under routine care conditions.

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PRE-TRAVEL HEALTH CARE AMONG PEDIATRIC U.S. MILITARY BENEFICIARIES

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An increasing number of children are traveling to developing countries, placing them at risk for travel-related infections. There is limited data comparing pre-travel care, exposures and illnesses in pediatric and adult travelers. We used a prospective, observational cohort of US Department of Defense beneficiaries traveling overseas (TravMil), to compare pediatric and adult travelers using a case-control methodology. Pediatric subjects were matched 1:1 with adult military dependents by travel region, malaria risk at destination, and duration of travel. Outcomes of interest included pre-travel preventive care and associated compliance, travel exposures, and reported illnesses. 83 pediatric and adult subjects were matched. The median ages of pediatric and adult subjects were 11 years (IQR: 7-16) and 63 years (IQR: 44-67) years, respectively. Common regions of travel included South-East Asia (42%), South/Central America (34%), and Africa (26%). Pediatric travelers was more likely than adults to visit friends and relatives (39% vs. 10%; $p < 0.05$). Travel related vaccine coverage rates were similar, except Hepatitis A (pediatrics: 90% vs. adults: 71%; $p < 0.05$) and B (84% vs. 48%; $p<0.05$). Malarone was the most common antimalarial prescribed in children and adults, with no difference in non-compliance. Poor compliance with skin insect repellent use (55% vs. 67%), and frequent skin exposures including insect bites (94% vs. 89%), animals bites or scratches (52% vs. 27%) were reported among pediatric subjects ($p<0.05$). Subjects < 10 years of age were less likely to be prescribed antibiotics (RR=0.65; 95% CI: 0.50-0.86) and anti-diarrheals (RR=0.09; 95% CI: 0.03-0.28) for TD self treatment than adults, despite similar rates of high risk behavior for TD acquisition. Strategies to improve compliance with preventive measures, and standardization of TD treatment guidelines are needed for pediatric travelers.

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IRON DEFICIENCY IS COMMON IN UGANDAN CHILDREN WITH SICKLE CELL ANEMIA

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African children have a high prevalence of sickle cell anemia (SCA), which causes significant morbidity and early mortality. Previous studies of iron deficiency (ID) and the degree to which it contributes to anemia in children with SCA in malaria endemic areas have produced conflicting results. Our objective was to determine the prevalence of ID and its relationship to erythrocyte parameters in Ugandan children with SCA living in a malaria endemic area. 205 Ugandan children <4 years of age with SCA were assessed for ID by testing plasma levels of four iron-related biomarkers (ferritin, soluble transferrin receptor (sTfR), C-reactive protein (CRP), and hepcidin) by ELISA. ID was defined using previously established cutoffs: (1) ferritin <12 µg/L if CRP <10 mg/L or ferritin <30 µg/L if CRP ≥10 mg/L; or (2) hepcidin <5.5 ng/mL. Iron markers collectively reflected low iron stores and poor erythrocyte iron availability. Median (IQR) values for ferritin, sTfR, hepcidin, and CRP were 12.2 µg/L (7.5-26.2); 7.2 mg/L (5.5-9.0); 7.8 ng/mL (1.2-23.2); and 6.7 mg/L (2.6-16.7), respectively. The prevalence of ID was high (62.9% by ferritin cutoff, 42.4% by hepcidin cutoff). Children with ID as defined by low ferritin had similar hemoglobin levels (mean (SD)) to children without ID (7.6 g/dL (1.0) vs 7.4 g/dL (1.0), $p=0.12$) but had a lower MCV (78 fL (10) vs 82 fL (8), $p=0.005$). The data provide strong evidence that ID is common in children with SCA in a malaria endemic area. Studies are needed to assess the clinical and neurodevelopmental consequences of ID in children with SCA in malaria endemic areas, and to assess whether iron supplementation can be given to children with SCA in malaria endemic areas without increasing their risk of malaria and other infections.

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ENTERIC PATHOGENS AND FECAL BIOMARKERS OF GUT INFLAMMATION IN ASYMPTOMATIC INFANTS AND IMMUNE RESPONSE TO ORAL POLIOVIRUS VACCINE

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Oral poliovirus vaccine(OPV) immunogenicity is sub-optimal, in developing countries with high enteric infection prevalence. Enteropathogen presence in asymptomatic children in such settings is hypothesized to lead to impaired oral vaccine efficacy. We studied the relationship of enteropathogens and biomarkers of gut inflammation in asymptomatic 6-11 month old Indian infants with the immune response to a dose of monovalent OPV3 (mOPV3). Infants included were from a randomized clinical trial evaluating effect of a short course of azithromycin on mOPV3 seroresponse (CTRI/2014/05/004588). Enteropathogen profile in these infants (n=729) was evaluated using Taqman array card assays for enteropathogens, including bacterial, virus and parasite targets, from stool samples collected on the day of mOPV3 vaccination. In a subset of infants (n=299), fecal biomarkers of gut inflammation like calprotectin, myeloperoxidase, alpha-1antitrypsin and neopterin were estimated. Immune response to a mOPV3 dose was assessed by serum neutralization assays for anti-poliovirus 3 antibodies on samples collected 21 days after vaccination. Significantly higher proportion of infants failing to seroconvert were found to be infected with one or more enteropathogens on the day of the vaccination (94.3% of PV3 seronegatives vs 86.8% of seropositives, OR=2.5, 95CI=1.43-4.55). Enteric viruses were found in a significantly higher proportion of seronegative infants compared to seropositives (62% vs 46%, OR=1.9, 95CI=1.4-2.59). The difference with respect to bacterial enteropathogens or intestinal parasites even at higher pathogen loads was not significant. Among enteric viruses, non-polio enteroviruses (NPEV) were significantly associated with non-response to mOPV3(44% in seronegatives vs 29% in seropositives, OR=1.9, 95CI=1.4-2.6). Levels of fecal biomarkers like calprotectin, MPO and alpha-1AT were higher among seronegatives compared to seropositives, however, this difference was not significant. To conclude, this study adds to the evidence of association of enteric viruses, specifically NPEV, with OPV non response in developing settings.

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DEMOLISHING ACCESS BARRIERS TO HEALTHCARE IN TWO DIFFERENT CHAGAS DISEASE SCENARIOS: ENDEMIC AND NON-ENDEMIC AREAS IN ARGENTINA

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Approximately 1.6 million people are infected with *Trypanosoma cruzi* in Argentina and less than 1% has access to etiological treatment. Within the country there are three distinct epidemiological scenarios that can be characterized for Chagas Disease (CD): 1) areas with a recent history of vector transmission (VT) currently controlled with entomological surveillance (S1); 2) areas with uncontrolled VT and/or without current entomological surveillance (S2), and 3) a historically non-endemic area (S3). In both in SI and in S3, it is necessary to conduct actions of timely diagnosis and treatment (D&T) of affected individuals; which is where this study was conducted. It is worth mentioning that in S2 these actions should not be promoted given the risk of possible re-infection through vector transmission. According to the recommendations of the Pan American Health Organization, a model of D&T was developed for the first level of healthcare. In order to achieve this, it was first necessary to provide primary healthcare centers with the technical capacity to be able to treat the patient in one place, minimizing the economic burden on the families and loss to follow-up. Mundo Sano participated in the training and motivation of the healthcare teams and provided the equipment to allow the centers to perform electrocardiograms in site. The process of D&T was free of charge (including clinical analysis, medical consultations and anti-parasitic drug). As of March 2016 a total of 12,096 people were diagnosed, of which 1,101 were positive for CD and 999 of these were treated. This experience shows the great potential of the first level of healthcare to provide access to D&T for CD.

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A NOVEL ELECTRONIC ALGORITHM USING HOST BIOMARKER POINT-OF-CARE TESTS FOR MANAGEMENT OF FEVER IN UNDER-FIVES IN RESOURCE-POOR SETTINGS (E-POCT): A CONTROLLED, NON-INFERIORITY STUDY

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Infections cause the majority of deaths in under-fives in resource poor settings. Management of febrile illnesses at outpatient level remains inadequate and antibiotic overuse is a great challenge. The objectives were to determine whether a novel, electronic algorithm using point-of-care test results (e-POCT) is noninferior to a validated electronic algorithm derived from IMCI (ALMANACH) in treating fever in under-fives and to compare the proportion of antibiotic prescription between the two algorithms. E-POCT is an electronic algorithm developed by our group based on current evidence of pediatric fever management. It is built into an android application, which guides through the entire consultation and recommends treatment based on a few clinical signs, as well as point-of-care laboratory tests. We performed a randomized, controlled non-inferiority study of patients aged 2-59 months presenting with fever (axillary temperature ≥37.5°C) at 10 outpatient clinics in Dar es Salaam, Tanzania. The primary outcome was a noninferiority comparison between e-POCT and ALMANACH on the proportion of clinical failure by day (D) 7 of follow-up. Noninferiority would be declared if the percent of clinical failure with e-POCT was no worse than the proportion of clinical failure with ALMANACH, within statistical variability, by a margin of 3% in a modified intention to treat analysis. The secondary outcome was the comparison between both arms on the proportion of antibiotic prescribed on D0. We enrolled 1583 patients into the e-POCT-, and 1581 into the ALMANACH-arm between December 2014 and February 2016 with lost to follow-up of 0.5% and 0.8%, respectively. The percent of clinical failure by D7 was 2.1 for e-POCT and 3.4 for ALMANACH. The 97.5% lower confidence limit was a -2.4 difference in percent, establishing noninferiority. The Antibiotics were prescribed at D0 in 10.9% of patients (95% confidence interval [CI] 9.4-12.5) using e-POCT, compared to 29.0% using ALMANACH (CI 26.8 - 31.3). In conclusion, e-POCT is noninferior to ALMANACH in terms of clinical outcome by D7 while significantly reducing (by 62%) the proportion of antibiotic prescription at D0.

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ACTIVITY OF CRUDE EXTRACTS AND CHROMATOGRAPHIC FRACTIONS OF *DANIELLIA OLIVERI* AND *PSOROSPERMUM FEBRIFUGUM* AGAINST ADULT *BRUGIA PAHANGI*

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Lymphatic filariasis (LF) caused by *Brugia malayi*, *B. timori*, and *Wuchereria bancrofti,* potentially affects an estimated 1.2 billion people, with over 120 million people infected, and about 40 million people disfigured and incapacitated by the disease. The disease manifestations include painful and profound disfigurement of the skin, lymphedema, elephantiasis and scrotal swelling which may eventually lead to permanent disability. Hence, individuals infected not only suffer physical disability, but also suffer mental, social and economic losses, thus resulting in depression, stigma and poverty. Current control of LF relies on mass drug administration either a combination of ivermectin and albendazole or DEC and albendazole. However, these drugs are only effective against the microfilariae (mfs), with limited activity against the adult worms, which may live for up to 18 years. Effective control is therefore hindered by the lack of adulticides (macrofilaricides). There is a pressing need for new macrofilaricidal compounds. Medicinal plants used in traditional medicine have been identified to play a potential role in the remedy of diseases. Preliminary studies in our lab showed crude extracts of *Daniellia oliveri* and *Psorospermum febrifugum* inhibited adult *B. malayi* motility, when the Worminator system was used. In this study, we screened crude extracts and chromatographic fractions of D. oliveri and I for activity against adult I, a suitable animal model for *B. malayi*. Remarkably, the active crude extracts had IC50 values in the range of 44 - 154 µg/mL, the cleaned-up extracts had IC50 values in the range of 27 - 331 µg/mL, and the active chromatographic fractions caused a 100% inhibition of motility when tested at 300 µg/mL. This indicates that, *D. oliveri* and *P. febrifugum* may serve as sources of lead compounds for the development of the much needed macrofilaricidal drugs.

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FACTORS IMPACTING THE DETECTION OF *BRUGIA MALAYI* DNA WITHIN THE EXCRETA/FECES OF EXPOSED MOSQUITOES

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Recent experiments have indicated that the DNA of both *Brugia malayi* and *Plasmodium vivax* parasites is detectable in the pooled excreta/feces of vector mosquitoes. Utilizing real-time PCR, proof-of-concept work has demonstrated the high-throughput potential of this alternative approach to molecular xenomonitoring (MX). Yet despite these encouraging results, excreta/feces testing remains largely unexplored, and further work is required to more completely evaluate this novel methodology and gage its operational feasibility. Accordingly, we have performed comparative studies, aimed at assessing the detectability of parasite DNA within the excreta/feces obtained from mosquitoes under a variety of experimental conditions. Analyses have evaluated the impact of mf-blood density on parasite detection, while also examining DNA detection rates at various time-points post-exposure. The potential for DNA detection within the voided material of recently blood-fed, gravid, and host-seeking mosquitoes has also been explored, helping to better inform future trapping efforts intended for excreta/feces collection. Furthermore, as non-vector species clear *B. malayi* rather than harboring its development, the detectability of parasite DNA within the excreta/feces of non-vector mosquitoes was evaluated and results were compared with those for vector hosts. While a number of important questions remain to be addressed, the results described here provide a foundation for future studies aimed at operationalizing this novel approach to MX.

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PLACENTAL EXPRESSION OF IRON TRAFFICKING GENES IN THE CONTEXT OF HUMAN HELMINTHIASIS

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Iron deficiency during pregnancy and early infancy is a leading cause of poor growth and neurocognitive delays in childhood. Pregnant women and children in low and middle income countries (LMICs) are at increased

risk for iron deficiency due to iron poor diets and extra corporeal iron loss in stool due to helminthiasis. Herein, we examined the expression of ferroportin (FPN) and ferritin, key genes involved in iron trafficking across the placenta. Ferritin is the main storage protein for intracellular iron and FPN has been implicated at the primary exporter for iron from the placenta into fetal circulation. We isolated RNA from whole placental tissue stored in RNAlater (n=72) collected as part of a randomized controlled trial in Leyte, the Philippines, evaluating the impact of Praziquantel treatment during pregnancy on birth outcomes. Total RNA was reverse transcribed using random primers, and FPN, ferritin and 18S gene expression assessed using quantitative real-time PCR. FPN and ferritin gene expression were highly correlated in this cohort. Neither FPN nor ferritin gene expression in the term placenta was altered by Praziquantel treatment or maternal anemia status. Women who were infected with T. trichuria displayed significantly lower levels of both ferritin and FPN gene expression in the placenta. In addition, maternal levels of hepcidin, a key regulator of iron metabolism, at 32 weeks gestation were negatively associated with FPN gene expression in the placenta, while cord blood hepcidin was not associated with either FPN or ferritin gene expression. FPN expression was also positively associated with gestational age, and both FPN and ferritin expression were positively associated with birth weight in this sample of infants. Taken together, these data suggest that maternal factors such as trichuria infection and high hepcidin levels may have a greater influence on transplacental iron flux than fetal factors. In addition, effective iron trafficking is instrumental to successful pregnancy, and placentas challenged to transport adequate iron may result in newborns with lower birthweight.

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EFFICACY OF SINGLE AND REPEATED ORAL AND SUBCUTANEOUS DOSES OF FLUBENDAZOLE IN *LITOMOSOIDES SIGMODONTIS* INFECTED JIRDS

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Flubendazole (FBZ) is a promising macrofilaricidal drug candidate for the elimination of onchocerciasis. Recently a new bioavailable oral formulation was developed and its efficacy was investigated in the jird *Litomosoides sigmodontis* model. Microfilariae-positive jirds were divided into 11 groups of 12 jirds each and FBZ was tested at single (40 mg/kg) or repeated (2, 6 or 15 mg/kg for 5 or 10 days) oral (OR) doses and at single subcutaneous (SC) injections (2 or 10 mg/kg). Positive controls received 5 SC injections at 10 mg/kg, negative controls remained untreated. Jirds were euthanized 8 weeks post treatment end for adult worm counts. Single and 5 x 10 mg/kg SC FBZ completely cleared the adult worms in all animals (100%). Single 2 mg/kg SC and 10 x 15 mg/kg OR FBZ reduced the adult worm burden by 94% and 90%, respectively; single 40 mg/kg OR and 5 x 15 mg/kg OR by 80 and 85%, respectively. At necropsy, all animals in the SC groups had no detectable microfilariae within the peripheral blood, while the oral FBZ treatment regimens reduced the microfilaremia in a dose and duration dependent manner. FBZ was measured in plasma by LC/MS/MS. After OR, AUC 0-24h increased dose proportionally from 2 to 40 mg/kg and was similar between days 5 and 10. After SC, FBZ was slowly released from the injection site and plasma levels remained constant up to necropsy. Results of this study demonstrate that single and repeated SC injections and repeated oral administrations of FBZ have an excellent macrofilaricidal effect and achieve efficacies of ≥ 90% adult worm reduction. Histopathological analyses of the remaining female adult worms could be considered to determine whether a permanent sterilization was achieved, which would guarantee that the transmission is stopped and microfilariae-driven pathology in Onchocerciasis is reduced.

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LACK OF SIGNIFICANT MICROFILARICIDAL EFFICACY OF PARENTERAL OR ORAL BIOAVAILABLE FLUBENDAZOLE FORMULATIONS IN A *BRUGIA MALAYI* MICROFILARAEMIC MOUSE MODEL

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Flubendazole (FBZ) is a validated macrofilaricide in preclinical models of lymphatic filariasis (LF) and onchocerciasis when administered by multiple subcutaneous injections. A new amorphous solid dispersion (ASD) formulation of FBZ, with improved oral bioavailability, has been developed by Janssen. This formulation is currently undergoing testing in rodent models of filariasis to evaluate its *in vivo* efficacy as a candidate macrofilaricide. Preclinical macrofilaricides also require evaluation of direct microfilaricidal activity, especially considering potential indications in loiasis co-endemic foci, where severe adverse events have been reported following ivermectin (IVM) treatment. The *Brugia malayi* microfilaraemic mouse model is a validated screen to test activity of filaricidal compounds in inducing rapid decline of circulating blood mf *in vivo*. The purpose of this study was to assess whether single dose oral FBZ ASD (2 and 40 mg/kg) has direct microfilaricidal efficacy in SCID mice intravenously infused with *B. malayi* mf. In parallel, efficacy of standard FBZ suspension given by injection was evaluated (10mg/kg x 5 days). Human bioequivalent oral IVM (0.2 mg/kg) induced 81% average reduction in circulating mf at +48h compared with pre-treatment levels (n=5, P=0.0003). Circulating mf did not significantly decline at +48h in either single oral dose regimens (12 or 49% reductions in 2 or 40 mg/kg dose groups) or multiple injections of FBZ (35% reduction). Reductions observed in FBZ groups were similar to reductions in vehicle control circulating mf at +48h (38%). After 7 days, microfilaraemias were reduced significantly in single dose IVM group only compared to vehicle controls (85% efficacy, n=5 / group, F=3.733, P=0.0234). FBZ groups showed 0% efficacy when compared with vehicle controls. In conclusion, this counter-screening model demonstrates a lack of direct microfilaricidal activity of parenteral or oral FBZ formulations against circulating *B. malayi*. It is therefore unlikely that such bioequivalent FBZ regimens would mediate substantial direct effects against bloodborne microfilarial infections of humans.

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INDUSTRIAL SCALE SCREENING OF 1.3 MILLION COMPOUNDS IDENTIFIED 14 NOVEL CHEMOTYPES AS PROMISING NEW LEADS FOR THE TREATMENT OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS: A COLLABORATION BETWEEN THE ANTI-WOLBACHIA CONSORTIUM AND ASTRAZENECA

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A screening collaboration between the anti-Wolbachia consortium and AstraZeneca has identified 14 novel areas of diverse chemical space with potential for ongoing development into treatments for lymphatic filariasis and onchocerciasis. This was accomplished through the first industrial scale screening of a 1.3 million compound collection housed by AstraZeneca against Wolbachia, the A·WOL drug target and endosymbiotic bacteria of the filarial worms which cause onchocerciasis and lymphatic filariasis.

Following a primary screen at 10μM in a model insect cell line (C6/36) stably infected with Wolbachia we obtained ~20,000 hits which were triaged through a chemoinformatic analysis to select 6,000 compounds for secondary dose response screening. Again chemoinformatics were used to cluster the resultant hits into 58 distinct areas of chemical space. Representatives from all 58 clusters were then screened at 5μM in a microfilarial (larval worm) assay. From these representatives 14 clusters demonstrated equivalent Wolbachia reduction to our gold standard drug doxycycline after 6 days *in vitro* exposure. These novel chemotypes, including one related to known oxazolidinone antibiotics, are extremely promising new leads against lymphatic filariasis and onchocerciasis, diseases which afflict 157 million people worldwide resulting in severe disability globally.

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THAT FEELING OF BEING OUT OF PLACE: A MICROFILARIAL TALE

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In, Alberta, Canada, a 27-year-old man presented to the Emergency Department with a complaint of pruritic swelling on his chest. His past medical history was unremarkable. He was born and raised on a farm in Sudan, and drank water from the river. At the age of 12, he fled to Kenya with his family, where he lived in a refugee camp, worked in construction, ate the food provided and drank water from a well. He immigrated to Canada in 2006 and has not returned to Africa since. His symptoms started in January 2016, when he noticed skin eruptions to his right chest. These eruptions were painful, pruritic and at times felt like slowly spreading movement underneath his skin. He presented to a local emergency department where he underwent a minor surgical procedure resulting in removal of a worm from the chest wall lesion. The worm was submitted to the laboratory for identification. Ten days later, the patient felt similar symptoms over his right eyelid; he returned to the hospital for removal of what turned out to be a second worm. He was referred to the Infectious Diseases Clinic. He was completely asymptomatic except for night sweats. His physical examination was unremarkable. There were no ocular findings and no serpiginous lesions on skin examination. Both worms were referred to US Centers for Disease Control and Prevention (CDC) for identification. Blood smear and stool were received in our laboratory; parasitic serology was referred to the National Centre for Parasitology (McGill). The patient was found to have a microfilarial load of >=8,000 microfilariae/ml. Coincidentally, his stool examination revealed rhabditiform larvae of Strongyloides stercoralis . His serology was negative for HIV and positive for Strongyloides sp. and schistosomiaisis (the latter possibly representing a cross-reaction or past exposure). *Loa loa* can live up to 17 years and is not considered endemic in Kenya. Most likely the patient acquired it while in Sudan. This case reports an unusual presentation of loiasis, further complicated by co-infection with S. stercoralis. It emphasizes the importance of obtaining a detailed travel history reaching far beyond the customary few years.

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ELIMINATION OF ONCHOCERCIASIS WITH IVERMECTIN: A VALIDATION OF THE EPIONCHO AND ONCHOSIM MODELS USING DATA FROM MALI AND SENEGAL

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EPIONCHO and ONCHOSIM are two independently developed models for the transmission and control of onchocerciasis. Both models have been used to explore the feasibility of eliminating onchocerciasis from Africa within the timeframes outlined by the World Health Organization (WHO) and endorsed by the 2012 London Declaration on Neglected Tropical Diseases. A recent comparison of projected timeframes to elimination by mass treatment with ivermectin highlighted similarities but also discrepancies between these two models that warranted further investigation and subsequent model refinement. Here we describe refinements to and re-fit the model to parasitological data from human populations in Cameroon and Ghana. We compare the refined version of EPIONCHO and ONCHOSIM in their ability to predict trends in infection prevalence from baseline to elimination in the three West African transmission foci in Mali and Senegal where infection was successfully eliminated circa 2007-2009. We also compare projected timeframes to elimination in terms of programmatic prevalence thresholds, stochastic fade-out and transmission breakpoints and evaluate the impact of uncertainty in key epidemiological and programmatic parameters. We conclude that both EPIONCHO and ONCHOSIM capture epidemiological trends towards elimination with sufficient accuracy to serve as useful decision-support tools for estimating when elimination is likely to be achieved in a variety of epidemiological and programmatic settings and for identifying foci where alternative intervention strategies might be required to reach the WHO targets.

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INTEGRATED LYMPHATIC FILARIASIS AND PODOCONIOSIS CLINICAL CASE MAPPING USING SMS MHEALTH TOOLS AND COMMUNITY NETWORKS IN HAWELLA TULA AND BENSA DISTRICTS OF ETHIOPIA

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Lymphatic filariasis (LF) and podoconiosis are disabling neglected tropical diseases (NTDs) that affect the world's poorest people and pose a significant economic burden. In Ethiopia, an integrated mapping project confirmed that 112 districts are LF-endemic, 345 districts podoconiosis-endemic and 53 districts are co-endemic for both diseases, however limited information is available on the number of clinical cases. Such information is crucial for the effective planning and delivery of a basic package of care. The aim of this study was to identify cases using a bespoke SMS reporting tool, MeasureSMS, in two co-endemic districts, Bensa and Hawella Tula (population 430,439), of the Southern Nations, Nationalities, and Peoples' (SNNP) Region. In July 2015, a total of 59 Health Extension Workers (HEWs) and four district supervisors were trained on how to identify lymphoedema and hydrocele cases in their catchment area (including multiple kebeles) and to report the information by SMS using their own, basic mobile phones. This data was then sent to a local

smartphone with the MeasureSMS app installed, and uploaded to a cloud server where the automatically collated data was accessible via a web browser. A total of 2,197 lymphoedema cases and 134 hydrocele cases were reported, with 42 cases reported as having both conditions. The mean age of all cases was 45.2 years, with 45.4% of lymphoedema and 100% of hydrocele cases being males. All HEWs reported cases with a mean of 40 cases per HEW, with a range of 3 to 210 cases across their multiple kebeles. To ensure data quality, 129 patients were randomly selected to be visited and verified by a trained clinician; 79.1% were confirmed as having the same condition as reported through SMS by the HEW. Network problems and power outages lasting up to two days were some of the challenges faced by the HEWs when using the mHealth tool in the field. Nevertheless, given the high numbers of cases reported in these districts, particularly of lymphoedema, MeasureSMS was shown to be a valuable mHealth tool to obtain patient estimates and guide the allocation of limited resources to those most in need.

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SCALING-UP A 'PACKAGE OF CARE' FOR LYMPHATIC FILARIASIS CASES IN MALAWI USING SMS MHEALTH TOOLS FOR MORBIDITY MAPPING, AND COMMUNITY HEALTH WORKER NETWORKS AND DISTRICT HOSPITALS FOR PATIENT CARE

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After six successful rounds of mass drug administration (MDA) for lymphatic filariasis (LF), MDA in Malawi was stopped in 2014 following nationwide Transmission Assessment Survey (TAS) results. With evident success in interrupting transmission, Malawi, with partner support is now focussed on morbidity management and disability prevention (MMDP) for those affected by clinical manifestations, namely lymphoedema and hydrocele. To begin to scale-up MMDP, three key activities have been implemented in the two most endemic districts, Chikwawa and Nsanje in the Southern Region, and planned in a third district, Karonga in the Northern Region. Firstly, case estimates were obtained using the SMS reporting tool, MeasureSMS, which is an efficient and cost-effective tool by which community health workers (CHWS) identify and report clinical cases of LF in their communities by SMS to develop a clinical database of cases. To then provide access to care for these identified cases, lymphoedema management training of CHWs and hydrocele camps were organised. In Chikwawa district, 369 lymphoedema cases (71 % female) and 986 hydrocele cases were identified by SMS with 11 cases reporting both hydrocele and lymphoedema. In Nsanje district, 265 cases of lymphoedema (77% female) and 866 cases of hydrocele were reported with 9 cases reporting both conditions. To date, 123 of the hydrocele cases have been visited and verified by a clinical to ensure data quality and accuracy. Of these, 99 (80.5%) were confirmed to have the same condition as was reported by the CHWs. In December 2015, 326 of these reported cases had their hydrocele operated at six hydrocele camps across Nsanje and Chikwawa districts with impact assessments conducted to demonstrate an improvement in quality of life. In total, 468 CHWs were trained on lymphoedema management (integrated with leprosy) and hydrocele referral in early 2016. These results demonstrate that Malawi has made significant progress in scaling up MMDP activities and will continue to move these activities forward with a commitment from the Ministry of Health and partners with the aim of meeting the 2020 global and national elimination goal.

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LESSONS FROM MASS DRUG ADMINISTRATION FOR THE ELIMINATION OF LYMPHATIC FILARIASIS (LF) IN AN URBAN SETTING IN HAITI

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The world is increasingly urbanized as 54% of the world's population lives in urban areas, including 57% of the population in Haiti. Urbanization has far-reaching health consequences, as these highly mobile and heterogeneous populations have varying experiences and perceptions of the health system. As lymphatic filariasis (LF) is endemic throughout Haiti, the NTD Control Program has a goal to eliminate LF as a public health problem by 2020 through mass drug administration (MDA). To achieve elimination, at least five rounds of MDA with ≥ 65% coverage must be completed for the population at risk. High coverage rates are necessary for MDA to effectively reduce LF prevalence to a level at which transmission is not sustainable. Peri-urban settings in Haiti typically require additional rounds of MDA. Two communes were selected, the peri-urban Croix-des-Bouquets and rural Thomazeau in the West department for a Knowledge, Attitudes and Practice (KAP)/coverage survey supported through USAID's ENVISION Project. A two-stage 30-cluster sample was used to ensure random selection. Data collection took 7 days and analyzed with STATA version 14. The survey coverage for Croix-des-Bouquets was 53.3% compared to reported coverage of 67.09%; 80% for Thomazeau (61.3% reported coverage). The peri-urban nature of Croix-des-Bouquets may influence how and why respondents participate in MDA. Croix-des-Bouquets is of interest as Haiti nears LF elimination, since success relies on high coverage urban MDAs. When asked how they heard about the MDA campaign, the most popular response was megaphones (35% Croix-des-Bouquets and 65% in Thomazeau). The survey also found that population movement and general mistrust of the health system and participating in projects supported by international aid, contribute to low coverage rates. Further, those in urban settings have access to a greater variety of media therefore diluting the effectiveness of MDA messaging. Focusing on more direct ways to reach the population and alternative social mobilization strategies tailored to specific needs of the urban community may help overcome these barriers.

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LESSONS FROM LYMPHATIC FILARIASIS TRANSMISSION INTERRUPTION IN HAITI: ARE FIVE ROUNDS OF ANNUAL MASS DRUG ADMINISTRATION (MDA) NECESSARY IN LOW-PREVALENCE SETTINGS?

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Lymphatic filariasis (LF) is endemic throughout Haiti. In line with World Health Organization's (WHO) LF elimination targets, the Haiti NTD Control Program (HNTDCP) has a goal to eliminate LF as a public health problem

by 2020. Currently WHO recommends 5 rounds of consecutive annual mass drug administration (MDA) with ≥65% coverage, after which a transmission assessment survey (TAS) can be carried out to determine whether MDA can be stopped. Ile de la Tortue, a commune in the Northwest department, is a remote island that had an antigen prevalence of 6.0% in 2000 and only 0.8% by 2006 sentinel site evaluation. Two rounds of MDA were conducted in Ile de la Tortue 2003 and 2005 with 110% and 87% reported coverage, respectively. MDA was then suspended due to resource constraints. In 2012, the HNTDCP with assistance from CDC and UND carried out TAS1 in order to determine if additional rounds of MDA were necessary to reduce antigen prevalence to below 2%. School-based TAS was carried out on a sample of 1,308 11-12 year olds, with 13 immunochromatographic card test (ICT) positive cases, which was below the calculated critical cut-off for sample size of 14. This older age group was used because school enrollment for 6-7 year-olds was significantly below the recommended 75%, and enrollment did not exceed 75% until the 11-12 age group. Further, using an older age group is appropriate in settings where MDA has not been recently carried out. In 2016, the HNTDCP and IMA World Health/ENVISION carried out a school-based TAS2 in Ile de la Tortue. A total of 928 6-7 year olds were tested, and 0 were ICT-positive. These results indicate that 12 years since the last treatment, there is no evidence of ongoing LF transmission and additional MDA is not indicated. The results suggest that 5 rounds of annual consecutive MDA may not be necessary in areas with adequate MDA coverage and low baseline prevalence. Further research is warranted to determine the number of rounds of MDA required, as reducing these could help resource-poor settings with low initial prevalence reach their 2020 LF elimination goals.

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PROJECTIONS OF ATTAINING ONCHOCERCIASIS ELIMINATION IN OGUN STATE, NIGERIA: A CROSS-SECTIONAL REPORT OF THE OV-16 SEROLOGY (RAPID DIAGNOSTIC TEST) AMONG CHILDREN BORN AFTER 10 YEARS OF TREATMENT WITH IVERMECTIN

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Evidence based studies using the Ov-16 serology among children below 10 years of age is one of the key components of the revised WHO course of action for indicating interruption of transmission of *Onchocerca volvulus* among human population receiving treatment with ivermectin. In view of this background, this study conducted between March and July 2015 investigated the sero-prevalence of onchocerciasis in endemic communities of Ogun State, Nigeria after 10 years of treatment with ivermectin. Using the Ov16 Rapid Diagnostic Test (RDT), 719 children between the age 5-9 years residing in 32 firstline communities in 8 endemic Local Government Areas (LGA's) provided whole blood specimen which were tested for IgG4 antibodies against the *O. volvulus* antigen Ov-16. Data were analysed using Pearson's Chi square in SPSS 20. Results showed a cumulative sero-prevalence of 21(2.9%), Relationship between age and prevalence was statistically insignificant ($p > 0.05$). Thirteen females and eight males were exposed to *O. volvulus* respectively. The low sero-prevalence recorded among children within the age range (5-9 years) born after the inception of ivermectin implementation implies that they may have had diminutive historic exposure. Although this finding is somewhat greater than the 0.1% threshold set by the guideline for this study population. The Information obtained will serve as a baseline serological information and a guide to prepare Ogun State for Post Treatment Surveillance (PTS) in the nearest future.

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INTEGRATING NOVEL PEPTIDES AND REPORTER NANOPARTICLES IN A RAPID TEST FOR ONCHOCERCIASIS

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This study describes a lateral flow assay (LFA) to detect exposure to *Onchocerca volvulus*. The assay features two innovations: a novel peptide biomarker and novel nanoparticles. The advent of high throughput peptide synthesis and micro-array technology enables new avenues for the discovery of diagnostic targets. Scanning the *O. volvulus* proteome for immunodominant linear epitopes revealed at least three highly immunogenic motifs, as reported previously. In parallel, we have introduced new plasmonic reporter nanoparticles to increase the analytical sensitivity of LFAs. These nanoparticles are gold shells, consisting of a gold layer deposited over a low-density silica gel core. The shells are blue to black and absorb visible light 35 times more efficiently than the red colloidal gold typically employed in LFAs. The increase in absorption translates into a stronger visual readout and an increase in analytical sensitivity. The two technologies were merged into a prototype LFA that employs gold shells to detect circulating antibodies to one of the recently discovered peptide motifs. A total of 20 plasma samples (FR3 repository) from individuals suffering from onchocerciasis and 16 samples from non-endemic healthy controls were tested i) in the Ov16 LFA, ii) in a peptide ELISA, and iii) in the peptide LFA using gold shells. The Ov16 LFA showed 85% and 100% sensitivity and specificity, respectively. The peptide ELISA gave 90% sensitivity and 100% specificity. The peptide LFA was 80% sensitive and 100% specific. The peptide LFA results correlated with the peptide ELISA data, and the four samples that scored negative in the LFA gave no or very low signal in the ELISA. Onchocerciasis samples lacking Ov16 antibodies did recognize the peptide in ELISA and LFA, and conversely, samples lacking antibodies against the peptide reacted in the Ov16 LFA. Thus, a peptide LFA was assembled which, when used in combination with the Ov16 LFA, and when tested on a small sample collection, detects antibodies to *O. volvulus* with a sensitivity of 100% and specificity of 100%.

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IMMUNOREACTIVITY OF AN ON*CHOCERCA VOLVULUS* LINEAR EPITOPE IN INDIVIDUALS FROM DIFFERENT REGIONS IN GHANA

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Using proteome-wide high-density peptide microarrays we have identified an immunodominant linear epitope, as reported previously. We have used peptide ELISA to assess the immunoreactivity of a 15-mer peptide 02-052 containing the identified motif 2, in individuals from Ghana. Plasma samples were collected from nodule-positive individuals (n=94), endemic controls (n=48), non-endemic controls (n=20) and lymphatic filariasis patients (n=45). A healthy control group of 10 samples from South Africa was included to assess specificity. All samples were tested on the Ov16 IgG4 lateral flow test (LFA) (Standard Diagnostics). None of healthy controls from South-Africa and non-endemic controls were positive in this test, while 24/48 endemic controls, 68/94 nodule-positive, and 12/45 LF patients were positive for Ov16 IgG4 antibodies. 'No peptide' controls were included for all samples to assess non-specific binding of antibodies (i.e. background). A cut-off for positivity was set at the average background + 3SD. None of the healthy control samples had detectable antibodies against the peptide. In the non-endemic control group, 3/20

were positive in the peptide ELISA. In the endemic control group, 17/24 and 14/24 of the Ov16 positive and negative individuals, respectively, were positive in the peptide ELISA. 40/68 of the Ov16 positive nodule-positive individuals, and 13/26 of the Ov16 negative nodule-positive individuals, had detectable antibodies against the peptide. Also in the LF group, 8/12 and 23/33 of the Ov16 positive and negative individuals, respectively, were positive in the peptide ELISA. In conclusion, the new *O. volvulus* linear epitope showed perfect specificity in healthy controls from South Africa. This peptide ELISA identifies more cases of exposed/infected individuals as compared to the Ov16 IgG4 test. Reactivity in LF patients residing in onchocerca endemic areas requires in depth analysis and will drive the use case for peptide LFA. This peptide has been used previously for development of a LFA.

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DEWORMING IN PRE-SCHOOL AGE CHILDREN IN NIGERIA: ARE THOSE WHO NEED IT THE MOST RECEIVING TREATMENT?

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Pre-school age children are at high risk for nutritional and growth impairments from soil transmitted helminths (STH) and yet, despite the World Health Organization's recommendation of periodic treatment for those in that age group in endemic areas, they are currently not receiving the deserved attention from national control programs. We examined the deworming status of pre-school aged children in 12 high prevalence states in Nigeria and whether those at increased risk of infection or with related symptoms received treatment. Children aged 12-59 months were selected from the 2013 Nigeria Demographic and Health Survey, a national representative sample survey of adults aged 15-49 years. Markers of risk for infection with STH were state prevalence levels (≤ 20%), socioeconomic status and other demographic variables. Symptomology was represented by the presence of stunting. Weighted logistic regression was used to determine the association of infection risk factors and stunting with mother's reports of whether children received treatment. Of the 3,062 children considered in the analysis, 39% received treatment for worm infections. Compared to children with normal height, severely and moderately stunted children were less likely to access treatment with OR=0.45 (95CI: 0.31 - 0.64) and OR =0.69 (95CI: 0.51 - 0.93) respectively. Uneducated mothers and fathers were less likely to report treating their children with OR = 0.52 (95CI%: 0.35 - 0.75) for mothers and OR =0.42 (95CI%: 0.25 - 0.72) for fathers. Poorer children were less likely to access treatment than the richest children with OR = 0.41 (95CI%: 0.26 - 0.64). The disparities in treatment access among stunted children as well as the potential influence of socio-economic factors call for increased need for deworming coverage of pre-school aged children in Nigeria. Deworming interventions can prevent children from becoming more stunted and may even reverse it. Control programs must identify the most-at-risk populations in order to leverage existing resources to decrease intestinal worm prevalence and break transmission.

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SITUATIONAL ANALYSIS OF NEGLECTED TROPICAL DISEASES MANAGEMENT INFORMATION SYSTEM IN ETHIOPIA

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Health Management Information System (HMIS) is critical for neglected tropical diseases (NTDs) services delivery and informed decision making. In Ethiopia the current situation of the NTD-HMIS is not clearly known. This study is therefore conducted to assess the existing NTD management information system and thereby generate evidence for formulating interventions for improving the system in Ethiopia. The situational analysis involved collection of both quantitative and qualitative data using structured self-administered questionnaire, observation and key informant interview among district monitoring and evaluation officers, district managers, health extension workers and regional NTD program managers. Data was analyzed by using Epi-Info version 3.5.4 and descriptive statistics was conducted using the software. A total of 11 NTD endemic regions in Ethiopia were included in this study. Of these, the largest proportions (66.6%) had no NTD team at regional level, thus the program was executed by focal person. About a third (33.1%) of respondent in the sample had not received a training workshop or technical briefing on monitoring & evaluation of NTDs. According to the NTD data management, it was reported that (83.3%) of the respondent were using simple Excel file to store their data, and the remaining (16.7%) were using paper based mechanism to keep. Regarding data quality assurance, all of the regions (100%) had not carried out data quality assessment. Findings for the NTD medicine supply and management revealed that most of the region (83.3%) had a system for tracking medicines remaining in stock and wastage in the region while only (16.7%) of the overall sampled had no tracking system. This study recommends that collaboration among the stakeholders on capacity buildings of management information system, supportive supervisions with timely and concrete feed backs, and establishment of functional NTD monitoring and evaluation working group at regional level is encouraged. Continual presence of standardized data collection tools and use of information technology for management information system should be given due attention.

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TREATMENT COVERAGE VALIDATION SURVEY AFTER A SCHOOL-BASED MASS DRUG DISTRIBUTION OF PRAZIQUANTEL AND MEBENDAZOLE IN SELECTED DISTRICTS OF ETHIOPIA

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The Ethiopian Ministry of Health is scaling up programs to deliver regular, large-scale schistosomiasis (SCH) and soil-transmitted helminths (STH) treatment for at-risk populations, through repeated rounds of preventative chemotherapy (PCT) using Praziquantel (PZQ) and Mebendazole (MEB). One of the key indicators of program success is program coverage. The aim the surveys presented here is to produce validated coverage estimates of reasonable precision following mass drug administration round in April 2015 in randomly selected district. To assess the coverage of mass drug administration in the dewormed districts of Ethiopia. A community-based cross-sectional study design was employed. Accordingly, 960 households from 96 kebeles found in the sampled 8 districts were selected. The survey covered districts in Amhara, Oromia and Southern Nations Nationalities and Peoples Region. Participation in the treatment campaign and other related Information was collected from school age children (SAC) between the age ranges of 5 to 14 years. 960 households were selected for the validation survey and a total of 1910 (97.2%) school age children (SAC) residing in the selected households between the age ranges of 5 to 14 years were interviewed. During the house hold visit, 55(2.8%) households were not available for interview. While 81.2% of the school age children interviewed were attending school, 18.8% were non-enrolled. 91% of the interviewed children were present in schools during the mass drug administration campaign. During the treatment campaign 84.9% and 85.3% interviewed SACs swallowed Praziquantel and Mebendazole respectively and there was no difference by gender. Our current finding shows that approximately 85% of school age children interviewed received the treatment and the coverage is very high in enrolled school age children. Based on these findings, we recommend creating mechanisms to

reach non-enrolled school age children and awareness in the community if targeting 2020 to control both schistosomiasis and soil-transmitted helminths in Ethiopia.

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TO INTEGRATE OR NOT TO INTEGRATE? DEVELOPING AN EVIDENCE-BASED TOOL FOR NEGLECTED TROPICAL DISEASE CONTROL

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While evidence from clinical and modeling studies have demonstrated the potential benefit of integrating vertical disease programs, few evidence-based tools exist to assist decision makers in evaluating and comparing approaches for integration of control measures, to determine the most impactful and cost-effective approaches for their setting. We have created an application available for mobile devices or on the web, with a simple user interface, to support on-the-ground decision-making for integrating disease control programs or their components, given local conditions and practical constraints. The model upon which the tool is built provides predictive analysis for the effectiveness of integration of schistosomiasis and malaria control, two common parasitic diseases with extensive geographical and epidemiological overlap, and which result in significant morbidity and mortality in affected regions. Working with data from countries across sub-Saharan Africa and the Middle East, we present here a proof of principle for the use of our tool in providing guidance on how to optimize integration of vertical disease control programs.

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SURGICAL MANAGEMENT OF MORBIDITY DUE TO LYMPHATIC FILARIASIS: HYDROCELE SURGERY IN HEALTH DISTRICT HOSPITALS IN MALI

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Mali is committed to national lymphatic filariasis (LF) elimination by 2020. The program has two main components: interrupting transmission of LF through mass drug administration and managing morbidity and preventing disability. Mali has achieved great progress toward LF elimination: to date, 31 of the 63 health districts (HDs) have reached the criteria to stop MDA. However, improvements in morbidity management and disability prevention are more difficult to achieve. Interventions for the surgical management of hydrocele and lymphedema management were introduced in 2012. These interventions are very demanding by the patients because of social impacts in their daily live. From 2014-15, the national program, with the support of HKI with funding from the End Fund, performed surgical treatment of 369 LF patients in 16 HDs. General practitioners performing surgery were trained on hydrocele case management and hydrocele cases were identified at the community level. Surgeons obtained informed consent prior to the surgical procedure and maintained patient records. A descriptive analysis to show the impact of hydrocele management was performed on 175 patients who were included in this analysis based on the completeness of the patient record. The median age of patients was 52 years and 75% (132/175) were married. The majority of patients 82.3% (144/175) did not report postoperative complications. The median duration of hospitalization was 4 days (ranging 2-55 days). Results showed that 32.6% (57/175) patients reported a considerable positive impact on their work and 44.6% (78/175) reported improved sex lives. Of those patients operated, 77% (54/70) indicated satisfaction after surgery and noted improvement in their daily lives. Dissatisfied patients had developed postoperative secondary infections that were managed with supportive therapy. This study shows that the management of hydrocele surgery can be done in a district hospital setting in Mali without major complications and achieve general patient satisfaction. Consequently, the LF morbidity management and disability prevention project will be expanded to other HDs.

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ACHIEVING THE ENDGAME: IMPROVING INTEGRATED CASE SEARCHES FOR GUINEA WORM DISEASE AND TRACHOMA TO ACHIEVE ERADICATION AND ELIMINATION TARGETS

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Guinea worm disease and trachoma are both neglected tropical diseases (NTDs) slated for eradication and elimination as a public health problem, respectively, by the World Health Organization. In 2010, The Carter Center, the Ghana Ministry of Health and local partners, carried out integrated case searches for rumors of possible cases of Guinea worm disease and persons with trachomatous trichiasis (TT), the end stage of trachoma. These case searches were conducted to meet eradication and elimination targets. The first series of case searches were carried out in four districts, which were searched community to community, with patients referred to a centralized surgical facility. This method of searching did not adequately cover the target population and resulted in low surgical uptake. The second series of case searches, which were conducted house-to-house with patients being offered immediate investigation of suspected Guinea worm disease cases and TT surgical care either in the home or an adjacent primary care facility. This method resulted in higher surgical uptake. The house to house immediate resolution approach was also shown to be more cost effective. The cost to investigate suspected cases of both diseases in the house to house immediate response approach was about USD$13.99 per case examined, compared to a cost of $19.78 per case examined in the community with referral to surgical facilities approach. A review of the two approaches showed significant cost differences and favorable outcomes to the house to house immediate response approach. This approach should be considered an option for disease "end game" where case searches are required to meet targets. This approach should be considered in an integration fashion with other NTDs to maximize cost saving and efficient use of resources.

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ARE WE REACHING EVERYONE AS WE MOVE FROM CONTROL TO ELIMINATION OF NTDS: FINDINGS FROM AN INTEGRATED TREATMENT COVERAGE SURVEY IN NORTHERN NIGERIA

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Countries like Nigeria have developed and are implementing programmes for integrated control of Neglected Tropical Diseases (NTDs). Sightsavers is leading consortium of partners in scale-up for elimination of NTDs in northern Nigeria. However, little data are available to verify reported coverages of previously conducted Mass Drug Administration (MDA). The purpose of this presentation is to validate the reported coverage from Community Drug Distributors (CDD) through integrated post-MDA