surface. In order to test whether antibodies to PfMAS170 could block merozoite invasion, growth of *P. falciparum* 3D7 parasite in the presence of anti-PfMAS170 antibody was tested. The anti-PfMAS170 antibody significantly inhibits the merozoite invasion to erythrocytes. Since anti-PfMAS170 antibodies inhibited merozoite invasion *in vitro*, we decided to investigate whether PfMAS170 is exposed to the human immune system in *P. falciparum*-infected individuals. The sera from *P. falciparum* infected individuals in Thailand reacted with the recombinant PfMAS170, indicating PfMAS170 is immunogenic in humans. Taken together, these results suggested that PfMAS170 plays important role in the merozoite invasion process. The C-terminal erythrocyte-binding domain is of interest for the development of blood-stage malaria vaccine.

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EVALUATING THE POTENTIAL IMPACT OF TRANSMISSION BLOCKING VACCINES AGAINST *PLASMODIUM FALCIPARUM* INFECTION ALONGSIDE EXISTING INTERVENTIONS

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Transmission-blocking vaccines are under development with the aim that they will reduce or interrupt transmission in malaria endemic settings when used alongside existing interventions. However, their utility will depend not only on their efficacy and durability, but also on characteristics of the transmission setting including the intensity and seasonality of transmission. We extended a published mathematical model to identify settings in Sub-Saharan Africa in which a TBV could interrupt transmission if implemented alongside existing interventions, and the characteristics required of the TBV and of the vaccination programme. In all settings repeated annual rounds of vaccination will be required, with more frequent rounds required if the duration of protection is shorter or if the initial transmission intensity is high. The protective efficacy of a typical TBV vaccine with a 1-year half-life is predicted to be substantially higher in seasonal settings compared to perennial settings with the same average transmission intensity, with greater efficacy achieved if the vaccination programme is aligned with the start of the transmission season. Overall the protective efficacy is predicted to be greatest in areas of low transmission but the number of cases averted greatest in areas of moderate transmission. Thus the optimal location to undertake Phase III trials for candidate vaccines would be in existing low to moderate transmission areas.

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IMMUNOGENICITY OF DNA VACCINES ENCODING PFS48/45, A *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING VACCINE ANTIGEN IN RHESUS MONKEYS BY *IN VIVO* ELECTROPORATION INCLUDING EVALUATION OF CODON OPTIMIZATION AND N-LINKED GLYCOSYLATION

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Antigens expressed on various sexual stages of *Plasmodium falciparum* are being pursued as targets of transmission blocking vaccines (TBV). These include gamete surface proteins (Pfs230 and Pfs48/45) and zygote/ookinete surface protein (Pfs25) expressed after fertilization in the mosquito midgut. Ingestion of antibodies against these antigens effectively blocks parasite development in the midgut. Efforts are underway to develop vaccines based on either recombinant proteins-adjuvant formulations or as DNA vaccines. While our laboratory has previously expressed Pfs48/45 as a functionally effective recombinant molecule in E. coli, structural complexity has continued to be a challenge for further development of an effective vaccine. The goal of this study was to investigate Pfs48/45 using DNA vaccine platform. The rationale is to develop a TBV that is: (i) technically less challenging in comparison to recombinant protein production, (ii) cost-effective, (iii) stable, and (iv) easy to manufacture. In addition, DNA vaccine platform allows a multivalent approach by combining several antigens. Our vaccine design included codon optimization of DNA sequence for optimum expression, in-vivo electroporation for enhanced immunogenicity, mutations to block any N-linked glycosylation in the expressed protein, and a heterologous prime-boost approach. Additionally, we evaluated a combination of DNA vaccines encoding Pfs48/45 and Pfs25. All 7 putative N-linked glycosylation sites in Pfs48/45 were mutated (N to D or K) based on available sequences of P48/45 orthlogs in various *Plasmodium* species. Rhesus macaques (N=4) were assigned to each of three groups and immunized (IM) with 3 DNA vaccine doses (2.5mg), using *in vivo* electroporation at 4 week intervals followed by a recombinant protein boost (50ug protein in Alum). Antibody titers were determined by ELISA and functional activity in mosquito membrane feeding assays. Results on various test parameters as well as outcome of combining two different TBV target antigens will be discussed. (Funded by AI47089, AI101427 and AI 103466).

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DISTRIBUTIONAL IMPACT OF RTS,S VACCINATION IN SUB-SAHARAN AFRICA: IMPLICATIONS FOR POLICY IMPLEMENTATION

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A growing body of evidence has demonstrated that many public health interventions developed to aid the poor are not reaching their intended target. These concerns are relevant for the introduction of a malaria vaccine, the most advanced of which is RTS,S, currently in Phase III clinical trials. Initial results indicate that RTS,S can provide modest protection against both clinical and severe malaria in young infants. We examine the potential distributional impact of the RTS,S vaccine in 6 African countries by evaluating differences in the relative risk of malaria against projected vaccine coverage and health benefits across beneficiaries grouped by socio-economic characteristics using an asset index. To accomplish this we first link country Demographic and Health Surveys with the distributions of entomological inoculation rate derived from the prevalence data assembled by the Malaria Atlas Program. We then combine information on transmission, access to health services and baseline vector control interventions coverage to assess the impact of vaccine deployment on disease burden and its distribution across different population groups using a stochastic simulator of malaria epidemiology and control. We estimate the extent of forgone health due to disparities in access to immunization services by simulating a scenario assuming immunization coverage of the group in the highest socio-economic quintiles for the whole vaccination cohort. We highlight the importance of malaria case management in sustaining the health gains achieved with the RTS,S by simulating vaccine impact at levels of highest wealth quintile for both immunization and case management coverage. Our findings suggest that substantial additional reductions in burden could be realized with RTS,S by targeting the underserved population with either extensive outreach or through innovative distribution channels. We further illustrate the gains in program effectiveness if vaccine deployment in combined with systems strengthening to improve access to malaria case management.

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DEVELOPMENT OF *PLASMODIUM FALCIPARUM* RETICULOCYTE BINDING-LIKE HOMOLOGOUS PROTEIN 2 (PFRH2) AS A BLOOD-STAGE MALARIA VACCINE CANDIDATE

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The *Plasmodium falciparum* reticulocyte binding-like homologous (PfRH) family of proteins are key determinants of different erythrocyte invasion pathways. Out of five functional PfRH proteins, we report that native PfRH2 undergoes processing yielding fragments that exhibit differential erythrocyte binding specificities. Consistent with previous PfRH2 knockout studies, the RBC binding specificity of native PfRH2 was sialic acid-independent, trypsin resistant and chymotrypsin sensitive. However, a smaller processed fragment bound erythrocytes with a different phenotype. To further characterize the processing sequence and localization of the resulting fragments, we have raised specific antibodies against different regions of the PfRH2 ectodomain. We have mapped the erythrocyte binding domain of PfRH2 to a conserved 40kDa N-terminal region (rPfRH240). Recombinant rPfRH240 bound to erythrocytes in a sialic acid independent, trypsin resistant, chymotrypsin sensitive manner, consistent with the binding of the native protein. PfRH2 antibodies against only the 40 kDa receptor binding domain were able to efficiently block erythrocyte invasion and further produced synergistic inhibition of erythrocyte invasion in combination with antibodies against other parasite ligands such as EBA-175 and AARP. A recent study has demonstrated that PfRH2 is naturally immunogenic in humans residing in malaria endemic regions and that PfRH2 antibodies exhibited the highest association with protection against malaria among a pool of 91 recombinant blood-stage antigens. We have developed and optimized a process for the production of rPfRH240 with cGMP specifications for it's clinical development. Thus, rPfRH240 is a major candidate antigen under the ICGEB portfolio for the development of a new generation combination based blood-stage malaria vaccine.

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INDUCTION OF IMMUNITY FOLLOWING VACCINATION WITH A CHEMICALLY ATTENUATED MALARIA VACCINE CORRELATES WITH PERSISTENCE OF A SUB-PATENT INFECTION

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Antigenic polymorphism presents a major hurdle for subunit malaria vaccine development. To overcome this obstacle we are developing whole parasite approaches using DNA alkylating agents to chemically attenuate blood stage parasites which will be used as vaccines. We have demonstrated that attenuated *Plasmodium chabaudi* or *P. yoelii* parasitized RBCs can induce immunity in mice. The RBCs in the vaccine had to remain intact for the induction of immunity and killed parasites did not induce immunity. To further understand the nature of immune induction as we move to a clinical trial, we have attempted to follow the fate of attenuated parasites post-inoculation. Mice were immunized once with 10^6 *P. chabaudi* pRBCs attenuated with 2μM tafuramycin A (TfA). qPCR and qRT-PCR were used to monitor parasite DNA and RNA in blood and various tissues. Adoptive transfer studies were used to investigate whether sub-microscopic levels of parasites could transfer immunity between animals. Surprisingly, parasite DNA was detectable for up to one week within the RBCs of vaccinated mice. In contrast, inoculation of mice with killed pRBCs did not result in persisting levels of parasite DNA in blood or tissues of recipient mice. Irraditated whole blood from vaccinated mice could adoptively transfer immunity to recipient mice. Furthermore, treatment of vaccinated mice with anti-malaria chemotherapy was able to reduce the level of immunity in vaccinated mice. Our results suggest that immunity in mice is dependent on a persisting sub-patent attenuated infection. These results have informed our strategy to develop a *P. falciparum* vaccine with a major goal of utilizing an attenuating dose of TfA that enables a persisting sub-patent infection.

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THE USE OF A TRANSGENIC RODENT MALARIA CHALLENGE MODEL FOR ASSESSMENT OF NOVEL LIVER-STAGE MALARIA VACCINES

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Malaria remains one of the most important global infectious diseases. At present there is no completely effective/licensed malaria vaccine. Most of the severe pathologies and deaths due to malaria are associated with *Plasmodium falciparum* (Pf) strain, and developing effective vaccines remains a priority. Unfortunately Pf does not infect small animals and a number of the Pf candidate vaccine antigens either differ from or are absent in rodent parasites, limiting pre-clinical efficacy studies in murine models. In this work we sought to rank-order the protective immune responses to several novel Pf vaccine candidates using a rodent challenge model. We first immunized mice with different Pf pre-erythrocytic vaccine antigens delivered using a viral vectored vaccine (ChAd63/MVA prime-boost) approach and tested the responses to these antigens in mice. For each antigen, we created transgenic *P. berghei* (Pb) parasites expressing the Pf vaccine-candidate gene of interest thus enabling a vaccine efficacy/challenge assessment *in vivo*. Consequently, we were able to perform a screening of 11 Pf vaccine candidates - and several others are in progress. We rank ordered these antigens based on protection studies in different mice strains, selecting the most promising ones to be taken forward for further development. We created two sets of mutants: (i) Pf candidate-antigen genes were expressed in sporozoite and liver-stage using the Pbuis4 promoter; and (ii) when the Pf antigen had a homolog in Pb, the Pb gene was replaced by its Pf equivalent and expressed under the corresponding Pb promoter. Both sets of mutants were used in the immunization-challenge studies. Antigen screening using this challenge model identifies PfLSA1 and PfLSAP2 as more protective than PfCSP or PfTRAP in Balb/c mice with high efficacy: 81.25% and 75% respectively. Also, these two antigens show good efficacy in CD1 outbred mice: (87.5%) and (70%) respectively. Based on results from our initial candidate antigen immunogenicity and efficacy ranking experiments we have also created double transgenic parasites that express different combinations of the most promising candidates. These reagents, we believe, are powerful tools that can help in the rapid assessment of multiple-antigen vaccine approaches.

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OPTIMIZATION OF NON-IV ADMINISTRATION OF A RADIATION-ATTENUATED SPOROZOITE MALARIA VACCINE IN A MURINE MODEL

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Radiation-attenuated sporozoites (SPZ) have been known to induce high-level protection against malarial infection for nearly 5 decades. This was

initially proven in mice with intravenous (IV) administration of rodent malaria SPZ, and was followed by successful immunization of humans by the bite of irradiated *Plasmodium falciparum* (Pf)-infected mosquitoes. It was recently reported that subcutaneous (SC) and intradermal (ID) administration of radiation-attenuated, aseptic, purified, cryopreserved PfSPZ Vaccine was poorly immunogenic and protective in a clinical trial. The same authors also reported that it required 7-10 times as many irradiated (irr) *P. yoelii* (Py) SPZ administered SC or ID as compared to IV to achieve similar protection in BALB/c mice. Subsequently, a clinical trial involving IV administered PfSPZ Vaccine demonstrated protection in 6/6 subjects against controlled Pf malaria infection. Many think it would be optimal if PfSPZ Vaccine was administered by a non-IV route. We utilized the PySPZ-BALB/c model system to systematically address how to optimize non-IV administration of irrPySPZ, using 3 doses of $2x10^3$ irrPySPZ administered IV as the gold standard. We assessed IM, SC, and ID routes of administration, and varied volume of administration (1-25 µL), number of sites of administration (2-30), number of doses (3-5), and number of irrPySPZ administered (2 - $7.5x10^4$ irrPySPZ). Protection > 80% was generally seen after IV administration. However, we were not able to achieve > 80% protection with the other routes. The SC route was superior to IM and ID routes, and injection in multiple sites with smaller volumes was superior to larger volumes in fewer sites, with the SC route achieving the highest protection at 56%. The complete results will be presented, as will plans for studies with higher doses and adjuvants. These results highlight the inefficiency of non-IV as compared to IV administration of SPZ. However, the data indicate that a systematic approach has the potential to significantly increase the efficiency of administration by non-IV routes.

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IMMUNE RESPONSES INDUCED BY AN ATTENUATED, WHOLE PARASITE, BLOOD-STAGE *PLASMODIUM YOELII 17X* MALARIA VACCINE

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We are investigating the protective efficacy of a chemically attenuated, whole parasite, blood-stage *Plasmodium yoelii 17X* vaccine in a rodent malaria model. Optimization of an immunization regimen using a chemically attenuated, asynchronous *P. yoelii 17X* vaccine has demonstrated that 3 doses results in protection in BALB/c mice against death, severe anemia and weight loss following homologous challenge. Furthermore, parasite burden is limited in the blood of immunized mice compared to control mice. Immune responses induced by vaccination are being characterized, with data demonstrating that 3 immunizations induces both significantly higher levels of proliferative cellular responses and antibodies compared to control mice. The induction of antibodies following vaccination is novel to the *P. yoelii 17X* model as they were not detected following vaccination with a chemically attenuated *P. chabaudi* vaccine. However, the precise role of these antibodies in vaccine-induced protection is still unclear as no protection was observed when passively transferred to naive mice followed by homologous challenge. Lymphocyte depletion studies were undertaken to investigate the role of $CD4^+$ and $CD8^+$ T cells in vaccine-associated protection, demonstrating a crucial role for $CD4^+$ T cells only. Following *in vitro* stimulation with parasitized red blood cells, IFN-γ and IL-2 were detected. The cellular origin of these cytokines is being investigated. The induction and longevity of memory T cells in vaccinated mice is being investigated. Preliminary data show significantly higher effector memory T cells are observed up to 3 months post-vaccination in the blood of vaccinated mice compared to control mice, with the significance waning by 6 months. No difference in central memory T cells was observed. Additionally, we are investigating whether cross-stage protection against sporozoite/ liver-stage challenge is induced by this blood-stage vaccine. Our data show that vaccination is capable of inducing activated $CD8^+$ T cells ($CD8^{lo}CD11a^+$), which are known to play a role in liver-stage immunity.

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THE RELATIVE IMPORTANCE OF MALARIA VACCINE PROPERTIES

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Currently many malaria vaccines directed at *Plasmodium falciparum* are under development and in clinical trials, with one candidate, RTS,S, in large scale Phase III trials across multiple sites in Africa. A policy recommendation from WHO is expected in 2015 on RTS,S and last year WHO announced their updated malaria vaccine roadmap with the strategic goals of developing both vaccines with protective efficacy of 75% against clinical malaria suitable for administration to appropriate at-risk groups in malaria-endemic areas, and vaccines that reduce transmission of the parasite and thereby substantially reduce the incidence of human malaria infection. In this new roadmap no length of protection is explicitly define and neither the period for which 75% protection must be achieved. Using simulations from an individual based stochastic model of malaria, we address the relative importance of coverage, vaccine efficacy and measures of duration of protection (such as half-life and shape of decay curve) for different outcomes, including reductions in transmission, parasitological and clinical effectiveness, and the overall public health impact at different time points. We investigate the drivers of optimal impact of pre-erythrocytic, blood-stage, and transmission blocking vaccines for these different outcomes, considering the coverage, target age-group and the initial transmission intensity. We consider the value of using alternative metrics that summarise the coverage, initial efficacy, decay shape and half-life as single parameters. These metrics could facilitate comparison across a portfolio of vaccine candidates, considering different endpoints measured in first-in-man challenge studies, Phase II, and Phase III clinical trials.

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REVERSIBLE CONFORMATIONAL CHANGE IN THE *PLASMODIUM FALCIPARUM*

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The *Plasmodium falciparum* circumsporozoite protein (CSP) is the only malaria parasite antigen to advance to phase 3 clinical trials as part of a virus-like particle vaccine identified as RTS,S. The CSP is filamentous and comprised of three dominant domains: a charged N-terminus that binds heparan sulfate proteoglycans, a central NANP repeat domain and C-terminus comprised of a thrombospondin-like type I repeat (TSR) domain. RTS,S which contains numerous NANP repeats and the TSR domain protects about 50% of children against clinical disease. Hypothetically, a second generation CSP vaccine might improve vaccine efficacy by incorporating the N-terminal domain that is absent in RTS,S. Using a panel of CSP-specific monoclonal antibodies, well-characterized recombinant CSPs, and label-free quantitative proteomics, we show here that native CSP is N-terminally processed in the mosquito host. The processed CSP undergoes a conformational change from a filamentous, open form to a closed form in the salivary gland, which masks the N- and C-terminal domains until the sporozoite interacts with hepatocytes in the liver as determined by inhibition of sporozoite invasion, *in vitro*.

Interestingly, in mice the cleaved N-terminal domain contains a T-helper cell epitope(s) that enhances the immunogenicity of recombinant full-length CSP. Our findings show the importance of understanding the unique biophysical nature of the CSP and its impact on vaccine design and suggest that the conformational change is due to a mechanical or molecular signal.

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DOUBLE-BLIND DOSE-ESCALATING RANDOMIZED CONTROLLED PHASE 1 STUDY IN MALARIA EXPOSED ADULTS OF THE SAFETY AND IMMUNOGENICITY OF PFS25-EPA/ALHYDROGEL®, A TRANSMISSION BLOCKING VACCINE AGAINST *PLASMODIUM FALCIPARUM* IN BANCOUMANA, MALI

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Transmission blocking vaccines (TBV) are a critical strategy for malaria elimination and eradication in Sub-Saharan Africa. A double blind, randomized, controlled Phase 1 clinical trial is being conducted to assess the safety and immunogenicity in malaria exposed Malian adults. The *Plasmodium falciparum* TBV vaccine, Pfs25-EPA/Alhydrogel®, contains a recombinant protein of Pf25 and a recombinant mutant non-toxic protein corresponding to sequence of ExoProtein A (EPA) of *Pseudomonas aeruginosa* with the adjuvant, Alhydrogel®. In May 2013, 120 healthy adult volunteers aged 18-45 years old living in the village of Bancoumana (or the surrounding area), Mali were enrolled into the study. Among the 120 participants, 20 (low dose of 16µg Pfs25-EPA/Alhydrogel® or control) have received 2 doses (Days 0 and 56) and 100 (high dose of 47µg Pfs25-EPA/Alhydrogel® or control) have received 3 doses (Days 0, 56, and 112.) Vaccinations started in May/June 2013 with the third vaccination occurring in September/October 2013 prior to the predicted peak of the transmission season. The fourth and final vaccine dose for the high dose group (47µg Pfs25-EPA/Alhydrogel® or control) is planned in September 2014. Vaccinations have been well tolerated. The related adverse events reported have been mostly mild or moderate injection site reactions and transient neutropenia cases which occurred both in Pfs25-EPA/Alhydrogel® and Euvax B/Hepatitis B vaccine (control) groups. In the group who received the high dose of 47µg of Pfs25-EPA/Alhydrogel®, antibody responses to Pfs25 increased with each subsequent dose of vaccine given, but dimished quickly following vaccination. Overall, Pfs25-EPA/Alhydrogel® TBV has been well tolerated and produced significant antibody responses in a malaria exposed adult population.

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TARGETING OUTDOOR MALARIA VECTORS USING ODOR-BAITED MOSQUITO LANDING BOX (MLB) EQUIPPED WITH LOW-COST ELECTROCUTING GRIDS

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Residual malaria transmission, especially the proportion that occurs outdoors, is now among the major obstacles in achieving malaria elimination goal. This outdoor transmission is attributed to outdoor mosquito bites by mosquitoes that are behaviorally resilient or resistant to existing indoor insecticidal interventions. Field experiments were conducted against free-flying wild mosquitoes, to evaluate an improved version of the recently developed odor-baited mosquito landing box (MLB), fitted with solar-powered low-cost electrocuting grids on its sides to rapidly kill even mosquitoes that only make very short contacts with the devices. Three MLBs were equipped with EC grids made from locally purchased mosquito racket zappers. One MLB was fitted with the EC grids on only one of its sides; another MLB had EC grids on two sides while the third MLB had EC grids on three of its sides. The three were comparatively evaluated using a 3 by 3 Latin square experiment, with outcome measure being average number of mosquitoes of different species. A total of 4986 dead mosquitoes were collected from the 3 odor-baited MLBs equipped with EC grids, 29% (1432) of which were from MLB with grid on one side, 35% (1738) from the MLB with EC grids on 2 sides while 36% (1816) were from the MLB with EC grids on 3 sides. More mosquitoes were caught from the MLB with more than one grid, which might be due to in the higher surface area of contact for mosquitoes with the EC grids. As targeting host-seeking mosquitoes with insecticide-based methods is increasingly challenging due to either behavioral or adaptive resistance of the mosquito vectors after prolonged use, MLBs equipped with low-cost EC grids regularly charged by solar energy, could have significant advantages, as it would also effectively control even those species that are behaviorally resilient or physiologically resistant to insecticidal interventions like long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) after prolonged use.

1011

EFFECTIVENESS OF FREE DISTRIBUTION OF INSECTICIDE TREATED NETS (ITN) IN RURAL HEALTH DISTRICT: RESULTS FROM CROSS SECTIONAL SURVEY IN NOUNA HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE, BURKINA FASO

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Malaria remains the global cause of morbidity and mortality with most of the burden being in sub-Saharan Africa. Insecticide Treated Nets (ITNs); one of the most effective strategies of Roll Back Malaria is currently rolled out on a large scale. However no much is known about the effectiveness of such a strategy in terms of coverage, use, and equity in protecting vulnerable groups.We used data from a cross-sectional household survey which was conducted in the Nouna Health and Demographic Site in 2012 after a large campaign of free ITNs distribution in 2010. The primary objective was to monitor household coverage in ITN and its use and acceptability. Data were collected from a total of 1050 households, selected using a three-stage cluster sampling procedure including 1202 pregnant women and 1114 children. Overall 97 % of households revealed a possession of at least one ITN in 2012 compared to 89% in 2009. In 2012, 69% of children have slept under ITN the last night compared to 25% in 2009. It was 69 % for pregnant women compared to 28% in 2009. The prevalence of presumptive malaria among under five years was 21% in 2011 compared to 20% in 2009. Meanwhile the malaria mortality decreased from 4% in 2009 to 2,7% in 2012 ($P<0.05$) in general population probably due to the high uptake of ITNs among vulnerable groups. However the household compliance to ITNS was high during rainy season (93%) than dry season (28.4%). 2.5% of general population reported a side effects dominated by pruritis (41.4%) and dyspnoea (32.9%). Although significant progress have been made to improve access of population to ITNs resulting in slight decrease of malaria morbidity due to high ITNs use in NHDSS, still much remains to do for achieving the MDGs goals. More attention should by pay to the side effects of ITNS which could play a role in adherence.

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INTEGRATED APPROACH TO MALARIA PREVENTION AT HOUSEHOLD LEVEL IN UGANDA: EXPERIENCES FROM A PILOT PROJECT

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Background Malaria is a major public health challenge in sub-Saharan Africa. In Uganda, malaria is the leading cause of morbidity and mortality especially among children under five years of age. This pilot project promoted prevention of malaria at household level using an integrated approach in 2 rural communities in Wakiso district, Uganda. This involved advocating and implementing several strategies in a holistic manner geared towards reduction in the occurrence of malaria. The specific strategies included installing mosquito proofing in windows and ventilators, draining stagnating water, closing windows and doors early in the evenings, and sleeping under ITNs. Methods The objectives of the project were to: carry out a baseline survey on malaria prevention; train community health workers and increase awareness on the integrated approach of malaria prevention; and establish demonstration sites using the integrate approach. Results The project conducted a survey among 376 participants which generated information on the knowledge, attitudes and practices of the community on malaria prevention. 25 community health workers were trained and over 200 members among the general population sensitized on the integrated approach of malaria prevention. 40 demonstration households using the integrated approach were established. Conclusion The results from this pilot project showed that the integrated approach to malaria prevention was well received by the study communities, which could be scaled up to more areas in Uganda and other malaria endemic countries.

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EFFECTS OF A NEW ODOR-BAITED MOSQUITO CONTROL DEVICE, THE MOSQUITO LANDING BOX, ON MALARIA VECTOR DENSITIES AND SURVIVAL INSIDE A SEMI-FIELD SYSTEM IN TANZANIA

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Malaria transmission is increasingly occurring outdoors due to changing vector behavior and effects of existing indoor insecticidal interventions. The commonly used Long Lasting Insecticide treated Nets (LLINs) and Indoor Residual Spraying mostly kill human-biting, indoor-feeding and indoor-resting mosquitoes. We assessed effects of a new odor-baited device that mimics humans, the mosquito landing box (MLB), which was recently developed for killing vectors outdoors to complement LLINs on vector densities and survival. Experiments were conducted in a semi-field system (SFS) in Tanzania for 40 nights. 400 unfed, laboratory-reared female *Anopheles* arabiensis mosquitoes were released in each chamber. Two MLBs, baited with nylon socks emanating human foot odor and CO_2 gas from yeast-molasses, were placed in one chamber, and another chamber left as control. The MLBs were dusted with either 10% larvicidal pyriproxyfen (PPF) powder, spores of entomopathogenic fungi (M. anisopliae IP46), or 5% pirimiphos methyl (PM). Human volunteers in each chamber collected and individually stored mosquitoes from their legs, or from walls of the SFS, using new collection tools for each specimen. The recaptured mosquitoes were assessed for contact with the MLBs by: 1) introducing them into beakers holding 10 3-4th instar An. arabiensis larvae and observing PPF effects, 2) observing the growth of fungus on mosquito carcasses, or 3) monitoring mortality rates (experiment with PM). In tests with PPF, 60% of mosquitoes recovered from human volunteer legs were contaminated compared to 6% in controls ($P<0.05$). 43% of mosquitoes found on walls in the treatment chamber were contaminated with fungus compared to 0% in controls ($P<0.05$). Lastly, in tests with 5% PM, daily survival of mosquitoes was significantly lower than in controls ($P<0.001$). The high contamination rates and reduced mosquito survival shows MLBs can significantly reduce densities and survival of malaria mosquitoes, thus being a potentially effective complementary intervention for use outdoors.

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IMPROVING MATERNAL AND NEONATAL HEALTH: COMPLEMENTARY ROLE OF THE PRIVATE SECTOR INCREASING UPTAKE OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY IN KENYA

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Malaria in pregnancy (MIP) is associated with poor pregnancy outcomes including maternal anaemia, intrauterine growth retardation and low birth weight. Kenya changed its policy on intermittent preventive treatment using Sulfadoxine Pyrimethamine (IPTp-SP) in 1998 however, IPTp coverage rates have remained low 4% in 2003, 14% in 2007, 15% in 2008 and 25 % in 2010. To increase the coverage rate, MCHIP supported malaria control and reproductive health divisions of the ministry of health, first to harmonize knowledge among service providers on provision of IPTp-SP in 2011, and second to train community health workers (CHWs) on sensitization of pregnant women to start early antenatal care (ANC) attendance in 2012. A community survey conducted in 2013 showed a significant increase in the proportion of pregnant women receiving two or more IPTp doses from 25% to 63%, the highest increase in IPTp uptake since 1998. Following the successful scale up of IPTp, one sub-county conducted an assessment of its health facilities to determine quality of data on ANC clients accessing IPTp-SP. A total of 15 (58%) out all 26 health facilities in the sub-county (public - 6 out of 8, faith-based - 2 out 3 and private - 7 out of 15) were selected. Data on new ANC clients, revisits and IPTp doses given was collected from the ANC registers. Among the assessed health facilities 13 (87%) out of the 15 were registering new ANC cases, revisits and provided IPTp-SP (public 6, faith based 2, private 5. One private clinic provided ANC services to revisits and IPTp2 doses only after the clients had been registered in public facilities, the second did not offer ANC services. In 2013 the government declared provision of free maternity services in public facilities but ANC clients have continued to utilize services from the private sector. This is an indication of the untapped potential in the private sector in increasing access to high impact interventions and importance of supporting the sector by all partners to provide these interventions. Such complementary efforts if implemented will not only result in enabling the country to move towards achievement of set targets but also improve pregnancy outcomes through reduction in effects of malaria in pregnancy.

1015

FACTORS AFFECTING INSECTICIDE TREATED NET USE AMONG CHILDREN UNDER AGE OF FIVE YEARS IN MAINLAND TANZANIA

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Recently, there has been successful universal coverage of Insecticide Treated Nets (ITNs) in households through the national campaigns. Despite the free-distribution and high coverage of ITN, gap in ownership and

usage remains a challenge in achieving the National targets. Data from the 2011-12 Tanzania HIV/AIDS and Malaria Indicator survey (THMIS) was used to assess the combined effect of child level factors, maternal factors, household factors and community factors on ITN usage among children under five years of age. Using logistic regression, associations between usage of ITN in children under five years (<5) of age and potential risk factors were analyzed. A total of 8624 children under five who slept under an ITN the night before survey were included in the analysis. Education level of mothers, age of the child <5 and number of children in a household were significantly associated with net usage among Children <5 years in adjusted model. Households with children aged 36-47 months [Odds Ratio (OR) =0.74; 95% CI 0.58-0.95], children aged 48-59 months (OR=0.75; 95%CI 0.57-0.99) were associated with decrease usage of ITN. Household with three or four children (OR=1.68; 95% CI 1.35-2.09), one or two children <5 years (OR=3.25; 95% CI 1.14- 9.29) and mothers who are educated (OR =1.3; 95% CI 1.09 -1.61) were associated with higher usage of ITN among children <5 years of age. To improve use of ITN among children <5 years, policies promoting women's education, education on malaria prevention methods to society especially women (mothers and care-givers of children) as well as distribution of nets must be coupled with sensitization about use should be encouraged and strengthened. However, further research and efforts are needed to address barriers and determine the strategies to increase the ITN usage.

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LIGHT-REGULATED BLOOD-FEEDING AND FLIGHT BEHAVIOR AND A LIGHT PHASE RESPONSE CURVE FOR THE *ANOPHELES GAMBIAE* MALARIA MOSQUITO

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Biting behaviors in anopheline mosquitoes are time-of-day specific, with a greater abundance of biting occurring during the dark phase of their photoperiod. We investigated how a single light pulse administered at the beginning of the night effected biting behavior. Additionally, *Anopheles gambiae* locomotion has a distinct circadian rhythm, characterized by nocturnal activity bouts. We investigated how precisely timed light pulses delivered throughout the circadian cycle can shift the activity rhythm, leading to the synthesis of an *An. gambiae* Phase Response Curve (PRC). To investigate biting inhibition, two incipient *An. gambiae* species (S and M molecular forms) were treated with white light (10 min, 150-800 lux) at the onset of complete darkness and the percentage taking a blood meal was recorded every 2 hr up to 8 hr. To produce an anchored PRC, S-form mosquitoes received a single 30 min pulse of light at various times during the immediate 24 hr transitioning from a light-dark cycle to constant darkness. The pulse significantly reduced biting tendency in the S-form mosquito for 2 hr after administration (at 0.20 hr and 2 hr), with variable responses observed at 4 hr, and no differences detected at 6 and 8 hr. Conversely, M form mosquitoes, were unresponsive to the light treatment, *i.e.* their biting tendency did not change (*n.s.*). For the PRC analysis, as seen in most other examined species, *An. gambiae* mosquitoes demonstrated distinct delays and advances in circadian phase when light was presented during the early and late subjective night, respectively. These data reveal a strain-specific effect of acute light treatment on biting behavior that is both immediate and sustained. The *An. gambaie* PRC is qualitatively similar to several model insect and vertebrate organisms. At present, insecticidal treated bed-nets designed to prevent mosquito-human contact and kill mosquitoes, are relied upon to prevent malaria transmission; as mosquitoes and malaria parasites are becoming increasingly resistant to insecticidal and drug treatments, respectively, there is a necessity for the development of innovative control strategies. The inhibitory and phase shifting effects of light may prove to be an effective tool in assisting with these strategies.

1017

NEW LINES OF *ANOPHELES GAMBIAE* REFRACTORY TO *PLASMODIUM FALCIPARUM*: SELECTION, CHARACTERIZATION AND SPECIFIC MARKER GENE SELECTION

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Anopheles gambiae is a principal vector of *Plasmodium falciparum* malaria in Africa. Some individual mosquitoes within a population are naturally refractory to infection. The only existing refractory line of *An. gambiae* (G3) melanises *P. falciparum* parasites, a refractory behaviour uncommon under natural transmission. Understanding common, non-melanising mechanism of natural refractoriness could be used for development of transmission blocking vaccines or GMO vector strategies. We have selected a new, non-melanising, refractory line of *An. gambiae* named GU-REF. GU-REF was selected for refractoriness to *P. falciparum* clone 3D7 over 11 generations of selection. At the same time, the GU-CON line was selected at random as a control for inbreeding effects. The refractory line was then tested for genotype specificity, parasite stages affected, timing of blood meal digestion after feeding on infected and uninfected blood, expression of candidate genes previously linked with refractoriness, and for fitness costs of refractoriness. GU-REF mosquitoes exhibit a significantly lower infection prevalence with *P. falciparum* compared to GU-CON and the parent Keele line of *A. gambiae*. The refractory behaviour is not specific to the parasite clone (3D7) used for selection, in that refractoriness is seen to an unrelated parasite, HB3. The refractory mechanism affects the parasite stages before the oocyst. GU-REF mosquitoes do not appear to exhibit fitness costs associated with refractoriness, as measured by fecundity. Protein digestion of the blood meal is slightly faster in GU-REF after an infectious blood-meal, compared to GU-CON. There is no difference in speed of digestion after a non-infected blood-meal. Analysis of candidate marker genes for refractoriness identified one locus with significant allele frequency differences between GU-REF and GU-CON and the parent line. We are currently developing a further line, GU-REF2, homozygous at this locus, to confirm its involvement in the refractoriness.

1018

A SCALABLE ASSESSMENT OF MALARIA TRANSMISSION IN THE STANDARD MEMBRANE FEEDING ASSAY USING TRANSGENIC GFP-LUCIFERASE *PLASMODIUM FALCIPARUM* GAMETOCYTES

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The development of drugs and vaccines to reduce malaria transmission is an important part of plans to eradicate the disease. The transmission reducing activity (TRA) of these agents is currently determined in the standard membrane feeding assay (SMFA), which is based on subjective microscopical read-outs which significantly limit the techniques throughput. Utilising a *Plasmodium falciparum* strain expressing the firefly luciferase protein, we present a luminescence based approach to SMFA evaluation that eliminates the requirement for mosquito dissections in favour of a simple approach where whole mosquitoes are homogenised

and examined directly for luciferase activity. Analysis of 6860 *Anopheles* stephensi mosquitoes across 68 experimental feeds shows that the luminescence assay was as sensitive as microscopy for infection detection. The mean luminescence intensity of individual and pooled mosquitoes accurately quantifies mean oocyst intensity and generates comparable TRA estimates. The luminescence assay presented here could increase SMFA throughput so that 10-30 experimental feeds could be evaluated in a single 96-well plate. This new method of assessing *Plasmodium* infection and transmission intensity could expedite the screening of novel drug compounds, vaccine candidates and sera from malaria exposed individuals for TRA.

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AN ASSOCIATION BETWEEN THE 1014F KDR ALLLELE AND *PLASMODIUM FALCIPARUM* INFECTION IN *ANOPHELES GAMBIAE* SENSU LATO

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Malaria vectors in Burkina Faso are highly resistant to pyrethroid insecticides but little is known about the impact of this resistance on malaria transmission. One approach to address this epidemiologically important relationship is to test for associations between known insecticide resistance mechanisms and infection with malaria parasites. Adult mosquitoes were collected over three years from four sites across Burkina Faso using pyrethrum spray catches, exit traps pit shelters. Genomic DNA of *Anopheles gambiae* s.l mosquitoes was identified to species and genotyped for the L1014F mutation. The circumsporozoite antigen was detected in mosquitoes using sandwich ELISA. *An. arabiensis* and *Anopheles gambiae* s.s were incriminated as vectors of *Plasmodium falciparum* with overall sporozoite rates of 3.8% for *An. arabiensis*, 4.4% for M-form, and 6.6% for S-form. The L1014F kdr allele was significantly associated with the sporozoite positive population of *An. arabiensis* s.l. (p=0.03).The current study shows a significant association between the presence of a kdr mutation and infection with the malaria parasite. By extending this work to include additional genes linked to pyrethroid resistance a clearer picture of the impact of resistance on malaria transmission will emerge.

1020

ELIMINATION OF MALARIA: THE CURRENT APPROACHES HAVE FAILED TO REDUCE MALARIA TRANSMISSION IN LOWLANDS IN WESTERN KENYA

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Insecticide treated bed nets (ITN) are a powerful tool in the control of malaria in Africa as most vectors there bite indoors at night. The World Health Organization recommended universal coverage of ITNs for all people at risk of malaria. The mass distribution of ITNs in Kenya in 2011 marked a milestone toward universal coverage. The study was conducted in six study sites in Western Kenya, three in the highlands and three in the lowlands from 2010 to 2013. ITN ownership and coverage were surveyed annually during the same study period. Mosquito vectors were collected monthly using the indoor PSC catch method, blood samples from school children aged between 6 and 15 years were collected in May and June each year using finger-prick method and malaria parasite prevalence was determined microscopically. Overall, 6,677 *Anopheles* were caught with 8,762 trap-nights; 4,018 blood samples were examined with 826 infections identified; 6,552 households with 20,267 individuals were surveyed for ITN ownership and coverage and 7,609 ITNs were surveyed. There was a marked increase in vector densities in four out of the six study sites but this depended on the vector species. Parasite prevalence increased in all the lowland sites while decreased in the highland sites. ITN ownership and coverage increased from an average of 64% in 2010 to 84% in 2013. This lead to significant decrease in indoor resting vector densities and in parasite prevalence in places where vector densities were low, however in the lowland regions an increase in the ITN coverage did not transform into a decrease in the parasite prevalence as the vector densities are high and there is a resurgence of An. funestus in the lowlands. The current malaria control strategies are not working in the Western Kenya lowlands as indicated by increased vector densities and malaria prevalence. Different or modified strategies are needed in order to achieve malaria elimination in the lowland areas in Kenya.

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FIELD EVALUATION OF YEAST-MOLASSES FERMENTATION AT A PRACTICAL SOURCE OF CARBON DIOXIDE FOR BAITING OUTDOOR DEVICES AGAINST HOST-SEEKING MOSQUITO VECTORS IN RURAL TANZANIA

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Odour baited traps have been recently developed and increasingly used for sampling and controlling disease vectors. Carbon dioxide (CO_2) gas is recognized as an important stimuli mediating host attractiveness to mosquitoes. Currently, the main sources of CO_2 for mosquito sampling include dry ice, sugar-yeast fermentation and bottled industrial CO_2, all of which are expensive, labor-intensive and impractical for regular field use, beyond small-scale experimentation. We assessed molasses-yeast fermentation as an alternative organic and locally available source of CO_2 for sampling mosquito vectors in Tanzania. Mosquito trapping experiments were conducted in the field by using *Ifakara* OBS baited with 3 different sources of CO_2 and an unbaited *Ifakara* OBS as control. In each trap, the CO_2 was produced in two separate pots by adding 40g in one pot and 75g of yeast in another, adding 0.25 liters of molasses in each pot, then mixing these with 1.5 liters of water. Attractiveness of the molasses-yeast CO_2 to mosquitoes was tested and compared with conventional industrial CO_2, yeast-sugar fermentation generated CO_2 and control, i.e. unbaited traps.We also assessed whether CO_2 generated from molasses yeast-fermentation can enhance the attractiveness of synthetic human-derived cues to mosquitoes. Traps baited with CO_2 obtained from molasses-yeast fermentation caught significantly more *An. gambiae* mosquitoes than traps baited with yeast-sugar CO_2 ($P \leq 0.01$) and unbaited traps (control) ($P \leq 6.5E\text{-}05$). The trap baited with industrial CO_2 had higher mosquito catches than trap baited with molasses CO_2 but was not statistically significant ($P \leq 0.75$). Addition of molasses derived CO2 to traps baited with human odour significantly increased trap catches. There were no significance difference on the catches between the traps baited with molasses derived CO_2 and traps baited with industrial CO_2 ($P \leq 0.6$). Yeast-molasses fermentation is an effective, locally available and cheap source of CO_2 for mosquito vector studies. The CO_2 could potentially be used to replace yeast-sugar CO_2 in odor-baited devices for mosquito sampling, so as to sustainably monitor disease vector and control in low income communities.

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USING MOSQUITO PROOF HOUSING TO PROTECT ITINERANT RICE FARMERS IN SOUTHEASTERN TANZANIA AGAINST MOSQUITO-BORNE INFECTIONS

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Poor housing increases exposure to insect bites and pathogens that these insects transmit. In rural south-eastern Tanzania, rice farming is a major economic activity, with most rice farmers relocating to their rice paddies in distant fields to tend to their crops for many days, weeks or months. While in the farms they live in semi-open improvised structures, which do not prevent mosquito entry, and indoor vector control tools such as insecticide treated bed nets (ITNs) and Indoor residual Spraying (IRS) cannot be effectively used, making these farming households disproportionately more vulnerable to nuisance biting and mosquito-borne diseases. We are exploring the use of portable mosquito-proof houses to protect these farmers while at their fields. Initial qualitative surveys through semi-structured interviews and participant observations assess community members' views and preferences regarding designs and use of the portable houses have been completed. The houses have been designed and initial prototypes ready for construction. The prototypes will be tested against farm house replicas that are currently used, to compare their protective efficacies in both semi-field and field settings, with human volunteers in each hut type. In the semi field, tests will be done in a 120m screened tunnel where the two hut types will be placed 50m apart, 500 3-5 days old female *Anopheles* arabiensis released in the middle and numbers of mosquitoes entering each hut recorded. In the field setting, the prototype will again be kept 50m away a farm house replica in an open field near mosquito breeding sites. Mosquito collections will be done using CDC light traps all night and indoor-resting catches in the morning to determine number of mosquitoes that enter the houses. Findings will be presented at the meeting. We expect that there will be significantly fewer mosquitoes entering the mosquito proof prototypes than the farm house replicas, and that by including community preferences into the design, these portable houses will be highly acceptable and scalable in these communities. The study will demonstrate potential of such low-cost interventions for effectively controlling mosquito-borne infections among itinerant communities, who are otherwise mostly disenfranchised from organized disease prevention campaigns

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COMPARATIVE EVALUATION OF A NEW EXPOSURE-FREE TOOL THE M-TRAP, AGAINST OTHER EXISTING SAMPLING TOOLS FOR OUTDOOR-BITING MOSQUITOES

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Outdoor sampling of disease transmitting mosquitoes is a major challenge for experts when monitoring pathogen transmission risk and evaluating new interventions. Human landing catches (HLC), a potentially risky and labour-intensive method requiring use of human volunteers to collect mosquitoes attracted to their legs, unfortunately remains the standard for sampling host-seeking mosquitoes outdoors and indoors. In recent years alternatives to HLC have been tried including Ifakara tent trap (ITT-B), Ifakara odour baited stations (OBS), Mosquito Magnet (MMX), and BG sentinel traps. We designed a new exposure-free mosquito trap, known as M-Trap, as an outdoor surveillance tool for disease-transmitting mosquitoes, and comparatively evaluated it against ITT-B, MMX, Ifakara OBS, BG Sentinel and HLC. The study was conducted in rural southern-east Tanzania during dry and rainy seasons. The experiment was a 5 by 5 latin-square design in which the different traps were randomly allocated to five locations >30m apart, and rotated such that at the end of any five experimental days, each trap type had been to each of the five locations at least once. In the first study, all traps except HLC were baited with the same synthetic mosquito attractant, while in the second experiment, human volunteers slept in only ITT-B and M-trap, whereas synthetic odour baits were used in MMX, Ifakara OBS and BG Sentinel traps. tests were conducted from18:00hrs to 06:00hrs nightly for 75 nights. A total of 9302 mosquito were collected comprising 66.8% (6212) *Mansonia* species, 22.2% (2066) Culex species, 6% (558) *Anopheles coustani*, 4.5% (415) *Anopheles arabiensis* and 0.2% (15) *Anopheles funestus*. Trap perfomances were ranked as follows: MMX (43.4% (4038)), M-trap (24.1% (2244)), BG Sentinel (17.9% (1662)), ITT-B (17.9% (1662)) and Ifakara OBS (9.4% (875)) of total mosquitoes caught There was consistency in the sampling proportionality between the MMX and M-trap catches for both Anopheline and Culicine mosquitoes. The M-trap is less efficient than MMX while it is more effective than BG Sentinel, ITT-B and Ifakara OBS and suitable for use in large scale surveillance, Also M-trap is less cost effective in terms of development than this other traps, and it can be locally available.

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KNOWLEDGE AND USE OF MALARIA PREVENTIVE MEASURES BY CAREGIVERS OF UNDER-FIVES IN RURAL SOUTHWESTERN NIGERIA

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Malaria is highly endemic in sub-Saharan Africa, and Nigeria is one of the worst hit countries causing about 30% of child mortality. Malaria is responsible for high infant and childhood mortality rates in this region. Several common and effective measures had been used to control malaria; and some African countries have reported up to 50% reduction in malarial cases. However, data from Nigeria have not shown significant reduction. This study therefore assessed the use of malaria preventive measures by caregivers of under-fives in rural South Western Nigeria. This was a cross sectional survey conducted in 2010 among 274 caregivers of under-fives who were selected using the cluster sampling technique. Logistic regression analysis was used to model for predictors of use of malaria preventive measures at 5% level of significance. About 86.1% of the caregivers were females and their mean age was 28.95±8.1 years. Almost all (92.7%) were married. They were largely unskilled workers 63.5%. About 95.6% of the care givers knew that malaria can be prevented and 78.1% had good knowledge of known malaria preventive measures. However, the use of malaria preventive measures was varied as only 19.7% used indoor residual spraying, 15.0% used anti malaria drug, 8.4% used personal and environmental hygiene, 8.0% used insecticide treated net and 6.0% used window net. Respondents who were professionals were more likely to have a good knowledge of malaria preventive measures $p<0.05$. The predictor of use malaria preventive measures was respondents who had a good knowledge of causes of malaria (OR 9.3, 95% C.I: 1.35-64.3). The knowledge of caregivers of under-fives about malaria infection and prevention were adequate, however, use of malaria preventive measures was poor. There is a need to provide more information and education on the causes of malaria and the need to use malaria preventive measures in order to prevent children from developing malaria.

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FACTORS RELATED TO THE USE OF LONG-LASTING INSECTICIDE-TREATED BEDNETS IN THE PERUVIAN AMAZON

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With the support of the Global Fund's Multi-Country Malaria Project PAMAFRO, Peruvian Malaria Program has incorporated long-lasting insecticide net (LLIN) distribution as important strategy to prevent and control malaria. From October to December 2010, 21174 LLIN Olyset Net® were delivered in 70 communities of San Juan district in the Peruvian Amazon, covering 32337 people (ratio: 0.65 LLIN/person). Then, from January to March 2012 a household survey was conducted in a representative sample of 400 households located in 20 communities, in order to identify factors associated with the usage of LLIN after one year of the LLIN delivery. Data about usage of LLIN and potential associated factors (demographics, availability of LLIN, knowledge of malaria and preventive measures) were collected through interviews with the head of the household, using a semi-structured questionnaire. Households with low LLIN usage were those, in which less than 80% of their members slept under a LLIN the previous night. Household vulnerable rate was determined by the sum of children under 5 years and pregnant women divided by the total number of household members. Ownership ratio was defined as the number of LLIN divided by the number of household members. Ownership ratio decreased from 0.70 to 0.52 resulting in LLIN retention of 73% after 1 year of intervention. Almost 75% of children under 5 years old and 80% of pregnant women slept under a LLIN the previous night. While increasing ages of household heads were associated with households with low LLIN usage (OR=1.024, IC95%=1.01-1.04); decreasing vulnerable household rates (OR=0.972, IC95%=0.96-0.99) and ownership ratios (OR=0.966, IC95%=0.96-0.98) were associated with households with low LLIN usage. Despite a reduction of LLIN ownership seemed to be an important determinant of low usage LLIN usage, LLIN usage remained high after 1 year of intervention in the targeted communities.

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A CASE-CONTROL STUDY OF THE EFFECT OF HOLES ON MALARIA INFECTION AMONG USERS OF LONG-LASTING INSECTICIDE-TREATED BEDNETS - MALAWI, 2013

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Insecticide-treated nets (ITNs) are the cornerstone of malaria prevention, but as holes develop, ITNs may be less effective. We conducted a case-control study among children 6-59 months old who visited an outpatient clinic in Liwonde, Malawi for an acute febrile illness and who reported consistently using an ITN during the prior two weeks to explore whether the number, size or location of holes reduced protection from malaria. Cases were children who were positive for *Plasmodium falciparum* by slide microscopy, while controls were microscopy negative. The ITNs used by participants were collected and mounted on a frame where the number, size category and location of all holes were noted by visual inspection; the WHO-recommended proportionate hole index (pHI) was calculated. Log-binomial models were used to relate the pHI, hole size category and hole location to malaria while controlling for potential confounders. A total of 105 cases and 171 controls were enrolled, and 276 Olyset-brand nets were evaluated. Overall, 86% of nets had at least one hole; 69% had at least one hole between thumb and fist size; 36% had at least one hole between fist and head size ; and 17% had at least one hole larger than a head. The median (mean) pHI was 86 (578), and 77% of ITNs were categorized as in good or acceptable conditions (pHI<643). In univariate analysis, having at least one thumb-to-fist-sized hole in the net roof was associated with malaria (prevalence ratio [PR] 1.43, 95% confidence interval [CI] 1.01, 2.03), but no associations with other net variables, including pHI, were found. In a multivariate model adjusting for potential confounders, having at least one thumb-to-fist-sized hole in the net roof remained significantly associated with malaria (PR 1.48, 95% CI 1.06, 2.05). The association may be explained by mosquitos' preference for resting on net roofs rather than sides. This is the first study to find that hole size and location impact malaria prevalence. Digital image analysis of ITNs in this study will further explore these relationships.

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THE CRY TOXIN OPERON *CLOSTRIDIUM BIFERMANTANS MALAYSIA* IS HIGHLY TOXIC TO LARVAL MOSQUITOES

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The management and control of mosquito vectors of human disease currently relies primarily on chemical insecticides. However, larvicidal treatments can be effective, and if based on biological insecticides they can also ameliorate the risk to human health of chemical insecticides. The aerobic bacteria *Bacillus thuringiensis* and *Lysinibacillus sphaericus* have been used for vector control for a number of decades. But a more cost effective use would be an anaerobic bacteria because of the ease with which these can be cultured. More recently the anaerobic bacterium, *Clostridium bifermantans malaysia* (Cbm), reportedly has high mosquitocidal activity, and a series of proteins were identified as potentially mosquitocidal. However, the cloned proteins showed no mosquitocidal activity. We show here that the four toxins in the Cry operon, Cry16A, Cry17A, Cbm17.1 and Cbm17.2, are all required for toxicity, and these toxins collectively show remarkable selectivity for *Aedes* rather than *Anopheles* mosquitoes, even though Cbm is more toxic to *Anopheles*. Hence toxins that target *Anopheles* are different from those expressed in the Cry operon.

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WORSENING SOCIO-ECONOMIC DISPARITIES IN INSECTICIDE-TREATED NETS (ITN) OWNERSHIP, ACCESS AND USE FROM 2006 TO 2011 IN ANGOLA

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Insecticide-treated nets (ITNs) are a key malaria control intervention as they significantly decrease malaria morbidity and mortality. However, in many malaria endemic countries, ITN coverage remains low and is inequitable among different socio-economic groups. In Angola, despite an increase in ITN use among children under five (from 18% 2006/7 to 26% in 2011) for example, it still remains far below the target of 85%. Using data from the two Malaria Indicator Surveys (MIS), this paper investigated change in equity in ITN ownership, access, and use among children under five and pregnant women between 2006/7 and 2011 in Angola. Lorenz Concentration Curve and Index (C-Index) was used to assess the magnitude of the disparities between wealth quintiles between both surveys. Disparities in ITN ownership, access and use worsened between socio-economic groups. ITN ownership was less equitable in 2011 (C-index: 0.17) compared to 2006/7 (C-index: 0.05). In 2011, 44% of households from the least poor wealth quintile owned at least one ITN compared to 15% in the poorest quintile. This disparity was less in 2006/7; 31% compared to 26% respectively. Similar results were found for ITN

access (C-index: 0.16 and 0.02), use among children under five (C-index: 0.17 and -0.004) and pregnant women (C-index: 0.14 and -0.06). Despite an increase in ITN ownership, access and use between 2006/7 and 2011 in Angola, inequity became greater, favoring the least poor quintiles. This could be due to different ITN distribution strategies implemented. Before the MIS 2006/7, there was a massive free net distribution campaign implemented in seven provinces. Afterwards, ITNs were mainly distributed through routine antenatal care. Universal coverage distribution began in 2010, but was limited to specific municipalities. ITNs therefore became more accessible to those living closer to facilities (most in urban areas) and who could afford them. A different strategy for ITN distribution that results in both higher and more equitable coverage is needed in Angola.

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THE IMPORTANCE OF PARASITE DENSITY IN ASSESSING THE EFFECTIVENESS OF TRANSMISSION-BLOCKING INTERVENTIONS FOR MALARIA

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Transmission-blocking interventions (TBIs) have the potential to reduce malaria transmission by targeting *Plasmodium* life-stages in the mosquito. However, the density-dependent effects of parasite numbers on malaria transmission remain poorly understood and may influence the effectiveness of TBIs. We fitted mathematical models to 2550 mosquito and 575 vertebrate host experimental infections, carried out as part of a laboratory population assay that simulates human-to-mosquito and mosquito-to-human infection over successive transmission cycles, to assess the influence of sporozoite and asexual parasite density on infection probability. Models categorised parasite density into groups, each with their own infection probability, and were compared with likelihood ratio tests (LRT). For mosquito-to-mouse transmission, the best model (LRT, $p = 0.023$) was the most complex, with the probability of infection increasing with the number of sporozoites present in the salivary glands following injection: 0 (1%); 1-10 (33%); 11-100 (54%); 101-1000 (64%); and >1000 sporozoites (88%). For mouse-to-mosquito transmission, the best model (LRT, $p = 0.0006$) also fitted infection probabilities that increased with asexual parasite density. To gauge the impact of this density-dependence on TBI assessment, we incorporated asexual parasite density into a chain binomial model of transmission, and fitted it to data from the laboratory population assay. Intervention effectiveness was assessed using effect size, the ability of a TBI to reduce the reproduction number of the parasite. The best model (LRT, $p = 0.00004$) showed that effect size was highest in mice with the lowest parasite density. This provides the first direct evidence that, in this laboratory model, parasite density influences the ability of TBIs to reduce transmission, and that TBIs are more likely to reduce transmission when parasite density is low. Current evaluations based on prevalence, and the relatively high parasite densities of laboratory assays, may, therefore, under-estimate the potential impact of TBIs.

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IMPACT OF A BEHAVIOR CHANGE INTERVENTION ON CARE AND REPAIR BEHAVIORS FOR LONG-LASTING INSECTICIDAL NETS IN NASARAWA STATE, NIGERIA

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Improvements in long-lasting insecticidal net (LLIN) durability have programming implications for national malaria programs. LLIN durability varies depending on user behaviors, household and environmental characteristics, and the strength and knitting pattern of the textile itself. A behavior change communication intervention was designed to promote household-level behaviors related to the care and repair of LLINs in Nasarawa State, Nigeria. The intervention was carried out in one local government area (LGA) in Nasarawa from October 2012 to April 2013, and again from October 2013 to April 2014. A second LGA served as a control area. The intervention included radio spots, local radio pronouncements, community events, and house-to-house visits. A baseline survey, nested within an ongoing ITN durability study using two-stage cluster sampling design, was conducted in April 2012 to measure LLIN ownership and existing patterns of net care and repair in both control and intervention LGAs, among 600 households. Control and intervention areas were similar at baseline in LLIN ownership and retention, net condition, observed repairs, and proportional hole index (PHI). Radio ownership was slightly higher in the intervention LGA. A midline survey conducted in April 2013 showed that in the intervention LGA, 28.2% (95%CI 21.4%-37.2%) of households had heard or seen at least one component of the BCC intervention, while in the control area, 12.3% (95%CI 8.1%-18.8%) reported exposure to care and repair messaging. The proportion of campaign nets with holes that had been repaired was not significantly different at midline, with 28.8% (95%CI 19.9%-39.6%) in the intervention LGA and 20.0% (95%CI 15.2%-25.9%) in the control LGA. An endline survey was conducted in April 2014 to determine whether exposure to the intervention contributed to any changes in care and repair behaviors, and whether changes in care and repair behaviors contributed to differences in net durability, measured by PHI. The results from this study will provide evidence as to whether BCC interventions have the potential to improve care and repair behaviors. These findings have implications for national malaria control programs' and donor agencies' programming decisions.

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CONTINUOUS DISTRIBUTION OF LONG-LASTING INSECTICIDAL NETS THROUGH SCHOOLS - RESULTS FROM A THREE YEAR EVALUATION IN CROSS RIVER STATE, NIGERIA

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Following a phase of mass campaigns for the distribution of Long-lasting Insecticidal Nets (LLIN) for malaria prevention in Africa South of the Sahara most countries now focus on establishing systems for continuous distribution of LLIN to sustain achieved gains. However, the primarily used channels of antenatal care and child immunization services alone are not sufficient to sustain universal coverage with LLIN. Schools have been considered as possible additional distribution channels, but to date no data exist to show the potential of such distributions. A pilot program of school-based LLIN distributions was undertaken in two districts in Cross River State, Nigeria in collaboration between the State Government's health and education departments of and the USAID NetWorks project funded by US President's Malaria Initiative. All students registered in two classes in public primary as well as two classes in public secondary schools were eligible to receive an LLIN. Distribution was organized by the education department of the districts and was undertaken in the second term of the school year. Distribution was sequentially rolled out starting in Obubra district (three distribution rounds in total) in early 2012 and in Ogoja district in 2013 (two rounds). By March 2014 a total of 55,000 LLIN had been distributed in 192 schools with a program efficiency (% of eligible children reached) of over 95%. In March 2014 an evaluation survey was undertaken to assess the impact of the school-based distributions on overall household LLIN ownership. In addition to the two implementation districts, one district which only had received LLIN through ANC services was included as a control site. In each of the districts 510 households (1,530 in total) were sampled in a cluster survey design. Data are currently processed and will be presented focusing on the success

in equitably sustaining LLIN coverage. A second focus of analysis will be the impact of behavior change communication in schools on the net use practices of exposed families.

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POTENTIAL IMPLICATIONS OF OUTDOOR-SLEEPING BEHAVIORS AND NIGHTTIME ACTIVITIES FOR MALARIA CONTROL IN THE UPPER WEST AND NORTHERN REGIONS OF GHANA

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Despite large-scale net distribution and indoor residual spraying campaigns, malaria rates in the northern regions of Ghana remain high. According to the Ghana Multiple Indicator Cluster Survey (2011), malaria prevalence rates for children under five were 52% and 48% in the Upper West and Northern Regions respectively. Nighttime activity, including outdoor sleeping, might be a possible explanation. In-depth interviews and nighttime observations were used to document outdoor sleeping and a variety of social, cultural, and economic activities that occur during night time in the Upper West and Northern Regions of Ghana. The study included 48 in-depth interviews with key stakeholders and 24 household observations at night with a total of 175 household members. Outdoor sleeping due to heat was reported and observed frequently among household members of all ages. Long-lasting insecticidal net (LLIN) use was observed to be low irrespective of whether people slept indoors or outdoors, in both regions. In addition to outdoor sleeping, a variety of outdoor nighttime activities were documented including cooking and other household chores, socializing both within the household compound and elsewhere, and studying both within the household compound and at night school classes. Funerals emerged as a common large-scale nighttime event with participants reporting that they attended funerals up to once a week. Nighttime behavior is key to understanding malaria transmission in Ghana and other malaria endemic countries. This presentation will discuss the potential contribution of outdoor sleeping and nighttime activity to increased risk of malaria and its potential implications for malaria control programs in Northern Ghana and beyond.

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HIGH ACCEPTABILITY BUT LOW ADHERENCE TO MALARIA PREVENTIVE MEASURES MAY LIMIT THE EFFECTIVENESS OF TOPICAL REPELLENTS

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The effectiveness of topical repellents in addition to long-lasting insecticidal nets has been evaluated at community level by an epidemiological trial in the forested and remote province of Ratanakiri, Cambodia. The introduction of repellents aimed at tackling residual malaria transmission induced by early and outdoor biting vectors. Ancillary to the trial, a mixed methods social science study on the acceptability and use of the topical repellents was conducted, combining ethnographic research, a cross-sectional survey and a structured observation study. In the cross-sectional survey (n=824), self-reported daily use was estimated at 52.4%. As local ethnic minorities have a multiple residence system (depending on the season and agricultural activities they live either in the village, at the forest farm or on the rice field), reported use was differentiated per location. The highest use was reported during deep forest activities (68.0%) such as hunting and logging, and the lowest use while residing in the villages (35.0%). During the structured observation study (n=1495) - relying on a respondent-independent method of collecting data - repellent use was 7,8% on the evening the observation took place. Alternative uses (including spraying on bed nets, on insects, on hair) were reported by the large majority of respondents (>90%), indicating a low consistent and continuous use of the repellent on the skin. The qualitative study, nevertheless, showed a high acceptance of the trial and the product. The perceived inconveniences and risks (bad smell, skin irritation, perceived toxicity), as well as a lack of 'habit' in the daily use of the repellent, contributed to the non-optimal use of repellents.

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INVESTIGATING THE POTENTIAL CIRCULAR EFFECT OF BED NET OWNERSHIP ON UNDER-FIVE MORTALITY RISK IN UGANDA

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Numerous studies have demonstrated the impacts of bed net ownership on mortality risk and there is clear agreement that bed nets are an effective malaria prevention intervention. However, it has been challenging to quantify the exact effect due to several factors including the lack of data on malaria specific mortality and the possible circular effect, whereby mortality risk might lead to increase ownership of bed nets. For households that experience the death of a child under five years of age, it can be a traumatic event that shapes uptake of health interventions. This study investigated whether this circular effect should be considered when analyzing the impact of bed net ownership on under five mortality risk. Data from the 2011 Uganda Demographic and Health Survey (DHS) was analyzed using logistic regression to assess whether death is associated with household ownership of bed nets. We hypothesized that households might be more likely to own nets after experiencing a death event in the household after controlling for other possible covariates. Analysis was restricted to households with at least one bed net. These households were retrospectively checked of death in the past 36 months for the survey dates to assess exposure to the predictor. Overall bed net ownership was 74% and of the households with bed nets, 12% experienced a death event. The results of the analysis show no association between death event and bed net ownership (OR=0.96, CI: 0.99;0.78), suggesting that a households decision to own a net is not driven by death. The results suggest that the circular effect is not necessarily affecting the estimate of mortality risk in relation to bed net ownership, however, this relation should be accounted for.

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LONGITUDINAL IMPACT OF INDOOR RESIDUAL SPRAYING (IRS) ON MALARIA PARASITAEMIA IN NORTHERN GHANA

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Malaria remains a major public health problem in Ghana, with an endemicity more pronounced in the northern savannah zone. The US PMI and the Ghana Health Services have been implementing an Indoor Residual Spraying (IRS) program in selected districts in the northern savannah zone of Ghana since 2008, as part of an initiative to reduce the disease burden in the country. This study was conducted between November 2010 and November 2013 to assess the impact of the PMI-

IRS program on malaria parasitaemia during the high transmission season in children under five in Bunkpurugu-Yunyoo District in northern Ghana. The district was sprayed with alphacypermethrin (a pyrethroid) in 2011 and 2012 at a rate of 25mg/m^2, and with pirimiphos methyl (an organophosphate), at an application rate of 1g/m^2 in 2013. The change of insecticide was as a result of declining susceptibility of local vectors to pyrethroids. During each household survey, probability proportional to size estimates (PPSE) were used to yield a minimum sample size of 1,311 children under 5 years old. All selected children were tested for malaria parasitaemia by microscopy, and a questionnaire was used to collect demographic, recent fever and bednet ownership and use information from caregivers. Trend analyses of prevalence of malaria parasitaemia showed a marginally significant decline from pre-IRS prevalence of 52.5% (95% CI: 50.1, 54.8) in November 2010 to post-IRS prevalence of 47.7% (95% CI: 45.5, 49.9) in October 2012 (p=0.013). After the change of insecticide in April 2013, malaria parasitaemia significantly declined further to 20.6% (95% CI: 18.4, 22.9) in October 2013 (p<0.001). Similar trends were observed in prevalence of reported fever and measured severe anaemia (hemoglobin < 7 g/dL) in the district. These findings support the policy change from pyrethroid to organophosphate for IRS in the Bunkpurugu Yunyoo District.

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PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM BETA-LACTAMASES IN *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES IN ACCRA, GHANA

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Extended-spectrum beta-lactamases are plasmid-mediated beta-lactamases capable of hydrolysing beta-lactams except carbapenems and cephamycins. Hence, ESBL-producing isolates limit therapeutic options and contribute to treatment failure. This study determined the occurrence of ESBL genotypes in *Escherichia coli* and *Klebsiella pneumoniae* and their antibiotic resistance profile in Accra, Ghana. Four hundred (400) *K. pneumoniae* and *E. coli* non-duplicate isolates were collected at Korle Bu Teaching Hospital and Advent Clinical Laboratories. The species identification, ESBL detection, MIC and antibiotic sensitivity testing were concurrently determined using Vitek 2 Compact System. The combined disc synergy method was used to confirm ESBL-producing strains. The genotypes of the ESBL-coding genes were determined using standard PCR reaction, agarose gel electrophoresis and bands visualization. The results showed that 202 (50.5%) of the bacterial isolates were ESBL-producers with increased resistance to amoxicillin/clavulanic acid (31.7%), nitrofurantoin (46.5), piperacillin/tazobactam (52.5%), tetracycline (70.8%), ciprofloxacin (79.7%), gentamicin (82.2%) and trimethoprim/sulphamethoxazole (97%). Of the 100 ESBL producers, CTX-M ESBL genes (90%) were dominant and 25% had TEM genes. None of the ESBL producers possesses SHV genes. Twenty (20%) of the isolates had both CTX-M and TEM genes. Of the 100 ESBL phenotypes, 78% and 2% were positive for CTX-M-1 group and CTX-M-9 group ESBL genes respectively. The CTX-M-type and TEM-type ESBL showed co-resistances to piperacillin/tazobactam, amoxicillin/clavulanate, ciprofloxacin, nitrofurantoin, ciprofloxacin, gentamicin, tetracycline and trimethoprim/sulphamethoxazole. Imipenem and amikacin were the *in-vitro* drugs of choice for treating CTX-M and TEM-type ESBL producers. It is vital to routinely detect ESBL-phenotypes in health facilities and implement appropriate antibiotic stewardship programs. There is the need for further studies into the clinical therapeutic outcomes to infections by CTX-M-type and TEM-type ESBL producers in Accra.

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MOLECULAR CHARACTERIZATION OF MULTI-DRUG RESISTANCE *STAPHYLOCOCCUS AUREUS* ON CLINICAL AND NON-CLINICAL SOURCES IN NIGERIA

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Staphylococcus aureus is a major pathogen associated with serious community-acquired and nosocomial diseases in all age group and of such a frequent microorganism obtained from clinical and non-clinical sources. Data on clinical identity, diversity, surveillance and new approaches in the molecular characterization of this pathogen in Nigeria are limited. 850 samples were collected from both clinical and non-clinical sources. The clinical source included the routine specimens of wound swabs, urine, stool, blood and sputum from the University Teaching Hospital Complex. The non- clinical samples were obtained from food handlers and food vendors at OAU campus restaurants and Ile-Ife centre community market respectively. The bacteriological analysis was carried out on the samples which was later cultivated on mannitol salt agar and incubated for 24-48 hours. Gram's reaction, catalase, Coagulase, DNAse tests and antibiotic susceptibility was carried out using disk diffusion technique.Plasmid isolation and detection of mecA,nucA, Panto van luekocidin genes and Random Amplified Polymorphic DNA(RAPD) by PCR amplification were also carried out.A total of 405 *Staphylococcus aureus* isolates comprising 230 (56.8%)from clinical and 175(43.2%) from non-clinical sources were recovered from 770 presumptive staphylococci. Prevalence of S.aureus infections and its incidence rate was higher in clinical than in non-clinical sources(p>0.05). All the 405 clinical and non clinical isolates were resistant to penicillin while 96% were sensitive to vancomycin. Among the clinical isolates, 4% were vancomycin intermediate resistant. All the non-clinical isolates were sensitive to vancomycin.Meanwhile,53% of the food handlers isolates were resistant to oxacillin. (44%) of the 50 representative screened clinical and non clinical isolates contained plasmid genes of varying molecular weight ranging from 562 to 23,490kb. 21(42%) contained nuc gene and 13(26%) mec gene, while 2(4%) clinical isolates had PVL gene. RAPD constituted 4(8%) of the clinical isolates. In conclusion, the study established clonal interrelatedness between the clinical and non-clinical isolates and confirm the importance of phenotypic identification of *S. aureus* by molecular techniques. Improved evaluation scheme should be put in place to combat the ineffectiveness of antimicrobial therapy due to high incidence of resistance among the isolates of *S. aureus* in Nigeria.

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BRUCELLA MELITENSIS METHIONYL-TRNA SYNTHETASE: A PROMISING TARGET FOR STRUCTURE-BASED DRUG DEVELOPMENT FOR THE TREATMENT OF BRUCELLOSIS

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Brucellosis is the most common zoonotic disease and disproportionally afflicts the most impoverished people. Its devastating impact on human lives is a result of both debilitating human illness and the loss of affected livestock. Brucellosis treatment is plagued by high failure and relapse rates despite long durations of therapy. New drugs have not been discovered for treatment of Brucellosis in decades. New and more effective drugs are greatly needed. Aminoacyl-tRNA synthetase (AaRS) enzymes are promising antimicrobial drug targets. These enzyme are necessary for protein synthesis and, therefore, bacterial growth. Bacteria resistance to antimicrobials is an enormous issue and one promising feature of anti-AaRS compounds is that there appears to be a profound

loss of fitness associated with bacteria resistance to the compounds. We have solved the crystal structure of *Brucella melitensis* methionyl-tRNA synthetase (*Bru*MetRS) and this allows for structure-based drug development. Furthermore, we have developed a working assay to screen for compounds that inhibit *Bru*MetRS and have identified a number of promising leads from screening a small compound library. *Brucella* is highly infectious and easily transmitted in the laboratory environment. One of the major barriers to drug development for Brucellosis is the necessary research precautions, including a BSL-3 laboratory. To further facilitate rapid screening of compounds for inhibition of *Brucella* growth via BrumetRS inhibition, we are using genetic manipulation to replace the MetRS gene in a nonpathogenic *E coli* with the structurally unique *Bru*MetRS gene. We are assessing for off-target effects by testing both the wild-type and mutant *E coli.* The dramatic difference of *E. coli* and *Brucella* MetRS will likely be reflected by compounds targeting *Bru*MetRS inhibiting *E. coli* growth of bacteria expressing *Bru*MetRS but not *Ecoli*MetRS. Lead compounds will later be tested directly against *Brucella melitensis* for confirmation of activity. This is a needed first step to developing a new drug for an ancient and neglected disease.

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SINGLE DOSAGE OF DOXYCYCLINE FOR PROPHYLAXIS AGAINST LEPTOSPIRAL INFECTION AND LEPTOSPIROSIS DURING URBAN FLOODING IN SOUTHERN THAILAND: A NON-RANDOMIZED CONTROLLED TRIAL

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Data on doxycycline for prophylaxis against leptospiral infection and leptospirosis during flooding is limited. During October 2010, a study was conducted in Hat Yai City of Southern Thailand, to explore the risk factors for leptospiral infection and leptospirosis among urban flood victims and investigate the protective efficacy of a single dosage of 200-mg doxycycline against the infection and developing disease. Of 641 participants, 600 received doxycycline while 41 did not due to fear of side effects and having a history of drug allergy. Twenty two participants were infected with leptospires and six developed leptospirosis. Having a laceration wound was significantly associated with both leptospiral infection (odds ratio [OR] = 37.20; P < 0.001) and leptospirosis (OR = 18.24; P = 0.003) whereas exposure to flood water more than 3 hours per day was significantly associated with only leptospiral infection (OR = 3.70; P = 0.038). Seventeen participants who received doxycycline were infected with leptospires compared to five who did not receive doxycycline, resulting in a protective efficacy of 76.8 % (95% confidence interval [CI] = 34.3 % - 92.0 %). Among participants with leptospirosis, 4 received doxycycline while 2 did not, a protective efficacy of 86.3% (CI = 9.8% - 98.2%). Among the participants with laceration wound, the protective efficacy for leptospiral infection was 92.0 % (CI = 81.2% - 96.6%) and for leptospirosis was 95.6% (CI = 78.2% - 99.3%). Among the participants exposed to flood water less than or equal to three hours per day, the protective efficacy for leptospiral infection was 89.2 % (95% CI 63.6 % - 96.67 %). Flood victims with laceration wounds and exposed for more than three hours per day should take precautions for leptospiral infection and leptospirosis. A single dosage of doxycycline could be considered as prophylaxis for victims of flooding.

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ACUTE UNCOMPLICATED FEBRILE ILLNESS IN CHILDREN AGED 2-59 MONTHS IN ZANZIBAR - ASSESSMENT OF INFECTIOUS DISEASE ETIOLOGIES AND INFECTIONS REQUIRING ANTIBIOTICS

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Understanding the causes of fever in areas of sub-Saharan Africa where malaria recently has undergone a rapid decline is key to ensure evidence based fever case management. We therefore studied infectious etiologies of uncomplicated febrile illness in 677 children aged 2-59 months managed according to Integrated Management of Childhood Illness (IMCI) at a rural primary health facility in Zanzibar, using point-of-care tests, radiology and multiplex real-time PCR analyses of nasopharyngeal (NPH) and fecal samples. Some 168 asymptomatic community controls provided NPH and fecal samples for PCR. The IMCI classifications were compared with final reference diagnoses established after study completion based on all available clinical and laboratory data. A majority of patients were classified according to IMCI either as non-bloody diarrhoea 164 (24%), or non-severe pneumonia 387 (57%), of which 42 (11%) were confirmed by chest x-ray. More than one pathogen was detected by NPH PCR in 592 (87%) of patients and 139 (83%) of asymptomatic controls. The most common final reference diagnoses in patients, were Influenza A or B (22%), respiratory syncytial virus (22%), rhinovirus (11%), streptococcal infection (7%), radiological pneumonia according to WHO criteria (6%) and Shigella gastroenteritis (4%). *Plasmodium falciparum* was diagnosed in two cases. Antibiotics were prescribed to 500 (74%) patients, but only 154 (23%) had a final reference diagnosis considered requiring antibiotic treatment of whom 24 (16%) did not receive antibiotics. The detection rate of pathogens in both cases and controls were high, indicating the complexity of determining infectious etiologies in acute uncomplicated febrile illness among children in the new context of low malaria transmission in Zanzibar, and further the difficulty of identifying infections requiring antibiotics.

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CONTACT INVESTIGATION OF MELIOIDOSIS CASES REVEALS REGIONAL ENDEMICITY - PUERTO RICO, 2010 AND 2012

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Melioidosis results from infection by percutaneous inoculation, ingestion, or inhalation of the saprophyte *Burkholderia pseudomallei*, and is associated with case-fatality rates up to 40%. Improved survival rates are attributed to early diagnosis and treatment with appropriate antimicrobials. Sporadic cases have been identified in Puerto Rico, where the incidence and epidemiology is unclear. Following identification of one fatal and one non-fatal melioidosis case in 2010 and 2012, respectively, contact investigations were conducted to identify risk factors for infection. Questionnaires were administered and serum specimens were collected from co-workers and persons living within 250 meters of cases' residences (neighborhood contacts) and from injection drug use (IDU) contacts of the 2012 case. Serum specimens were tested for evidence of prior exposure to *B. pseudomallei* by indirect hemagglutination assay (titer

≥1:40). Serum specimens were collected from 51 and 60 individuals associated with the 2010 and 2012 cases, respectively. None of the co-workers were seropositive for anti-*B. pseudomallei* antibody, whereas 2 (5%) of 40 and 12 (23%) of 52 of 2010 and 2012 neighborhood contacts were seropositive, respectively, and 67% (2 of 3) of IDU contacts. Of all seropositive persons, 39% reported no travel outside of Puerto Rico. Characteristics significantly associated with seropositivity were reporting skin wounds, sores, or ulcers (adjusted odds ratio [aOR] = 4.6; 95% confidence interval [CI]: 1.2-17.8) and IDU (aOR=18.0; 95% CI: 1.6-194.0). Sporadic reports of melioidosis and high seropositivity in case contacts suggest at least regional endemicity in Puerto Rico. Increased awareness of melioidosis among clinicians, laboratories, and public health professionals is needed to improve case identification, initiate appropriate antimicrobial therapy, and facilitate case reporting in Puerto Rico.

1042

NOVEL VACCINE CANDIDATES TO PREVENT INFECTION WITH GROUP A STREPTOCOCCUS

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Streptococcus pyogenes (group A streptococcus, GAS) infection leads to a wide array of clinical manifestations ranging from uncomplicated self-limiting infections such as pharyngitis and impetigo to severe invasive diseases including deep soft tissue infection, sepsis, and streptococcal toxic shock syndrome. If untreated, streptococcal infection can also lead to post-infection sequelae of rheumatic fever and rheumatic heart disease. We have developed candidate vaccines based on a conserved peptide from the M protein (J8) and on a recombinant fragment of the streptococcal interleukin-8 protease virulence factor, Spy-CEP. Using a novel skin challenge model in mice that we have developed and which closely mimics the human condition, we have been able to show that vaccination with J8 can protect against pyoderma caused by multiple strains of GAS and can also protect against septicemia that develops post skin infection. Long term immunity is dependent on memory B cells, T cells and neutrophils. We were thus concerned that the vaccine might not protect against the virulent CovR/S strains of GAS that up regulate Spy-CEP, thus potentially limiting neutrophil ingress to the site of infection. We show that vaccine-induced immunity to these strains is in fact significantly reduced; however, co-vaccination with a recombinant fragment of SpyCEP induces antibodies that restore IL8 function and which are able to fully restore the level of protection against CovR/S mutants. J8 is currently in a Phase I clinical trial and J8/Spy-CEP is being developed as a second-generation vaccine to prevent infection with hyper-virulent strains of GAS.

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CHARACTERIZATION OF BLOODSTREAM INFECTIONS FROM HOSPITALIZED PATIENTS IN IQUITOS, PERU

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Bloodstream infections, especially those associated with central line devices, are associated with increased length of hospital stay and substantially increased morbidity and mortality. The identification and characterization of pathogens associated with such nosocomial infections is critical to establishing control programs, particularly in resource-limited settings. Accordingly, in this study we characterized bloodstream infections in two major hospitals located in Iquitos, a jungle city in the Amazon Basin of Peru. A total of 336 hemocultures were performed on patient samples obtained between June 2011 and March 2014. Of the samples tested, 58% were from adult patients ($p<0.0001$) while the remaining 42% were from children under the age of 18. All samples were cultured and screened according to the guidelines and criteria established by the Centers of Disease Control and Prevention (CDC) in the United States. A total of 11% (36/336) of the samples tested were deemed positive for bacteremia. *Klebsiella pneumoniae* and *Staphylococcus aureus* were the most common bacteria isolated and were present in 10 isolates each, accounting for a combined total of 56% of positive samples. In addition, 9 isolates of *Acinetobacter* (25%), 3 isolates of *Enterobacter* sp. (8.3 %), 2 isolates of *Pseudomonas aeruginosa* (5.6%) and one isolate of *E. coli* and *Serratia marcescens* were also identified. *Klebsiella pneumoniae* showed 100% resistance to ceftriaxone, gentamicin and ticarcillin while 30% of *S. aureus* and 11% of *Acinetobacter spp.* were resistant to methicillin and imipenem, respectively.

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SEVERE BUBONIC PLAGUE WITH SEPTIC SHOCK AND ACRAL NECROSIS IN CENTRAL OREGON

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A 59 year-old man acquired bubonic plague from a cat bite in central Oregon in June 2012. He developed secondary septicemia, probable plague pneumonia, severe septic shock, disseminated intravascular coagulation, acute renal failure, and acute respiratory failure. His clinical course was complicated by the need for renal replacement therapy, mechanical ventilation, and vasopressor drug support, and he ultimately developed acral gangrene of all of his fingers and toes. Despite renal failure, he was successfully treated with gentamicin and high volume hemofiltration and was discharged after a lengthy hospital course. Save for the loss of essentially all of his digits, he made a good recovery including return of normal renal function. The case highlights options for antibiotic therapy for plague in the context of various clinical conditions, the role of renal replacement therapy in severe sepsis, and challenges with antibiotic dosing during high volume hemofiltration. We also review additional recent plague cases in Oregon and cat-associated plague infections in humans.

1045

CLINICAL CHARACTERISTICS AND ETIOLOGIES OF ACUTE CENTRAL NERVOUS SYSTEM INFECTIONS IN CHILDREN AND ADULTS ADMITTED TO RURAL AND URBAN HOSPITALS IN KENYA: PRELIMINARY FINDING, 2011-2014

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Acute central nervous system (CNS) infection is an important cause of hospital admission of children and adults in Sub-Saharan Africa with high mortality, but diagnostic testing is often limited, hindering treatment and prevention strategies. We investigated etiologies among inpatients with suspected acute CNS infection at one rural (Siaya District Hospital [SDH]/Western) and one urban (Mbagathi District Hospital [MDH]/Nairobi) hospital in Kenya. Clinical data and diagnostic specimens were collected from eligible patients from November 2011 to November 2013 at SDH and from January 2014 to March 2014 at MDH. Cerebrospinal fluid (CSF) and blood samples were tested by routine microbiology, chemistry and cytology/hematology. CSF specimens were examined for antigens of bacterial pathogens and *Cryptococcus neoformans* and were stored for multi-pathogen testing using a real-time polymerase chain reaction (PCR) assay platform. Malaria was ruled out by blood smear. CSF was collected from 340 patients (60% aged <5 years, 54% male) and 145 patients (40% aged <5 years, 50% male) at SDH and MDH, respectively. Of 153 (32%) participants with known HIV status, 92 (60%) were positive. Common presenting symptoms included seizures (66%), neck stiffness/

nuchal rigidity and/or bulging fontanel (51%) and fever (88%). A positive malaria blood smear was obtained from 27% of participants at SDH and 11% at MDH (P<0.0001). Abnormalities of CSF chemistry and/or cytology were observed in 33% and 66% of specimens from SDH and MDH, respectively (P<0.0001). A pathogen was identified from CSF or blood in 14% of participants, including *Cryptococcus neoformans* (7%), *Neisseria meningitidis* (3%, MDH only), non typhoidal salmonella (0.9%), *Haemophilus influenza* (0.2 %) and *Streptococcus pneumonia* (0.2%). Additional clinical and PCR testing is ongoing. These preliminary findings demonstrate a high burden of cryptococcal and bacterial meningitis among patients hospitalized with suspected acute CNS infection in Kenya. Marked rural-urban differences are observed and should be evaluated.

1046

COMPARISON OF TWO MOLECULAR DIAGNOSTIC APPROACHES USING SERUM AND BUFFY COAT FOR RAPID DIAGNOSIS OF ACUTE LEPTOSPIROSIS

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Leptospirosis (lepto) has a high incidence in the tropics and is often clinically indistinguishable from other causes of fever. Early diagnosis is required to ensure appropriate therapy. Culture and serology (MAT) are slow and insensitive. PCR methods hold promise, but few assays have been evaluated clinically, and the optimal sample is unclear. We compared a newly developed quantitative real-time PCR (qPCR), targeting *rrn* and pathogen-specific *LipL32* (and including an internal control), with a previously described qPCR targeting *rrs*, using serum and buffy coat (BC) DNA from stored admission blood. 59 Lao patients diagnosed with lepto by culture (n=19), qPCR (n=20), and/or MAT (n=20) between 2008-2012 were identified retrospectively. Mean symptom duration was 5.2 days. 83 controls included other infections (n=78), or no diagnosis (n=5). Using culture as gold-standard, *rrn-LipL* was 73.7% sensitive and 98.7% specific with serum, and 57.9% and 97.5% respectively with BC; *rrs* PCR was 89.5% sensitive and 95.2% specific with serum, and 78.9% and 95.2% respectively with BC. Using the 59 lepto cases as gold-standard, *rrn-LipL* was 39% sensitive with serum and 30.5% with BC; *rrs* PCR was 44.1% sensitive with serum and 42.4% with BC. Performance of the PCRs did not differ significantly (using serum, p=0.58; BC, p=0.25), and performance of serum and BC did not differ significantly (for *rrn-LipL*, p=0.44; *rrs* PCR, p=0.86). Mean Ct values were lower for BC (suggesting higher bacterial loads) but not significantly (for *rrn-LipL*, p=0.21; *rrs* PCR, p=0.28), and did not correlate with duration of illness. PCR inhibition was seen in 12.5% of BC extracts (indicated by failed internal control), but never with serum. In conclusion, PCR has the potential to rapidly diagnose acute lepto. There was no significant difference in performance of the 2 PCRs, however, *rrn-LipL* had the advantages of a pathogen-specific probe and internal control. Inhibition seen with BC emphasises the importance of the internal control, and supports use of serum as the optimal sample. Due to the lack of a perfect gold-standard, Bayesian latent class modelling may help determine true performance characteristics of lepto PCRs.

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KATRINA COUGH: SYMPTOM COMPATIBLE WITH EARLY DIAGNOSIS OF LEPTOSPIROSIS?

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Katrina Hurricane (2005) victims made extensive complaints regarding symptoms of a cough during 2006 described as the 'Katrina Cough'. Coughing is a symptom compatible with an early diagnosis of leptospirosis. This symptom is important because victims of Katrina were exposed to environmental, occupational and zoonotic conditions favorable to leptospirosis. New Orleans had no integrated surveillance system that included diagnostics for leptospirosis in place prior to hurricane Katrina. .Extreme weather conditions influence patterns of the disease. During the early 50's leptospiral case histories from Grady hospital in Atlanta, Georgia were reviewed. This reflected decades of important growing concern regarding epidemiological and public health problems caused by leptospirosis in the United States. leptospirosis is an acute febrile zoonotic disease first observed clinically by Adolf Weil in 1886. Drs. Inada, Ido, Hoki, Kaneko and Hiroshi in 1916 established 'The Etiology, Mode of Infection, And Specific Therapy' of the disease. Hideyo Noguchi described the morphology and nomenclature of the infection naming it Leptospira icterohemorrhagiae in 1918. The predominant vector for leptospira is found in the urine and feces of rodents. Infections are also transmitted through the bites or scratches of diseased rodents. The infection is also found in: raccoons, foxes, pigs, dogs and other mammals. Evidence based guidance is recommended for early diagnosis of the infection to avoid misdiagnosis and progression to Weil's disease with possible fatal outcomes. We did a select literature search of medical journals between 1922 and 1958 from Pub Med and the archives of Medical Journals for case histories on leptospirosis. Mapping and weather data are used to examine influence on surveillance and progress of the disease. Temperature and humidity are important factors for the survival of Leptospira. We used radiological images to review the systemic progression of the infection. Risk factors for leptospirosis were evaluated in various settings. A network of sentinel surveillance sites are required in order to develop appropriate interventions for populations at higher risk of developing the disease in the United States. Long term funding must be allocated so that active and passive public health programs have funds from all levels of government to foster the demands of the work.

1048

DOES HIGHER MASS ANTIBIOTIC TREATMENT COVERAGE FOR TRACHOMA REDUCE CHLAMYDIAL INFECTION AMONG CHILDREN? RESULTS FROM PRET-NIGER

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Programs distribute mass oral azithromycin in an effort to eliminate the ocular strains of *Chlamydia trachomatis* which cause trachoma. In trachoma-endemic areas, WHO guidelines recommend 3 annual mass antibiotic distributions with a target of at least 80% coverage. It is unclear if higher coverage would be more effective and worth the additional effort. Here, we compare a single day treatment aiming for 80% coverage (standard) to subsequent distribution days aiming for greater than 90% coverage (enhanced). 24 communities in Matamaye District, Niger, were randomized to either standard or enhanced coverage and infection was assessed biannually until 36 months. A random sample of up to 100 children (0 - 5 years of age) in each of the 24 communities were swabbed for the presence of conjunctival chlamydia Amplicor PCR. The mean antibiotic coverage was 70.6% per community in the standard arm and 88% in the enhanced arm. At baseline, the prevalence of chlamydial infection among children was 20.2% (95% CI: 9.6% to 30.8%) in the standard arm and 22.1% (95% CI: 11.4% to 32.7%) in the enhanced arm. The clinical activity among children was 27.0% in the standard arm and 28.4% in the enhanced arm. At 36 months, the prevalence of chlamydial infection among children was 4.6% (95% CI: 0.0% to 9.5%) in the standard arm and 7.1% (95% CI: 2.7% to 11.4%) in the enhanced arm. The clinical activity among children was 7.1% in the standard arm and 8.9% in the enhanced arm. Correcting for baseline, we were unable to demonstrate a statistically significant difference between standard

and enhanced treatment (p = 0.41). This study suggests that additional efforts to achieve higher mass antibiotic treatment coverage may not add significant benefit.

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PROPHYLAXIS AND DISEASE TRENDS OF TROPICAL DISEASES (ALTITUDE SICKNESS, MALARIA, DIARRHEA) AMONGST TRAVELERS TO PERU

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Travelers to foreign countries are subject to country-specific ailments of which many are preventable. This study attempts to determine the current trends in altitude sickness, malaria, and diarrhea prophylaxis amongst travelers to Peru. The participants of the study include students and travelers from around the world that participate in study abroad and tourism in Lima, Tumes, and Cuzco. In particular these sites include the Tropical Medicine Institute in Lima and UPCH-UTMB Collaborative Research Center in Cuzco, which have close relations with the University of Texas Medical Branch. The specific objective of the study is to examine quantitative and qualitative data on the use of prophylaxis for altitude sickness, malaria, and diarrhea amongst the travelers. The study will look at both allopathic and naturopathic remedies, as well as, disease prevention strategies amongst the travelers. Moreover, the frequency of the ailments with and without the various prophylaxis remedies will be determined. Results from the study help dictate travel consultation suggestions to optimize future prophylactic treatment.

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PILOT STUDY TO ASSESS THE PREVALENCE OF CHAGAS DISEASE IN PREGNANT WOMEN FROM LATIN AMERICA IN A LOS ANGELES COUNTY HOSPITAL

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Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, causes the most important parasitic disease burden in Latin America, where an estimated 8-10 million persons are infected. Approximately 24 million persons born in the countries in which CD is endemic currently reside in the U.S. and roughly 300,000 of these immigrants are thought to have chronic CD. Though transmission by domestic vectors is still the most important route of infection in disease-endemic areas, congenital transmission is the most important transmission route in non-endemic countries. Rates of congenital transmission from untreated infected mothers range from 2-12% in published studies. Previous studies demonstrate considerable information regarding CD prevalence among pregnant women in Latin America, however information about the prevalence of CD in pregnant women immigrants residing in the U.S. is largely lacking. This study of prevalence of CD in pregnant women from Latin America was performed at Olive View-UCLA Medical Center (OV-UCLA MC), a Los Angeles County hospital, from 4/2008 -5/2011 under the auspices of the Center of Excellence for CD. Enrollment criteria for the study were pregnant patients who had lived in a Latin American country for at least 1 year. 300 consecutive patients were screened for Chagas disease using both an immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Testing was performed by the Center for Disease Control and Prevention (CDC). Subjects were 15-46 years old with a median age of 32 years old. The countries of origin where subjects spent 1 year or more were Mexico 233 (77.7%), El Salvador 34 (11.3%), Guatemala 20 (6.7%), others 13 (4.3%). The average time of residence in country of origin was 14 years versus average time of residence in US of 17 years. A total of 1 subject was positive (0.33%) by both serological tests. The positive subject was a 36 year old female from rural El Salvador who migrated to US 19 years prior. Our study demonstrates a prevalence of CD in pregnant women immigrants from Latin America residing in the U.S. lower than expected, though the small sample size makes conclusive determination not possible. Further larger scale studies are needed to obtain a better idea of prevalence in this population, especially given the high-risk of congenital transmission.

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PRE-TRAVEL HEALTH CARE FOR PEDIATRIC TRAVELERS: EXPERIENCE FROM THE TRAVMIL COHORT

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Practice guidelines and prospective data regarding pediatric travel medicine are limited. We investigated the pre-travel health care and illnesses encountered by children enrolled in TravMil between 1/2010 and 7/2013. 64 children were enrolled at 3 military travel clinics (5% of all enrollees [n=1379]): 61 were enrolled pre-travel, and 3 enrolled post-travel (due to a travel-related illness). Pediatric travelers were visiting friends and relatives more frequently than adults (31% vs 14%; p< 0.05). The median trip duration for children was 17 days (IQR: 12-25) and the most common destination was South/Central America & Caribbean (n=26). Coverage rates for travel related vaccines (for those at risk) were similar among children and adults: Japanese encephalitis 71% vs 55%; yellow fever: 65% vs 85%; meningococcus: 100% vs 96%; typhoid: 95% vs 96% (p>0.05 for all). Malarone was the most common antimalarial prescribed (56%; n=22/39) regardless of the duration or location of travel. Rates of partial or non-compliance with chemoprophylaxis were similar among children and adults (13.3% vs 23%; p=0.54). Children < 10 years of age were less likely to be prescribed antibiotics (69% vs 97%; RR: 0.07 [95% CI: 0.01-0.61]) or antidiarrheals (7% vs 78%; RR 0.09 [95% CI: 0.02-0.34]) for self-treatment of traveler's diarrhea (TD) compared to children ≥10 years. Rates of travel-related illness were similar among children and adults: TD: 15% vs 24%; undifferentiated fever 3% vs 2%; influenza-like illness 12% vs 17% (p> 0.05). Three patients were enrolled post travel: 1 each with malaria, influenza-like-illness, and suspected dengue. None had been seen for a pre-travel evaluation. Significant differences in travel characteristics and pre-travel health care exist based on the age of travelers. Children < 10 years were less likely to receive medications for TD self-treatment despite similar rates of TD among pediatric and adult travelers. Strategies to improve utilization and standardization of pre-travel healthcare in pediatric travelers are needed.

1052

A SYSTEMATIC REVIEW OF THE EFFECTS OF ARTEMETHER-LUMEFANTRINE ON GAMETOCYTE CARRIAGE AND DISEASE TRANSMISSION

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Despite significant advances made in the prevention and treatment of malaria in recent years, the current success still falls short of the World Health Organisation (WHO) goals for malaria control and elimination. For elimination strategies to be effective, all parasite lifecycle targets including disease transmission have to be addressed. Rapid and effective reduction of infectious parasite reservoir and gametocyte carriage is therefore critical. Currently, artemisinin-based combination therapies (ACTs) form the cornerstone of WHO-recommended treatment for uncomplicated *P. falciparum* malaria, and in combination with other control intervention measures will play a pivotal role in elimination programmes due to their gametocytocidal properties. There is irrefutable epidemiological evidence of reductions in malaria incidence and transmission in African

regions since the introduction of these agents. A systematic review of 62 articles published between 1998 and January 2014 was done which compares effects of the ACT artemether-lumefantrine (AL) on gametocyte carriage and malaria transmission with other ACTs and non-ACTs. AL was assessed based on its widespread usage as 'gold standard' treatment for uncomplicated *P. falciparum* malaria in several African countries and the high number of clinical trials that have evaluated the product. The impact of AL on population gametocyte carriage and the potential future role of AL in malaria elimination initiatives are also considered. Despite the inherent difficulties in comparing data from a range of studies that utilised different diagnostic approaches to assess baseline gametocyte counts and differences in study designs, the gametocytocidal effect of AL was proportionately consistent across the studies reviewed, suggesting that AL will potentially play a vital role in treatment and elimination of malaria. However, the specific place of AL is the subject of ongoing research and will be dependent on its use in combination with other intervention measures, rational use of the product, interaction with other medicinal agents *in situ*ations of malaria co-infections and comorbidities, as well as demographic differences. Use of ACTs like AL in malaria elimination strategies will therefore require balancing potential increased roll out with rationale use and protection against resistance development.

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THE EFFECT OF AN ENHANCED ANTENATAL CARE PACKAGE FOR PREVENTION OF MALARIA AND ANEMIA IN PREGNANCY IN GHANA

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Efficacious antenatal care (ANC) interventions for malaria and anemia have been implemented for over two decades. However, the prevalence of malaria and anemia remains high among pregnant women due to sub-optimal uptake of these interventions. Subject participation in their own health care can improve health outcomes by improving adherence to treatment. We hypothesized that if pregnant women participated in their ANC, this would improve their adherence to ANC recommendations and promote better health outcomes. A cluster randomized, controlled trial was conducted to assess the effect of pregnant women's participation on the risk of anaemia and parasitemia 4-8 weeks after enrolment. We also assessed the feasibility and acceptability of the intervention. In the intervention group, ANC staff showed women their rapid malaria test (RDT) and Hb colour scale (HCS) results and explained their significance; the control group received only routine care. The overall mean age, gestational age and Hb concentration at baseline were 26.4 years, 17.3 weeks and 110 g/l respectively and similar in each group; 10.7% had asymptomatic parasitaemia; 74.6% owned an ITN, 48.8% slept under it the night prior to enrolment. The prevalence of anemia after 8 weeks was 51%, (95%CI: 37.2-64.7) in the control group and 52%, (95%CI: 42.8-61.5) in the intervention group. The corresponding figures for parasitemia were 6.5%, (95% CI: 3.1-9.9) and. 6.2%, (95%CI: 4.0-8.2). Integration of the HCS and the RDT into the ANC system was feasible and acceptable. Pregnant women who saw their own blood being tested believed the results and felt motivated to act to improve their health. Although ANC staff and pregnant women perceived some improvement in pregnant women's adherence to ANC recommendations, their enhanced participation in ANC did not have any effect on the prevalence of malaria or anemia. The introduction of the use of RDT into routine ANC in Ghana at the time of the study and implementation challenges may have contributed to the lack of impact of the intervention on anemia or parasitemia.

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FACTORS ASSOCIATED WITH COMPLICATED MALARIA IN COLOMBIA

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Colombia is an area of low transmission for malaria but with environmental and social conditions that favor its transmission principally in the Pacific coast. Between 2009 and 2013 2.099 cases of complicated malaria were reported. The incidence of complicated malaria increased from 14.68/1.000 in 2012 to 21.19/1.000 in 2013. A retrospective case-control study in a 1:2 proportion was conducted, in Tumaco, Cali and Buenaventura. The sample size was 60 cases and 120 controls, patients with positive thick blood smear between the period 2009 -2013 were included. The cases were selected base on the national clinical malaria attention guideline, adding transminases greater than 80 as a severity criteria to asses hepatic disfunction, controls were patients without severity (complication) criteria. 88.3% (159) of patients originated from Tumaco, 5.0% (9) from Buenaventura, and 6.67% (12) from Cali. 50.0% (30) of the cases were female. In the controls 46.6% (56) corresponded to women and the remaining percentage to men; regarding the distribution of parasite specie, 43 cases corresponded to P.falciparum, 16 to P.vivax, and 1 was mixed. With respect to the controls, 85 were P.falciparum, 32 were P.vivax and 3 were mixed malaria. In the bivariate analysis identified risk factors were chills OR 3,44 (IC 95%: 1,14; 10,42), coluria OR 3,49 (IC 95%: 1,02; 11,95), jaundice OR 4,00 (IC 95%: 1,72; 9,24), thrombocytopenia less than 100.000 platelets/mm3 OR 10,77 (IC 95%: 3,73; 31,10), and transfer from an institution of low medical complexity OR 3,79 (IC 95%: 1,75; 8,21). In the multivariate analysis continued as risk factors chills OR 7,24 (IC 95%: 1,00;52,45), janduice OR 4,86 (IC 95%: 1,75;13,40) and thrombocytopenia OR 5,95 (IC 95%: 1,84; 19,22). Even though jaundice was identified as a risk factor we did not establish any associations with laboratory studies like bilirubin o transaminase values due to lack of information in the medical records, which was the principal limitation of the study due to its retrospective nature.

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OPPORTUNISTIC INTESTINAL PARASITES DURING CHRONIC MALNUTRITION IN URBAN AND RURAL AREA OF MADAGASCAR: A PROBLEM IN TROPICAL AREA?

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In low income countries, malnutrition-related immunosuppression is suspected to pave the way of opportunistic protozoan infection. A study was conducted to better understand the burden of intestinal parasite carriage in malnourished versus control children in Madagascar. Children hospitalized with severe acute malnourished (SAM) and diarrhoeal were enrolled in hospital as well as children from rural area attending dispensary for diarrhoea. These last children (n=508) were enrolled for a two years follow up. Anthropometric measurements were registered. Stools analysis was done using specific stainings for detection of opportunits in microscopy and pathogens were typed by PCR methods. In hospital 246 children were treated for malnutrition out of which 43 children had diarrhoea. Opportunistic pathogens were frequent with mainly yeast in abnormal quantity (51%), microsporidia (21%), *Cryptosporidium hominis* (7%) and mixed co-infestations. In rural area, 273 children attended dispensary for diarrhea. Out of which 138 malnourished children and controls were selected for further analysis. Children between 12 and 24 months were the most affected by chronic malnutrition (45.7% of children). Wasting did not differ according to age, gender, but differs between villages (p=0.02). Opportunists where found in 24.6% of chronic

malnutrition and 14.4% in controls. Opportunistic carriage (i.e. yeasts, microsporidias, cryptosporidia) was linked to brachial perimeter under 105 mm (p=0.029). In multivariate analysis, factors associated with this carriage were i) growth retardation (5x increase in risk p=0.004), non-exclusive breast-feeding, and hygiene (absence of soap). *Cyclospora cayetenensis* and *Isospora beli* were also found in these children at low rate. Overall, this study described for the first time opportunistic intestinal infections in children with malnutrition in Madagascar. It highlights the role of chronic malnutrition in carriage of cryptosporidies, whereas microsporidies were more frequent during SAM. Implication of these findings must be discussed.

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A RURAL FOCUS OF LEPTOSPIROSIS IN MADAGASCAR?

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Leptospirosis is due to bacteria belonging to more than 20 species. All animals especially rats can be chronic carriers of the bacteria and the human disease is often due to professional exposure and/or to contact with contaminated fresh water. Clinical expression can be mild which could explain under-estimation of the burden. The Mascareignes islands are endemic areas but in Madagascar only a couple of human cases were reported mostly in travelers. In highlands of Madagascar, the use of zebus for rice cultivation could be associated with transmission in an Asian like setting. To explore this setting 111 livestock in 28 villages of the district of Moramanga were investigated. In the same time, farmers' families were blood sampled, as well as an equivalent number of peoples randomly selected in households of the vicinity but without cattle. All the cattle and humans were geolocalized for cluster analysis. 670 zebus were sampled, out of which 81 (12% from 43 livestock) were ELISA IgG positive for leptospirosis. Additionally, 25 zebus suffering from symptoms related to leptospirosis were lepto-IgG negative. 70 positive serums (out of 81) were tested in microagglutination technic (MAT) and 10% were positive. In the same time 21 urines from the 81 IgG positive zebus and 11 blood samples from the 25 sick animal were obtained and tested with a Leptospirae sp 16S-PCR. Respectively 10 and 3 samples were positive from urines and blood samples. Sequencing of two PCR products from urines confirmed Leptospira interrogans. In households 530 subjects were enrolled (329 farmers/201 controls) with an overall lepto-IgG prevalence of 4.5%. Subjects from households with positive zebu had higher IgG prevalence than those with negative ones (7.2% vs 4.2%, p=0.3) but even more than those without zebus (7.2% vs 1.5%, p=0.004). In this setting zebus may not be the unique source of contamination of humans and in deep analysis of risks factors for leptospirosis is now in process. However this study highlights the circulation of the bacteria in rural area of Madagascar and the presence of L interrogans in cattle.

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NON-INVASIVE MANAGEMENT OF MADURA FOOT WITH ORAL POSACONAZOLE

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Madura Foot is a chronic granulomatous subcutaneous fungal (eumycetoma) or bacterial(actinomycetoma) infection with high disease burden. Estimation of the global burden of madura foot is difficult but most cases have been reported in the "Mycetoma belt" i.e. from countries between 30N and 60 S of the Equator. Traditionally,maduramycosis of the foot has been treated with surgical debridement, which leads to permanent disfigurement of the limb. Non-invasive management with long-term antimicrobials alone has been reported in the past but, almost always the cost of antimicrobials, increased rates of non-compliance and resistance against antifungals have discouraged physicians from this approach. We report a case of biopsy proven eumycetoma of the foot in a young Somali refugee presented with right foot pain and swelling for 7 years and "tiny watermelon seeds" extruding out of the "puncture wounds" on the sole of the right foot, which was successfully managed without the need for any kind of surgical intervention with oral posaconazole and ciprofloxacin.

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SENSITIVE REAL-TIME PCR DETECTION OF PATHOGENIC *LEPTOSPIR*A SPP. AND A COMPARISON OF MOLECULAR METHODS FOR THE DIAGNOSIS OF LEPTOSPIROSIS

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Leptospirosis remains under-diagnosed due to a non-specific clinical presentation and limitations of available diagnostics. Bacteria of the genus *Leptospira*, the causative agents of leptospirosis, are categorized into pathogenic and saprophytic species, though the clinical import of making this distinction remains unclear. Our group recently developed a real-time PCR (rtPCR) for the detection of all Leptospira species, which is included in a multiplex diagnostic for an undifferentiated febrile illness (the UFI assay). While the UFI assay proved more sensitive than conventional PCRs for *Leptospira*, it was not evaluated against another rtPCR and it was unclear if similar results could be obtained with an assay for pathogenic Leptospira. In this study, we present the development and evaluation of an rtPCR for the detection of pathogenic *Leptospira* species (the pathogenic rtPCR) that targets the same region of the 16S gene as the UFI assay. The linear range of the pathogenic rtPCR extended from 7.0 to 2.0 log10 copies/µL, with a lower limit of 95% detection of 29 copies/µL. Thirty-nine cultured Leptospira isolates, representing 7 species and 23 serovars, were tested. All isolates were detected using the pathogenic rtPCR except for two strains of L. biflexa, which produced no signal. Clinical samples from 65 patients with suspected leptospirosis were tested using the pathogenic rtPCR and a reference 16S rtPCR originally reported by Smythe, et al, 2002. All 65 samples had previously tested positive using the UFI assay; 62 (95.4%) samples tested positive using the pathogenic rtPCR (p=0.24). Twenty-four (36.9%) samples tested positive in the reference 16S rtPCR, which was significantly less sensitive than the UFI assay or pathogenic rtPCR (p<0.0001 for both comparisons). In conclusion, both the pathogenic Leptospira rtPCR and the UFI assay proved significantly more sensitive than the rtPCR used for comparison. Future studies are needed to determine the impact of more sensitive Leptospira detection on both patient care and epidemiologic surveillance.

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VITAMIN D AND OSTEOARTHRITIS IN IRAQI WOMEN

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Osteoarthritis (OA) is a biomechanical process whereby joints respond pathologically to mechanical stress, resulting in cartilage degradation and changes in subchondral bone. OA has many known risk factors including gender, obesity and aging. Low levels of vitamin D may exert its effect on OA through synergistic bone density loss (osteopenia) and impairment of the ability of bone to respond optimally to insults. Hypovitaminosis D is a global medical problem even in regions where oral intake is sufficient and sunlight is abundant because subtle degrees of malabsorption of vitamin D can occur independent of adequate vitamin D intake. To explore

the role of vitamin D in Iraqi women with knee OA, we enrolled 45 females with the diagnosis of OA and an equal number of age matched healthy controls. OA was diagnosed according to American College of Rheumatology (ACR) criteria for OA and subject body mass indexes (BMI) were recorded according to the WHO classification of BMI in 3 groups: normal weight, overweight and obese. Radiological grading was assigned according to Kellegren and Laurence radiological classification for OA. Vitamin D levels were measured using an ELISA assay (normal range > 30 ng/ml). Our study found that the vitamin D levels in OA patients ranged from 0.309 - 0.641 whereas the range in controls was 0.261 - 0.386. Vitamin D deficiency was prevalent among both OA cases and controls, but Vitamin D levels were inversely proportional to BMI. Vitamin D deficiency was more severe among females in the obese and overweight groups than those with normal BMI. Lower levels of vitamin D were observed in OA subjects with radiological grades III and IV disease compared to those with grade II. In summary because vitamin D deficiency was so prevalent among almost all study subjects, it was difficult to establish a clear association between vitamin D deficiency and OA. Also, there is debate about the possible variability of different methods used to determine vitamin D levels. However, the magnitude of the observed hypovitaminosis D problem in Iraqi women warrants considerable attention to all possible causes and treatment options.

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AN UNUSUAL PRESENTATION OF AMEBIC LIVER ABSCESSES IN A RETURNING TRAVELER

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Amebic liver abscess (ALA) is uncommon in the USA. Clinical presentation is usually with fever and abdominal pain and is predominantly in men. Rupture of ALA is a rare but life threatening complication if left untreated. Patients usually have leukocytosis and deranged liver function tests. Serology can be positive in >90% 7 days after exposure. Needle aspiration is indicated only for impending rupture or to aid diagnosis and reveals trophozoites in 20%. We present an unusual case in a female returned traveler initially thought to have only community-acquired pneumonia (CAP). A 53-year-old female presented with cough and fever for 3 weeks having spent 4 weeks in India. She had low-grade fever, wheeze and cough for 1 week in India where she was treated unsuccessfully with azithromycin. Following her return to the US she also developed vomiting, anorexia, malaise and vague right upper quadrant abdominal pain. She was markedly distressed with fever of 102ºF, tachycardia, tachypnea and right upper quadrant tenderness. Her white blood count was 19,000/µl (neutrophils 82%) and hematocrit 27.9%. Liver function tests were normal. Chest X-ray showed minor linear patchy density in the left lung base. Ceftriaxone and azithromycin were initiated for possible CAP and she was admitted. Ultrasound abdomen subsequently demonstrated an 8 cm hypoechoic, septated, thick walled mas in the left lobe of liver. Computed tomography (CT) revealed 3 cm and 8 cm abscesses. Thick pus were drained under CT guidance. Microscopy of the pus showed no trophozoites or bacteria and culture was negative. Around 700 cc of pus were drained over the next 2 weeks. She was given metronidazole and ciprofloxacin. Antibody to *Entamoeba histolytica* was positive so ciprofloxacin was stopped. A follow-up CT scan showed marked reduction in size of the abscesses and the patient fully recovered. ALA in this patient was not initially suspected due to the respiratory presentation and normal liver function. Without a low threshold for imaging, this potentially life threatening diagnosis could have been overlooked.

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DETECTING APPROXIMATE REGIONS OF LUNG CANCER AND FLUID USING CHEST X-RAY SCREENING IMAGES

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Lung cancer is a leading cause of death in Iraq and despite active research in computer-aided diagnostic tools, development of an automated screening process for lung cancer is extremely challenging. The mortality rate for lung cancer is high in part because early detection is difficult, along with technical factors in capturing images or experience in x-ray interpretation all contribute to this problem. Therefore, the aim of our research is to develop an image-based coarse level texture descriptor to distinguish between cancerous regions and fluid filled regions in chest radiograph (x-ray) images of the lung. The presence of a significant amount of fluid in the lung is also associated with a high mortality rate. We present a computer-based approach for automatic detection of cancerous and fluid regions in the lung using chest radiographs. The image morphology approach using Marker-Controlled Watershed Segmentation method is used to isolate the cancerous and fluid regions within the lung tissue boundary. Different methods were used to enhance the X-ray images prior to computing the first order texture analysis histogram used for feature extraction. Our textural analysis method is shown to be capable of distinguishing between normal and cancerous cases, normal and fluid cases, as well as between cancerous and fluid cases. Such approaches will be useful for developing image-based automated lung cancer screening systems.

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ULTRASOUND AND CHEST X-RAY IN AN ADULT PATIENT WITH DENGUE

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The pathognomonic feature of dengue hemorrhagic fever (DHF) is a transient increase in vascular permeability resulting in plasma leakage. Several studies assessed ultrasound as a tool in gauging disease severity in detecting plasma leakage into tissues and body cavities. Most of these studies were done in children and information on ultrasound study on adults was limited. We conducted a prospective study to recruit adult patients above 18 years presenting with acute fever from August 2011 to September 2012 to Communicable Disease Center, Singapore. Demographic, clinical and laboratory data were collected. Ultrasound assessment included pericardial, pleural and abdominal cavities including gall bladder wall thickness, liver, spleen and ascites on enrolment visit, recovery (4-7 days from enrolment) and convalescent visit (3-4 weeks). Postero-anterior chest X-ray (CXR) together with right lateral decubitus view was done on the same day as ultrasound. Plasma leakage (clinically detected fluid accumulation, hematocrit [Hct] change>20% and hypoproteinemia) and DHF were defined using 1997 World Health Organization criteria. Of 110 recruited patients, 74 had laboratory confirmed dengue, 11 probable dengue and 25 were dengue negative. Among confirmed dengue cases, median age was 34 years, 98% were males, and 24% were hospitalized. Median duration of fever on enrolment was 6 days (range: 2-10). Fluid was detected by ultrasound in 2 cases and CXR in 10 cases. There were 19 cases with plasma leakage (0 clinical fluid accumulation, 1 HCT change and 19 hypoproteinemia) and 11 with DHF. One ultrasound and 6 CXR detected fluid in those with hypoproteinemia; one patient with HCT change and hypoproteinemia had fluid on CXR. Of 11 DHF cases, fluid was detected by CXR in 4 cases (3 on visit 1, 1 on visit 2) and ultrasound in 1 case (on visit 2). Detection of fluid by imaging was uncommon in our adult dengue cohort. Its utility in severe adult dengue needs further study.

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SERUM BIOCHEMICAL AND HEMATOLOGY VALUES IN *ONCHOCERCA VOLVULUS* INFECTED PEOPLE IN NORTHEAST DRC, LIBERIA AND GHANA DETERMINED PRE-TREATMENT IN A CLINICAL STUDY COMPARING THE EFFECT OF MOXIDECTIN AND IVERMECTIN

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For a Phase 3 study comparing the effects of a single dose of moxidectin and of ivermectin in 941 males and 531 females, including 79 children 12-17 years, 1499 people underwent baseline serum biochemical and haematological examinations. All had ≥10 *Onchocerca volvulus* microfilaria/mg skin and 876/1465 (60%) who underwent stool examination with a single sample Kato Katz test were infected with at least 1 type of intestinal helminth. None of the people suffered from an acute disease at the time of testing. The interquartile range of the laboratory values (i.e. the range within which the values for 50% people screened lay on either sides of the median) were: Albumin: 37.2-43.0 g/L, Alkaline phosphatase (>20yrs): 72.4-108.2 U/L, Bilirubin: 5.2-11.0 umol/L, Creatinine: 60.3-78.6 umol/L, GGT: 18.2-41.7 U/L, Glucose: 4.5-5.3 mmol/L, LDH: 210.0-278.0 U/L, Protein: 77.3-88.0 g/L, SGOT-AST: 23.1-35.1 U/L, SGPT-ALT: 18.0-31.1 U/L, Urea: 1.8-3.1 mmol/L, WBC: 5.70-8.62, x10E9/L Basophiles: 0.01-0.03 x10E9/L, Eosinophiles: 0.44-1.47 x10E9/L, Lymphocytes: 1.90-3.17 x10E9/L, Monocytes: 0.39-0.75 x10E9/L, Neutrophils: 2.64-7.15 x10E9/L, Platelets: 206-316 x10E9/L, Hematocrit Female: 36-43 %, Male: 40-48 %, Hemoglobin Female: 12-14 g/dL, Male: 14-16 g/dL. There were no substantial differences between the values obtained in the four different areas in which participants were recruited: Nord-Ituri and Nord-Kivu in DRC, Lofa county in Liberia and Nkwanta district in Ghana. These values in an essentially adult population are within generally accepted 'normal ranges' for 'healthy subjects'. Given that 'chronic' or temporary infections are frequent in many African populations, the data obtained can contribute to defining more locally-relevant 'normal laboratory values'.

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A TAQMAN ARRAY CARD (TAC) FOR DETECTION OF CENTRAL NERVOUS SYSTEM INFECTIONS IN KENYA

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Infections of the CNS are often associated with meningitis, encephalitis or meningoencephalitis and may result in significant morbidity and mortality. In Kenya, the Ministry of Health reviewed hospital-based surveillance and concluded that hospitals in Kenya failed to diagnose large numbers of meningitis cases, probably due to technical challenges of diagnosis. To mitigate diagnostic challenges, we developed encephalitis TaqMan Array Card (TAC) which enables simultaneous detection of 21 pathogens associated CNS infections. These targets includetwo amoebae, 13 virus, and 6 bacteria. An intrinsic control (RNAase P*)* was included to monitor the extraction and amplification efficiency and assure the validity of testing results. Nucleic acid was extracted and assayed on TAC using TaqMan Fast Virus 1-Step kit (Life Technologies, Carlsbad, CA) on a ViiA 7 Real time system. Validation of the pathogen specific assays included evaluation of the linearity and range, limit of detection, sensitivity and specificity of each assay with pathogen specific nucleic acids. Transcripts of recombinant plasmids were developed to serve as positive controls for the assays. Each assay displayed linear amplification of the target nucleic acid, detected 5 copies or less of target genomes, and was specific for its target pathogen. This TAC will be deployed and evaluated as a rapid screen for organisms associated with CNS infection in surveillance and/or clinical care settings.

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ETIOLOGIES OF ACUTE FEBRILE ILLNESS AMONG ADULTS ATTENDING OUTPATIENT CLINICS IN DAR ES SALAAM, TANZANIA

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Fever is one of the most frequent cause of attendance at outpatient level. Beyond malaria, little is known about etiologies of fever in adults which urge clinicians to overprescribe antimicrobials. We aimed at investigating precise causes of acute febrile episodes in adults attending outpatient clinics in urban Tanzania. Consecutive patients >18 years with tympanic temperature >37.5°C were recruited. Detailed medical history and clinical examination were done and blood taken to perform rapid tests for malaria, Dengue, Typhoid, HIV, Cryptococcus, Tuberculosis, Streptococcus A, Syphilis and rota/adenovirus, as well as blood cultures, serological and molecular analyses. All had nasal/throat swabs taken for PCR. Chest X-rays were performed when WHO criteria for clinical pneumonia were met. Urine culture, stool culture and other investigations were performed according to pre-defined algorithms. All final diagnoses were based on pre-defined criteria. 400 patients out of a total of 500 have been recruited up to April 2014. Preliminary results showed that 36% were HIV infected

(prevalence in the general population: 12%). Causes of fever (prior to serologies/PCR results) were: 41% acute respiratory infections (16% URTI, 10% tuberculosis, 7% radiological pneumonia, 3% Pneumocystis jiroveci (PCP), 3% tonsillitis, 2% COPD exacerbation), 9% typhoid, 7% occult bacteremia, 4% malaria, 4% urinary tract infection, 3% diarrhea, 3% sexually transmitted infections (STI), 2% chickenpox, 2% Cryptococcus, 1% skin infection, and 24% unknown at this stage. In the subgroup of patients with severe illness (of which 47% were HIV infected), radiological pneumonia, diarrhea and occult bacteremia were overrepresented, while in the subgroup of HIV infected patients with illness of any level of severity, it was the case for tuberculosis, PCP, STI and Cryptococcus. These results provide for the first time an accurate picture of the diversity of causes of fever in African adults. Results of molecular analyses will provide further insight on respective contribution of bacteria versus viruses, a critical issue to design decision charts for the appropriate management of fever and rational use of antibiotics.

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CHILDHOOD MORTALITY IN COMMUNITIES TREATED WITH AZITHROMYCIN FOR TRACHOMA CONTROL IN NIGER

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Childhood mortality in communities treated with azithromycin for trachoma control in Niger Trachoma control programs utilize mass azithromycin distributions to treat ocular *Chlamydia trachomatis* as part of an effort to eliminate this disease world-wide. Antibiotics are provided to target the ocular strains of chlamydia that cause trachoma, but may also have some effect against diarrhea, upper respiratory infections, and malaria_frequent causes of childhood mortality in trachoma-endemic areas. One previous trial in Ethiopia suggested that childhood mortality was reduced in communities treated with oral azithromycin. Here we monitor childhood mortality in a large mass treatment trial in Niger. We conducted a community randomized clinical trial for trachoma from April 2010 - August 2013. Communities were selected from 6 health centers (Centre de Santé Intégrée or CSIs) and were eligible for inclusion if they had an estimated total population of 250 to 600 persons, generally encompassing 50 to 100 children. 48 communities were randomly assigned to annual treatment of the entire community or biannual treatment of children only. Deaths of children under age 5 were identified and trained interviewers conducted verbal autopsies in Hausa, the local language, with the next of kin using the 2007 WHO standard verbal autopsy questionnaire in order to determine the cause of death. A total of 351 of 2632 children under age 5 (13.4%, 95% CI 11.6% - 15.3%) died in the communities treated annually; whereas 287 of 2493 children (11.4%, 95% CI 10.1% - 12.7%) died in the communities treated biannually (p=0.07). Thus, fewer deaths were reported in the biannually treated communities, although the difference was not significant. Verbal autopsies have been conducted and data from the two treatment arms will be compared during Summer 2014.

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INCIDENCE OF NON-MALARIA FEVERS IN A HIGH MALARIA ENDEMIC AREA OF GHANA

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With the role out of diagnostic tests that allow the rapid diagnosis of malaria in febrile patients, the need for a better understanding of the epidemiology of non malaria fevers (NMF) is now increasingly important. In this study, we investigated some of the risk factors associated with NMF in a malaria endemic area of Ghana. A prospective birth cohort study was carried out among 1855 newborns between 2008 and 2011; 4028 episodes of NMF were detected during one year of follow up. Diagnoses in infants with NMF included illnesses associated with respiratory (43.9%), gastrointestinal (32.6%), or cutaneous (12.8%) systems. The incidence of all episodes of NMF (first and subsequent) was 1.55 per child- year (95% CI 1.51, 1.60). Infants born in households of lower socio-economic status experienced a higher incidence of NMF compared with those from households of higher socio-economic status [adjusted hazard ratio 1.33 (95% CI 1.14, 1.56), [P<0.01]. The incidence of NMF was higher in infants from rural communities compared to those from urban areas [adjusted hazard ratio 1.33 (95% CI 1.02, 1.32), [P=0.02]. Similarly, the incidence of NMF was higher among infants living further from health facilities compared with those living close to health facilities [adjusted hazard ratio 1.24 (95% CI 1.12, 1.38), P<0.01]. Placental malaria was not associated with the incidence of NMF [adjusted hazard ratio 0.97 (95% CI 0.88, 1.07), P=0.49]. The incidence of NMF is high in this region of Ghana, especially in poor, rural communities.

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EFFECT OF ALENDRONATE ON SERUM GHRELIN LEVEL IN OSTEOPOROTIC POSTMENOPAUSAL WOMEN

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Osteoporosis is a systemic skeletal disorder where net bone resorption exceeds bone formation and is characterized by low bone mass and density and micro-architectural deterioration of bone tissue. Osteoporosis leads to increased bone fragility with a consequent increase in fracture risk. Ghrelin a 28-amino acid peptide hormone that is synthesized primarily in the stomach and released during fasting, is an osteoblast mitogen that may regulate the activity of human bone cells. This study was designed to evaluate the effect of an anti-resorptive drug alendronate, a nitrogen containing bisphosphonate, on serum ghrelin levels. Twenty-three postmenopausal women with mean age, 64±8.3 years and diagnosed as osteoporotic patients based on bone mineral density (BMD) measurements using Dual X-ray Absorptiometry (DXA). The study was conducted at the Ibn Seena teaching hospitals in Mosul, Iraq. Patients were treated with alendronate tablets (PMS-Alendronate, Canada) 70mg once weekly and followed for three months between November 2011 and March 2012. Serum ghrelin hormone concentration was measured pre- and post-treatment using a commercially available Enzyme-Linked Immune Sorbent Assay (ELISA) diagnostic kit (MyBiosour, USA). The results showed that three months post treatment with the drug alendronate led to a statistically significant (p>0.05) increase of 21.4% in the basal serum level of ghrelin peptide hormone and that there was a significant inverse

association of ghrelin with body mass index (BMI). The implication of increased serum ghrelin levels on osteoporosis in postmenopausal women is being further investigated.

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BAYESIAN GEOSTATISTICAL MODEL-BASED ESTIMATES OF GEOSPATIAL DISTRIBUTION OF SOIL-TRANSMITTED HELMINTHIASIS AND ALBENDAZOLE TREATMENT REQUIREMENTS IN NIGERIA

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The control of soil-transmitted helminthiasis (STH) in Nigeria, using preventive chemotherapy, has become imperative in the light of global fight against Neglected Tropical Diseases (NTD). We provide for the first time Bayesian model-based risk maps to facilitate planning, targeting of control activities and surveillance. Disease prevalence data were derived from National STH surveys carried out in 2011. The data were geo-referenced and collated in a geographical information system (GIS) database for the generation of STH point prevalence maps. Bayesian geostatistics methods using advanced variable selection with remotely sensed environmental covariates, was used to model the spatial risk of STH for Nigeria. STH is currently endemic in 20 of 36 states of Nigeria including Abuja. Infections were found in 513 unique locations out of 555 different survey locations. Hookworm infection was found in 482 (86.8%) locations covering 20 states, ascariasis is endemic in 305 (55%) locations in 16 states and trichiuriasis is endemic in 55 (9.9%) locations in 12 states. ascariasis and hookworm infection are co-endemic in 16 states, while the three species are co-endemic in 12 states. The prevalence range for ascariasis was 1.6% to 77.8%, 1.7% to 51.7% for hookworm and 1.0% to 25.5% for trichiursis. Model-based predicted prevalence of ascariasis, ranged from 0.1% to 82.6% with a mean prevalence of 2.9% (95% confidence interval (CI): 2.90-2.93%), while hookworm infection ranges from 0.7 to 51.0% with a mean prevalence of 7.9% (95% confidence interval (CI): 7.86-7.91). Land surface temperature and dense vegetation are the significant covariate influencing the spatial distribution of STH in Nigeria. Prevalence estimates adjusted for school-aged children in 2011, showed that ascariasis is <10% in all the 36 states including Abuja, while hookworm infection is >10% in 8 states and <10% in 29 states. The model estimated that 40.1 million school-aged children are at risk of STH in Nigeria, requiring 80.2 million albendazole tablets annually for treatment. These maps and its associated predictions would help in accelerating the control of STH in Nigeria.

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PODOCONIOSIS PATIENTS' WILLINGNESS TO PAY FOR TREATMENT SERVICES IN NORTHWEST ETHIOPIA: POTENTIAL FOR COST RECOVERY

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Podoconiosis is non-filarial elephantiasis (neglected tropical disease) of the lower legs. It is more commonly found in tropical Africa, Central and South America, and northwest India. In Ethiopia, a few non-governmental organizations provide free treatment to podoconiosis patients, but sustainability of free treatment and scale-up of services to reach the huge unmet need is challenged by resource limitations. We aimed to determine podoconiosis patient's willingness to pay (WTP) for a treatment package (composed of deep cleaning of limbs with diluted antiseptic solution, soap, and water, bandaging, application of emollient on the skin, and provision of shoes), and factors associated with WTP in northwestern Ethiopia. A cross-sectional study was conducted among randomly selected untreated podoconiosis patients (n=393) in Baso Liben woreda, northwestern Ethiopia. The contingent valuation method was used with a pre-tested interviewer-administered questionnaire. The majority of podoconiosis patients (72.8%) were willing to pay for treatment services. The median WTP amount was 64 Birr (US$ 3.28) per person per year. More than one-third of patients (36.7%) were willing to pay at least half of the full treatment cost and 76.2% were willing to pay at least half of the cost of shoes. A multivariate analysis showed that having a higher monthly income, being a woman, older age, being aware of the role of shoes to prevent podoconiosis, and possession of a functional radio were significantly associated with higher odds of WTP. The considerable WTP estimates showed that podoconiosis treatment could improve sustainability and service utilization. A subsidized cost recovery scheme could reduce treatment costs and more feasibility integrate podoconiosis treatment service with other NTDs and the government's primary health care system.

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PREDICTORS OF NON-COMPLIANCE WITH MASS DRUG ADMINISTRATION FOR SCHISTOSOMIASIS CONTROL IN WESTERN KENYA - THE SCORE PROJECT

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Mass drug administration (MDA) is being used to control schistosomiasis and other neglected tropical diseases of public health significance. However, achieving optimal community participation during implementation remains challenging. When a critical proportion of the population fails to participate in MDA, a potential reservoir for the parasite is left untreated, opening the opportunity for resurgence of the infection and reducing the probability of successful transmission control. This study was designed to identify predictors of non-compliance with MDA in western Kenya in villages with ≥25% prevalence of schistosomiasis to develop more effective health educational and delivery strategies. We used a population based unmatched case-control study design nested within a cross sectional household survey employing a structured questionnaire administered to 550 heads of households. Both univariate and multivariate analyses were used to identify the independent predictors of non-

compliance. Two hundred and forty respondents (44.9%) reported being non-compliant. By univariate analysis, non-compliance was significantly associated with the household head not asking the community health workers (CHWs) questions about the program, crude odds ratio (COR) 10.3, 95% CI [6.61-15.93], not having heard about the program COR 2.4 [1.6-3.6], and low schistosomiasis risk perception COR 3.1 [1.89-5.03]. In a logistic regression model, the odds of being non-compliant significantly increased amongst household heads who perceived their CHW not to be doing good work during the MDA exercise; adjusted odds ratio (AOR) 4.9, 95% CI [1.82-13.74], heads of households who lacked knowledge about schistosomiasis control methods AOR 7.5 [3.3-16.8], and those who did not know how the CHW was selected AOR 2.5 [1.3-5.0]. In order to improve compliance with praziquantel MDAs, effective strategies should be identified to ensure CHWs are well-trained and supervised to ensure quality service provision. Health education is also necessary to increase the knowledge levels of the disease in the community.

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THESE PEOPLE HAVE USED US AS RUBBER STAMPS: QUALITATIVE DESCRIPTION OF COMMUNITY PARTICIPATION IN WATER AND SANITATION ACTIVITIES IN THE CONTROL OF BILHARZIA IN NYALENDA B, AN INFORMAL SETTLEMENT IN KISUMU CITY, WESTERN KENYA

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The burden of disease caused by schistosome and soil-transmitted helminthes (STHs) infections is recognized as a major public health problem .A key ingredient to the success of the control interventions is community participation, which not only enhances the control efforts, but also guarantees their sustenance. Despite evidence from Community Directed Intervention (CDI) studies that community participation has brought great successes many policy makers and implementers have often neglected this aspect. Eight key informant interviews (KIIs) and eight focus group discussions (FGDs) categorized by gender and age were conducted. In addition, data on NGOs dealing with water and sanitation activities in Kisumu was collected from the NGO registration Board (Kisumu office). Qualitative data was organized into themes and concepts and analyzed using Atlas.ti. Most participants felt that project implementers did not involve them in key levels of project implementation. This in turn led to unsustainable projects and unacceptance from the community. Participants identified structures in the community that could be used as avenues of engaging the community in improving water and sanitation, for instance use of organized groups such as youth groups, gender based groups, farmers groups, merry-go rounds, and HIV support groups. Factors mentioned that hinder community participation included negative attitude from community members, poor monitoring and evaluation strategies, limited disclosure of project details to community members, and over-dependence from the community. Poor drainage systems, low latrine coverage, broken pipes and leakage of the sewerage systems were the leading factors associated with poor water and sanitation conditions. For effective community participation in water and sanitation activities, a multi-pronged paradigm is required that incorporates change of attitude from the community, information sharing and consultation, improved monitoring and evaluation, transparency and accountability.

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DETERMINING THE RISK OF TRANSMISSION OF MALARIA AND LYMPHATIC FILARIASIS IN A POST-MASS DRUG ADMINISTRATION SETTING IN CHIKWAWA DISTRICT, RURAL SOUTHERN MALAWI

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Malawi is a lymphatic filariasis (LF) and malaria co-endemic country where these diseases also share the same vectors *Anopheles gambiae* and *Anopheles* funestus. A high transmission focus for LF occurs in the rural southern part of the country, Chikwawa district with a microfilaria (MF) baseline prevalence of 35.9%. Here, five annual rounds of mass drug administration (MDA) of anthelminthic drugs were completed in 2012, a WHO recommended strategy to interrupt LF transmission. Long lasting insecticide-treated bednets (LLINs) for malaria prevention were also rolled out by the ministry of health. In our first study, we did a survey of LF prevalence, LF-associated morbidity, MDA and LLIN coverage in a small geographical area in Chikwawa district ten months after the fifth round of MDA was distributed by the Ministry of Health. 795 individuals were surveyed from six villages with an antigen prevalence of 15% (12.4% in females, 20% in males, p = 0.006). Prevalence of adult filarial worm antigen measured using rapid ICT cards ranged 4.1 - 38.5% and the prevalence of night blood microfilaria (MF) was estimated to range 0-7.5%. Median MF density was 4 MF/20 µL (range: 0.3-58.5 MF/20 µL). Self-reported MDA coverage in the fifth round ranged 69.2-90.2% and household LLIN coverage ranged 74.5 - 92.2%. 99% of 665 people that owned at least a net reporting usage the previous night before the survey. MF prevalence dropped from baseline 35.9% to range 0-7.5% after MDA,attributed to MDA and LLIN scale up. It is unknown whether the relatively low MF densities observed are sufficient to sustain transmission. To investigate this, in May 2014, we shall randomly sample 40 households for a questionnaire survey and mosquito surveys using CDC light trap and human landing catch methods to check the prevalence of LF and malaria in mosquitoes using PCR. The questionnaire will allow us to determine any gaps in intervention coverage, identify reasons for non-compliance and identify associated household risk factors for mosquito biting and LF and malaria transmission. Results will be due end of July 2014.

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DIGITIZED DATA FROM RURAL AFRICA: A FIELD APPLICATION OF OPEN DATA KIT (ODK) TO COLLECT BASELINE HEALTH DATA IN EASTERN SUDAN, NEW HALFA LOCALITY

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The collection of health survey data is often a challenge in developing countries. Although there are some of available health data in these countries, many are often not enough for research purposes or useful as a basis for health interventions. Hence, usually, when researchers or organizations grasp of health needs in target areas, health survey data need to be collected before meaningful intervention. Recently, a free and open-source set of tools named Open Data Kit (ODK) developed by a research team in the University of Washington is available. In brief, the ODK simplifies three steps of data collections: designing a questionnaire form, data collections with Android devices, and aggregating collected data. Herein we present our field application of ODK in Sudan to collect baseline health data for Schistosomiasis, Leishmaniasis and human immunodeficiency virus and the acquired immune deficiency syndrome (HIV/AIDS). We conducted the study in New Halfa, Eastern Sudan, and randomly selected 10 villages. Basically, 10 households (HH) per village

were randomly selected using the expanded programme of immunization (EPI) sampling method developed by the World Health Organization. We administrate questionnaire to members in each HH. The study was conducted between 22 February and 3 March 2014. We visited 100 HHs, and administrated 485 questionnaires. Majority of HHs had pit latrines or better toilet facilities (n=83), but 17 HHs did not have any such facilities. Self-reported Schistosomiasis prevalence was 21.6% (n=105), and 16 members experienced blood urine. Self-reported Leishmaniasis cases were few (n=1) although only 17.3% of residents used bednet during the previous night of the survey for malaria and Leishmaniasis prevention. Regarding HIV, many participants were unaware of Voluntary Counseling and Testing (VCT) cervices in their area (89.6%, n=435). We showed the results of health data collections using the ODK and EPI sampling method. This combination made it easy to collect data in a short period. We believe that ODK is a quite useful tool for data collections in a country with a poor health system such as Sudan.

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OVERALL PERFORMANCE OF THE INTERNATIONAL TRACHOMA INITIATIVE 1999-2012

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Trachoma is the world's leading infectious cause of blindness. The WHO recommended approach for trachoma control is the comprehensive SAFE strategy (Surgery-Antibiotics-Facial cleanliness-Environmental improvements, e.g., access to safe water, latrine building), coordinated by the WHO Alliance for Global Elimination of blinding Trachoma by the year 2020 (GET 2020). More than 20 organizations in the International Coalition for Trachoma Control (ICTC) work together to assist countries. The International Trachoma Initiative (ITI) coordinates the 'A' component of SAFE, the donation of azithromycin (Zithromax®, Pfizer) to countries for annual mass drug administration (MDA). We reviewed ITI's performance since the start of the program. From 1999 to 2012, 24 countries conducted MDA with Zithromax® for ≥1 years, increasing from 2 countries in 1999, to 20 in 2012. In total, 291,105,608 doses of Zithromax® were distributed, increasing from 677,401 in 1999 to 47,911,058 in 2012. Collection of 2013 distribution data is ongoing, 6 additional countries were scheduled to start MDA in 2013 (Central African Republic, Chad, Guatemala, Guinea, Mozambique, Solomon Islands). 7 countries (Ghana, Iran, Morocco, Myanmar, Oman, the Gambia and Vietnam) have reported to WHO the achievement of the ultimate intervention goals. ITI has been working on fine-tuning and streamlining procedures, to increase transparency, and make it easier to apply for and distribute Zithromax®, while maintaining quality standards and controls. We anticipate a shift from reporting process to reporting impact, with more detail down to the district level. Challenges encountered include conflict and post-conflict situations, resource limitations in endemic countries, uncertainty about disease burden (currently being assessed through the Global Trachoma Mapping Project), costs and need-to-treat. The trachoma community is developing diagnostics and strategies for MDA impact surveys, MDA stopping decisions, and post-MDA surveillance. Coordination with the 'S', 'F' and 'E' components of SAFE is needed. ITI has become a successful drug donation program, and a partner among partners in the ICTC. The progress towards GET 2020 is encouraging, but further upscaling is needed to eliminate blinding trachoma by 2020.

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NEGLECTED TROPICAL DISEASE (NTD) COMMUNICATIONS WORKSHOPS FOR HEALTH PROFESSIONALS AND JOURNALISTS IN NTD ENDEMIC COUNTRIES, TACKLING FEAR AND NEGLECT, SHARING KNOWLEDGE, BUILDING BRIDGES

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Informing the public and policy makers about NTDs is a task shared by health professionals and the media. Interaction between these groups is essential, yet often limited and difficult. Not enough and/or incorrect information reaches target audiences. NTD programs have been challenged by misinformation and rumors. We addressed these issues by organizing dedicated NTD communications workshops for health professionals and journalists in NTD endemic countries. The workshops were meant to 1) help health professionals build skills needed to effectively engage with and inform the media, the public and policy makers about NTDs, and 2) to inform journalists about NTDs and the health professionals that work on NTDs, and explain how, why, and on what the public should be informed. The aim is to build rapport and trust, increase awareness and support, reduce misinformation, and reduce the fear that many health professionals have of engaging with the media. From 2010 to 2013, we conducted 5 workshops in 4 NTD endemic countries. Local communications companies and NGOs were involved in planning and organizing. Health professionals and journalists first worked separately, reviewing their own communications, advocacy and/or reporting work, to identify and develop key messages, and identify key audiences (public, donors, media). Health professionals practiced media interviews that were videotaped and played back with critiques. The 2 groups then engaged with each other, and went on a joint field visit. Health professionals and journalists began to appreciate each other as partners. The workshops and field visits led to several local publications about NTDs. Health professionals said that learning how to speak simply and clearly about NTDs was very useful. We are planning NTD communications workshops in additional NTD endemic countries. This model, and the lessons we learned during development, may be useful for other disease programs. We will create a toolkit that countries can use for advocacy and NTD communications plans, combined with training and/or remote technical assistance.

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ASSESSMENT OF SUPPLY CHAIN MANAGEMENT (SCM) SYSTEMS FOR NEGLECTED TROPICAL DISEASE (NTD) DRUGS IN CAMEROON, MALI, TANZANIA AND UGANDA

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The rapid expansion of NTD control activities has not been without pharmaceutical and health system challenges. The purpose of the four country assessment of the pharmaceutical and supply chain management of NTD control programs was to identify the gaps and make recommendations for strengthening the system. Qualitative and quantitative information concerning NTD medicine availability and management were collected using structured assessment tools, interviews of healthcare workers, and direct observations at sample sites at national, regional, districts and peripheral facilities. The questionnaires focused on availability of treatment guidelines, procurement, distribution, and stock management practices, as well as the adverse drug reaction reporting, disposal of expired products, reverse logistics challenges following MDA and integration with relevant national services and structures. Along the supply chain several NTD medicines were not documented in certain depots due to poor stock management practices. Donated NTD medicines sourced from the manufacturers makes quality issues less of a concern. However, there are concerns on the downstream quality of NTD medicines

as the medicines come in loose pills and are distributed from containers. The outdoor nature of the MDA's increases the chances of exposure of the medicines to humidity, heat, dust, and other unhygienic situations. NTD programs had difficulty getting information on the actual amounts of medicines distributed by the national medical store to the districts. Likewise, information on persons treated, doses dispensed and balance of stock medicine from MDA sites and districts is not always submitted on time and the quality of data not reliable, making planning and forecasting difficult. The assessment identified both strengths and weaknesses in the different aspects of NTD pharmaceutical management. Following the assessment, two post-assessment workshops were conducted for the purpose of disseminating the assessment report and to reach consensus with key stakeholders and partners on the way forward.

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SIGNIFICANT VARIATION OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS PREVALENCE ACROSS ECOLOGICAL AREAS IN NORTHERN BENIN: CALL FOR GENDER FOCUS AND LOCALIZED INTERVENTIONS

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Schistosomiasis and soil-transmitted helminthiasis (STH) infections are widespread in sub-Saharan Africa and constitute a public health problem in Benin, but limited data are available on prevalence among vulnerable populations. To address this gap, a survey was conducted to determine the prevalence of schistosomiasis and STH among schoolchildren from eight communes in northern Benin. Parasitological investigations were conducted in May 2013. Urine and stool samples were taken from 850 schoolchildren ages 8 to 14 years in 17 selected primary schools from eight communes within three ecological areas (Departments), using appropriate techniques, i.e. urine filtration and Kato-Katz. The results showed that *S. haematobium* was the most prevalent schistosome species (42.59%), followed by *Schistosoma mansoni* (2.24%). Hookworm was the most prevalent STH, with a mean prevalence of 20.71%, followed by *Ascaris lumbricoides* (3.76%) and *Enterobius vermicularis* (0.59%). There was a significant variation of schistosomiasis and STH prevalence across schools, and ecological areas. *S. haematobium* and hookworm were found in all communes surveyed, however *S. mansoni* was present in four communes with the highest prevalence in N'Dali (15.00%); *A. lumbricoides* was present in 3 communes and *E. vermicularis* was found only in the commune of Ouaké. Hookworm prevalence for males and females was high in the commune of Ouake (46.40% and 27.20% respectively) which also harbors a high prevalence of *S. haematobium* (58.40%). The moderate-to-high hookworm prevalence indicates a need for vigorous deworming campaigns with particular focus on women of child bearing age. Variation of schistosomiasis prevalence across villages in the same ecological zone (department) indicates the presence of highly focal schistosomiasis infection. This underscores the importance of localized interventions based on village-specific findings as opposed to extrapolating results from one village to the whole ecological zone.

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MULTIPLEX SEROSURVEYS AS A TOOL FOR INTEGRATED NEGLECTED TROPICAL DISEASES (NTD) SURVEILLANCE

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A key strategy for control and elimination of five Neglected Tropical Diseases (NTDs): lymphatic filariasis (LF), onchocerciasis, schistosomiasis, soil-transmitted helminthiasis (STH), and trachoma is preventive chemotherapy (PC) through mass drug administration (MDA). The impact of MDA is usually monitored by parasitological assessments or clinical examination. There is often geographic overlap of NTDs, and populations in these areas are regularly exposed to a variety of other diseases. Disease control programs require routine monitoring and evaluation (M&E), but coordinated M&E is rarely conducted and can be a challenge. Antibody-based tests may facilitate integrated M&E. A multiplex bead assay (MBA) that detects antibody against multiple antigens using a single blood sample has been developed. A total of 935 serum samples collected from individuals (1-85 years) living in communities in Mbita district, western Kenya were tested for antibody responses to 36 antigens covering a variety of diseases including all five PC NTDs. This area in western Kenya is highly endemic for *Schistosoma mansoni* and at variable risk for STH, but is not believed to be at risk for LF, onchocerciasis or trachoma. It is also an area of intense malaria transmission. Antibody responses to two *Schistosoma* spp. antigens were significantly associated with intensity of *S. mansoni* infection assessed by stool examination ($p<0.001$). Although antibody responses to a *Plasmodium falciparum* antigen were not associated with blood film results, MBA results indicated high levels of infection or exposure to *P. falciparum*, but very low levels of antibody to *P. vivax*. Minimal levels of antibodies were detected for LF, trachoma and onchocerciasis. Additionally, antibody responses to tetanus toxoid were low, indicating insufficient coverage of routine vaccinations. Preliminary multiplex results are consistent with traditional measures such as stool examination and indicate the use of MBAs has the potential to provide an integrated platform for M&E in complex public health settings.

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HEALTH SYSTEMS MODELLING - DEMONSTRATING THE POTENTIAL IMPACT OF DIAGNOSTIC AND TREATMENT INTEGRATION OF HUMAN AFRICAN TRYPANOSOMIASIS USING DIFFERENT HEALTH SYSTEM STRUCTURES

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Human African trypanosomiasis (HAT) is a Neglected Tropical Disease targeted for elimination. The declining prevalence of infection will change the demands on health systems to effectively detect cases. Detection of HAT currently relies on vertical surveillance programs where patients are identified in their villages and then required to travel long distances to HAT treatment centres (HTC). New diagnostics and interventions could change the future of service delivery of case detection and treatment; as local services would reduce out-of-pocket (OOP) expenditures and the inconvenience of travelling long distances. It is proposed that the integration of programs into the local health centres (LHC) could be modelled to forecast outcomes related to service delivery, patient accessibility, time spent in the system and resources used with current and new interventions. A discrete-event simulation (DES) health systems model has been developed using SIMUL8®. The model simulates patients' movement through the health system within a specified area. Different health system structures of both integrated (E.g. inclusion of local health centres) and non-integrated (E.g. vertical surveillance programs)

approaches were constructed in the model. Data from current and new diagnostic and treatments have been simulated through the model in order to measure the impact of switching from a non-integrated to integrated health system. Preliminary results suggest that integrated systems with new technologies will increase accessibility, decrease patient wait times but also require additional costs for training and for improving health infrastructures at the local level. An integrated health system could lead to improvements in coverage of treatment and reducing inequity in access to HAT treatment. While the initial additional costs of these interventions could be offset by savings in OOP payments, affordability to health systems should be carefully assessed. The analysis shows that health systems' modelling is an informative tool for investment decisions regarding an integrated approach.

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CO-ENDEMICITY OF *SCHISTOSOMA HAEMATOBIUM* WITH *S. MANSONI* AND SOIL TRANSMITTED HELMINTHS IN SOUTH NYANZA, WESTERN KENYA

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Multiple parasite infections are believed to increase susceptibility to coinfection with other parasites, playing a vital role in the development of morbidity. There is a paucity of information on *Schistosoma haematobium* and polyparasitism in south Nyanza region, western Kenya. This cross-sectional survey determined the co-endemicity and distribution of *S. haematobium* with *S. mansoni* and STHs (*Ascaris lumbricoides* and *Trichuris trichiura*) among 3,487 children aged 7-18 years in 95 primary schools in south Nyanza, western Kenya. Helminth eggs were analyzed from single urine (for *S. haematobium*) and stool (for *S. mansoni* and STHs) samples by centrifugation and Kato-Katz, respectively. Schools and water bodies were mapped using Geographical information system and prevalence maps generated using ArcView GIS Software. Overall, 91.7% (95% CI = 89.9-93.2%) children were infected with a single parasite species, while 7.9% (95% CI = 6.4-9.6%) and 0.5% (95% CI = 0.2-1.1%) children harbored dual and triple species infection respectively. Prevalence of *S. haematobium* and *S. mansoni* dual coinfection with any other parasites was 16.9% (95% CI =13. 3-21.4%) and 19.1% (95% CI =15.8-23.1%) respectively. Of the four parasite species, only *A. lumbricoides* infections were positively associated with both *S. mansoni* (P = 0. 0026) and *S. haematobium* (P = 0. 0295) infections. *S. haematobium* - *S. mansoni* coinfections occurred near Kayuka pond and Kamenya dam in Rachuonyo district, Katumo, Osani and Wachara pond in Homabay district, and along the Ongoche River in Migori district. The prevalence of *S. haematobium* monoinfection (7.2%, 95% CI = 6.4-8%), *S. mansoni* monoinfection (12.3 %, 95% CI = 10.4-12.5%) and *S. haematobium*- *S. mansoni* coinfection (1.2%, 95% CI = 0.9-1.6%) was highly skewed, with less than 10% prevalence was observed in 78%, 63% and 97% of all the schools respectively. There was no significant difference in infection intensity between mono and coinfections. Although the overall distribution and prevalence of *S. haematobium* - *S. mansoni* coinfections was generally low, understanding their geographical distribution is important especially for disease control programs.

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DEVELOPMENT OF A MARKOV TRANSITION PROBABILITY MODEL TO PREDICT CHANGES IN SCHISTOSOMIASIS INFECTION FOLLOWING TREATMENT

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The last decade has seen significant progress in the large-scale control of schistosomiasis and soil-transmitted helminth (STH) infections. Even greater expansion is required to achieve the coverage and morbidity reduction targets set for 2020. A crucial tool in this scale-up will be the ability to monitor the impact of control programmes. Specifically, being able to identify areas not responding to treatment as expected will allow adjustments to be made to the programme design and help ensure their longer-term success. However, to date, there are very few tools available that would allow the identification of such areas whilst at the same time being user-friendly. An STH Markov model developed recently at the World Health Organization (WHO) used data from Vietnam to predict changes in STH prevalence following successive rounds of deworming treatment. In addition, a user-friendly interface was also developed to help ensure the model is used as widely as possible by programme managers. Data collected by the Schistosomiasis Control Initiative and its country partners from several countries in sub-Saharan Africa have enabled the validation of this model for STH infection, its extension to include schistosomiasis infection, and the addition of robust confidence intervals around the predicted changes in prevalence. It is hoped that the output of this model could potentially provide an early warning of where treatment campaigns are not achieving their aims (for example due to poor coverage, adherence, or putative resistance) and enable programme managers to make the necessary changes to meet the expected targets. The performance of the model will be discussed, with particular reference to the utility of stratifying the model outputs by parasite species, location, underlying endemicity, and host age. In addition, we will discuss the results of a model comparison exercise between the predictive capacity of the Markov model and other models currently available.

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FEASIBILITY AND ACCEPTABILITY OF INTEGRATING FACE WASHING MESSAGES INTO ONGOING HAND WASHING CAMPAIGN FOR THE CONTROL OF NTDS: LESSONS FROM A SCHOOL-BASED PROGRAM IN TURKANA, KENYA

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Hand-washing is a key intervention in the prevention of a number of infectious diseases, including some Neglected Tropical Diseases (NTD). Improvements in facial cleanliness are also an important aspect of trachoma control, as outlined in the WHO endorsed SAFE strategy and both need to be delivered at scale in order to achieve the elimination of blinding trachoma by 2020. However, little is currently known about the best approach to improve face-washing practices and whether it is practical to integrate face-washing messages into current large scale hand-washing campaigns. This presentation aims to highlight the lessons learnt from the design and testing of an integrated face and hand-washing school based behaviour change campaign implemented in a water poor environment in Loima County, Turkana, Kenya. The intervention is an interactive school-based behaviour change programme integrating face washing messages into an on-going hand-washing campaign, delivered jointly by Sightsavers, Unilever and the London School of Hygiene and Tropical Medicine. The presentation will focus on the use of formative research in the design of face-washing messages and materials and on the assessment of their feasibility and acceptability to the local communities. The assessment of the pilot project will apply qualitative methodologies, including observations of hygiene practices both in school and in the community, participatory focus group discussions with children, caregivers and teachers and semi-structured interviews with key stakeholders. We will also present how the findings from this pilot work will be used to modify and scale up the proposed intervention across other NTD-endemic regions of Kenya and how we will evaluate its impact on improving and sustaining face-washing practices in these communities.

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EVALUATION OF INTEGRATED MASS DRUG ADMINISTRATIONS FOR NEGLECTED TROPICAL DISEASES IN MADAOUA DISTRICT, NIGER

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Neglected Tropical Diseases (NTDs) are a group of debilitating illnesses that affect the lives of more than one-sixth of the world's population. Preventive chemotherapy partnered with health education is the primary strategy in the control and elimination of NTDs. In December 2012, the Niger Ministry of Health carried out an integrated mass drug administration (MDA) with the goal of eliminating lymphatic filariasis (LF) and trachoma and controlling soil-transmitted helminthiasis (STH) and schistosomiasis. Six months after the MDA, a coverage and knowledge, attitudes and practices (KAP) survey was carried out in the Madaoua district, Niger using the WHO-recommended two staged probability cluster survey design. In each of the selected households, coverage data were collected from all persons, while the KAP survey was administered to one randomly-selected adult (≥14 years). A total of 293 households in 30 villages participated in the surveys, with 1711 persons interviewed for coverage and 291 adults for KAP. Overall, 80.2% (95% CI: 78.2-82.1) of those surveyed reported taking at least one medication; 75.5% of men and 83.7% of women reported taking a medication during the 2012 MDA. Surveyed coverage of Ivermectin (60.0%; 95% CI: 57.1-62.9), albendazole (71.3%; 95% CI: 69.0-74.3) and praziquantel (65.6%; 95% CI: 62.8-68.4) was significantly lower than the reported coverage (96.9%, 96.9%, and 86.0%, respectively), while the reported coverage for azithromycin (72.2%) was confirmed by the survey (71.8%, 95 CI: 69.3-74.1). KAP respondents reported that they had heard of LF (66.0%), STH (93.8%), schistosomiasis (72.2%) and trachoma (86.6%), but only 24.0%, 51.8%, 56.2% and 57.9%, respectively, knew at least one symptom. Of 46 respondents who had heard of LF, only 2 respondents (4.3%) knew it was transmitted via a mosquito, and of those who had heard of schistosomiasis, 70.9% believed that one is infected by the sun or heat. There was no significant association between participation in the MDA and knowledge of the NTDs. Knowledge of someone with a NTD did increase the odds of participation: men who knew someone with hydrocele were 4.3 times (95% CI: 4.1-4.4) more likely to take medication. MDA participation appeared not to be affected by the low level of knowledge. With the exception of Ivermectin, drug-specific coverage was adequate.

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DETERMINING WHERE TO MAP FOR TRACHOMA: LESSONS FROM UGANDA'S CLASSIFICATION TOOL

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Trachoma (Chlamydia trachomatis) is the leading cause of preventable infectious blindness. Ten million people are at risk in 36 of Uganda's 112 districts, with 1.1 million people estimated to be infected. After mapping all highly-suspected districts, the National NTD Control Program turned their focus on prevalence surveys in districts that shared a border with known endemic districts. Population-based prevalence survey (PBPS) is the WHO-recommended methodology for trachoma baseline surveys, but cost, time, and logistics prohibit execution of these surveys. In Uganda, the estimated cost of a two-week PBPS is $11,000 per district. Instead of immediately conducting PBPS, the national program used a trachoma classification tool in 8 districts in Eastern Region in order to prioritize where a PBPS should be conducted. Open-ended interview guides were developed to conduct key informant interviews at regional and district health facilities. A tool was developed in line with the Health Management Information System (HMIS) health unit database to collect clinical record data. Districts were classified as suspected if they met each of the following criteria: more than 10 cases of TT reported per year for two or more years; at least 3 health staff at 5 or more health facilities identifying TT as a problem in the catchment area; a shared border with an endemic district (TF in children 1-9 years old ≥10%). In each district, the classification tool cost $1000 per district and took 3 days for a team of 4 people to complete the methodology. Two districts met all three criteria and were classified as suspected; these districts were prioritized for PBPS. Two more districts showed evidence of being endemic for trachoma. A recommendation was made to conduct PBPS in these four districts to validate the methods used in the classification tool. Final analysis is expected in May 2014. In districts with suspected low TF prevalence, this trachoma classification tool offers programs a simple way to prioritize districts in which to conduct the more resource-intensive PBPS.

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MARKED CHANGES IN MATERNAL PARASITIC INFECTIONS IN KWALE COUNTY (2006-2014)

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In the developing world, maternal pre-natal parasitic infections can place a high burden on pregnancy outcomes, including potential adverse effects on neonates. We evaluated changes in malaria, *Schistosoma haematobium*, hookworm, and *Trichuris trichiura* prevalence in two serial cohorts in the same population. One cohort was recruited in between 2006 and 2009 and the other between 2013 and 2014. Pregnant women were recruited from the antenatal clinic (ANC) at Msambweni District Hospital in Kwale County. For both cohorts, maternal venous blood was drawn at first ANC visit and maternal peripheral, cord, and placental blood were examined at delivery for malaria parasites by light microscopy. Maternal stool and urine samples were collected at the first ANC visit and at delivery. Stool samples were tested for hookworm and *T. trichiura* infections using Ritchie's concentration method. Urine was evaluated for presence of *S. haematobium* using Nuclepore filtration. Among the 4 infections evaluated, only malaria prevalence was observed to have increased both at ANC enrollment (60%) and at delivery (50%) during the interval between studies. By 2013-14, *T. trichiura* infection prevalence had increased at ANC enrollment (36%) but was decreased at delivery (72%). *S. haematobium* and hookworm prevalence decreased by 30% and 80%, and by 60% and 30%, respectively, at ANC enrollment and at delivery. The increased prenatal malaria burden was unexpected and may be an indicator of operational failures of the current malaria control efforts. On the contrary, the current soil transmitted helminths (STH) control efforts seem to be bearing fruit. This decline STH infection may be attributable to the ongoing national school-based de-worming programme. Similar population-based studies on parasitic infections are necessary for monitoring and evaluation of the current control measures.

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ANTIBODY-BASED MULTIPLEX TESTING AS A PLATFORM FOR INTEGRATED SURVEILLANCE OF NEGLECTED TROPICAL DISEASES AND OTHER INFECTIONS OF PUBLIC HEALTH INTEREST

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Neglected Tropical Diseases (NTDs) are debilitating parasitic and bacterial diseases that affect 1.4 billion of the world's poorest people. Several NTDs are targeted for global elimination and control through programs centered on mass drug administration (MDA). An integrated surveillance strategy would be valuable to assess infection prevalence and program impact in areas of geographical overlap of NTDs. To investigate the utility of multiplex antibody assays for integrated disease and program surveillance, sentinel sites were established in two NTD-endemic, treatment-naïve communities in Morrupula district, Mozambique. Baseline serologic, parasitological and clinical indicators were assessed in a sample of individuals aged >12 months (n=1423) in 2013 prior to the first NTD treatment. Preliminary analysis has been conducted on 302 serologic samples that were tested by multiplex for antibody responses to a panel of 36 antigens for NTDs, malaria, water-borne and vector-borne parasites. Multiplex results demonstrate antibody responses against multiple NTDs and malaria, confirming the high prevalence of lymphatic filariasis (LF), *Schistosoma haematobium*, trachoma, *Ascaris lumbricoides* and *Plasmodium falciparum*. Antibody responses to antigens for LF (Wb123, Bm14, Bm33NS), trachoma (pgp3, CT694), and malaria (Pf MSP-1) were correlated with results from conventional diagnostic tests. Antibody responses in children to LF antigens were detected before ICT and microfilaria (Mf) responses, indicating that antibody provides a more sensitive measure of exposure. Multiplex analysis additionally provided novel observations on diseases that were not assessed using conventional tests and on which little or no data exist. High levels of reactivity to a number of vector-borne or water-borne pathogenswere detected. From these preliminary baseline analyses, we believe that antibody-based multiplex tests can provide important epidemiologic information about disease burden and actionable disease surveillance data for national health programs that includes but is not limited to data on NTDs.

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A SENSITIVE AND REPRODUCIBLE *IN VIVO* IMAGING MODEL IN MICE FOR LATE STAGE HUMAN AFRICAN TRYPANOSOMIASIS FOR EVALUATION OF ANTI-TRYPANOSOMAL DRUGS

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In the past decade 3 pre-clinical murine models have been used for the evaluation of new drugs for stage II human African trypanosomiasis (HAT) involving the parasites *Trypansoma brucei brucei* GVR35 and AnTat1 and *T. brucei rhodesiense*. Although there are currently two novel drugs in clinical trials, there remains a need for proven treatments with potency, pharmacokinetic and safety profiles that are required for new HAT chemotherapy, not to mention a short treatment regimen. Incomplete knowledge of the metabolic status and drug sensitivity of trypanosomes in the central nervous system (CNS), and the long murine CNS model has hindered drug development. Here we report the generation of highly bioluminescent parasites and their use in an *in vivo* imaging model of stage II African trypanosomiasis. Bloodstream forms of the chronic model strain GVR35 were transformed with a construct designed to express "red-shifted" luciferase. Using the standard 21 day treatment model in CD1 mice, we were able to identify CNS infection after treatment with berenil. By using the bioluminescence model in a drug relapse experiment with a stage II drug, early relapse could be identified when no peripheral blood parasitaemia could be detected. We provide evidence that the model can be used to reduce the current 180-day experiment and also provide dose-response data.

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THE EFFECT OF CIVIL WAR ON CUTANEOUS LEISHMANIASIS ("ALEPPO BUTTON") IN ALEPPO CITY, SYRIA

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In the ancient northern Syrian city of Aleppo, CL has been present for hundreds of years (if not longer), where it is known as the "Aleppo evil", "Aleppo ulcer", "Aleppo boil", or "Aleppo button" which is Cutaneous Leishmaniasis. Aleppo ulcer is a disfiguring condition that disproportionately occurs on the face, especially of young people. It typically lasts one or 2 years before the lesion heals spontaneously, and is often known locally as "one-year sore". However, in many cases specific anti-parasitic chemotherapy can hasten the healing process and improve clinical and cosmetic outcomes. A major problem with one-year sore is that the scar can produce permanent disfigurement of the face. It is well known about the rise and fall and then a rise again in the incidence of the disease in the city of Aleppo. During the 1950s the number of cases of CL fell after an insecticide campaign aimed at controlling malaria, but it then rose again during the 1960s. However, CL was mostly controlled during the 1980s.There is no doubt that the areas of Syria affected by the civil war are experiencing an increase in cutaneous leishmaniasis, and this will also be seen in the refugee camps in Jordan and Turkey. This is due to garbage collection, open sewage, and poverty which promote the habitats of Phlebotomus_ sandflies that transmit CL. Interestingly, a clinical trial conducted prior to the current civil conflict found that use of insecticide-treated bednets (ITNs) could prevent CL in Aleppo. Recently, WHO reports out of Syria indicate the emergence of epidemic cutaneous leishmaniasis in the besieged city of Aleppo, adding further to the misery there, perhaps the international community needs to focus on refugees and refugee encampments to ensure local control and patient access to treatments.

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TRIPLEX METHODOLOGY (FC-TRIPLEX-IGG1) FOR THE DIFFERENTIAL DIAGNOSIS OF CHAGAS DISEASE, LOCALIZED CUTANEOUS LEISHMANIASIS AND VISCERAL LEISHMANIASIS

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Conventional serological tests for Chagas disease (CD) are routinely used for blood banks screening. However, non-negative result for CD screening in blood banks does not guarantee the presence of *Trypanosoma cruzi* infection, since other infectious diseases, such as Leishmaniasis (LEISH) can contribute to the occurrence of false positives results. In this work, we present a nonconventional serological approach for the simultaneous detection of anti-*T. cruzi,* anti-*Leishmania chagasi* and anti-*L. braziliensis* IgG1, using a differential Alexafluor647-fluorescent parasite staining in a single flow cytometry platform. The method, named FC-TRIPLEX Chagas/Leish IgG1, uses the percentage of Phycoeritrin-fluorescent positive parasites (PPFP) as indicative of seropositivity upon the use of specific cut-off edges. The method is based on an inverted detuned algorithm applied starting with the analyses of anti-*L. chagasi* positivity at 1:32,000 (PPFP>60%) to define the diagnosis of Visceral Leishmaniasis (VL). Samples

with anti-*L. chagasi* reactivity <60% guide to the next algorithm step consisting of the analysis of anti- *T. cruzi* positivity at 1:2,000 (PPFP>50%) defining the diagnosis CD. Samples with negative anti- *T. cruzi* reactivity (PPFP<50%) are forwarded to the final algorithm step with the analysis of anti-*L. braziliensis* reactivity at 1:1,000 with PPFP>60% defining the Localized Cutaneous Leishmaniasis (LCL) diagnosis and PPFP<60% excluding these three Trypanosomatidae infections. A proof concept carried out using a range of well characterized sera samples from VL, CD and LCL showed correct results in 76 out of 80 tested samples reaching outstanding global accuracy and 95% of overall performance of FC-TRIPLEX Chagas/Leish IgG1 array. The occurrence of 5% of incorrect diagnosis (false negative results in 1/20 CD and 1/20 LCL and false positive results in 2/20 non-infected carriers) underscore the remarkable performance of the methodology. Alexafluor-647 differential brightness as well as the parasite antigenicity were preserved up to one year at distinct storage conditions (RT, 4°C and -20°C). In conclusion, our data suggest that the outstanding performance of FC-TRIPLEX Chagas/Leish IgG1 array for the differential diagnosis of CD and LEISH shall contribute to the elucidate the false positives results frequently observed in conventional tests currently used for serological screening in blood banks.

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DEVELOPMENT OF ORAL EFLORNITHINE CHEMOTHERAPY FOR HUMAN AFRICAN TRYPANOSOMIASIS

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Human African trypanosomiasis (HAT), a neglected tropical disease, threatens millions of people in sub-Saharan Africa. Without treatment, sleeping sickness is fatal. The majority of cases are diagnosed during the second stage of disease, after parasites (*Trypanosoma brucei gambiense* and *T. b. rhodesiense*) have crossed the blood-brain barrier and infected the central nervous system. Current eflornithine-based chemotherapies for second stage HAT are unsatisfactory, due to a complex regimen of intravenous infusions required to overcome inadequate oral bioavailability and poor BBB permeation. We hypothesized that an intercellular junction-modulating peptide ECP1 can improve the BBB permeation and gut absorption of eflornithine. ECP1 was relatively stable ($t_{1/2}$>5.5 h) after a 4-h incubation at 37°C in the simulated gastric fluid with pepsin, in the rat small intestinal mucosal scrapings and in the rat plasma. Using MDCK cell monolayers, ECP1 (1.0 mM) increased eflornithine permeability by 5-fold, whereas a scrambled peptide (ECP1scr) did not have any effect. In rats, co-administration of ECP1 (50 mg/kg) with eflornithine (100 mg/kg) orally increased plasma C_{max} and AUC of eflornithine by 40-60%, compared to vehicle or ECP1scr. Furthermore, using *in situ* rat brain perfusion, ECP1 (1.0 mM) increased the concentrations of eflornithine in various parts of the brain (e.g., cerebellum, hippocampus, frontal cortex and choroid plexus) by 85-390%. Further experiments are currently underway to determine eflornithine brain concentrations after co-administration with ECP1 orally in rats and to demonstrate that co-administration of ECP1 could improve the oral efficacy of eflornithine in rodent models of first and second stage HAT. If successful, oral ECP1-eflornithine formulation could positively impact the elimination of HAT.

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THE EFFICACY OF THREE COMPOUNDS ISOLATED FROM CVP005B LEAVES AGAINST LABORATORY AND FIELD STRAINS OF *LEISHMANIA* SPP.

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Leishmania spp. are parasitic protozoans that cause Leishmaniasis which is characterized by disfigurement, morbidity and mortality. Chemotherapy, the main form of control is undermined by toxic effects of available drugs and emerging drug resistance. The use of traditional medicine to treat infections is very common in Africa. CVP005B is one of the popular medicinal plants in West Africa. Although several research groups have reported on CVP005B to have anti-protozoa (e.g. Trypanosome and Leishmania) properties, no compound(s) have been assigned the responsibility for this activity. Our research group first identified novel compounds, ML-2-2, ML-2-3 and ML-F52, active against trypanosome by *in vitro* assay-guided purification from CVP005B leaves. These compounds share the same side chain but two of them, ML-2-2 and ML-F52, have the same functional group. This study was therefore aimed at finding out if these anti-Trypanosoma active compounds also possess anti-Leishmania properties. ML-2-2 and ML-F52 were found to have anti-Leishmania activities, by microscopic observation, with Minimum Inhibition Concentration (MIC) values of 2.87 µM and 2.87 µM respectively, while ML-2-3 had no activity on Leishmania culture for up to 72 hrs incubation. Comparison of the efficacies of these compounds against field strains of *Leishmania* showed MIC values of 4.17 µM and 2.60 µM for ML-2-2 and ML-F52 respectively. These data suggest that the functional group of ML-2-2 and ML-F52 might be crucial for their activity against *Leishmania*, and that could be effective against diverse *Leishmania* field strains. Investigations are underway to determine the molecular mechanisms of the activities using FACS analysis, Immunohistochemistry and Western Blotting.

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NOVEL COMPOUNDS ISOLATED FROM CVP005B LEAF EXTACT SHOW STRONG ANTI-TRYPANOSOMAL ACTIVITY *IN VITRO*

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African Trypanosomiasis, a devastating disease in Africa, is caused by the kinetoplastid parasite, *Trypanosoma brucei* sp. Due to drug inefficacy against the late stage of Human African Trypanosomiasis, toxicity and resistant parasites, development of novel chemotherapy is urgently needed. Africa has a long history of the use of traditional medicinal plants and the WHO reports about 80% of people relying on traditional medicines as their first-line treatment. CVP005B, a famous and one of the main medicinal plants in use in West-Africa, has previously been reported to have anti-trypanosomal activity by several research groups, though

active compounds were still unknown. This study identified compounds responsible for anti-*Trypanosoma* activity in CVP005B. Bioassay-guided fractionation and purification of CVP005B crude extract led to the isolation of 3 novel active compounds, "ML-2-2", "ML-2-3" and "ML-F52". They shared the same side chain and two of them had same functional group. FACS analysis of the Nexin assay revealed that ML-2-3 and ML-F52 induced apoptosis in *T. b. brucei* (GUTat3.1 strain), whereas ML-2-2 did not. FACS analysis of Multi-caspase assay in ML-2-3-treated trypanosomes also indicated the involvement of the caspase cascade in apoptosis signaling in trypanosomes. Cell cycle assay revealed alteration in G2/M phase in ML-2-3-treated parasites. Further investigation into the phenotypic differences in ML-2-2-, ML-2-3- and ML-F52-treated trypansomes by immunohistochemistry and western blot analysis using α-tubulin antibodies and flagellum marker, PFR-a, showed: nucleus fragmentation only in ML-2-3-treated trypanosomes which is a marker for apoptosis and could be a confirmation of the Nexin assay results. ML-2-3 and ML-F52 suppressed the expression of both α-tubulin and PFR-a in parasites, while ML-2-2 showed no effect. ML-2-3 and ML-F52, so far show very promising prospects for development of new anti-trypanosomal drugs whilst, ML-2-2 may be investigated for its usefulness for other scientific purpose(s).

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IN VITRO ACTIVITY OF NATURAL PRODUCTS AGAINST *LEISHMANIA* AND *TRYPANOSOMA CRUZI*

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In Brazil, the tropical neglected diseases leishmaniasis and Chaga's disease are causes of great impact on public health. The drugs currently used to treat these conditions have limitations concerning cost, efficacy and safety, making the search for new therapeutic approaches urgent. Natural products (NP) and their derivatives are important sources of new molecules that can be used for drug development. In the search for new leishmanicidal and tripanocidal drugs, we have carried out a screening for *in vitro* activity of various NP and two naphthoquinones stood out: eleutherine and naphthothiophenquinone. To evaluate the leishmanicidal effect we used THP-1-derived macrophages infected with *Leishmania amazonensis*. After incubation, infected cells were stained with hematologic dye and macrophages were counted to assess the percentage of infection. Amphotericin at 1.08μM was used as control. For the tripanocidal assay we used cultured mouse fibroblasts infected with *Trypanosoma cruzi* Tulahuen strain transfected with the β-galactosidase gene. After incubation, the enzyme substrate was added and the absorbance was measured at λ_{570}nm. Benznidazole was used as control at 3.81μM. The toxicity of the compounds was evaluated in THP-1 cells using alamarBlue™. Eleutherine reduced *Leishmania* infection to 4.5% at 70μM and *Trypanosoma* infection to 49% at 140μM. It decreased cell viability by 2% at 140μM. Naphthothiophenquinone showed a strong leishmanicidal activity, reducing the infection to 2.5% at 93μM. The tripanocidal activity was already described. It decreased cell viability by 16% at 196μM. In conclusion, eleutherine and naphthothiophenquinone are better leishmanicidal than tripanocidal agents. Moreover both compounds showed low toxicity to the THP-1 cells. The next steps are to investigate the cellular targets and mechanisms of action of these NP.

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PCR-BASED DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN TWO ENDEMIC VILLAGES FROM PERU AND BOLIVIA: AN ARTICULATED STRATEGY FOUNDED ON LOCAL HEALTH PROMOTERS AND CONVENTIONAL SAMPLE SHIPPING

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Diagnosis of cutaneous leishmaniasis (CL) is critical for appropriate management since confirmatory diagnosis and identification of causative species are recommended due to differences in parasite pathogenicity and drug sensitivity. Smear examination has a poor sensitivity even under expert examiners whereas identification of species is nowadays impossible in rural conditions. We implemented an articulated strategy for PCR diagnosis based on samples obtained and shipped by local health promoters (LHP) in two villages from Peru and Bolivia. LHP normally obtaining smears were trained to obtain samples using cytology brushes and apply and interpret Leishmanin skin test (LST). Smear examination was performed following local procedures whereas two clinical samples (brushes and lancet scrapings) were collected, preserved in absolute ethanol and shipped at environmental temperatures to reference centers for further PCR. A lesion was defined as CL if two of the following procedures were positive: smear, LST, or PCR on lancets. One hundred and twelve individuals were enrolled from whom 115 lesions were sampled. Sixty nine lesions fulfilled definitive CL criteria whereas 44 were positive by smear examination, 61 by LST, 63 by PCR of lancets, and 67 by PCR of cytology brushes. Sensitivity and specificity under ROC curve analysis were 64% and 89% for smear, 91% and 80% for LST, 91% and 93% for PCR on lancets, and 97% and 85% for PCR on cytology brushes. PCR in non-invasive samples was the diagnostic procedure with the highest sensitivity and specificity in comparison to smear examination and LST. This procedure has been demonstrated to be a valid diagnostic tool in controlled and real conditions with the additional potential to be easily performed by minimally trained personnel. Our strategy including LHP can be an interesting alternative to replace conventional diagnostic methods with the additional advantage of identifying causative species and establishing the geographic distribution of Leishmania parasites in endemic regions of Latin America.

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NATURAL PRODUCTS DRUG DISCOVERY FOR TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS: NEW DRUG LEADS FROM A NATURAL PRODUCTS FRACTIONS LIBRARY

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Human African Trypanosomiasis (HAT) or sleeping sickness is caused due to infection with Trypanosoma brucei. Current drugs for treatment of HAT suffer from severe toxicities and require intramuscular or intravenous

administrations. Situation is further aggravated due to emergence of drug resistance. There is an urgent need of new drugs effective orally against both stages of HAT. Natural products offer an unmatched source for bioactive molecules with new chemotypes. However, natural product extracts present several challenges with respect to modern drug discovery programs. Polyphenols (vegetable tannins), often present in considerable quantities in ethanol extracts of plants, can cause false-positive results in both enzymatic and cellular screening procedures due to non-selective enzyme inhibition and changes in cellular redox potential. The chemical diversity found in a single extract may represent several different classes of molecules that exhibit different (and sometimes opposing) biological activities. The biologically active compounds may be present in crude extracts at extremely low concentrations, below the detection threshold for bioactivity screening. A high throughput fractionation of natural product extracts was done to encounter these problems. A library of >60,000 natural product fractions has been generated through a high throughput fractionation paradigm. Total 7379 fractions from 510 plant extracts were screened *in vitro* in both Trypanosoma brucei assay and cytotoxicity assay using differentiated THP1 cell lines. 454 active fractions were identified with more than 50% inhibition and subjected to dose-response evaluation. 285 anti-trypanosomal fractions were confirmed with IC50 values of <10 µg/mL: 22 with IC50 < 2µg/mL, 105 with IC50 between 2 and 5µg/mL and 158 with IC50 between 5 and 10µg/mL. Only 13 fractions showed toxicity against THP1 cells. The most active fractions namely Ledum groenlandicum c2 (0.75 µg/ml), Hippeastrum reticulatum c3 (0.9 µg/ml), Psychotria berteroana c4(0.98 µg/ml), Muntingia calabura c7(0.57 µg/ml), Eucalyptus robusta c3(0.92 µg/ml) and Myrsine coriacea c14(0.84 µg/ml) were further analyzed for QC-UPLC-MS/MS data and showed several new antitrypanosomal drug leads with novel pharmacophores.

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LEISHMANIASIS IN SURINAME - NEW INSIGHTS INTO A NEGLECTED DISEASE

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According to text books, leishmaniasis manifests in Suriname as cutaneous leishmaniasis (CL) caused by *Leishmania guyanensis*, which can be adequately treated with pentamidine. Sand fly vectors and possible reservoir are not well known. A Suriname - Netherlands research consortium studies in an integrated approach several aspects of the disease in order to gain more in depth knowledge of CL in the country and to contribute to a control programme for leishmaniasis in Suriname, which is currently not available. The research programme comprises three projects: 1) Biological aspects of parasite and vector; 2) Clinical aspects of CL, and 3) Medical anthropology. Biological research has revealed that, next to *L. guyanensis*, at least three other species are present in Suriname, including the muco-cutaneous leishmaniasis-(MCL-)causing *L. braziliensis*. Medical doctors (also in non-endemic countries!) treating cases from Suriname for CL must be aware that next to CL, also MCL could be contracted. This finding has therapeutic implications since the first-line recommended treatment for *L. braziliensis* infections is not standard in Suriname. Furthermore, at least three, for Suriname new, sand fly species have been identified, and molecular analysis revealed that these can be infected with *Leishmania*. Reservoir studies on dogs are on-going. Clinical research has demonstrated that pentamidine may not be efficacious for all cases of CL, as around 25% cases of treatment failure are observed, and alternative treatment regimens are being explored. Effect of treatment can be well monitored over time by using a recently developed RT-PCR method that can predict treatment outcome. There is an excellent correlation between parasite load at week 6 and treatment outcome at week 12 after initiation of treatment. Medical anthropology revealed that stigmatization of infected individuals may not be a major problem in the social acceptability of the disease in Suriname, in contrast to other countries. Many non-conventional methods, including the use of dangerous chemicals, are practiced to treat CL, in particular in the interior of Suriname. Reasons for failing treatment adherence are being studied and will be presented.

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A FULLY INTEGRATED PARTNERSHIP PERFORMING DRUG DISCOVERY TOWARDS VISCERAL LEISHMANIASIS: PART 2

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GSK Kinetoplastid DPU and University of Dundee with support from the Wellcome Trust have formed a 5 year partnership to conduct drug discovery within kinetoplastid diseases (Visceral Leishmaniasis, Chagas disease and Human African Trypanosomiasis). This collaboration has made significant progress in the first 3 years, which has resulted in the identification of a lead optimisation series for Visceral Leishmaniasis through phenotypic screening. It is estimated that Visceral Leishmaniasis causes over 20,000 deaths per year world-wide. Current drugs suffer from multiple issues such as lack of efficacy and unacceptable levels of toxicity. Part 1, by Paul Wyatt from Dundee University, will describe work that resulted in the identification of a series that fulfils lead optimisation criteria for Visceral Leishmaniasis. This novel series is one of the few reported globally to show oral efficacy in an acute *in vivo* mouse model against Visceral Leishmaniasis. Part 2, by Tim Miles from GSK, will concentrate on the lead optimisation and progression of this series. As a number of issues were highlighted through critical path screening that have been overcome (*i.e.* solubility and exposure). Hence a discussion of medicinal chemistry strategies to solve these issues within a phenotypic screening setting will be discussed. The current set lead compounds within this series are being evaluated for pre-candidate selection.

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SPECIES DISCRIMINATION BETWEEN *LEISHMANIA PANAMENSIS* AND *L. GUYANENSIS* FROM CLINICAL SAMPLES OF COLOMBIAN PATIENTS VIA PCR-RFLP

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The discrimination between two genetically related *Leishmania* species, e. i. *L. panamensis* and *L. guyanensis*, has been accomplished by the amplification of a region of the gene coding for the heat shock protein 70kDa (Hsp70), followed by restriction fragment length polymorphism (RFLP) assays using the BccI enzyme. Here we determined the efficiency of this experimental approach for gene amplification and species discrimination, from isolated parasites as well as clinical samples. We included reference strains, 30 parasites isolates from patients and 30 clinical samples from needle aspirates, dermal scrapings or skin biopsies obtained from patients diagnosed for cutaneous or mucocutaneous leishmaniasis. *Leishmania* parasites isolated from patients and clinical samples were previously identified as belonging to the *L. guyanensis* complex (*L. panamensis*/*L. guyanensis*), by a PCR-RFLP assay using the gene encoding mini-exon sequence and the restriction enzyme HaeIII. The 30 isolated *Leishmania* were further identified by isoenzyme analysis. A 1422bp sequence within the Hsp70 gene was amplified using the following primers: (Forward 5'-GACGGTGCCTGCCTACTTCAA-3') (Reverse 5'-CCGCCCATGCTCTGGTACATC-3'). Amplification reaction mixtures where then treated with BccI restriction enzyme for RFLP analysis. The positive reaction was obtained in 100% of the cultured parasites and in 30% of the clinical samples. Each amplicon showed either *L. panamensis* or *L. guyanensis* RFLP pattern and there was a 100% correspondence

between isoenzyme and PCR-RFLP analyses. Even though these results confirm the ability of this technique to discriminate between the two species, it also suggests that the amplification efficiency from clinical samples is limited. This could be due to the low parasite load typical of clinical samples especially in cases of mucocutaneous Leishmaniasis or the size of the amplicon. Given the clinical importance of these two *Leishmania* species discrimination, further optimization of the technique should be pursued.

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ACCURATE DIAGNOSIS OF SLEEPING SICKNESS BY TARGETING THE TRYPANOSOME'S SPLICED LEADER RNA

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Trypanosomatids transcribe their genes in large polycistronic clusters that are further processed into mature mRNA molecules by trans-splicing. During this maturation process a conserved spliced leader RNA (SL-RNA) sequence of 39 bp is physically linked to the 5-prime end of the pre-mRNA molecules. Among the trypanosomatids are three major human pathogens: *Trypanosoma brucei*, *T. cruzi* and *Leishmania*. Their trans-splicing mechanisms have been extensively investigated for understanding eukaryotic cell biology but the SL-RNA has never been explored as a diagnostic target, although this molecule has several attracting diagnostic features. It is a short non-coding RNA sequence that is conserved but unique for each species and is present in each mRNA molecule in the cell. Importantly, mRNA is considered as the best surrogate marker for viable organisms. In this study, we investigated this SL-RNA molecule for its diagnostic potential using reverse transcription followed by real-time PCR. As a model we used *T. b. gambiense* that causes sleeping sickness in west and central Africa. We showed that the copy number of the SL-RNA molecule in one single parasite is at least 8600. The lower detection limit of the SL-RNA assay in spiked blood samples was 100 trypanosomes per mL of blood and in the same range as for DNA based tests. We also showed that we can detect the trypanosome's SL-RNA in the blood of sleeping sickness patients recruited in Guinee with a sensitivity of 92% (95% CI: 78%-97%) and a specificity of 96% (95% CI: 86%-99%). For the first time, we explored the SL-RNA as a molecular target for next-generation diagnostics in diseases caused by trypanosomatids. Evaluation of the assay for the assessment of cure after treatment of sleeping sickness is ongoing. We will present the design of the SL-RNA assay, the experimental proof of concept and the accuracy of the test for sleeping sickness diagnosis and cure assessment.

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RETINAL CHANGES IN VISCERAL LEISHMANIASIS

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In visceral leishmaniasis (VL), retinal changes have previously been noted but not described in detail and their clinical and pathological significance are unknown. A prospective observational study was undertaken in Mymensingh, Bangladesh aiming to describe in detail visible changes in the retina in unselected patients with visceral leishmaniasis. Patients underwent assessment of visual function, indirect and direct ophthalmoscopy and portable retinal photography. The photographs were assessed by masked observers including assessment for vessel tortuosity using a semi-automated system. 30 patients with VL were enrolled, of whom 6 (20%) had abnormalities. These included 5 with focal retinal whitening, 2 with cotton wool spots, 2 with haemorrhages, as well as increased vessel tortuosity. Visual function was preserved. These changes suggest a previously unrecognized retinal vasculopathy. An inflammatory aetiology is plausible such as a subclinical retinal vasculitis, possibly with altered local microvascular autoregulation, and warrants further investigation.

1102

EVALUATION OF THE POINT OF CARE TEST INBIOS CHAGAS DETECT PLUS IN BOLIVIA

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Trypanosoma cruzi causes Chagas disease, which affects an estimated 7-8 million people. Chagas disease is endemic throughout Latin America, with the highest prevalence in Bolivia. Conventional diagnosis requires an experienced laboratory. We evaluated the Chagas Detect Plus (CDP) kit (InBios, Seattle WA), a rapid immunochromatographic assay for antibodies to T. *cruzi*, in both hospital and community settings. Performance of CDP was compared to conventional diagnostic tests including indirect hemagglutination assay (IHA), immunofluorescent antibody test (IFA), and enzyme-linked immunosorbent assay (ELISA). Confirmed infection required positive results by at least 2 conventional assays. Specimens from 3 studies in Bolivia were used: prospectively evaluated specimens from a study of congenital Chagas disease in Camiri Municipal Hospital (n=277) and a hospital-based study of cardiac biomarkers in Santa Cruz (n=108), and archived specimens from a community study in endemic villages of Gutierrez municipality (n=200). CDP was performed in finger stick blood and serum from 385 individuals, and in 200 archived serum specimens from the community study. CDP showed sensitivity / specificity of 96.2% [92.7-98.4] / 98.8% [95.9-99.9] in whole blood, and 99.3%

[97.5-99.9] / 96.9% [94.2-98.6] in serum. There were no differences by sex, age group or study population. For comparison, recombinant ELISA showed sensitivity / specificity of 94.8% [90.7-97.5] / 99.4% [96.6-100.0]. Lysate ELISA showed sensitivity / specificity of 100% [97.9-100] / 100% [97.9-100]. CDP demonstrated excellent sensitivity and specificity in our study population. Sensitivity was higher and specificity slightly lower in serum than in whole blood. The CDP is simple to use and reliable for both hospital settings and field sites. These rapid tests may also be used for practical, accurate maternal screening to identify neonates at risk of congenital transmission.

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IMPACT OF PEDIATRIC AND ADULT ACUTE MALNUTRITION ON VISCERAL LEISHMANIASIS RK39 DIAGNOSTIC TEST RESULTS AND CLINICAL OUTCOME IN THE SUDAN

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In the Sudanese town of Tabarakallah, Gedaref state, Médecins sans Frontières and the Ministry of Health of Sudan treated 2053 patients with primary Visceral Leishmaniasis (VL) between January 2010 and January 2014. Given the high rates of malnutrition, we questioned its impact on performance of rK39 rapid diagnostic tests (RDT) and on clinical outcomes (relapse and case-fatality rates) of primary VL patients. The rK39 RDT was used as first-line test, but due to its limited sensitivity in this region, patients with negative rK39 RDT also had a Direct Agglutination Test (DAT) performed, with lymph node aspirate (LN) restricted to borderline DAT results. Patients were treated with sodium stibogluconate, plus paromomycin since 2011, and liposomal Amphotericin B for severe cases, and followed-up for 6 months. We calculated global acute malnutrition (GAM) using WHO standards adapted to age: under 5 years (n=362) GAM was 53% (95%CI: 49.1-56.9%) weight-for-height <-2SD, including 23.3% (19.9-26.6%) of severe acute malnutrition (SAM); age 6-59 months: GAM 16.0% using middle-upper-arm-circumference (MUAC) <125mm, (including 4.7% SAM using MUAC<115mm); age 5-19 years (n=1202): 60.6% (58-63.1%) using BMI-for-age <2SD; adults (n=489): 27.3% using BMI<17 kg/m^2. Out of 2053 primary VL patients, 1880 (91.6%) were diagnosed by rK39 RDT, and 173 (8.4%) with negative rK39 were confirmed by DAT or LN. For ages 6-59 months (best age for MUAC homogeneity), rK39 was negative in 7.4% of GAM and 8.6% non-GAM children (p=0.78). For adults, rK39 was negative in 11.20% of GAM vs. 8.48% of non-GAM (p=0.37). In the pediatric population GAM or SAM based on MUAC at admission was not significantly associated with higher case-fatality rates (OR: 0.71(95%CI: 0.16-3.28), p=0.66), despite a slight trend in SAM (8.33%) vs. non-SAM (6.64%, p=0.82), unlike descriptions from conflict regions. In contrast, adult case-fatality rates were strongly increased from 5.7 to 18.6% (OR: 3.79 (1.71-8.41)) in GAM patients, independently of HIV-positivity which was low (1.34% of 1657 HIV tested). Odds of relapse with GAM were comparable in adults (OR: 0.97(0.38-2.54)) and children (OR: 0.96(0.21-4.41), p=0.96). In conclusion, no significant rK39 false-negative difference was detected in malnourished patient, so it is safe to rely on rK39 RDT for primary VL field diagnosis. Secondly, acute malnutrition increases mortality nearly fourfold in adults with VL in this stable setting.

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EFFECT OF ZINC, COPPER AND IRON *IN VITRO* ON TH1 AND TH2 CYTOKINE RESPONSE OF PERIPHERAL BLOOD MONONUCLEAR CELLS TO *LEISHMANIA* SP. INFECTION

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An appropriate balance between pro-inflammatory and anti-inflammatory cytokines that mediate innate and adaptive immune responses is required for effective protection against human leishmaniasis and to avoid immunopathology, this immunological balance may be influenced by micronutrient deficiencies. We investigated the effect of zinc, copper and iron, in the production of Th1 and Th2 cytokines *in vitro* by Peripheral Blood Mononuclear Cells (PBMC) from patients with cutaneous leishmaniasis (CL) with therapeutic failure (Resistant) or patients who responded successfully to treatment (Sensitive), and if these trace elements might be involved in the immune response towards the parasite.Patients with CL living in Bolivia, an area highly endemic for Leishmania sp. were enrolled into the Resistant and Sensitive groups mentioned about. Measurement parameters of the immune response were: production of IFN-γ and IL-13 as markers of Th1 and Th2 response respectively, by peripherical blood mononuclear cells (PBMCs) stimulated with Antigen soluble leishmania (SLA) under different conditions of nutrients (Zn, Cu and Fe). The data obtained indicate that zinc, copper and iron are associated with a significant decrease in INF-γ response by PBMC the patients Resistant and Sensitive, as compared to PBMC stimulated only with SLA ($P < 0.05$). Production of IL-13 remained low and similar in both groups. These results show that: i) the specific immune response of Resistant and Sensitive patients is polarized toward TH1. ii) It necessary to know more about this elements trace how possible therapeutic administration in this pathology *in vivo*. iii) Environmentally or genetically determined increase in Cu, Zn and Iron levels might augment susceptibility to infection with intracellular pathogens, by causing decrease in INF-gamma production.

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NON-SEROLOGICAL DETECTION OF *TRYPANOSOMA CRUZI* BIOMARKERS IN MURINE DRUG DISCOVERY MODELS OF CHAGAS DISEASE

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Trypanosoma cruzi, a bloodborne parasite, is the etiological agent of Chagas disease. Following an infection in the host, patent parasitemia is detectable in the blood, and is termed as the acute phase. This is followed by a chronic phase where parasite levels in the blood are difficult to estimate. For the treatment of Chagas disease, currently prescribed drugs, Benznidazole (Bz) and Nifurtimox display multiple side effects and led to early termination of the treatment regimen by patients. Additionally, these drugs have not been show to result in sterile cure in patients. Thus new drugs are needed for disease mitigation. However, a lack of standardized universally acceptable assays that demonstrate parasitological or sterile cure in animal models have hampered development of new drugs. To address these issues, we envisaged the detection of antigens secreted by parasites (*T. cruzi* Excreted Secreted Antigens or TESA), as a diagnostic for Chagas disease. We have demonstrated recently that aptamers (short RNA molecules) can detect parasite antigens circulating the blood of infected mice using an Enzyme Linked Aptamer (ELA) assay. Here we show the ability of the ELA assay to detect these TESA biomarkers in *T. cruzi* infected mice, treated with the drug (Bz). Of the 7 aptamers tested, Apt-29 was able to detect circulating biomarkers in infected mice treated with Bz during the acute phase and in mice treated during the chronic phase of disease. For the acute phase Apt-29 ELA assay (100% +ve) was as good

as Blood PCR (100% +ve) but significantly better than tissue PCR (11% +ve in heart tissue and 89% +ve in skeletal muscles by PCR), to establish treatment failure in mice. In the chronic phase, Apt-29 ELA assay (100% +ve) was significantly better than blood PCR (46% +ve), heart tissue PCR (38% +ve) or even skeletal muscle PCR (92% +ve), to identify treatment failure in mice. These results indicate that ELA assays may be useful in Chagas drug discovery and can provide longitudinal data indicating reduction in parasite load upon treatment in mice models of Chagas disease. Disclaimer: "The findings and conclusions in this abstract have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy"

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NANOTECHNOLOGY TO DNA DETECTION OF *TRYPANOSMA CRUZI* FROM URINE BY REAL TIME PCR

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In recent years there has been an explosion of interest in developing applications involving nanotechnology. Chagas' disease is a neglected tropical disease caused by *Trypanosoma cruzi* and constitutes a serious public health problem for Latin America. Accurate diagnostics are research priorities and the polymerase chain reaction has been proposed as a sensitive laboratory tool for detection of *T. cruzi* infection and monitoring of parasitological treatment outcome, the urine is a valuable noninvasive sample and some studies reported the presence of DNA fragments in urine. In our work we explored the application of microparticles for DNA detection of *T. cruzi* from urine by RT-pcr. The NIPAm/allylamine microparticles showed efficient results in DNA capture in urine samples. We infected urine samples with DNA and applied the NIPAm/ allylamine microparticles and continued with DNA extraction from micronanoparticles, we recovered 93.5 % of DNA from parasites. Finally we tested in 10 urine samples from guinea pig infected with *T. cruzi*. Our results using Rt-pcr from microparticles showed sensitivity values of 87.5 % and specifity 100%. We consider the use of microparticles could be as biomarker through DNA in urine samples

1107

IS LEPTOSPIROSIS AN OCCUPATIONAL DISEASE IN THAILAND?

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The temporal and spatial trend of human leptospirosis in Thailand were explored using statistical models. Several potential factors related to environment, human-animal interaction and human behaviors which are traditionally associated with exposure to the pathogen were investigated. Multiple data sources were deployed including: spatially stratified surveillance of cases between 2010 and 2012; satellite images on flooding; number of potential animal reservoirs spatially explicit livestock population density. We combined this to novel data collected for this study on human contact pattern, level of knowledge about the disease and self-protection practices. We found no significant temporal trend of leptospirosis over the study period. Spatially, leptospirosis occurred repeatedly and predominately in northeastern Thailand. Flooding has previously been assumed to have a significant influence on leptospirosis incidence but for Thailand this was not the case. Statistical analysis showed inconsistent patterns of incidence rate ratio (IRR) of flooding across years and regions. However, the number of buffaloes per district was significantlyassociated with human leptospirosis. From the survey, we found that 75% of population in the study area was farmers who routinely have close contacts with their animals. Their contact with the environment was also extremely high during the rice season when they spent, on average, 6 hours per day in water with only 50% wearing protective footwear. Indeed the seasonal rise and fall of reported leptospirosis cases over time can be explained by differences in seasonal exposure as many rice farming activities and correlates with temporal flooding events. We conclude that there is strong evidence to support leptospirosis being an occupational disease in Thailand rather than one associated with flooding and environmental factors alone. This information will have implications for public health policy to control and treat this disease in Thailand.

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DETECTION OF VIRULENCE GENES ASSOCIATED WITH *SALMONELLA* SPP. ISOLATED FROM RAW ANIMAL FOOD AND HUMANS

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Disease-causing potential in *Salmonella* requires the coordinated expression of complex arrays of virulence factors that allow the bacterium to evade the host's immune system. The TTSS encoded by SPI-2 is an important component of the virulence strategy of *Salmonella* and contributes to systemic infection and replication within macrophages. The presence of virulence encoding genes factors was determined in a total of 30 *Salmonella* isolates by PCR targeting (ssaT, sseB, sseF, sseG, , sseD, sseC) of SPI-2 and invH (SP1-1) virulence determing genes. The presence of ssaT, sseB, sseG, sseC genes was (100%) and (93.3%) sseF, sseD, while sseG were not detected in any of the isolates tested. This study reports detection of virulence genes in *Salmonella* isolated from clinical, raw food samples, including chicken and seafood from Nigeria and India.

1109

THE EFFECT OF HUMAN MOVEMENT PATTERNS ON EXPOSURE TO *PLASMODIUM KNOWLESI* IN SABAH, MALAYSIA

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The zoonotic malaria parasite *Plasmodium knowlesi* is the most common cause of human malaria in the heavily deforested region of Kudat in Sabah, Malaysia. Although human movements into macaque and mosquito habitats determine the risk of exposure to *P. knowlesi* and cases have been reported in men, women and children, little is known about individual mobility patterns relative to different land cover types. As part of an integrated programme, this study is investigating the role of individual local movements in the transmission of *P. knowlesi* and exploring the hypothesis that deforestation and resulting habitat fragmentation have led to increased contact between people, mosquito vectors and primate hosts at forest edges. This paper reports data from an ongoing study using GPS tracking devices to map movement patterns of individuals living in two areas where *P. knowlesi* transmission is occurring: a highly deforested area (Matunggong, Kudat) and a less disturbed area (Limbuak, Pulau Banggi). The study incorporates a seasonal, cross-sectional design. During pre-

defined two-week periods randomly selected individuals are asked to carry a tracking device at all times and to record when macaques are sighted. Over the same period, longitudinal mosquito sampling (using human landing catches) is being carried out within representative land cover types. This paper presents data from the first 6-months of human movement monitoring and describes the methods that will be used to combine metrics for mobility with entomological data to assess the probability of exposure to *P. knowlesi* and identify characteristics of individuals and land use types associated with an increased probability of exposure.

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RISK FACTORS FOR OUTBREAKS OF ANTHRAX IN LIVESTOCK IN BANGLADESH

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Anthrax is endemic and annually causes outbreaks in Bangladesh. The purpose of this study was to identify risk factors for animal anthrax to guide measures to prevent outbreaks among livestock. Between October 2012 and October 2013, we conducted case-control studies in cattle and goats in four districts of Bangladesh where human cutaneous anthrax outbreaks were identified by government officials. Case-animals were defined as ruminants with an epidemiological link to a human cutaneous anthrax case with evidence of anthrax by either Gram stain and McFadyean reaction and/or characteristic appearance of colonies growing on blood agar medium. We enrolled as controls all ruminants on the 10 farms closest to the case-farm that reported no illness in their animals in the three days before the outbreak at the case-farm. We interviewed owners of all enrolled ruminants using a structured questionnaire to record their feeding, grazing and vaccination histories. We estimated the association between animal exposures and anthrax infection with 95% confidence intervals (CI) using bivariate and multivariate logistic regression, accounting for farm-level clustering. We enrolled 47 case-animals from 33 farms and 403 controls from 180 farms. Compared to controls, infected animals were more likely to be <24 months of age (57% vs. 37%, p=0.02), feed on green grass cut or pulled up from agricultural lands (69% vs.33%, p=0.003), and graze on agricultural lands for a longer period of time in the 24 hours preceding onset of the animal outbreaks (4.3 vs. 1.3 hours, p<0.001). Case-animals were less likely to be vaccinated against anthrax during the past year compared to controls (15% vs. 53%, p<0.001). On multivariate analysis, being fed green grass cut or pulled-up from agricultural lands was independently associated with anthrax infection in ruminants (adjusted odds ratio [AOR]=3.0, 95% CI: 1.1-8.3) and anthrax vaccination in the past year was protective (AOR=0.15, 95% CI: 0.05-0.39). Cut or pulled up grass from agricultural lands can be contaminated with soil containing anthrax spores and this is a potential source of anthrax infection for ruminants. Considering the challenges of avoiding the use of green grass mixed with soil contaminated with spores, we suggest identifying and addressing barriers to increased vaccine coverage.

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KNOWLEDGE, ATTITUDES AND PRACTICES ABOUT RABIES MANAGEMENT AMONG HUMAN AND ANIMAL HEALTH PROFESSIONALS IN MBALE DISTRICT, UGANDA

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For the past five years (2007-2011), Mbale District registered the highest number of suspected and confirmed rabies cases in Eastern part of Uganda (587 animal bites and 19 deaths in humans on average annually) and many factors could have contributed to this. The aim of this study was to assess the knowledge, attitudes and practices (KAP) of animal and human health professionals and also to establish the level of relationship within KAP towards rabies management so as to inform responsible authorities to effectively mitigate the problem. A cross-sectional study was conducted between December 2012 and March 2013 among 147 randomly selected animal and human health professionals in Mbale District. Of these, only16 were animal health professionals. Quantitative data was obtained using a semi-structured questionnaire while qualitative data was obtained from 4 Focus Group Discussions (FGDs) using an FGD interview guide and 2 Key Informant (KI) interviews using a KI interview guide. Quantitative data was entered into Epiinfo version 3.5.1 and proportions computed while qualitative data was summarised into themes and sub-themes. Of all the respondents, only 44.22% (n=65) had sufficient knowledge about rabies while 25.2% (n= 37) had positive attitudes towards rabies management. Nearly half of the respondents (49.7%, n= 73) had limited good practices. Respondents knowledgeable about rabies were more likely to have positive attitude towards rabies management (OR=3.65; 95% CI: 1.60-8.3) while respondents with positive attitudes, were more likely to have good practices towards rabies management (OR: 2.22; 95% CI: 1.01- 4.86). Respondents had low knowledge, negative attitude and limited good practices of rabies management in the District. Regular refresher trainings about rabies to broaden staff knowledge and improve their attitudes and hence practices of rabies management should be conducted by the District leaders. Adoption of "One Health" approach for rabies control should be instituted to reduce the incidence of the disease in the District.

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PREVALENCE AND RISK FACTORS TO HUMAN AND ANIMAL BRUCELLOSIS IN MUBENDE DISTRICT-UGANDA, 2013

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Brucellosis is a highly contagious bacterial zoonosis and annually affects more than 500,000 people globally. In Africa the prevalence is 16.2% and 18-24% in Uganda. Humans contract Brucellosis by direct contact with infected animals and consumption of unpasteurized milk and its products. Brucellosis is endemic in Uganda and specifically Mubende district. Increase in number of cases reported to ministry of health between May to November 2011 from Mubende district with no comprehensive report on risk factors to explain the increase in confirmed cases initiated a study to examine prevalence and main risk factors for both human and animal brucellosis in the district. Unmatched 1:1 case control study done. We extracted Brucellosis cases biodata from laboratory records and actively searched for them in communities where 52 cases out of the target 100 were enrolled. Fifty two Controls enrolled from neighborhood considering age and sex. A structured questionnaire administered to both cases and controls. Adult cases and controls interviewed in person and children, their parents responded to questionnaires after consent. Cattle above two years and goats above one year were randomly selected and bled. Seroprevalence determined using cELISA test. Data entered in Epi info version 5.3.1 and exported to Stata Version 9.0 for analysis. Human Brucellosis seroprevalence was 31%. Seroprevalence of Brucellosis in cattle was 11% at animal level and 38% at herd level. In goats, prevalence was 36% and 58% at animal and herd level respectively. Significant risk factor associated with brucellosis in humans, consumption of undercooked meats [OR=8.3, 95%CI: (1.4-48.1)]. Use of protective wear while handling animals and products was protective [OR=0.04, 95%CI: (0.01-0.84)]. History of animal abortions on farm [OR=7.9, 95% CI: (1.4-45.7)] was found a significant animal risk factor to Brucellosis. The increase in number of human cases in Mubende district was due to consumption of undercooked meat and failure to use protective wear while handling animals. The high prevalence in animals associated with history of abortions on farms. Government should strengthen sensitization on Brucellosis, regular testing of herds and Brucellosis considered a differential in animal handling communities.

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SEROPREVALENCE OF RIFT VALLEY FEVER, Q-FEVER AND BRUCELLOSIS IN RUMINANTS ON THE SOUTHEAST SHORE OF LAKE CHAD

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The seroprevalence of Rift Valley Fever (RVF), brucellosis and Q-fever among domestic ruminants on the southeast shore of Lake Chad was studied. The study area consisted of two parts including mainland and islands. On the mainland, the study was conducted in 9 randomly selected villages and camps. On the islands, samples were collected from all four available sites. A total of 985 serum samples were collected and 924 were analyzed using ELISA for RVF. A total of 561 samples collected from islands were analyzed using ELISA for Q-fever and both ELISA and Rose Bengal Tests (RBT) for brucellosis. The apparent seroprevalence by species was 37.8% (95% C.I: 34.2 - 41.3) in cattle, 18.8% (95% C.I: 12.3 - 25.2) in goats and 10.8% (95% C.I: 3.0 - 18.5) in sheep. For brucellosis and Q-fever, only cattle samples from islands were analyzed. For Q-fever, the apparent seroprevalence was 7.8% (95% C.I: 5.6 - 10.1). For brucellosis, the RBT showed a prevalence of 5.7% (95% C.I: 3.8 - 7.6) and ELISA showed 11.9% (95% C.I: 9.3 - 14.6) with κ value of 5.3 showing a moderate agreement between the two tests. This study confirms the presence of the three diseases in the study area. More research is required to assess the importance for public health and conservation of the Kouri cattle breed.

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SOURCE TRACKING *MYCOBACTERIUM ULCERANS* INFECTIONS IN THE ASHANTI REGION, GHANA

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Although several studies have associated *Mycobacterium ulcerans* (MU) infection, Buruli ulcer (BU), with slow moving water bodies, there is still no definite mode of transmission. Ecological and transmission studies suggest variable number tandem repeat (VNTR) typing as useful tool to differentiate MU strains from other Mycolactone Producing Mycobacteria (MPM).Determining the genetic relatedness of clinical and environmental isolates is seminal to determining reservoirs, vectors and transmission route. This study source-tracked MU infections to specific water bodies by matching VNTR profiles of human isolates to those in the environment. Environmental samples were collected from 10 water bodies in four BU endemic communities in the Ashanti region, Ghana. Animal trapping identified 5 mice with lesions characteristic of BU. Four VNTR markers in MU Agy99 genome, were used to genotype environmental isolates and those from 15 confirmed BU patients within the same study area. Length polymorphism was confirmed with sequencing. MU was present in the 3 different types of water body but significantly higher in biofilm samples. Four MU genotypes designated, W, X, Y and Z were typed in both human and environmental isolates. Other reported genotypes were only found in water bodies. Our findings suggest that patients were infected from community associated water bodies. Further, we present evidence that small mammals within endemic communities could be susceptible to MU infections and may be acting as reservoirs.

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INFLUENZA A AMONG SWINE AND DUCK POPULATIONS IN RURAL BACKYARDS WITHIN TROPICAL WETLANDS IN GUATEMALA, 2013

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Some ecosystems may serve as hotspots for the emergence of influenza A virus, and surveillance in such areas may detect novel strains before these become a substantial threat to human populations. In the present project, we searched for Influenza A among duck and swine raised in rural backyards in wetlands where aquatic migratory birds have previously tested positive for influenza A. We conducted monthly surveys of domestic animals to in the villages of Candelaria and Monterrico, Municipality of Taxisco, on the pacific coast of Guatemala. Serial samples were collected from ducks and swine to detect current or past infections of Influenza A. At each visit, we collected nasal swabs and sera from each swine and traqueal, cloacal and sera from each duck. Viral antigens were initially screened by rRT-PCR in swab samples, and positive samples were subsequently sub-typified by HI. Total antibodies against Influenza A were measured by ELISA in sera. In February 2013, we conducted a census of all backyard animals in the two villages, and identified 102 swine and 123 ducks which we followed monthly during April-August 2013 for a total of 377 swine- and 449 duck-visits. . A total of 754 swine and 1,635 duck samples were tested through rRT-PCR and/or ELISA. We identified Influenza A among 3% (12/377) of swine nasal swabs and 0.5% (2/377) of their sera; HI testing indicates reactivity to pH1N1 subtype. We identified Influenza A among 2% (10/546) of duck trachea-, 0.7% (4/546) of cloaca-, and 2.9% (16/543) sera-samples. Influenza A antigen and antibody positivity in swine ranged from 0.0 to 5.9% and 0.0 to 2.0 %, and in ducks from 1.0 to 6.2 % and 0.0 to 4.3%, respectively. The antigen positivity in ducks seems to show an increasing trend from April to August 2013, while no trend is evident in the case of swine. Additionally 18 animals, with undifferentiated signs, suggestive of respiratory disease, were tested and none were positive for Influenza A This is the first longitudinal study of Influenza A in backyard animals, and although the percent of infected backyard animals is low it may represents a public health risk for the householders specially those in charge of their husbandry. Additional studies are needed to better define seasonal fluctuations and their association with environmental and ecological variables. Moreover, viral isolation and subsequent nucleic acid Posequencing could reveal characteristics of their pathogenicity, relationship and origin.

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INCREASED MORBIDITY AND MORTALITY IN DOMESTIC ANIMALS FED GROUND AND BITTEN FRUIT IN BANGLADESHI VILLAGES AND THE IMPLICATIONS FOR BAT BORNE ZOONOTIC DISEASE TRANSMISSION

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Consumption of bat bitten fruit by domestic animals is a proposed pathway for the introduction of new zoonotic pathogens and is the most likely explanation in a 1998 Nipah virus outbreak on Malaysian pig farms. We hypothesized that consumption of fruits bitten by bats and dropped to the ground leads to increased morbidity and mortality in domestic animals due to bat related pathogens. As part of a study on Nipah virus, teams collected data on animal health and feeding practices from

randomly selected Bangladeshi households in villages inside and outside Nipah endemic areas from 2011 to 2013. Farmers were asked about deaths in the two-month period prior to the survey and illness in the year prior. We used mixed effects models controlling for village clustering, per capita household resources, and herd size to examine if farmers allowing animals to eat ground fruit or actively feeding bitten fruit were more likely to report sick or dead animals. The analysis included 206 villages, 5081 households, 9254 cattle, and 4265 goats. 30% of farmers reported that their animals consumed ground fruit. Compared with farmers who denied their animals ate ground fruit, farmers who reported consumption were more likely to report morbidity in their cattle (42% vs 36%, OR=1.2, CI 1.0-1.5, p=0.02) and in their goats (13% vs 9%, OR=1.8, CI 1.0-2.2, p=0.04). 20% of farmers reported that they actively fed bitten fruit to their herds. Compared with farmers who did not feed bitten fruit, farmers feeding bitten fruit were more likely to report morbidity in their goats (42% vs 31%, OR 1.6, CI 1.2-2.1, p<0.001) as well as mortality (13% vs 9%, OR 1.8, CI 1.2-2.7, p=0.004). The percentage of farmers reporting illness in their goats increased with more frequent feedings: 31% of farmers denying feeding bitten fruit reported illness compared with 42% feeding no more than 2 times/week (OR 1.5, CI 1.2-2.1, p=0.002) and 49% feeding >2 times/week (OR 2.4, CI 1.2-4.9, p=0.015). Feeding ground and bitten fruit is associated with increased mortality and morbidity in goats and cattle. This could be due to the transmission of bat pathogens. Given the close interface between humans and their animals, this represents a potential pathway from bats to human populations. Future serologic studies and pathogen detection aimed at these animals and their human owners may provide an efficient way to observe spillover events.

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HUMAN MONKEYPOX DISEASE SURVEILLANCE AND TIME TRENDS IN THE DEMOCRATIC REPUBLIC OF CONGO, 2001-2013

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Human monkeypox (MPX) is a zoonotic orthopoxvirus that causes a serious smallpox-like illness. The majority of MPX cases are reported in rural villages, located in or near heavily forested areas in the Democratic Republic of Congo (DRC). In DRC, MPX is a reportable disease at the national level (Ministry of Health, Division of Disease Control - Integrated Disease Surveillance and Response (IDSR) system). However, no analyses have been completed to-date looking at the passively collected data on suspected cases of MPX. Assessments of surveillance systems can help shape recommendations and have broad policy implications for disease surveillance systems, especially in low-income settings. We used available data from the IDSR system on MPX-suspected case counts received from every health zone on a weekly basis for an in-depth analysis of yearly and weekly trends, and factors which could influence reporting. A time series analysis will be used to assess the effect of seasonality on the number of suspected cases reported. If detected, additional analyses will be conducted to determine if the seasonality found is actually seasonal variation in MPX occurrence or seasonal variation in disease reporting. In order to examine this effect, other reportable diseases with or without seasonal patterns will be used in place of MPX as the outcome. Between 2001 and 2013, an increase in suspected cases of MPX was reported through the IDSR: 19,437 suspected cases were reported, with 269 of 514 (52.3%) health zones reporting. Additionally, 326 suspected deaths due to MPX were reported (CFR=1.7%). When all years (2001-2013) are collapsed, the average number of suspected cases reported per week is 34 (min=0, max 360). Weeks 9-11 and 42-43 have the highest average number of suspected cases, while weeks 51-52 have the lowest reporting. Apparent increases in reported annual MPX cases, and trends in weekly reporting, may be artifacts of improvements in disease surveillance. Further analyses should examine such trends, providing critical information for prevention and control strategies and suggesting areas of improvement for future data collection efforts.

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TUBERCULOSIS INFLUENCES THE PROGRESSION OF NEUROLOGIC DISEASE IN HTLV-1 INFECTED SUBJECTS

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The human T cell lymphotropic virus type 1 (HTLV-1) has a high prevalence in Central and South American. The HTLV-1 associated myelopathy or tropical spastic paraparesis (HAM/TSP) is observed in less than 5% of the cases but a large percentage of infected subjects have neurologic involvement mainly of overactive bladder. HTLV-1 increases from 2 to 4 fold the risk for tuberculosis (TB) but it is not known if TB influences HTLV-1 infection. We determine in a cohort of HTLV-1 infected subjects the prevalence of active or past history of TB, perform tuberculin skin test (TST) and evaluate if TB influences neurologic disease associated to HTLV-1. TB was defined by past or present history of TB or by X-ray showing scar lesions characteristic of TB. Participated of this cross-sectional study 190 HTLV-1 infected individuals presenting in our outpatient clinic for HTLV-1 from April of 2010 to March of 2012. They were evaluated for present or past clinic manifestations of TB and a neurologic examination, chest X-ray and a TST was performed. TB was detected in 39 (20%) of the subjects and latent TB (LTB) in 76 (58,9%). Of the 39 patients with TB 28 had history of the disease. Among them, 28 had pulmonary TB, one had lymph node TB and two pleural TB. In 9 (36%) of the 25 patients with past history of TB the RX was normal at the time of the present study. In patients who had lesions of TB in the X-rays there was no major sequel. The TST was positive in 108 (56,8%) of the 190 patients. There was a predominance of males (51,3%) among TB patients than in LTB (38%) and without TB (20%) groups (P=0.02)). Moreover HAM/TSP was strongly associated with TB but not with LTB or without TB (P=0.02). The chance of findings TB in HAM/TSP patients was 3.5 fold higher than in LTB. The frequency of HAM/TSP was similar in patients with history of TB and in patients who had the diagnosis based on the TST and chest X-ray. While severity of TB does not appear to be influenced by HTLV-1, TB influences progression of neurologic disease to HAM/TSP.

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IMPACT OF MATERNAL MALARIA AND HYPERGAMMAGLOBULINEMIA ON TRANSPLACENTAL TRANSFER OF RSV NEUTRALIZING ANTIBODIES IN COASTAL PAPUA NEW GUINEA (PNG)

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Passively acquired RSV neutralizing antibody (Ab) protects against RSV-associated lower respiratory illness, but placental malaria (PM) and hypergammaglobulinemia can interfere with transplacental transport of

immunoglobulins. To determine efficiency of transport in the context of PM and hypergammaglobulinemia, 313 paired maternal and cord sera from 2 cohorts in *P. falciparum* and *P. vivax*-endemic areas of coastal PNG were tested: the Alexishafen cohort (PM= 59%, 2005-08, 157 pairs) and the FIS cohort (PM=9.6%, 2012-13, 156 pairs). RSV Ab was measured in maternal and cord blood obtained at delivery by 60% complement-enhanced plaque reduction neutralization assay (60% PRN). Cord to maternal titer ratios (CMTR) were calculated for each pair. Maternal IgG was measured by radial immunodiffusion and hypergammaglobulinemia defined as total IgG ≥1700 mg/dL. Impaired and highly impaired transport were defined as CMTR <1.0 and <0.8 respectively. Maternal titers varied substantially (range, 11-7,150 Alexishafen; 19.5 -2,259 FIS). Impaired transport occurred across the spectrum of titers. In the Alexishafen cohort, impaired and highly impaired transport were observed in 34% and 17% of pairs respectively, and in 33% and 18% respectively in the FIS cohort. Rates were nearly identical despite substantial differences in PM prevalence. Analysis of impaired transport by PM status revealed no statistically significant differences in either cohort. Hypergammaglobulinemia was detected in 54% of Alexishafen mothers and was significantly associated with impaired transport (OR=2.2 [95% CI 1.06 - 4.67), p=0.02). However, hypergammaglobulinemia was detected in only 8% of FIS mothers and was not associated with impaired transport. In summary, we observed impaired transplacental transport of RSV PRN Ab in coastal PNG, but these data suggest that PM was not the primary driver. Further work is needed to determine the effect of hypergammaglobulinemia and assess other factors that may impair transport of RSV PRN Ab. These data may have important implications for future maternal RSV immunization programs.

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FACTORS ASSOCIATED WITH FAILURE IN SMEAR POSITIVE PULMONARY TUBERCULOSIS: USING SYMPTOMS PLUS SPUTUM SMEAR AND CHEST RADIOGRAPHY

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Successful outcome of smear positive pulmonary tuberculosis (PTB) is necessary to control the spread of this contagious disease. A retrospective study was conducted to identify outcomes and factors associated with failure in smear positive PTB. The target population was adult, HIV negative, smear positive PTB patients treated with standard category I regimen in new cases or category II regimen in retreated cases at Prasarnmit Hospital, Bangkok, Thailand between 2003 and 2012. Of 297 patients, the outcomes were cure in 229 (77.10%), complete in 5 (1.69%), failure in 16 (5.39%), transfer out in 29 (9.76%), default in 18 (6.05%), and no death. Failure cases were compared with successful ones (cure and complete) and analyzed by Epi Info version 3.4.3. Age of more than 50 years old ,sputum smear 3+ at diagnosis and drug resistance were significantly associated with failure (p-values 0.002, 0.002, and <0.001, respectively). Symptoms such as cough, fever, haemoptysis, chest pain and weight loss were not significantly associated with failure. By using a combination of symptoms with sputum smear in analysis, patients presenting with cough, fever or haemoptysis with a smear of 3+ had a higher risk of failure when compared with those having a smear of 1+/2+ (p-values 0.004, 0.007, and 0.004, respectively). Cavitary lesions in chest radiography (CXR) at diagnosis were not significantly associated with failure (p-value 0.206). When combining symptoms with CXR, only patients complaining of haemoptysis with cavitary lesions were 8.54 times more likely to fail when compared with hemoptysis without cavitary lesions (p-value 0.038). Although we did not reach the target of an 87% success rate of smear positive PTB recommended by the WHO, we were able to identify the risk factors of failure by using symptoms plus simple laboratory tests which might be useful in resource limited areas.

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PREVALENCE OF LATENT TUBERCULOSIS INFECTION AND ASSOCIATED RISK FACTORS IN AN URBAN AFRICAN SETTING

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The aim of the present study was to determine the prevalence of Latent Tuberculosis Infection (LTBI) and the associated risk factors in an urban African setting. This is a secondary analysis of data of a house-to-house cross-sectional survey of chronic cough that was conducted from January 2008 to June 2009. The survey included residents aged ≥15 years who were members of households visited. For our study we considered all participants who were tested with Tuberculin skin testing (TST) using the Mantoux method (183 with chronic cough and 100 without chronic cough). The primary outcome was latent TB infection (LTBI), defined as a TST with induration ≥10mm. The potential risk factors considered were; age (15-24, 25-34 and ≥35 years), being employed or a student, sex, marital status, smoking status and chronic cough. Bivariate and multivariable logistic regression analyses were used to assess the risk factors associated with LTBI. The overall prevalence of LTBI was 49% [95% CI 44-55]. Stratifying by age, and using the youngest category as a reference, the risk of LTBI increased with age (25-34, OR (95%CI) =2.03(1.78, 3.52); >=35, OR (95%CI) =3.33(1.77, 6.24). Furthermore, being a student or employed OR (95%CI) =1.88(1.17, 3.04); male OR (95%CI) =1.80(1.08, 2.98) and previously OR (95%CI) =2.22(1.13, 4.35) or currently married OR (95%CI) =2.11(1.18, 3.78) were associated with increased risk. On multivariable logistic regression analysis, age 25- 34, OR (95%CI) = 1.94(1.12, 3.38); ≥35 years OR (95%CI) = 3.12(1.65, 5.88) and being a student or employed OR (95%CI) = 1.72(1.05, 2.81) were found to be associated with LTBI. The prevalence of LTBI was high in this urban African setting. Older age and being a student or employed were factors associated with LTBI suggesting cumulative risk with age and a potential underlying risk related to expansion of one's social network outside the home. Our results provide justification for TB infection control interventions like LTBI screening and preventive treatment programs.

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INFLUENZA VIRUS IN SEVERE ACUTE RESPIRATORY INFECTIONS AT INTENSIVE CARE UNITS AND RESPIRATORY SPECIALIZED UNITS IN PERU. CASE CONTROL STUDY

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Acute respiratory tract infection, especially due to influenza virus, remains one of the most significant causes of morbidity and mortality worldwide. Two types of syndromes may be cause by influenza A virus: 1) Milder upper respiratory tract disease or "influenza-like illness" (ILI), typically defined as sudden onset of fever (oral temperature ≥38°C or axillary temperature ≥37.5°C) and either cough or sore throat for 7 days, and 2) Severe acute respiratory infection (SARI), typically defined as ILI plus dyspnea and need for hospitalization. We conducted a matched case-control study in three civilian and two military hospitals in Peru to determine risk factors for SARI versus ILI due to influenza virus. Cases were patients of all ages with SARI and controls were ambulatory patients with ILI. Cases and controls were matched by hospital, age group (<5, 5-14, 15-

29, 30-44, 45-59, ≥60), and date of enrollment, with a 7 day maximum between cases and controls. Oropharyngeal swab and/or aspirate bronchoalveolar samples were taken and tested for influenza viruses by RT-PCR. From October 2012 to March 2014, 1374 subjects were enrolled in the study (687 in each arm).The mean age was 13.9, and the median was 2 years old, 67% under 5 years of age, and only 9% age 60 years or older, with 50% males. Influenza A virus was diagnosed in 187 patients 77 in cases and 110 in controls. SARI and ILI were equally as frequent in persons infected with influenza A H1N1 pdm09 virus (OR 1.08, 95%CI 0.62-1.89). However, SARI was significantly less frequent than ILI in persons infected with influenza A H3N2 (OR 0.36, 95%CI 0.21-0.62) and influenza B (OR 0.18, 95%CI 0.08-0.38) viruses. Factoring all sub-types of influenza virus infection, respiratory (OR 1.61, 95%CI 1.26-2.04), cardiovascular (OR 2.54, 95%CI 1.71-3.88), and other premorbid chronic diseases (OR 2.50, 95%CI 1.81-3.45) were risk factors for SARI. We conclude that influenza A H1N1 pdm09 virus is a more frequent cause of SARI than influenza A H3N2 and B viruses. We plan to next extend this analysis to other non-influenza respiratory viruses.

1123

AN EXAMINATION OF POTENTIALLY SUPPRESSIVE INFLUENCES ON INFLUENZA VACCINE EFFECTIVENESS IN THE U.S. MILITARY

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The Armed Forces Health Surveillance Center (AFHSC) assesses influenza vaccine effectiveness (VE) in the US military and select civilian populations annually and semiannually. VE estimations frequently differ between service members and civilians, with higher VE estimates seen among the later. In addition, VE estimates have also varied by type of vaccine administered, with inactivated virus vaccines consistently out performing live attenuated vaccines among non-recruit military members. We propose several possible explanations for these differences. First, an important issue that may impact analysis of military members' VE is their consistently high vaccination rates (for example, in 2013-2014, over 96% were reportedly vaccinated). This could prevent valid comparisons between vaccinated and unvaccinated cases and controls since there are so few unvaccinated individuals in the analyses. Second, military members are required to be vaccinated each year. Thus, extensive vaccine-induced antigenic exposure takes place on a consistent base. Repeated exposure to these vaccines may diminish immunological response and, therefore, may lead to diminished VE. Third, the US military starts vaccinating very early in the season, typically three to four months before the Northern hemisphere usually sees a peak in influenza cases. It is theorized that the lower VE in military members could be due to a waning immunity (i.e., protective effects of the vaccine diminish quickly over a period of 3-6 months), perhaps leaving this population unprotected at the peak of the influenza season. For recent seasons, 40-50% of our military population received vaccines with live, attenuated influenza virus which have been shown to be less effective compared to inactivated virus vaccines. Data will be presented to examine potential vaccination waning within a single influenza season, and to examine the potential impact of high vaccination rates for US military members on statistical models designed to evaluate VE.

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EFFECT OF HELMINTH INFECTION ON TUBERCULOSIS DISEASE SEVERITY

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The global burden of disease caused by tuberculosis (TB) remains high, with an estimated 12 million prevalent cases in 2012. There is a clear need to identify factors that alter the severity of TB disease and potential infectiousness to others. Recent data suggest an association between helminth infections and mycobacterial disease. This pilot study aims to describe differences in TB severity in persons with TB disease and helminth infection compared to TB disease alone. We evaluated persons with suspected pulmonary TB presenting to local TB clinics in Vitoria, Brazil and analyzed data for those with culture-confirmed pulmonary TB. We collected demographic and symptom information, sputum AFB smear and culture including colony counts, and a postero-anterior xray of the chest; up to three stool ova and parasite examinations were performed for each person. To date, we have data on 14 people. Of these, 11 (79%) were male, the median age was 28 years (range 18-72) and four (29%) had helminths in their stool, including hookworm (n=4), *Schistosoma mansoni* (n=1), and *Strongyloides stercoralis* (n=2). Those with TB and helminth infection had more people living in their house than those with TB alone (7 vs 5.4; p=0.01). Those with TB and helminth infection also tended to have more than 200 mycobacteria on colony counts compared to those with TB alone (100% vs 50%; OR=9.00; p=0.17) and involvement of ≥3 lung zones on chest xray (100% vs 60%; OR=6.23; p=0.26), but the differences were not significant. The small sample size limits our findings, but we have started to identify potential trends in clinical parameters between those with TB and helminth infection compared to TB disease alone, with the former potentially exhibiting more severe clinical disease. Further enrollment is ongoing in order to confirm these results, power the study to achieve statistical significance, and perform multivariate analyses. If the study findings hold true, treatment of helminth infections could serve as a way to reduce the severity and infectiousness of TB disease.

1125

STRENGTHENING DISEASE SURVEILLANCE AND OUTBREAK RESPONSE CAPACITY OF HUMAN AND ANIMAL HEALTH LABORATORY SYSTEMS THROUGH IMPROVED SUPPLY CHAIN, COLD CHAIN AND INFECTIOUS WASTE MANAGEMENT PRACTICES

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Under the One Health approach to detecting, preventing, and coordinating the response to zoonotic disease transmission between the human and animal health sectors, the USAID | DELIVER PROJECT, Task Order 6, supported assessments of the human and animal health laboratory supply chains for influenza surveillance and outbreak response in three high-risk Emerging Pandemic Threats (EPT) countries - Indonesia, Uganda, and Vietnam. The results highlighted the need to standardize and improve logistics, cold chain, and infectious waste management procedures and practices in the laboratories and throughout the specimen collection, storage, and transport systems. In response to the identified needs in these three critical areas, the USAID | DELIVER PROJECT: • designed and implemented a laboratory logistics system for a national influenza-like illness (ILI) and severe acute respiratory infection (SARI) surveillance program to ensure reliable supply and quality of laboratory

reagents and specimen collection supplies for routine surveillance and diagnosis of suspected cases of disease outbreaks; and timely availability of personal protective equipment (PPE) and other infection prevention and control supplies to protect health worker safety in the event of an outbreak • developed and implemented standard operating procedures (SOPs) to improve cold chain monitoring practices and maintenance of cold chain equipment for proper storage and transport of specimens from the field and at the reference laboratories • developed SOPs for effective and safe management of biomedical waste in the laboratory. A consistent supply of the appropriate laboratory reagents and specimen collection supplies is needed to ensure specimen quality and to provide timely and accurate detection of disease outbreaks. In addition, to protect health worker, patient, and field staff safety, it is equally important to ensure the availability of PPE and other infection prevention and control supplies at laboratories, healthcare facilities, and field-based collection sites.

1126

UTILITY OF STRING TEST AND STOOL FOR DIAGNOSING PULMONARY TUBERCULOSIS USING GENE XPERT MTB/RIF

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Until recently, diagnosis of pulmonary tuberculosis (pTB), has relied on smear microscopy and culture, however microscopy is only 40-80% sensitive and diagnosis via liquid culture requires at best 7-14 days. While Xpert® MTB/RIF has significantly advanced the field of TB diagnostics, it fails to yield a diagnosis in 33-45% of smear-negative cases. Therefore, in cases in which sputum samples are challenging to obtain or in paucibacillary disease, complementary means of diagnosis are needed. We therefore compared sputum to string test and stool in 13 patients with suspected pTB having received <72 hours of anti-tuberculosis therapy (ATT). String test was performed as previously described: the capsule was swallowed and the trailing end taped to the cheek until removal by gentle traction after 4 hours. Stool was processed by two low-technology (not requiring centrifugation) methods, one using sugar flotation and the other using TB MicroSense Beads ®. Of the 13 patients with suspected pTB, 8 had culture-confirmed pTB including 2 with smear-negative pTB. The string test was well tolerated, with a median and mean Wong Baker score of 2. The Xpert from the string test was positive in 100% (8/8) of culture-confirmed pTB including both cases of smear-negative pTB. Stool was collected in 10 of 13 participants before 72 hours of ATT. Using both stool methods, Xpert detected 7 of 7 cases, however 30% of stool specimens were read as invalid. The sugar method detected 3 of 7 cases compared to 5 of 7 by MicroSense beads. Specificity was 100% for both the stool and string test including 2 cases of Non-Tuberculous Mycobacteria. This pilot highlights the potential utility of Xpert on specimens obtained from string test and stool. The string test had 100% concordance with sputum culture-confirmed pTB and does not require electricity or a trained respiratory therapist, making it an attractive means of obtaining respiratory specimens in resource-limited settings. With improved methods to remove the fibrous material, stool has the potential to be a non-invasive means of pTB diagnosis.

1127

RELATIONSHIP BETWEEN NEWS ABOUT INFLUENZA AND ILI OUTBREAKS. A SENTINEL STUDY IN PIURA, PERÚ

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ILI outbreaks reported in the sentinel sites relates to movement of new strains of virus or susceptibility of individuals, among others. Our country has not studied the relationship between the news about influenza and number of cases of ILI sentinel surveillance. A retrospective study (2013) in Piura was performed to determine the association between the publication of news about H1N1 influenza pdm09 and the number of visits for ARI / ILI at sentinel site. In one year the number of cases of ILI, ARI and news published in 6 newspapers is recorded. The correlation between the number of published news per week with the number of cases of ARI / ILI and relationship with two outbreaks of ILI is calculated. A total of 4,172 IRA cases and 1,316 of ILI, 86 cases of Influenza A and 36 influenza B are reported by RT; ILI cases correlate with influenza A virus ($p < 0.05$). Also 373 news on influenza A H1N1 pdm09 are published; the news content is 25.3 % of cases, 23.9 % of deaths, 23.7 % of prevention, 17% of the influenza vaccine, and 9.4 % on other issues related to Influenza A; 85 % of stories about cases and 95 % of deaths were from other cities. Total news published correlates with ILI, but not with the IRA cases. News of most newspapers correlate with ILI cases, but only one with ARI. ILI cases correlate with the news of flu cases, deaths from influenza and prevention issues ($p < 0.05$). Also shows that after a peak news happens peak ILI. At first outbreak of ILI in late summer 11 news (average 2.06 - range 0-6) per week spread over Influenza A virus , at the second in late winter diffuse 331 (average 22.07 , range 0 to 90). In the first outbreak diffuse 1/11 news on cases, no of deaths, 3/11 on vaccines, 3/11 for the prevention and 4/11 others; in the second 87/331 on cases, 85/331 deal deaths, 55/311 on the vaccines, 77/331 on prevention and 27/331 others. There are significant differences in both outbreaks: in the first outbreak the average age is 18.44 (SD 19.39) years, the influenza A H3N2 virus predominates, go elsewhere 40.5 % of ILI cases, detected 61.8% of cases of influenza A with rapid tests; in the second, the average age is 20.99 (SD 19.01), Influenza A H1N1 pdm09 predominates, go elsewhere 55.5 % of ILI cases, 38.0 % of cases of influenza A with rapid test is detected. Therefore there is a relationship between the increase in influenza A H1N1 pdm09 news and features of ILI outbreaks as older age, lower proportion of influenza virus identified and greater attention seeking ILI at sentinel site.

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KNOWLEDGE AND PRACTICES REGARDING TB PREVENTION IN MEDICAL STUDENTS FROM A PUBLIC UNIVERSITY OF LIMA, PERU

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Medical students are in high risk for nosocomial transmission of tuberculosis (TB) during their clinical practices. Adequate knowledge about TB and practices of biosecurity measures, particularly use of respiratory protective masks, are important to prevent nosocomial infection. This is the first study about knowledge and practices in Peruvian medical students. We aimed to describe the knowledge and practices regarding TB among medical students from a public university in Lima, Peru. In December 2012, a self-administered questionnaire-based survey was carried out in third-year medical students from San Marcos National University during the first clinical course of their career. Information was obtained about sociodemographic profile, knowledge about symptoms and ways of TB transmission, and use of masks (N95 or similar). Among 110 respondents, only 50% correctly recognized cough, coughing up blood, fever, night sweeting, fatigue and weight loss, as main symptoms of TB. In addition, 56.36% correctly answered that coughing, sneezing and speaking can transmit TB, and 60% incorrectly considered to share meals, drinks, utensils or cups, as ways of TB transmission. During the 15 weeks that lasted their clinical practice in teaching hospitals, 4% never used any respiratory protective mask, 55% used 1-5 masks, 30% used 6-14 masks, and only 11% used an optimal number of masks (≥15). Those findings suggest that an important proportion of Peruvian medical students are not aware of the main symptoms and routes of TB transmission. Furthermore, many students engage in risky behaviors: 89% used a suboptimal number

of masks during their clinical practice. Moreover, most of them have incorrect knowledge associated to TB stigma. We recommend active learning experience to improve knowledge and promote use of respiratory protective masks.

1129

PREVALENCE AND TRENDS OF RESISTANCE TO FIRST LINE DRUGS IN A HIGH TUBERCULOSIS BURDEN AREA IN LIMA, PERU

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The last Peruvian national surveillance of drug resistance for tuberculosis (TB) included isolates from 2005-2006 and reported high resistance patterns. However, TB prevalence in Peru varies greatly by region, with highest TB concentration in Lima. Few information exist about resistance patterns in these areas. This study reports drug sensitivity prevalence and trends from 2007-2011 in San Juan de Lurigancho (SJL), the most populated district of Lima and one of the highest reported cases of TB. Data from respiratory symptomatic TB suspects enrolled in parallel diagnostic trials during 2007-2011 were included. Isolates from TB patients; confirmed by Löwenstein-Jensen (LJ) cultures and positive Capilia test; were processed for drug susceptibility test (DST) using proportion method in LJ for isoniazid (INH) rifampin (RIF), streptomycin (SM) and ethambutol (EMB). Wayne test was performed for pyrazinamide (PZA). Results were categorized according to previous TB history and divided in two periods (2007-2008, 2009-2011). Annual-trends of resistance prevalence for each drug were evaluated. TB was confirmed in 1027 patients, all with final DST result. Pan-susceptible isolates were 695 (67.7%). Overall resistance isolates were: INH 207 (20.2%), RIF 119 (11.6%), EMB 91 (8.9%), SM 239 (23.4%), PZA 40 (4%), and multidrug resistance (MDR) were 105 (10.2%). In 780 naïve TB patients, resistant patterns were: INH 133 (17.1%), RIF 61 (7.8%), EMB 60 (7.7%), SM 167 (21.4%), PZA 16 (2.1%), and MDR 54 (6.9%). In naïve TB group, comparison of DST patterns during the 2 evaluated periods showed a not significant decrease in RIF, SM, PZA and MDR, and a not significant increase in INH. Increase in resistance patterns was founded (p=0.003) for EMB with 24/453 (5.3%) vs 36/327 (11%) with an increasing annual trend (p=0.0034). We found a significant increasing trend for EMB resistance. Special concern on resistance surveillance should be encouraged especially in TB hot spots like SJL.

1130

MOLECULAR DIFFERENTIATION OF *ENTAMOEBA* SPP. AMONG CAMEROONIAN HIV PATIENTS

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Entamoeba histolytica is an important cause of dysentery. *Entamoeba* spp. has been reported to colonize with increased frequency among HIV positive individuals. Routine microscopic examination of stool sample is most widely used technique but microscopy alone has low sensitivity and it is insufficient for differentiation between *Entamoeba* spp. Molecular techniques are newer methods which are currently used for the identification of *Entamoeba* species. The present study was planned to investigate the *Entamoeba* species and its genotypes by gene sequencing for the confirmation of microscopic findings of stool samples of HIV positive patients of Cameroon. Twenty eight stool samples diagnosed positive for *Entamoeba* spp. by microscopy were collected from Cameroonian HIV patients and studied for the differentiation of *Entamoeba* species. DNA was extracted from infected stool samples and used to amplify a part of the genus *Entamoeba* small-subunit ribosomal RNA gene (16S-like SSUrDNA) as well as the serin riched *Entamoeba histolytica* protein gene and chitinase gene. The 16S-like SSUrDNA was sequenced to identify the other species that could not be done by PCR and for the differentiation of *E. histolytica* from *E. dispar* and *E. moshkovskii*. Sequence analysis identified six different species of *Entamoeba* which were related to *Enamoeba*; *E. histolytica* (27.59%), *E. dispar* (13.79%), *E. moshkovskii* (3.45%), *E. coli* (20.69%), *E. hartmanni* (6.9%), *E. polecki* (10.34%), and *E. struthionis* (10.34%). The phylogenetic analysis within the sequences of *E. histolytica* isolates showed that two distinguishable variants are present among Cameroonian HIV patients. Thus, there is a possibility that specific genotypes are more prevalent among HIV positive patients and molecular diagnosis is of utmost importance in establishing the correct diagnosis of amoebic dysentry.

1131

ESTABLISHING WHETHER *GIARDIA INTESTINALIS* HAS A PROTECTIVE ROLE AGAINST THE INCIDENCE OF DIARRHEA AND ITS ASSOCIATED MORBIDITY, DURING THE FIRST 24 MONTHS OF LIFE IN A BIRTH COHORT IN RURAL TROPICAL ECUADOR

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Giardia intestinalis is a common protozoal infection (approx. 2.8 billion cases worldwide/year).The aim of this research is to establish whether *G. intestinalis* has a protective role against the incidence of diarrhoea and its associated morbidity.The study included sub-cohort of 195 children from the ECUAVIDA study.Stool samples have been collected at 7, 13, 18, and 24 months and samples were collected whenever the child has an episode of diarrhoea.PCR was used to detect *G. intestinalis*-specific DNA in the stool samples.Exposure for stunting/wasting was a positive stool sample collected at 13 and 24 months. Exposure for incidence of diarrhoea between 7 and 13 months was a positive stool sample collected at 7 months of life when child is asymptomatic and for incidence of diarrhoea between 13 and 24 months will be a positive sample collected at 13 months. The outcome observed is the number of acute diarrheal episodes each separated by 7 days free of diarrhoea. Anthropometric measurements of length and weight were taken at 13 and 24 months. Length-for-age, weight-for-age and BMI-for-age Z scores were calculated. Student t tests, Pearson's Chi-Squared analysis and uni- and multivariate regression analysis were carried out for the statistical analysis of results.There was no significant difference in the mean number of ADEs in those with and without Giardia in the two age-groups. Multivariate regression analysis revealed no significant associations between *G. intestinalis* infection and the incidence of acute diarrhoeal episodes between 7-13 months (RR=0.508, CI 0.165-1.562, p=0.237) and 13 to 24 months (RR=1.078, CI 0.694-1.676, p=0.738). Furthermore, no significant relationship could be established between Giardia infection and stunting. However, children who had *Giardia* infection at 7 months were less likely to have stunted growth at 13months, thus infection with Giardia demonstrated a protective effect, which remained when adjusted for age and sex. *G. intestinalis* infection was not associated with the incidence of diarrhoea in this cohort. There were no significant associations demonstrated between *G. intestinalis* infection and stunting/or BMI-for age scores. *Giardia* was significantly predictive of wasting in children at 13 months.This study questions the status of giardiasis as an important parasitic disease and suggests a review may be needed into treatment protocols in the rural tropics.

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CLINICAL EVALUATION OF *E. HISTOLYTICA* QUIK CHEK - A RAPID CASSETTE IMMUNOASSAY FOR THE SPECIFIC DETECTION OF *ENTAMOEBA HISTOLYTICA* IN HUMAN FECAL SPECIMENS

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Entamoeba histolytica is responsible for 100 million cases of amebiasis annually, causing diarrhea, dysentery, and colitis. Microscopic examination of fecal specimens for the presence of *E. histolytica* can approach 10% sensitivity. False positive results occur due to the morphologically-identical and non-pathogenic *E. dispar,* which can be 3-10 times more prevalent. Immunoassays and laboratory PCR assays have improved diagnosis; however, no test has combined a rapid format with specific identification of pathogenic *E. histolytica*. Two commercial microwell ELISAs provide specific detection of *E. histolytica*, but all other immunoassays suffer from poor specificity due to co-detection of *E. dispar*. These data describe results to date for an ongoing clinical evaluation of the *E. HISTOLYTICA QUIK CHEK* (*EHQC* - TECHLAB®, Inc.), the first rapid cassette-based immunoassay for the specific detection of *E. histolytica* in human fecal specimens. The *EHQC* and the ProSpecT *Entamoeba histolytica* microwell ELISA (*ProSpecT*, Remel) are being compared to the *E. HISTOLYTICA II* microwell ELISA (*EHII*, TECHLAB®, Inc.) for specific detection of *E. histolytica* in human fecal specimens from an endemic site in Bangladesh and a US clinical reference laboratory. To date, 160 retrospective specimens tested include *Entamoeba* spp. negatives, *E. histolytica* positives and *E. dispar* positives. Immunoassay-discrepant specimens are further evaluated by *Entamoeba* spp. specific PCR. Compared to *EHII* and PCR, the *EHQC* displays 99% correlation and the *ProSpecT* displays 91% correlation; 13 of the 14 *ProSpecT* discrepants are due to PCR-confirmed *E. dispar* positives. Analytical testing with cultured *E. histolytica* trophozoites determined a limit-of-detection of 586 trophozoites/mL of original specimen for *EHQC* and 1172 trophozoites/mL of original specimen for *ProSpecT*. The *ProSpecT* assay was the only test that reacted with cultured *E. dispar*. The *E. HISTOLYTICA QUIK CHEK* provides a rapid format for sensitive and specific detection of pathogenic *E. histolytica* without non-pathogenic *Entamoeba* cross reactivity.

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IN VIVO DISTRIBUTION OF MYELOPEROXIDASE IN A BALB/C MOUSE MODEL OF AMEBIC LIVER ABSCESS

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The invasive protozoan Entamoeba histolytica is the etiologic agent of amebiasis. There is great debate among researchers whether the pathogenic mechanisms of *E. histolytica* cause amebic liver abscess (ALA) or are principally employed by the ameba for survival and proliferation in the host organism. It is known that the majority of individuals are resistant to *E. histolytica*, and that in resistant individuals an appropriate immune response clears pathogens. The BALB/c mouse model is used to explore the mechanisms of resistance to *E. histolytica*. Neutrophils are the most abundant cells of the inflammatory response and the principal killers of amebae. These immune cells have various components with anti-amebic activity, such as myeloperoxidase (MPO). In an earlier work we demonstrated that MPO binds to *E. histolytica* and induces morphological changes. The aim of the present study was to explore to the importance of the participation of MPO in the resistance to hepatic tissue damage during the pathogenesis of amoebiasis in the BALB/c mouse model. BALB/c mice were inoculated with *E. histolytica* and then sacrificed at 3, 6 and 12 h post-inoculation. In order to study the presence of MPO, liver samples were processed for immunohistochemical MPO analysis. The *in situ* expression of MPO was also studied by qRT-PCR in order to determine the expression of this enzyme in the ALA. At 3 h post-inoculation, amoebae were surrounded by neutrophils stained for the MPO enzyme. At 6 h, inflammatory foci composed of neutrophils were positive for the presence of MPO. At 12 h of ALA evolution, acute and chronic inflammatory cells were labeled for MPO. The qRT-PCR of MPO mRNA revealed the expression of the enzyme in the ALA tissue. The results demonstrate the induced expression of mRNA for MPO in immune cells of hepatic lesions, suggesting that this enzyme is synthesized in response to the amoebic infection. Therefore, the resistance of the mice to *E. histolytica* probably lies in the nonspecific immune response, and MPO activity is apparently important in this sense.

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NAEGLERIA SP. IN TOURIST PONDS OF LIMA, PERU

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Naegleria fowleri causes primary amoebic meningoencephalitis, which affects immunocompromised and immunocompetent persons, and has a mortality rate near 100%. A recognized source of infection is direct contact with contaminated water, such as ponds, lakes, thermal baths or pools. Ponds of Chilca (also known as "La Encantada", "La Mellizera" and "La Milagrosa") are located 68 km south of Lima, Peru. Ponds of Chilca are tourist attraction that receives about 300,000 visitors a year because people believe in the healing properties of their water. The purpose of this study was to isolate *Naegleria* sp. from the water of the three ponds of Chilca and to provide information about the potential risk for the health of residents and tourists. Thirty water samples were collected in one liter containers, ten for each pond: half from surface water and half from one meter deep. After sedimentation, each sample was separated into two tubes with non-nutritive agar 2% with *Escherichia coli* inactivated at 56°C, and was cultured for 14 days at two temperatures, one at environmental temperature (20-25°C) and the other at 37°C. It was observed through the microscope at 10X and 40X. *Naegleria* sp. cysts were found in 40% (12/30) of samples. Forty-two percent of positive samples (5/12) were from "La Milagrosa"; 33% (4/12) were from "La Encantada" and 25% (3/12) were from "La Mellizera". Proportion of positive samples by each pond was 50% (5/10) in "La Milagrosa"; 40% (4/10) in "La Encantada" and 30% (3/10) in "La Mellizera". Approximately 67% (8/12) of positive samples came from deep water. This finding suggests that ponds of Chilca could be a source of transmission of *N. fowleri*, and there is a potential risk of disease in people and tourists who dive into their water. Therefore, we recommend immediate preventive activities.

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ENTAMOEBA DIVERSITY IN DISADVANTAGED BANGADESHI CHILDREN

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Entamoeba histolytica is the causal agent of amebic diarrhea and dysentery in young infants in developing countries. The recent identification of a novel *Entamoeba* species *E. bangladeshi* and the discovery that *E. moshkovski* could also cause diarrhea instigated a study

into diversity of *Entamoeba* spp in children living in an urban slum in Dhaka, Bangladesh. In this study a next generation sequencing approach was used to characterize nineteen samples that by microscopy contained ameboid trophozoites or cysts but which could not be assigned to an *Entamoeba* species by conventional qPCR or immunodiagnostic methods. Broad range primers (adapted for next generation sequencing) were used to amplify the 18S rDNA region previously used for taxonomic assessment in the *Entamoeba* genus. However to provide more detailed information, broad range primers targeting regions within the actin gene and heavy subunit of the Gal/GalNac Lectin were used to develop a multilocus typing system. One of the sequenced samples (8170) contained a novel *Entamoeba* variant. While the 18S region of the sequenced DNA was identical to that of the *E. bangladeshi* gene, the actin and Gal/GalNAc transmembrane regions were markedly divergent. The biological effect of these changes is unclear at present (the variations in the novel *Entamoeba* resulted in either no changes in the encoded amino acids or a conserved substitution) and currently we have no evidence that either *E. bangladesi* or *Entamoeba-8170* have the capacity to cause disease. The significance of this finding is that we have proven that an unplumbed diversity exists in the *Entamoeba* species circulating in Bangladesh. Preliminary evidence suggests that structural re-assortment is common in the parasitic *E. histolytica,* and the finding that several closely related species exist in endemic populations suggest that these could provide a reservoir of diversity that the parasite could draw on.

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PREVALENCE OF INTESTINAL PARASITES IN SCHOOL-AGED CHILDREN WITHIN THE FRAMEWORK OF THE FIGHT AGAINST NEGLECTED TROPICAL DISEASES (NTDS) IN THE CITY OF LOMÉ, TOGO

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In Togo in 2009, a nationwide survey of the prevalence of soil-transmitted helminths (STH), schistosomiasis and trachoma was conducted and, based on the results, nationwide MDA was started in 2010. The region of Lomé, which includes the capital city and its environs and represents around one fifth of the population of Togo, was considered a low-risk zone for these neglected tropical diseases (NTDs) based on available data and was excluded from the national mapping, Yet a high prevalence of NTDs has been found in other countries in urban areas that were assumed to be low-risk,. and school-aged children constitute a risk group for many intestinal parasites. In order to not lose the opportunity to treat exposed children in this region, we conducted a study to determine the prevalence of intestinal parasites in the school-aged population. Lomé region's five districts were each considered as separate ecological entities, based on their specific geographic and climatic characteristics. In each district, 5 primary schools were randomly selected and 30 children from each of the third, fourth, fifth and sixth grades were tested. The Kato-Katz method was performed to identify STH species on fresh stool samples collected from the children on site on the morning of the survey day. Direct examination using Lugol's solution was added to identify protozoa for the children in the 3rd and 6th grades. Across the five districts, 2,944 school-aged children aged 6-15 years, representing 25 schools, were tested. The mean prevalence of STH was relatively low, 5% across all sites, ranging from 1.5% to 8.6% at district level; prevalence also varied with the child's sex. The prevalence increased with age and with grade level. Protozoa were found in 52.2% of the 1,465 children tested and were represented primarily by *Entamoeba histolytica* (51.3% of all children examined). Double and triple infections were noted in 10% of cases. *Ancylostoma duodenale* and *Necator americanus* were the species of STH most frequently identified (3.4%) and infection was light in 80% of cases. *Schistosoma mansoni* was found in 0.3% of cases. The findings of this survey confirm that it is not indicated to extend integrated MDA to Lomé region, but instead highlighted the high prevalence of protozoa, and in particular of *E. histolytica,* in the school-aged population. These results demonstrate the need for a national strategy to address the high prevalence of intestinal protozoa in school-age children.

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HISTOPATHOLOGICAL DESCRIPTION OF PROTOZOANS AND HELMINTHS IDENTIFIED IN SMUGGLED TURTLES AND TORTOISES IN BANGKOK

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In January of 2014, upwards of 500 tortoises and turtles were confiscated from the bags of a single smuggler at Bangkok's International Suvarnabhumi Airport. Many of these animals died during the process and complete necropsies were performed. Histopathologic diagnosis of parasites observed in tissue section remains one of the more challenging mechanisms of disease identification, however it also allows for a rapid holistic mechanism of diagnosis of parasitic disease. Histopathological evaluation of all submitted tissues revealed a wide range of nematodiasis, trematodiasis, and protozoal diseases in various organ systems. In each case, the genus of the parasites was identified through descriptive microscopic criteria and through consultation and concurrence with other board certified veterinary pathologists. The identified parasites included renal myxozoanosis, intestinal ascaridiasis, multiorgan spirorchiasis, multiorgan coccidiosis, suspected biliary serpinemiasis as well as other unidentified intestinal nematodes. Microscopic descriptions of helminths hinged on location within the body, concurrent tissue pathology, measurements of size, external ornamentation, lateral chords and alae, type of musculature and hypodermis, presence or absence of a body cavity and gastrointestinal tract, and various features of the gastrointestinal tract and reproductive organs as well as ova or larvae. Protozoal identification similarly was governed by phenotypic characteristics of the parasite in tissue sections, to include location in tissue and concurrent pathology, size, shape, number, shape and location of nuclei as well as a wide range of minute identifying structural features. Without speciating the parasites it is impossible to assess their zoonotic potential, however their introduction into a naïve environment presents the possibility for the emergence of disease in novel hosts or even of epizootic outbreak. While most of the focus remains on the potential spread of viral diseases such as H5N1 influenza virus or monkeypox, parasitic infections can be host adapted, clinically silent and as such present a more subtle and insidious etiology. To our knowledge, this is the first histopathological survey of parasitic disease of smuggled testudines.

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DETECTING *CRYPTOSPORIDIUM*: A COMPARISON OF MICROSCOPY, IMMUNOFLUORESCENCE, RAPID TESTS AND RT-PCR

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Cryptosporidium is the cause of numerous outbreaks of intestinal illness due to contaminated water, especially recreational water, in first world countries and world wide is a major cause of diarrheal disease, particularly in children under five years of age. *Cryptosporidium* passes easily from

person to person due to a low infective dose, high numbers of oocysts shed from infected individuals, resistance to common disinfection methods, oocysts that are both immediately infective after passage and are hardy in the environment. There is only one drug that has been FDA-approved for treatment. Nitazoxanide is up to 75% effective in eliminating *Cryptosporidium* and can take up to five days to alleviate the diarrhea associated with infection. There are no vaccines or prophylactic treatments for cryptosporidiosis. Thus detection of the parasite is key to identifying sources of outbreaks and taking special measures to stop the spread of infection. Traditionally detection has been *via* acid fast staining and microscopy. However, microscopic methods are labor intensive and require specially trained personnel. In response to a need for easier means of diagnosis, rapid test that use immunochromatographic methods have been developed to detect parasite antigens. While these are approved for clinical use, in practice the sensitivity and specificity of some rapid tests has been found to be disturbingly low. To evaluate detection methods we compared results from clinical specimens tested by, staining and microscopy, immunofluorescent microscopy and a rapid test. Further, we developed a real time PCR assay and that was used to resolve discrepant results. The RT-PCR assay specifically identifies *C. parvum* and *C. hominis*, the two species that cause the majority of infections in humans, and also detects other species at the genus level. A comparison of the sensitivity and specificity of the methods will be discussed along with identification of unusual species.

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CRYPTOSPORIDIUM INFECTION IN RURAL GAMBIAN CHILDREN: EPIDEMIOLOGY AND RISK FACTORS

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The Global Enteric Multicenter Study (GEMS) documented *Cryptosporidium* as the 3rd commonest pathogen of moderate-to-severe diarrhoea (MSD) in children age <5 years. We investigated the epidemiology and risk factors for *Cryptosporidium* diarrhoea among children enrolled in GEMS from an enumerated population in rural Gambia. We recruited MSD cases (Dec. 2007- Dec. 2010), and cases with both MSD and less severe diarrhoea (LSD) (Nov. 2011-Nov. 2012) presenting at health centres, along with 1-3 controls matched for age, sex and community within two weeks of recruitment of cases. A questionnaire generated information on socio-demographic, water, sanitation and presence of animals in the compound. Each subject provided a stool sample to identify enteropathogens, including *Cryptosporidium* by immunoassay. We described the prevalence of *Cryptosporidium* in diarrhoea cases and their controls. Case-control analysis determined the association of risk factors with *Cryptosporidium* positive diarrhoea compared to matched controls negative for *Cryptosporidium.* We enrolled 1938 cases (1381 MSD, 557 LSD) and 2969 matched controls; 231 (11.9%) diarrhoea cases and 141 (4.7%) controls were positive for *Cryptosporidium*. Most *Cryptosporidium* diarrhoea cases (198/231, 86%) were aged 6-23 months and presented during the May-October rainy season (188/231, 81%). *Cryptosporidium* prevalence was similar between MSD and LSD (12.1% vs. 11.5%, p=0.711). Independent risk factors for *Cryptosporidium* diarrhoea were the compound having a cow (aOR 2.9, 95% CI 1.6-5.2), a cat (aOR 2.0, 95% CI 1.1-3.7) or rodents (aOR 2.1 95% CI 1.1-4.0), consumption of stored drinking water (aOR 4.1, 95% CI 1.8-9.7), and rainy season (aOR 25.2, 95% CI 4.4-146.0). In conclusion, continued surveillance is essential to assess the *Cryptosporidium* burden. Sheltering animals outside the compound, improved hygienic practices and treatment of drinking water should reduce *Cryptosporidium*-associated diarrhoea.

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DETECTION OF *NAEGLERIA FOWLERI*, *ACANTHAMOEBA* SPP. AND *BALAMUTHIA MANDRILLARIS* IN FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUES BY REAL-TIME MULTIPLEX PCR

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The free-living amoebae (FLA), *Naegleria fowleri*, *Acanthamoeba* spp. and *Balamuthia mandrillaris*, can cause significant human ocular and/or central nervous system (CNS) infections with high associated morbidity and mortality. There have been several recent efforts to develop molecular methods (e.g., multiplex PCR, isothermal amplification) for the diagnosis of FLA in CSF, fresh tissue and other clinical specimens with excellent sensitivity and specificity (e.g., triplex PCR assay described by Qvarnstrom et al, Centers for Disease Control and Prevention; reported limit of detection of 1 organism per sample). However, the performance of such assays has been less rigorously evaluated using formalin-fixed paraffin-embedded (FFPE) tissue, an important specimen type that presents challenges for DNA recovery. Twenty-eight human corneal or brain FFPE specimens with FLA infection diagnosed by histopathology (gold standard) as well as 11 FFPE human tissues from patients not suspected to have FLA infection were tested in duplicate by multiplex PCR (adapted from Qvarnstrom et al). Tissue sections (50-60 µm) were digested with proteinase K followed by DNA extraction using the Roche MagNA Pure system. Nucleic acid amplification and detection were performed using the Roche LightCycler 480. Twenty-two of the 28 positive FFPE specimens were detected by PCR (sensitivity 78.6%), with all organism-specific positive results matching the corresponding histopathologic diagnoses. One false positive Acanthamoeba sp. result was detected in a section of brain also confirmed to have *Naegleria fowleri* by histopathology and PCR but no positives were detected in the negative FFPE control specimens (specificity 91.7%). Given the ubiquitous environmental distribution of *Acanthamoeba*, this may represent exogenous contamination of the tissue or paraffin block. No inhibition was noted in the PCR reactions based on amplification of an internal control in each reaction. Although sensitivity was reduced using FFPE specimens, the Qvarnstrom et al. triplex FLA PCR assay may serve as a valuable tool for detection and confirmation of FLA infections in ocular and CNS specimens when fresh specimens are not available.

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SEROPREVALENCE OF TOXOPLASMOSIS AMONG CHILDBEARING WOMEN IN BAGHDAD DURING 2013

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Toxoplasmosis is a global parasitic disease caused by the protozoan *Toxoplasma gondii,* with estimates that up to a third of the global population and up to 95% in some subpopulations have been infected. Official assessment in the United Kingdom places the number of new infections at about 350,000 per year in the UK. In Iraq toxoplasmosis is widely endemic but often underreported since not all cases seen in private clinics are reported to the Ministry of Health. During the years 2009 to 2013, the official incidence of toxoplasmosis was reported to have doubled from 692 to 1390. We designed a study to estimate the incidence of toxoplasmosis in women of childbearing ages between 14 and 45 years old and to determine risk factors associated with seroconversion rates. There were 950 subjects recruited for this study and blood samples were collected at four health centers including two in Baghdad, one in Alkarkh and another in Al-Rusafa. Samples were tested for IgG and IgM specific antibodies by ELISA. Demographic data for each subject was collected

and stored in a database, including personal information and a series of questions related to possible risk factors. Our study revealed that the mean age of a patient with positive ELISA response for *T. gondii* was 24 years. There were 72 subjects who were IgM positive and 159 IgG positive. IgG or IgM antibodies indicative of toxoplasmosis were found in 89.9% of house wives. There was no significant difference between observed rates of seroconversion between students and employees (4.5% and 4.8%, respectively), and 0.8% did not complete the questionnaire. There was an association between positive ELISA responses and contact with stray house cats (43.3%), eating unclean vegetables (13.9%), eating outside the house in restaurants (12.6%) and failure to regularly wash hands (19.5%.) In conclusion, our study confirmed the high incidence of toxoplasmosis in Iraq, the public health importance of this disease, and identified some possible risk factors for disease transmission in women of childbearing age. This information will be very useful for new public health education campaigns and control programs throughout Iraq.

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DETECTION OF "EARLY WARNING" MONITOR MICE USING PROTEOMIC CLINPROTOOL ALGORITHM ESTABLISHED BY ACUTE SCHISTOSOMIASIS JAPONICA MICE

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The objective of this study was to establish proteomic ClinProTool algorithm with different expressed peptides in sera of acute Schistosomiasis japonicum mice for a new rapid and accurate detection method for "early warning" monitor mice. The acute schistosomiasis japonica infection mice was generated, sera peptides were enriched from the infected and control group by MB-IMAC Cu kit separation(Bruker Daltonics GmBH), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and ClinProTool algorithm were used to generate the proteomic pattern based on the differential expressed peptides. The infected "early warning" guard mice were simulated in our labrotory, sera from different infectiosity(1, 2, 4, 6, 10, 14, 18, 22 cercaria post-infection) and different infection time (every week) were collected, after serum peptides-peaks acquired by mass spectrometry and applied with ClinProTool algorithm, which was used to generate the proteomic pattern. Sera peptides were captured in the mass range from 800 to 20 000Da, seven peaks with a clear difference in amplitude were detected between the 5-weeks post-infection and control groups, 4 peaks with mass charge ratio (m/z) of 3493, 2869, 2024 and 4965 were down-regulated and 3 peaks with a m/z of 9068, 2082 and 4533 were up-regulated in infected mice($P<0.01$). Proteomic pattern was established with the seven difference peaks. The result also showed high sensitivity and specificity of the proteomic pattern. In weeks 1 to 4 post-infection, characteristic proteomic patterns could be detected in 5%(1/20), 37%(7/19), 79% (15/19) and 85%(17/19) of the infected mice, whereas ELISA testing resulted in positive results from week 3 onwards. The infectiosity assay showed 28%(2/7), 50%(4/8), 83%(5/6) positive in 4, 6, 10 cercaria post-infection groups, respectively. And 100% positive in 14, 18, 22 cercaria post-infection groups, All T. gondii control sera were detected *S. japonicum* negative. MALDI-TOF MS coupled with peptide enrichment can be a desired method in detecting the biological markers of schistosomiasis in a mouse model. ClinProTool algorithm could be a good method for the rapid detection of "early warning" guard mice in monitoring Schistosomiasis in China. The result also cast a fundamental research in schistosomiasis in the level of peptide and protein.

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RAPID ASSESSMENT OF SCHISTOSOMIASIS RISK FOLLOWING AN EARTHQUAKE IN SICHUAN, CHINA AND IMPLEMENTATION OF PREVENTION MEASURES

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On April 20, 2013 a magnitude 7.0 earthquake struck Ya'ancity, located in Sichuan, China.The earthquake caused a large number of casualties, altered the ecological and social environment and damaged medical treatment health facilities. The impacted areas were historically endemic areas for schistosomiasis transmission, raising concerns about disease reemergence. We conducted a rapid assessment of schistosomiasis risk in the area impacted by the earthquake and used the results of the assessment to rapidly deploy surveillance and control measures.The assessment included a comprehensive literature review, an analysis of schistosomiasis surveillance data in the earthquake-affected counties, and a field investigation. Based on our initial risk assessment,we formulated and carried out schistosomiasis surveillance and control measures including 1) locating displaced person settlements in areas so as to minimize exposure risk, 2) constructing carefulexcrement management systems in the displaced person settlements 3) carrying out snail control (molloscicidingor black plastic)in high-risk environments, 4) conducting health education, 5) increasing patient monitoringand treatment, and 6) expanded chemotherapy.We conducted an evaluation 40 days following the earthquake,including snail surveys,human infection surveys and health education assessments. We found no infected snails or acute Schistosomiascases in humans, but the average density of *Oncomelaniahupensis* snails was higher than observed before the earthquake (4.1snails/m^2 vs. 0.3 snails/ m^2). In the short term, the implementation of effective prevention and control measures may have helped to reduce the risk of schistosomiasis transmission in an area impacted by a natural disaster.However the rise in snail populations and the history of disease transmission in the region suggests health education, snail control and surveillance for schistosomiasis should continue.

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

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Schistosoma japonicum is the causative agent of schistosomiasis in the Philippines with 6.7 million people living in endemic areas and 1.8 million having direct exposure through daily water contact activities. As a zoonosis *S. japonicum* infects over 40 mammalian species, including water buffalo which have been shown to be major reservoir hosts in China. In the Philippines, water buffalo (carabao) have been considered unimportant hosts due to low prevalence and infection intensity found in previous studies. High prevalence of *Fasciola gigantica* has also been reported from bovines in the Philippines. Previous studies on F. hepatica and *S. mansoni* have suggested that cross-protection occurs while anecdotal evidence from the Philippines suggests this might also be the case for *S. japonicum* and *F. gigantica*. To examine the role of bovines in *S. japonicum* transmission human and bovine (cattle and carabao) stool samples from six barangays from Northern Samar, the Philippines, were collected. Bovine samples were examined with an improved microscopy technique, the formalin-ethyl acetate sedimentation (FEA-SD), and qPCR analysis, while human samples were examined by Kato-Katz (KK) in addition to qPCR. High *S.*

japonicum prevalence was found in humans when using qPCR (90.2%), while KK showed a much lower prevalence (22.9%). High prevalence was also found in bovines when using FEA-SD (62.1%) and qPCR (81.7%). Intensity of infection was higher for cattle (geometric eggs per gram 8.3) than carabao (gmepg 4.7). The Bovine Contamination Index was calculated using the combined carabao and cattle arithmetic epg (7.8) and showed that each bovine was excreting an average of 195,000 eggs into the environment daily. Bovines also had a high prevalence of *F. gigantica* infection by both FEA-SD (96.0%) and qPCR (95.3%) techniques. The identification of bovines as a major reservoir host for *S. japonicum* in the Philippines suggests that bovines should be includes in control programs by chemotherapy and/or vaccination to reduce the burden of disease due to schistosomiasis in the Philippines.

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URINE MICROALBUMINURIA, PROTEINURIA AND MICROHAEMATURIA ARE MARKERS OF UROGENITAL SCHISTOSOMIASIS-RELATED MORBIDITY IN INFANTS AND PRE-SCHOOL CHILDREN

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Urogenital schistosomiasis, caused by *Schistosoma haematobium* is highly prevalent in Africa. Only recently has the burden of disease in preschool children and infants aged 5 years and below began to be quantified and recognized as a significant attribute in childhood health and development. The tools for diagnosing infection in this age group are less sensitive and those for detecting morbidity poor. In addition, there is a paucity of studies assessing the performance of the available diagnostic tools for infection and morbidity in this age group. The objectives of this study were; to determine the reliability of currently available tools for measuring schistosome-related morbidity and to investigate the effect of antihelminthic treatment on these markers. The study was conducted in an endemic area in Zimbabwe. We examined several indicators using urine albumin-creatinine ratio (UACR), dipsticks, questionnaires and clinical examination at baseline in 298 children (1-5years, n=104; 6-10years, n=194) to identify morbidity markers most attributable to urogenital schistosomiasis. Microalbuminuria assessed from UACR and dipstick microhaematuria and proteinuria were significantly associated with infection. A single dose of praziquantel was given to study participants and showed 95.3% cure rate and 96.1% egg reduction rate at 12 weeks. The effect of treatment on these 3 identified markers was assessed in 92/298 children who received successful curative treatment. Prevalence of microalbuminuria at baseline (45.7%; 95%CI: 35.3-56.0%) significantly decreased (χ^2=39.1; P<0.001) to 1.1% (95%CI: 0.0-3.2%) after treatment. Similarly, there was a significant reduction in proteinuria post-treatment, but no change in microhaematuria. In conclusion, our study showed that microalbuminuria, proteinuria and microhaematuria are useful schistosomiasis-related morbidity markers in untreated children. These findings are important for planning, monitoring and evaluation of schistosome control programmes.

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CHRONIC SCHISTOSOMIASIS: COMPARISON OF THE EFFECTS OF TWO ROUNDS OF MASS DRUG ADMINISTRATION (MDA) VS ONE ROUND OF MDA ON PHYSICAL FITNESS

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Chronic schistosomiasis has been associated with reduced physical fitness, but additional evidence of the benefits of praziquantel treatment is needed. The aim of this study was to assess the impact of one and two rounds of mass drug administration (MDA) on physical fitness. The study was carried out among school aged children who were grouped into two cohorts as part of a larger investigation of most effective ways to provide MDA. A cohort that had received one round of treatment at school (SBT) was compared to another cohort that had received community wide treatment (CWT) two times in two years. At baseline and after two years, standardized quality controlled methods were used to determine helminth infections (Kato-Katz technique), hemoglobin levels, anthropometric measurements (weight and height) and physical fitness (20m shuttle run test). In this area of high transmission, there were no significant decreases in *S. mansoni* prevalence or intensity of infection in either SBT or CWT groups from baseline to the second year. However, significant increases in both *P. falciparum* infections ($p < 0.001$) and anemia ($p = 0.002$) were observed in both treatment cohorts. At two years after baseline, the proportion of children demonstrating wasting ($p < 0.001$) was decreased but physical fitness as measured by maximal aerobic capacity (VO2 max) was also significantly lower than at baseline in both treatment arms. Thus, we did not see a benefit of schistosomiasis treatment on physical fitness by either treatment approach. However, because of the high transmission levels of *S. mansoni* and *P. falciparum* during the follow up period, any praziquantel treatment benefits may have been masked.

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DETECTING MULTI SCHISTOSOME SPECIES DNA IN SINGLE URINE SAMPLE BY LAMP: A NOVEL DIAGNOSTIC TEST FOR SCHISTOSOMIASIS

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Schistosomes are easily transmitted and multiply considerably so if control strategies based on targeted mass drug administration (MDA) are to succeed it is essential to have a simple to operate sensitive and accurate test. As the control programs operating become more and more effective in reducing the parasite burden in the individual, the issue of diagnostic sensitivity will become more critical in the assessment of program effectiveness. Over the past several years we have demonstrated that parasite specific DNA can be detected in human urine by PCR when some specimens are apparently negative. Importantly this method does not require stored urine, but is effective in detecting and amplifying DNA from urine residue on coarse filter paper that is dried after filtration and can be stored for several months without freezing and easy to transport. In the current study we assessed the efficacy of *Schistosoma mansoni* and *S. haematobium* specific DNA detection from 86 urine residues both by PCR and loop mediated isothermal amplification (LAMP) collected in Ghana in an area of low to moderate endemicity. We also compared the DNA extraction techniques by standard extraction kit and field usable LAMP PURE kit and have evaluated these procedures on species-specific DNA detection. With *S. haematobium* all three methods showed similar sensitivity and specificity when compared with PCR amplification (100%). For *S. mansoni* sensitivity was highest for LAMP amplification (100%) than PCR and LAMP PURE (99% and 94%). The LAMP PURE extraction produced false negatives which require further investigation for this field usable extraction kit. Overall high positive and negative predictive values (90% - 100%) for both species were also indicative of a highly robust approach. The same pattern was observed when stratified for sex specific analysis. We have demonstrated a robust measure of prevalence of both species when compared with the classical examination of urine or stool. Our approach of using urine sediment for integrated diagnosis of schistosomiasis and a common DNA extraction procedure with LAMP can be an effective means to detect low intensity infection and would enhance the effectiveness of surveillance and MDA control programs of schistosomiasis.

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PRAZIQUANTEL 40 MG/KG IN CHILDREN, CLINICAL EFFICACY AND SAFETY, A META-ANALYSIS

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Children carry most of schistosomiasis burden: school-aged children are the main target group of preventive chemotherapy (PC); preschool-aged children are treated on confirmation of infection. The objective of this aggregated data meta-analysis is to evaluate the efficacy and safety of PZQ 40mg/kg, the WHO recommended dose, on intestinal and urinary schistosomiasis in children, and to assess if efficacy varies with age. Thirty-five studies were identified through a systematic literature search, enrolling 12562 preschool- and school-aged children and adolescents, of whom 11564 were assessed within 8 weeks post-treatment. The average attrition risk of bias was acceptable (91%). 6186 (54%) were treated with PZQ tablet at 40 mg/kg. Of these, 45% (n=2765) were infected with *Schistosoma mansoni* and 7% were preschool-aged children. Calculated median age was 10 years (range 1 to 19 years). The overall cure rate (CR) and egg reduction rate (ERR) obtained with PZQ 40 mg/kg were respectively: in *S. mansoni* 75.0% (95%CI 70.2-79.6, n=2754), and 92.3% (95%CI 88.4%-95.3%, n=1927); in *S. haematobium* 76.6% (95%CI 67.4%-84.7%, n=2673) and 93.9% (95%CI 88.8%-99.0%, n=1856); in *S. japonicum* 94.7% (95%CI 92.1%-98.0%, n = 406) and 95.0% (95%CI 90.1%-99.9%, n=203); and in *S. haematobium/S. mansoni* 67.6% (95%CI 53.6%-82.4%, n=372) and 98% (95%CI 90.9%-99.9%, n=54). A multivariate mixed-effect model with random effect on study site shows a significant relationship between age and CR for *S. mansoni* (p=0.001) and *S. japonicum* (p=0.001), but not *S. haematobium*. Age has no effect on ERR. PZQ proved safe across ages, with only mild transient reported adverse events. There is no clear evidence that the PZQ dose should be adapted to age, especially if the objective of the intervention is morbidity reduction in the context of PC (efficacy assessed by ERR rather than CR). However, data for PZQ tablet on preschool-aged children are limited to one country and *S. mansoni*, and studies reported on broad age-categories, making it difficult to derive conclusive estimates for age effect in small children.

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STRATIFIED WORM BURDEN APPROACH TO MODELING SCHISTOSOME TRANSMISSION IN AT-RISK POPULATIONS

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Schistosoma infection is widespread in many countries of the world, and WHO has made control of schistosomiasis a priority among tropical diseases. The infection is transmitted between human and snail hosts and multiple biological and ecological factors contribute to its spread and persistence. Mathematical modeling could help to design more efficient and cost-effective transmission control strategies. Conventional approaches to modeling macroparasite transmission have not sufficiently accounted for its complexity. We are developing new approaches that accommodate many important features of infection, such as highly aggregated worm distribution in host populations and model/data uncertainties. An earlier work has successfully applied these methods for prediction of schistosomiasis control in coastal Kenya. The current project extends the applicability of this earlier work - particularly in reference to heterogeneous host demographics and density dependent effects on worm fertility and mortality. We have also addressed diagnostic data uncertainty. New calibration procedures have now been developed and tested with data collected from several endemic areas. This has allowed us to estimate the essential biological and environment parameters of Schistosoma infection in structured population groups, and apply our model to analyze probable outcomes of control interventions in different settings. The new transmission model will next be implemented by the ongoing Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project for comparison trials of several proposed 'cost-effective' integrated control strategies.

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LONG-TERM EFFECT OF MASS CHEMOTHERAPY, TRANSMISSION AND RISK FACTORS FOR *SCHISTOSOMA MANSONI* INFECTION IN VERY LOW ENDEMIC COMMUNITIES OF VENEZUELA

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The prevalence of *Schistosoma mansoni* infection in Venezuela has changed from high to low mostly due to successful control activities including mass chemotherapy and molluscicide applications. This study examined the impact of mass chemotherapy on *Schistosoma mansoni* transmission and risk factors for infection 12 years after administration of praziquantel in Venezuela. Two relatively isolated rural communities where studied, one with snail control (Manuare) and the second without (Los Naranjos). A cross-sectional survey of randomly selected households included 226 (Manuare) and 192 (Los Naranjos) consenting participants. *Schistosoma mansoni* prevalence was determined using a combination of coprological (Kato-Katz) and serological (circumoval precipitin test, alkaline phosphatase immunoassay and Western blot) tests. Data on epidemiological and socioeconomic risk factors were obtained through individual structured interviews. Univariate analysis and multivariate logistic regression models identified independent risk factors for infection. Water sites were examined for the presence of *Biomphalaria glabrata* snails. Only one participant was positive by coprology. The overall prevalence according to the combined tests was 32.7% in Manuare and 26.6% in Los Naranjos. A lower prevalence (12.7% in Manuare and 13.2% in Los Naranjos) was found in children <12 years of age representing those born after mass chemotherapy. Variables associated with infection in both communities were older age (>25 years), contact with specific water sites, and being a farmer/non-specialized worker. In conclusion, mass treatment with praziquantel applied once to endemic communities led to an important and long-lasting sustained reduction of *S. mansoni* infections independent of the application of snail control. A degree of low active transmission of *S. mansoni* persisted in the treated areas which was associated with similar factors in both communities.

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SCHISTOSOMAISIS PREVALENCE IN RELATION TO THE PROXIMITY OF LAKE MALAWI

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Large freshwater bodies such as the great lakes of East Africa have long been considered key areas of transmission for schistosomiasis. Lake Malawi in particular is used as an example to highlight the risks of schistosomiasis and hot spot areas for transmission. To date there has been no study which evaluates the relationship between living in close proximity to Lake Malawi and risk of infection, or how the lake contributes to transmission within the country. Here we present findings from the re-assessment of five lakeshore districts which have benefitted from four rounds of preventive chemotherapy with praziquantel from the Ministry

of Health. These districts were targeted initially due to the presumption they are at greater risk of transmission. This survey was conducted firstly to identify the areas at greatest risk of infection, secondly to establish the relationship between prevalence of schistosomiasis and living in proximity to the lakes and finally the future frequency of treatment in line with World Health Organisation recommendations. A total of 2440 children from 75 schools were sampled. Fifteen schools from each of the five lakeshore districts were selected by stratifying distance to lake 0-5 kilometres (km), 5-15km and >15km. Thirty children from each school, 15 boys and 15 girls, aged 10-14 years were randomly selected and tested for *Schistosoma haematobium* using urine filtration and *S. mansoni* by Kato Katz. Probability of infection with either *S. haematobium* or *S. mansoni*, in relation to both school level characteristics, including proximity to the lake, as well as pupil characteristics such as age, sex and treatment with praziquantel in the last year were assessed. Overall 9.0% were infected with *S. haematobium* and 5.2% with *S. mansoni* varying dramatically by district; 4.85%(95% CI = 0-9.77) in Nkhata Bay to 22.46%(95% CI = 9.48-35.44) in Zomba despite previous rounds of treatment. Contrary to previous findings, *S. mansoni* was found to have a significantly higher prevalence as you move away from the lake (OR=0.33, 95% CI 0.11-0.97). This relationship was not consistent across species or between the districts accentuating the hazards of predicting at-risk areas based on the presence of large water bodies. This study has highlighted the need for comprehensive mapping during decision making for treatment strategies, in this case within Malawi, due to the heterogeneous nature of schistosomiasis infection.

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ASSESSMENT OF MASS TREATMENT FOR SCHISTOSOMIASIS IN KWAZULU - NATAL PROVINCE, SOUTH AFRICA

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It is estimated that 700 million people worldwide and 5.2 million people in South Africa are in need of annual treatment for schistosomiasis. In accordance with current policy, a Department of Health (DoH) in KwaZulu-Natal Province, South Africa, aimed to reach 75 percent treatment coverage in a mass treatment campaign (MTC) of schools in a schistosomiasis-endemic area. A cross-sectional study was designed to explore the implementation, coverage, challenges and limitations of a DoH MTC in a middle income country. The study was conducted by exploring nurses' and research team records, school enrolment lists and parental consent forms. Slightly more than 10 000 learners in 43 primary and high schools were treated, achieving treatment coverage of 44.3%. A median of two schools per day were visited over the course of 39 days. We found that older learners, being male and being in a large school were independent significant predictors for low treatment coverage. Our results indicate that coverage would likely increase through improved consent procedures and repeated schools visits. Further information is needed on how to increase compliance in teenagers, males and pupils in large schools.

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PERFORMANCE OF MICROHEMATURIA IN THE DETECTION OF *SCHISTOSOMA HAEMATOBIUM* IN CHILDREN FROM BENGO, ANGOLA

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Urinary schistosomiasis is endemic in Angola and for effective control measures, an efficient, quick and cheap means of diagnosis should be incorporated. The use of reagent strips for detecting microhematuria has long been recommended as a cheap and accurate proxy for schistosomiasis diagnosis. However, their performance levels do vary according to the underlying population prevalence. Moreover, whether or not these methodologies still turn in good performances after mass treatment with Praziquantel also needs determining. A cross-sectional study of children was completed to evaluate the performance of microhematuria in detecting *Schistosoma haematobium* infections in Bengo, Angola, both before treatment with praziquantel and then again in the follow-up (1 month and six months after treatment). Urine samples from 504 children were tested for microhaematuria with Combur-Test (Roche) reagent strips followed up by egg microscopy analysis. A total of 277 boys and 227 girls were analyzed at the baseline with 400 from this sample reanalyzed one month after treatment and 419 six months after. The prevalence of the infection was 64.5% in the sample area, classifying this area as of high prevalence. One month after treatment the prevalence was 29.5% while six months later the rate stood at 45.1%. The application of reagent strips was found to vary both in terms of sensitivity (90.8% at the baseline, 66.1% one month after and 81.5% six months after) and in specificity (69.3% at the baseline, 76.6% one month after and 79.1% six months after). There were no significant differences in the gender based performance. A significant correlation was found between the intensity of infection and the concentration of hematuria in all three samples ($r=0.620$; $r=0.434$; $r=0.419$, $p<0.001$, respectively). Recourse to urine strips in community studies, in endemic areas, where resources and specialized diagnostic methodologies are scarce, thus presents itself as an easy and economic technique with good sensitivity and specificity. Moreover, this method makes possible highly precise estimates of the degree of intensity of *S. haematobium* infection.

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SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHES REMAPPING SURVEY IN THE REPUBLIC OF YEMEN

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Schistosomiasis or Bilharzia (both uro-genital and intestinal) and soil-transmitted helminths (STHs) are widespread in the Republic of Yemen with an estimated 3 million people (of the total population of 24 million) infected with schistosomiasis and a similar number with STH The Yemen Ministry of Public Health and Population have completed three years of the current six year programme (2010-2015) to control schistosomiasis and STHs nationwide Following two/three treatment rounds, a nationwide remapping survey have been conducted and led by local researchers at the Parasitology Department at the University of Sana'a The aims of the remapping are to update the map of the distribution of SCH and STH in the country, to set the treatment approach for the remainder of the current programme, and to inform the need for future control activities, including those that may help aim for elimination of infection. As part of the remapping surveys approximately 90,000 school-aged children from 2,600 schools from 333 districts in the country were surveyed between March and May 2014 Hemoglobin levels have also been recorded from all these individuals in order to provide the richest dataset on anemia ever

collected in the country The results of these surveys will be presented and discussed, against the backdrop of the significant operating challenges experienced in Yemen.

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FEMALE GENITAL SCHISTOSOMIASIS: MORPHOLOGIC CHARACTERISTICS OF ABNORMAL BLOOD VESSELS

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Female genital schistosomiasis (FGS) manifests with deposition of *Schistosoma haematobium* eggs in the genital mucosa. This results in the appearance of sandy patches and abnormal blood vessels. The former manifestation is considered pathognomonic to the disease whereas the significance of the latter is still unknown. In the literature, the blood vessels have been described as reticular, branched, circular, convoluted (cork-screw shaped) and having uneven calibre. In this study we wanted to analyse these features objectively using computer image analyses on morphology. In a study on young women with FGS in South Africa, we selected colposcopic images in which the clinicians had indicated the presence of abnormal blood vessels (n = 29) characteristic of FGS. An equal number of negative endemic controls was selected based on clinical findings. Data on blood vessel morphology was extracted using computer image analysis. The morphologic features were analysed by fractal dimensions (a measure of complexity), identification of closed loops (circularity), clusteredness (distance between vessels), number of vessels and size of vessels. We found that vessel size, clusteredness and circularity were significantly associated with the clinician's identification of abnormal blood vessels ($p = 0.036$, $p = 0.046$ and 0.049, respectively). However, we found no association with fractal dimension ($p = 0.957$) or number of vessels ($p = 0.889$). Using clusteredness alone, it was possible to classify the images with a precision of 72.4 %. Adding vessel size or circularity to the classification model did not improve the precision. Automated computer analysis of blood vessels can help decipher the specific features of abnormal blood vessels in FGS. Furthermore, this could possibly be used as part of a diagnostic tool based on image analysis. Further studies are required to look at other morphologic features of abnormal blood vessels in FGS, such as open loops (semi-circular structures) and uneven calibre.

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THE ROAD TOWARDS SUSTAINABLE CONTROL OF SCHISTOSOMIASIS IN THE DEMOCRATIC REPUBLIC OF CONGO: PRE-ASSESSMENT OF STAFF PERFORMANCE AND MATERIAL RESOURCES IN ENDEMIC REGIONS.

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Schistosomiasis is a poverty-related disease affecting more than 200 million people in developing countries, 85% of them in Sub-Saharan Africa. Since a long time, schistosomiasis has been known to be endemic in certain provinces of the Democratic Republic of Congo. However, recent or hard figures to support these observations are not available, and the most recent national prevalence data available were generated over 30 years ago. Recently, the Ministry of Health adopted a national plan against schistosomiasis. For this program to be implemented in an efficient and sustainable way, data on schistosomiasis in the DRC urgently need to be updated. The present study assessed the knowledge and practice of health staff on schistosomiasis as well as the availability of material resources for diagnosis and management of schistosomiasis in two endemic provinces in the DRC. We performed structured interviews with staff from 35 health care facilities in 9 health zones (HZ) of Kinshasa and 2 HZ in Bas-Congo. Schistosomiasis was reported to be present in all of the included HZ. Health workers could name the most important symptoms of schistosomiasis such as bloody diarrhea and hematuria. Interestingly, health workers in rural Bas-Congo were more accurate than those in urban Kinshasa in citing more advanced/long term symptoms of schistosome infection such as ascites and hematemesis. Kato-Katz and urine filtration were not available in any of the health facilities. Diagnosis and treatment mainly relied on reported symptoms. Knowledge on schistosomiasis did not differ between rural Bas-Congo and urban Kinshasa. Fees for consultation, diagnosis and treatment however, were three times higher in Kinshasa than in Bas-Congo. Diagnosis of schistosomiasis in health care facilities in Bas-Congo and Kinshasa is mainly symptom-based. Though knowledge on schistosomiasis among health staff appears sufficient, substantial efforts still have to be made to improve the availability of diagnostic tools and treatment. Reinforcement of the wavering health system would be the first step on the challenging road towards sustainable control of schistosomiasis in a country fighting a heavy burden of schistosomiasis and many other neglected tropical diseases.

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HIGH PREVALENCE OF S*CHISTOSOMA MANSONI* IN THE HEALTH ZONE OF KASANSA, DEMOCRATIC REPUBLIC OF THE CONGO

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School-aged children suffer the most from Schistosomiasis infections in Sub Saharan Africa due to poverty and limited sanitary conditions. Surveillance of disease burden is recommended and 20-year-old prevalence data needed urgent updating in the Democratic Republic of Congo. Epidemiological and parasitological study was carried out in 2011 in Health zone of Kasansa in Democratic Republic of Congo. Six health areas were included in the study. In each health area, one primary school was selected. Kato-Katz and direct microscopy examinations were performed in school-aged children. High *Schistosoma* prevalence levels (82.7%) were found in the Health Zone of Kasansa and certain study areas presented prevalence levels reaching nearly 100%. These results demonstrate that *S. mansoni* infection is a bigger problem than anticipated and there is an urgent need to implement effective control measures.

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COMPARATIVE STUDY OF THE ACCURACY OF DIFFERENT TECHNIQUES FOR THE LABORATORY DIAGNOSIS OF SCHISTOSOMIASIS MANSONI IN LOW ENDEMICITY AREAS

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Schistosomiasis constitutes a major public health problem, and estimates suggest that 200 million people are infected worldwide. Barra Mansa, Rio de Janeiro State, Brazil, has an estimated prevalence of 1%. Areas of low endemicity (ALE) represent a new challenge for the helminth control because infections occur with low parasite load (<100 eggs per gram of feces), causing a decrease in sensitivity of parasitological techniques. To compare the performance of the techniques of Kato-Katz (KK), Hoffman, Pons and Janer (HH), ELISA-IgG and ELISA-IgM, Indirect

Immunofluorescence Technique (IFT) and qPCR technique in samples of serum and feces (qPCR in feces and serum) using the *Circumoval Precipitin Test (COPT)* as reference. An epidemiological survey, in a randomized sample of residents in five neighborhoods of Barra Mansa/RJ was undertaken to obtain stool and sera samples. A cross-sectional study conducted from April to December 2011, using a probabilistic sampling that collected 610 fecal samples and 612 serum samples. The laboratory diagnostic techniques used were: KK and HH, ELISA-IgG and ELISA-IgM, IFA-IgM, COPT, qPCR-feces and qPCR-serum. We obtained the following results from different diagnostic techniques: KK and HH, 0.8% (n=5); ELISA-IgG, 11.6% (n=71); ELISA-IgM, 21.4% (n=131); IFA-IgM 15.8 (n=97); RPO 5.4% (n=33); qPCR-feces, 9.8% (n=60) and qPCR-serum, 1.5% (n=9). ELISA-IgM (21.4%) presented the highest positivity while the techniques of HH and KK were the least sensitive to indicate the presence of infection (0.8%). In comparison with COPT, except for qPCR-serum, all other techniques showed a statistically significant difference in positivity ($p<0.05$) and high accuracy (from 82% to 95.5%). The lack of adequate surveillance in areas of low endemicity of schistosomiasis may turn them into areas of medium and high endemicity. This study presents a control perspective, pointing to the possibility of using these combined laboratory tools in the diagnosis of schistosomiasis in low endemicity areas.

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SCHISTOSOMIASIS IN DEMOCRATIC REPUBLIC OF CONGO: A LITERATURE REVIEW OF THE LAST SIXTY YEARS DATA

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Since a long time, schistosomiasis has been known to be endemic in the Democratic Republic of Congo (DRC). However, hard figures or detailed epidemiological data are scarce, seriously hampering control. A literature search was conducted in PubMed to identify relevant original articles related to schistosomiasis in the DRC, published between January 1955 and January 2014. This was completed by non-peer-reviewed publications and unpublished data from experts and researchers. Key indicators, including the prevalence and intensity of infection, schistosomiasis-related morbidity and the distribution of schistosome species were retrieved. The search yielded 38 records, of which only 5 (13.1%) were published in the last twenty years. Endemicity of schistosomiasis was described in regions within 10 of the 11 provinces of DRC. Three species of *Schistosoma* were reported: *S. mansoni*, the most widespread species, followed by *S. haematobium* and S. intercalatum (Zaïre or Congo strain), which has a restricted distribution. They are co-endemic in many endemic areas, and hybridization of *S. haematobium* and S. intercalatum has been reported in Kinshasa. The prevalence of schistosomiasis varied greatly between regions and, within these regions, between different villages ranging from 0.6% up to 95%. In Kinshasa, the capital of the country, the level of endemicity has declined to under 10% over the years and is currently low. However, in rural areas, the endemicity is still either moderate or high, with great intra-regional variability. Schoolchildren and mine workers were the two most infected groups with, in some areas, prevalences exceeding 90%. Hepatosplenomegaly, urinary tract lesions, anemia and stunting were commonly reported in schistosomiasis endemic areas of the DRC such as Bas-Congo, Kinshasa and Kasai. Still, the epidemiology of schistosomiasis and its distribution have not been sufficiently explored in the DRC. Data summarized in the present review are limited to certain endemic areas only, most of them are simply outdated, and some present methodological limitations. This review discusses the knowledge gaps regarding schistosomiasis in DR Congo. It also highlights the need for effective control strategies, as well as for updated epidemiological data through well designed studies.

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COMMUNITY PERCEPTIONS, ATTITUDE, PRACTICES AND TREATMENT SEEKING BEHAVIOR FOR SCHISTOSOMIASIS IN LAKE VICTORIA ISLANDS IN UGANDA

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Over 200,000 people, most of them infected with *Schistosoma mansoni* inhabit 150 islands in Lake Victoria in Uganda. Although a programme to control the disease has been ongoing since 2003, its implementation in islands is inadequate due to high transport costs on water. In 2011 and 2012, the Global Network for Neglected Tropical Diseases (GNNTD) through Schistosomiasis Control Initiative (SCI) provided financial support to ease treatment delivery on the islands and over that period, therapeutic coverage has been increasing. We conducted a study with an objective to assess community awareness of existence of the disease, its signs, symptoms, causes and transmission, as well as attitude, practice and health seeking behavior. This was a cross-sectional descriptive study which used pre-tested interviewer administered questionnaire among purposively selected individuals in schools, health facilities and communities. Frequency distribution tables, graphs and cross-tabulations were the main forms of data presentation. Our results showed that there are numerous challenges that must be overcome to achieve effective control of schistosomiasis in the islands. Many people especially young men are constantly on the move from island to island in search for richer fishing grounds and such groups are commonly known to miss treatment by mass chemotherapy. Unfortunately case management in health facilities is very poor; health facilities are few and understaffed mainly with unskilled personnel who are overburdened by other illnesses such as malaria and HIV and the supply of praziquantel in health facilities is inadequate. Furthermore, sanitation is appalling, with no clean water and community knowledge about schistosomiasis is low even among biomedical staff. Rather than elimination, our results indicate that the programme should continue to target morbidity control beyond the 2020s until preventive measures have been instituted. The government should provide adequate trained health workers and stock praziquantel in all island health facilities.

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SURVIVAL AND GROWTH OF *VIBRIO CHOLERAE, ESCHERICHIA COLI* AND *SALMONELLA* SPP. IN WELL WATER MICROCOSMS

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Faecal contamination of drinking water is believed to be responsible for recurrent cholera outbreaks and many other waterborne diseases in north-Cameroon. However, very few studies report the isolation of faecal bacteria from drinking water in this region. Little is also known about the survival and growth of bacterial pathogens in well water, largely consumed by local populations. The ability of strains of faecal bacteria (Vibrio cholerae, Escherichia coli ATCC 25922 and four strains of Salmonella isolated respectively from well water, pig, poultry, and human urine in Garoua) to survive or grow in well water microcosms was compared. Water samples were obtained from two wells in Garoua (north-Cameroun). Autoclaving at 121°C for 15 min and filtration through 0.2 µm filter were used to make microcosms. Microcosms were constituted of unfiltered-autoclaved-; filtered-non-autoclaved- and filtered-autoclaved well waters. Bacterial strains were inoculated at initial cell

concentration of 3 Log10CFU/ml. All strains were able to survive/grow in used microcosms, and a maximal concentration of 5.61 Log10CFU/ml was observed. Survival abilities were strain-and-microcosm-dependent. The declines were more pronounced in filtered-non-autoclaved water than in the other microcosms. *E. coli* and *Salmonella* sp (Poultry strain) lowered to undetectable levels (<1Log10CFU/ml) after two days of water storage. *Vibrio cholerae* decreased over time but surviving cells persisted for longer in filtered-non autoclaved water from well W1 (1.91 Log10CFU/ml) and well W2 (2.09 Log10CFU/ml). Competition for nutrients and/or thermolabile anti-microbial substances synthesized by "ultramicrocells" or by the autochthonous bacteria retained by the filter might affect the bacterial survival.

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HOUSEHOLD AIR POLLUTION AND ITS RESULTANT HEALTH EFFECTS IN RURAL KENYAN WOMEN

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Household air pollution caused by the burning solid fuels for cooking and heating, is a major cause of respiratory morbidity for nearly a third of the world's population. 84% of Kenyan households use solid fuels for cooking. Studies have suggested that reducing HAP may improve respiratory health in women and children. We conducted a stove intervention trial to evaluate the respiratory benefits of reducing HAP by replacing traditional stoves with fuel-efficient, low emission local stoves. We conducted a pre-post intervention trial in 50 randomly selected households in Western Kenya. Fifty women aged over 18 years, willing to allow a home assessment for HAP, accept a new improved stove, and spending at least 4 hours per day in the cooking area of the house were enrolled. One month before stove replacement demographic, smoke exposure, respiratory morbidity and cooking practices data were collected also direct observations and measurements of cooking spaces. Spirometry with bronchodilator challenge, blood pressure, oximetry, hemoglobin and anthropometry were conducted also 24-hr mean PM2.5 levels using the pDR1000 passive sampler; Relative Humidity using HOBO data logger and 24-hr mean CO using Easylog USB CO Monitor. We designed, fabricated and replaced traditional stoves with the Eldoboma stove. It is inexpensive, durable dependable and locally acceptable. It was found to have very low emissions of PM 2.5 and CO in laboratory testing. Mean age of the women is 34 years, 94% are married and 74% are farmers. Mean years of education is 9. They started cooking at the mean age of 13.6 and had spent an average of 20 years cooking. They cooked an average of 3 meals daily. Thatch (56%) and tin (44%) were the most common type of roofing material. Mud and thatch was the most common type of wall (52%). The kitchens had one permanently opened door and window. None of the women were currently smoking. Exhaled carbon monoxide was 4.3 ppm. Percentage of carboxyhemoglobin was 0.82% . Arterial carboxyhemoglobin saturation; 5.76%; Arterial Oxygen saturation 96.12%, Perfusion Index 4.2%, systolic blood pressure 126.43 mmHg, and diastolic 80.8 mmHg. Kitchen PM2.5 was 8145.73 µg/m3 and Carbon Monoxide level was 55.76 ppm. At baseline, indoor levels of PM2.5 and CO were much higher than in many prior studies of HAP. Women spending ≥4 hours in poorly ventilated kitchens using a traditional cookstove had abnormally high levels of SpCO, likely due to HAP exposure.

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INTEGRATING WATER, SANITATION AND HYGIENE WITH COMMUNITY-BASED NUTRITIONAL COUNSELING IN FOND DES BLANCS, HAITI, 2013-2014

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Reducing the burden of malnutrition through community-based nutritional counseling programs has shown promising impact in Haiti and worldwide. In Haiti, the prevalence of acute malnutrition is low (4.1%), but chronic malnutrition, based on rates of stunting, remains high (23.4%). Poor water, sanitation, and hygiene (WASH) conditions worsen malnutrition: 9% of the population has piped water, 36% of the population travels ≥ 30 minutes to retrieve drinking water and 63% of households in rural areas have an unimproved or no toilet. Insufficient water and sanitation infrastructure contributed to the rapid spread of epidemic cholera in Haiti in 2010 and to its subsequent persistence. To reduce the risk of cholera and other diarrheal diseases and to educate mothers about locally available nutrition, we integrated a 2-week community-based nutritional counseling program for undernourished children <5 with distribution of WASH education and kits (safe water container, a household water chlorination product [*Aquatabs*], and soap). During three monthly home visits, community health workers emphasized educational messages, collected anthropometric data, and distributed refills of soap and *Aquatabs*. We surveyed 103 families before program implementation. At baseline, 86 (84%) families used an improved water source for drinking, 96 (93%) had ever tried a water chlorination product, and 60 (58%) had no toilet facilities. All 103 (100%) families demonstrated correct handwashing procedures. While 95 (92%) reported using chlorine product that day, only 7 (7%) of baseline water samples contained residual free chlorine. In 288 monthly follow-up visits completed in the first half of the 6-month follow-up period, 177 (60%) of water samples contained residual chlorine. This evaluation suggests that the integration of community-based nutritional counseling and WASH incentives is feasible and acceptable, and may help motivate improved WASH behaviors.

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DETERMINANTS OF PESTICIDE EXPOSURE AND NEUROBEHAVIORAL IMPACT OF SUBSISTENCE FARMERS IN THE GAMBIA

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Pesticide use greatly impacts the health of agricultural communities, including both beneficial and detrimental impacts. There is increasing concern regarding the widespread use of pesticides and their potential impacts on public health. Acute high-level exposures to certain insecticides have well-known adverse neurobehavioral (NB) effects. Chronic exposures have more subtle effects which are harder to measure and evidence is limited on the NB aspects of low-level exposures to insecticides. We assessed levels of chronic pesticide exposure and effects on NB performance of subsistence farmers. NB tests were administered to rural residents in the Upper River Region of The Gambia. Participants (N=158, ages 18 - 40 years) completed eight NB tests to assess attention, memory, response speed, and coordination. Questionnaires were administered to participants on sociodemographic characteristics and agricultural and home pesticide use. Among participants who had ever directly used agricultural pesticides (N=77, 58%), practices that were potentially main determinants of pesticide exposure were duration and frequency of use, lack of personal protective equipment use (58%), mixing techniques (bare hands/leaves, 27%, and jerry can, 22%), application methods (hands,

40%, and hand-held sprayer, 23%), and hygiene practices (not bathing or changing clothes after use, 42%, and not washing hands before eating, 42%). These exposure determinants were weighted individually by six subject matter experts to create exposure scores that included frequency and duration of use to estimate exposure levels of participants. The average age of the studied population was 28 years and 48% of males and 63% of females had never been to school. Females had statistically significant higher exposure scores than males and certain ethnicity groups had statistically significant higher exposure scores. The results of such studies are critically important especially in developing countries where adverse health effects could be the greatest due to lack of protective measures and regulations.

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RAINWATER CATCHMENT AND PURIFICATION SYSTEM FOR IMPOVERISHED COUNTRIES

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Access to clean drinking water is one of the principle health needs faced by people living in rural Honduran communities. In a survey done by Virginia Commonwealth University's Global Health and Health Disparities Program (GH2DP), in one rural community (Lomitas) only 22% of respondents (11/50) had access to private water faucets. The majority of respondents obtained their water directly from the river (62% or 31/50). According to GH2DP's microbial testing, the river does not meet the drinking water standards set forth by the World Health Organization (WHO). VCU's chapter of Engineers Without Borders (EWB) has created a rainwater catchment system to allow individual households in Lomitas to collect clean drinking water that meets or exceeds the drinking water standards set forth by the WHO. The goal is to deploy a system that can be set up and maintained by local inhabitants without extensive training. This novel, clean-drinking water apparatus is sustainable and comprised of inexpensive and readily available materials such as tarps, water hoses, polyvinyl chloride (PVC) pipes, and polyethylene terephthalate (PET) bottles. We are currently studying the rigor of our rainwater catchment system through exposure to several extreme environmental conditions, through assessment of possible microbiological hazards, and through the determination of potential effectiveness for rural communities with sufficient rainfall.

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POST-EARTHQUAKE DRINKING WATER SURVEILLANCE IN THE OUEST DEPARTMENT OF HAITI DURING THE GREAT HAITIAN CHOLERA EPIDEMIC: THE MODERN DAY JOHN SNOW

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The 2010 earthquake in Haiti led to thousands of deaths, destruction of drinking and waste-water infrastructure and displacement of millions into temporary camps with little sanitation and hygiene. Even worse, the introduction of *Vibrio cholera* in October, 2010 resulted in a massive cholera outbreak that spread rapidly throughout Haiti. Once it was recognized that many displaced persons lacked access to water and the outbreak likely resulted from consumption of contaminated surface water, thousands of wells were installed by Non-Government Organizations. However, despite an ongoing epidemic, no water quality data was collected from these wells to verify their safety. To determine if the wells were a source of exposure, 359 sources of drinking water in the Leogane flood basin, located at ground zero of the earthquake, were screened for *V. cholerae* and fecal coliform bacteria. While no toxigenic strains of *V. cholerae* were identified, non-toxigenic *V. cholerae* was isolated from six water sources. Of these, all contained fecal coliforms and displayed significant clustering near the major highway, which was hypothesized as a major route of dissemination the cholera outbreak. Over 80% of unimproved water sources (WHO classification) had the presence of fecal coliforms along with 25% of the recently installed improved water sources, which showed increases in contamination after hurricane Sandy. This study provides evidence that the source of transmission was most likely from person to person and from consumption of surface water sources, which our research group has recently identified to be contaminated with toxigenic *V. cholerae* O1. The results also suggest that while drilling wells in emergency situations is a necessity, the long-term sustainability of those wells will likely require the support from the national government in the form of monitoring maintenance, and not by a fragmented patchwork of NGOs. If *V. cholerae* has become endemic, improvements in water and sanitation infrastructure might be the only way to eliminate the transmission of cholera in Haiti.

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INTRODUCTION OF INNOVATIVE LOW-COST FOOD BANKING SCHEME AS A MEANS OF PREVENTING MALNUTRITION AMONG UNDER FIVE CHILDREN AMONG FARMERS IN RURAL COMMUNITIES

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Malnutrition among other health problems in under-five children, contributes about 50% of all child mortality in Sub-Saharan Africa. Malnourished child during the first five years of his/her life leads to slowed physical and mental development. A child's physical growth, cognitive development and overall performance depend much on feeding of the child during the first five years. This is a preventable condition, among other solutions food security within the household is among them. So we intend to introduce the use of low-cost food banking schemes as a means of reducing and preventing malnutrition among children during their first five years. We intend to conduct cross sectional study in five villages with a sample 50 households among farming communities. Mothers and care givers will be recruited from the houses that have under five children. Malnutrition diagnostic test will be performed to all children understudy to get their status. Mothers and care givers will identify all food products which are locally available then will be trained on nutritional foods for children as well as proper feeding practices and food storage techniques. Monitoring of food types given to the children, feeding patterns and food availability within the household will be conducted. All this is to ensure children get the required nutrients from the local available food products within the areaFrom the provided training and knowledge sharing on nutritious foods, feeding patterns and storage techniques, we expect the following: 1) knowledgeable mothers and care givers on nutritional foods for their children, 2) improved feeding pattern of under five children and 3) improved storage facilities from the locally available means to ensure availability of at least 3 meals per day and food security within these households. Actual results will be presented during the conference. In conclusion. mothers and care givers knowledge on nutritional foods and feeding pattern for children are among the important entities in reducing child malnutrition without forgetting the role played by food security.

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THE IMPACT OF HYDRATION ON COGNITION AMONG SCHOOL CHILDREN: RESULTS FROM A RANDOMIZED CONTROL TRIAL IN ZAMBIA

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Access to drinking water during the school day may improve children's ability to learn through the positive effect of hydration on attention,

concentration, and short-term memory. The link between dehydration and lower cognitive performance has been well-established among adult populations and among school children in high-income settings. However, until recently this relationship has not been evaluated among children in low-resource settings. We conducted a randomized control trial to investigate the relationship between cognition, hydration, and water consumption among children residing in a water-scarce setting. The protocol for the study was adapted from a pilot conducted in Mali. We visited five schools in Chipata province, Zambia, for one day each. Pupils were randomly assigned within each school to receive either a bottle of drinking water that they could refill throughout the day (water group, n=149) or were not provided with supplemental water and could only access drinking water that was normally available at the school (control group, n=143). We assessed hydration in the morning and afternoon using urine specific gravity (USG) measured with a portable refractometer. Children were considered dehydrated if their USG exceeded 1.015. In the afternoon, we administered six cognitive tests to assess short-term memory, concentration, visual attention, and visual motor skills. Independent samples t-tests were used to compare cognitive test scores between the water and control groups, and linear regression was used to compare hydration level and cognitive test score. Mean morning USG was 1.018 for both water and control groups. Afternoon USG increased among the control group (1.022) and decreased among the water group (1.006). Mean scores for one of the cognitive tests were significantly higher among the water group. There was no significant difference in mean scores between the water and control groups for any other test, and there was no significant correlation between afternoon USG and any test scores. Results show that moderate dehydration among school children is prevalent and increases throughout the day in the absence of supplemental water, and increased access to water decreases dehydration prevalence. There is some evidence that hydration improves cognitive test performance, but we found no clear association.

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THE IMPACT OF SCHOOL-BASED WATER, SANITATION AND HYGIENE IMPROVEMENTS ON THE PRESENCE OF BLOOD ANTIBODIES FOR ENTERIC AND NEGLECTED TROPICAL DISEASES AMONG SCHOOL CHILDREN IN MALI

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The role of improvements in water, sanitation, and hygiene (WASH) on the reduction of enteric and neglected tropical diseases among children and adolescents is well supported. However, health impact evaluations of WASH interventions in low-resource settings are limited by the existing, and often biased, expensive, or laborious methods and tools used to measure diarrheal and NTD incidence. We piloted a novel, objective method for evaluating the impact of school WASH improvements on enteric and neglected tropical diseases incidence among pupils in Mali. Capillary blood in the form of dried blood spots (DBS) was collected from 400 students attending beneficiary schools of comprehensive school-based WASH program (intervention), and 400 students attending schools that did not receive any WASH improvements (control). Using a Luminex multiplexing assay, we will analyze the DBS for blood antibodies for a range of enteric and neglected tropical diseases. Levels of antibodies will be compared between pupils in intervention and control schools to provide biological evidence of the school WASH program impact on pupil health. Data collection will conclude in May, and DBS antibody analysis will be complete by June. Results from this pilot study will be used to assess the feasibility of using the Luminex multiplexing assay to detect disease incidence among school-aged children (SAC) - a novel age range for this approach - to identify the leading pathogenic causes of enteric and neglected tropical diseases among SAC, and to quantify the impact of a comprehensive school WASH program on blood antibody levels among SAC. Additionally, results may inform power and sample size calculations for future research.

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EFFECT OF SANITATION CONDITIONS AND HYGIENE ON THE PREVALENCE OF ENTERIC PATHOGENS IN AN IMPOVERISHED COMMUNITY IN THE PERUVIAN AMAZON

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Diarrheal diseases are the fourth most common cause of death worldwide and the second most important factor in global disease burden (WHO, 2011) and represent an important target for global health interventions. Many enteric parasites are transmitted by the fecal-oral route via waterborne sources and can lead to diarrhea or nutritional deficiencies. We hypothesized the prevalence of the roundworms *Ascaris lumbricoides* and *Trichuris trichiura*, and the protozoans *Entamoeba coli*, *Giardia species*, and *Cryptosporidium parvum* is higher in households without quality sewage treatment, treated water, proper water storage, and good hand washing practices than in those households that have access to and utilize these services and hygiene practices. We conducted this prospective observational study in Belen, an urban, riverside slum in the Amazon Basin in Iquitos, Peru. Interviews were used to assess source of drinking water, water treatment, and sanitation practices and environmental sanitation concerns as well as qualitative drinking water satisfaction. Ova and parasite analysis of stool and total coliform determination of drinking water was performed for each household surveyed. 64% of stools sampled had 1 or more parasites, with *A. lumbricoides*, *Giardia spp.*, *E. coli*, *T. trichiura*, and *C. parvum* at 34%, 18%, 14%, 8%, and 0% prevalence, respectively. Water source, water storage, and sanitation methods were not associated with parasite infection or water coliform presence. Water treatment method was significantly associated with *E. coli* prevalence, as was the presence of cats near the household. Seeking repeated treatment for diarrhea was also linked to *E. coli* infection. Coliform contamination of drinking water was significantly correlated with the number of children under 5 living in the household. These results suggest that parasite infection is common in this community. Future studies are needed to define the roles of drinking water, environmental contamination, and microorganism carriage in the etiology of gastrointestinal illness in this population.

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THE VALUE OF FRAGMENTED FORESTS FOR MITIGATING PATHOGENS IN SURFACE WATER IN RURAL UGANDA

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While the value to human health of ecosystem services is well established, questions remain regarding the scales at which these services operate. In particular, it is not clear whether forest fragments provide the same benefits to human health that are expected of undisturbed forests. One crucial ecosystem service provided by intact forests is water filtration. This process is highly relevant to human health, especially in the developing world, where water treatment is often nonexistent and forest fragmentation is common. This pilot study takes place in the region bordering Kibale National Park in western Uganda, which was once continuous forest; now, large swatches have been cleared for smallholder agriculture, and only small fragments of forest remain. In the villages surrounding these fragments, as in much of the developing world, lack of clean drinkable water is a major public-health problem. These forest fragments thus represent an ideal model system in which to examine the potential of forest fragments to improve the quality of surface water with regard to pathogens. To examine the effect of forest fragments on water-borne pathogens, I sampled streams and wells inside, upstream and downstream of representative fragments. Control samples were taken from intact forest in the park. Along with basic water-quality data, three complementary indicator systems of pathogen contamination (coliform

bacteria counts; adeno- and polyomaviruses; and complete bacterial metagenome analysis) provide a standard, well-established benchmark of fecal contamination, a sensitive, source-species-specific indicator system, and a snapshot of local bacterial communities. Preliminary findings indicate a clear mitigating effect of forest fragments on loads of fecal coliforms in surface water, while the effects on other quality indicators are more complex. Such combinations of water-quality data with respect to land use are uncommon and provide novel insights into connections between human health and the altered environment.

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THE BUMPED KINASE INHIBITOR, 1561, IS EFFECTIVE AGAINST EXPERIMENTAL TOXOPLASMOSIS

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Toxoplasma gondii causes severe brain and eye disease. Current drugs for *T. gondii* are limited by toxicity. Bumped kinase inhibitors (BKI) selectively inhibit calcium-dependent protein kinases of the apicomplexan pathogens *T. gondii*, cryptosporidia and plasmodia. We have recently shown that the BKI, 1294, is metabolically stable, orally bioavailable and effective in a mouse model of acute toxoplasmosis at 100 mg/kg and 30 mg/kg given for 5 days. At the 63rd annual meeting of the American Society for Tropical Medicine and Hygiene, we will describe a BKI, 1561, that has greater efficacy than 1294. In 2 independent experiments (n=8,) a one time oral dose of 10 mg/kg given 48 hours after intraperitoneal *T. gondii* inoculation reduced the burden of infection of the Type 1 RH strain of *T. gondii* in the brain and spleen by 72% and 99%, respectively, compared to a vehicle only control. 1561 administered as a loading dose of 20 mg/kg followed by 4 daily doses of 5 mg/kg reduced the level of *T. gondii* infection in mice below the limits of detection (n=4.) Burden of infection in the brain and spleen was evaluated with quantitative real time PCR. The burden of infection was also evaluated in the peritoneal fluid with fluorescent microscopy of RH strain *T. gondii* expressing yellow fluorescent protein and was found to be reduced by 98% with a single oral dose of 10 mg/kg and was below the limits of detection in the group of mice given 20 mg/kg once followed by 5 mg/kg for 4 days (n=4.) The efficacy of 1561 is in part due to its favorable pharmacokinetics. The serum concentration of a 10 mg/kg dose in mice is 4.7 μM after 24 hours and 3.61 μM after 48 hours. The serum concentration of a 20 mg/kg dose at 24 hours was 11.05 μM. These findings show that 1561 is highly effective against experimental toxoplasmosis and is an outstanding candidate for further investigation as an anti-*Toxoplasma* drug.

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MULTIPLEXED RECOMBINASE POLYMERASE AMPLIFICATION DETECTION OF INTESTINAL PROTOZOA USING LATERAL FLOW STRIPS

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Protozoan infections of *Cryptosporidium*, *Giardia*, and *Entamoeba* are increasingly being recognized as important causes of diarrheal episodes and are associated with growth and cognitive impairment. While each is treatable, the treatments differ. However, the clinical presentations are similar, underlying the importance of accurate diagnostics. Current diagnosis relies heavily on stool smear, which even in the best hands is neither sensitive nor specific. We have developed isothermal nucleic acid amplification assays that can detect low-level infections as well as PCR, but does not require expensive equipment that is often unavailable in low-resource settings. The recombinase polymerase amplification (RPA)- *Cryptosporidium* assay demonstrates a limit-of-detection (LOD) comparable to or better than PCR (100 parasites/ml stool). Similarly the RPA- *Giardia* assay has a LOD that compares well with established PCR assays (1,000 parasites/ml stool). The RPA- *Giardia* assay was field tested in the highlands of Peru using 111 stool specimens collected from rural communities. For specimens containing low and medium concentrations of DNA (90/111 specimens), the sensitivity was 93% and the specificity was 94%. The RPA- *Entamoeba* assay is currently undergoing bench-top testing and, like the other RPA assays, demonstrated a LOD equivalent to the gold standard PCR. We are currently working on integrating the three assays with our low-resource DNA extraction protocols to build a multiplex assay that can detect all three parasites on a single lateral flow strip.

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CRYPTOSPORIDIOSIS: EPIDEMIOLOGY AND CORRELATES OF IMMUNITY IN BANGLADESHI CHILDREN

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.Cryptosporidiosis has been identified as a leading cause of moderate-to-severe diarrhea in infants worldwide. Human immunity to *Cryptosporidium spp*. is thought to be T-cell mediated, however acquired immunity to this parasite has not been defined. The role of humoral immunity has not been established, though our group has demonstrated the protective effect of maternal breast milk IgA on breastfeeding infants in Bangladesh. In this community-based prospective cohort study, we aim to describe the natural history of *Cryptosporidium spp.* infection and identify correlates of mucosal and humoral immunity in slum-dwelling Bangladeshi children. Children were enrolled at birth and followed for two years, with active surveillance for diarrheal illness. Stool samples were tested for *Cryptosporidium spp*. using real time qPCR. Enzyme-linked immunosorbent assay was used to test for serum anti- *Cryptosporidium* IgG and fecal anti- *Cryptosporidium* IgA. Anthropometric measurements were taken every 3 months. We followed 392 children from birth to age two. Almost 80% of children had been infected with *Cryptosporidium spp*. Asymptomatic infection (75% of children) was more common than diarrheal infection (25% of children). Higher parasite burden, as measured by quantitative real time PCR, was associated with diarrhea rather than asymptomatic infection (T-test, $p < 0.0001$). Using multivariable regression analysis, we found that children with asymptomatic *Cryptosporidium spp.* infection during the first two years of life were significantly more likely to have growth stunting at age two, compared to children who were never infected ($p = 0.035$). Positive anti- *Cryptosporidium* serum IgG at 12 months of age was associated with lower risk of infection during the second year of life (log-rank test, $p = 0.033$). No protective effect was seen with positive fecal anti- *Cryptosporidium* IgA. We also found that over 90% of samples tested were of *C. hominis* subtype, which is consistent with previous reports. In summary, the burden of *Cryptosporidium spp.* infection in Bangladeshi children is largely subclinical, but is associated with significant growth faltering. This is the first study to demonstrate that *Cryptosporidium spp.* infection associated with diarrhea is related to higher parasite burden. Our findings suggest that human immunity to *Cryptosporidium spp*. may be acquired, which has important implications for the potential for vaccine development.

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RNA-SEQ-BASED STRUCTURAL ANNOTATION AND REGULATORY MOTIF DISCOVERY IN *THEILERIA PARVA*, AN APICOMPLEXAN PARASITE OF CATTLE IN SUB-SAHARAN AFRICA

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Understanding the biology of transmission, colonization and pathogenesis is essential to the rational development of efficacious therapeutics and vaccines against Apicomplexa parasites. Two critical steps that greatly bolster this process are the full structural annotation of their genomes and the identification of the full complement of transcription factor binding sites. Many apicomplexans have high AT nucleotide content and gene-dense genomes, which often thwart the accurate characterization of gene structures and gene regulation. Transcriptional regulation remains poorly understood in these pathogens, which are remarkable in their lack of canonical transcription factors and regulatory motifs. With ever more apicomplexan genomes being sequenced and annotated, including those of human and livestock parasites, there is a need for novel approaches to these issues. Theileria species are distinctive for their very high gene density and apparent lack of enrichment for the binding site of AP2 transcription factors, believed to be principal transcription factors in Apicomplexa. Therefore, *Theileria parva* is ideal as a model system for the development of novel approaches to gene structure annotation and of techniques that can give insight into transcriptional gene regulation in Apicomplexa. RNA-seq technology is particularly well suited to address this problem, providing the ability to identify the precise genomic coordinates of the start and end of transcripts and of introns, as well as relative expression levels. Leveraging RNA-seq data extensively, in standard and novel ways, we re-annotated the *T. parva* genome, identifying 121 new genes, altering 48% of the existing gene structures, and discovering three regulatory motifs. Experiments are ongoing to identify additional regulatory motifs and to determine the applicability of these approaches to other Apicomplexa, such as Babesia and *Cryptosporidium* species.

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PROFILING OF AGENTS AGAINST *BABESIA DIVERGENS*

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Babesiosis is a tickborne zoonotic disease found worldwide. There has been a recent increase in both the total number of human cases as well as the number of patients presenting with severe disease. The limitations of established treatment regimens, especially in immunosuppressed patients, are well recognized with prolonged treatment and frequent recurrences. In addition, parasitemia can be slow to respond to treatment in these patients, thus complicating management. B divergens in particular is noted to be difficult to treat with medications alone and red blood cell exchange transfusions are frequently required. To date, alternative treatment regimens have primarily been chosen empirically, based on activity against other apicomplexan parasites. We have been using an *in vitro* SYBR Green-based assay originally developed for malaria parasites to evaluate the anti-parasitic activity of various compounds against *B. divergens*. Compared to parallel assays in *P. falciparum*, the majority of agents were much less potent when tested against B divergens, with notable exception of agents previously shown to be effective against various species of Babesia; imidocarb, diminazene and atovaquone. We are currently exploring both cytostatic and cytocidal profiles of various agents as well as characterizing interactions between different drug combinations.

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INVASION BY ORGANELLAR DNA GENERATES STRAIN-SPECIFIC DIFFERENCES IN THE NUCLEAR GENOME OF *TOXOPLASMA GONDII*

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Toxoplasma gondii is one of the most successful zoonotic parasites, essentially capable of infecting any warm-blooded animal. We have determined the extent of mitochondrial (NUMT) and plastid (NUPT) DNA fragment assimilation by the *T. gondii* nuclear genome. While NUMTs have been reported in numerous eukaryotic species they typically constitute a very small fraction of the genome. *T. gondii* has ~13,000 organellar DNA insertions. This is the highest number of insertions and NUMT density (~1.66%) ever reported; ~10 times more than the honeybee genome and almost 100 X greater than is observed in the human genome. The NUMT fragments originate from all regions of the mitochondrial DNA and are distributed across all of the *T. gondii* chromosomes. Careful examination of the regions flanking insertion sites suggests these NUMTs are independent insertions and are not post-integration amplifications. Age estimation of the nucleotide insertion sequences reveals integration events spanning the last 20 million years suggesting that acquisition of organellar DNA by the *T. gondii* nuclear genome is a continual process. Comparison to the closely related species (28 million years separation), Neospora caninum revealed a much lower NUMTs density (~0.71%) in N. caninum and revealed very few conserved NUMTs between the two species. Comparisons of NUMTs between sequences of several *T. gondii* strains and the N. caninum genome sequence reveals that most insertions decay rapidly and that the insertion rate is high. Most interestingly, we have identified strain-specific NUMTs in every strain examined thus far suggesting a possible role for NUMTs in strain-specific biology and diversification. We are currently expanding this analysis to the 62 available strains to obtain a comprehensive picture of the scope, rate and potential implications for this aspect of genome evolution. We have preliminary evidence suggesting NUMTs upstream of genes could foster occasional functionalization and regulate gene expression.

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GIARDIA-BACTERIAL INTERACTIONS: DEPLETION OF MICROBIOTA FACILITATES GROWTH IN A MURINE MALNUTRITION MODEL OF GIARDIASIS

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Giardiasis affects up to 90% of children within the first year of life in resource-limited settings. The clinical manifestations of endemic pediatric Giardiasis range from protection against acute diarrhea to associations with persistent diarrhea and development shortfalls. While pathogen and host variables are known to affect *Giardia* pathogenesis, complex interactions between nutritional factors and microbiota may be highly influential in determining Giardiasis outcomes. Using a C57Bl/6 mouse model, protein energy malnutrition was recently shown to accentuate *G. lamblia*-induced growth faltering through 15 days post-infection. Concurrently, there was altered villus architecture and mucosal inflammatory response in malnourished compared with nourished *Giardia*-infected animals. In the current study, 10^6 *G. lamblia* H3 cysts were used to infect 5-week-old male C57Bl/6 mice sustained on a 2% low-protein diet. Mice were given an antibiotic cocktail containing ampicillin, neomycin, and vancomycin administered in drinking water beginning 7 days prior to and continuing throughout infection. Animal weights were compared to infected mice given water without antibiotics. RT-PCR was

used to confirm infection. In a separate experiment, mice on antibiotics were challenged with *G. lamblia* H3 cysts 35 days prior to challenge with 10^10 enteroaggregative Escherichia coli strain 042. The ampicillin, neomycin, vancomycin cocktail depleted stool microflora that remained suppressed with continued administration through 21 days. *G. lamblia* H3 cyst infection caused growth faltering in non-antibiotic treated mice compared with uninfected controls ($p<0.01$ d13-d14), and compared with antibiotic-treated *G. lamblia* infection ($p<0.05$ d5, $p<0.01$ d6, $p<0.001$ d7, and $p<0.001$ d8-14). Duodenal burden at 15 days post-infection by RT-PCR revealed similar burden of parasites irrespective of antibiotic treatment. In *G. lamblia*-enteroaggregative E. coli 042 co-infection models, growth faltering was worse in the co-enteropathogen infection ($p<0.05$ d8, d10) than with either single infection when compared with uninfected controls. Elucidating the mechanisms accounting for growth faltering in Giardiasis as related to microbiota and malnutrition may help to improve our understanding of this enigmatic parasite and its influence on co-pathogen infections, enteropathy, and childhood development.

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DETERMINATION OF LOCAL FECAL CONTAMINATION OF SURFACE WATER BY MICROBIAL SOURCE TRACKING AND ASSOCIATION WITH SCHISTOSOMIASIS RISK IN BRAZIL

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Schistosomiasis is to a degree a disease of contact with fecally contaminated surface waters, rather than ingestion. Since it is a chronic infection, schistosomiasis may be amenable to analysis of the quantitative relationship between fecal contamination and risk of infection. In order to examine this relationship, we surveyed an endemic community in rural Bahia, Brazil that straddles a shallow river. All residents were examined for eggs and samples taken from the major water contact sites. Nearly all of the population (98.3%) uses an indoor toilet, but only 44% flush to a septic tank. The remainder discharge to the river. We validated two widely used markers of human fecal contamination, human *Bacteroides* and *Lachnospiraceae* by pyrosequencing and qPCR of local stool samples. We observed that the concentration of these human-associated stains dramatically increased in the downstream portion of the village. Using map algebra in ArcGIS, we developed a model for individual infection risk based on the distance of a person's home to the nearest contact site and the concentration of human fecal contamination at this point. This model explained ~50% of the risk of infection. Following treatment of those infected in 2009, 89% were confirmed egg-negative. At follow-up in 2012, prevalence had declined by 48% and intensity by 44%. Those infected were again treated in 2012 and reexamined in 2013 when prevalence fell by 32% and intensity by 30%. Reinfection rates were 33.7 and 18.4%, in 2012 and 2013 respectively. Incidence was 22.0 and 12.9%. In both 2012 and 2013, all new infections were confined to the most downstream section. Genetic differentiation analysis showed that the reinfecting populations in individuals were moderately differentiated from their pretreatment populations (mean $D = 0.055$ and 0.077, respectively). This confirmed that these were new infections rather than localized absence or failure of treatment. Models of schistosome transmission as well as control planning might be improved by considering local concentrations of fecal contamination of surface water.

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SURPRISING INTERACTIONS BETWEEN SCHISTOSOMES AND AMPHISTOMES IN KENYAN *BIOMPHALARIA PFEIFFERI*: AMPHISTOME DEPENDENCE ON AND DOMINANCE OF SCHISTOSOME INFECTIONS, WITH SOME IMPLICATIONS FOR SCHISTOSOME CONTROL

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Schistosoma mansoni is commonly transmitted by *Biomphalaria pfeifferi* in streams in western Kenya. Also transmitted by *B. pfeifferi* in these streams are amphistomes, trematodes that mature as adults in domestic ruminants. Because amphistomes are common (some of our collections retrieve 25% or more snails shedding amphistomes), and because their intramolluscan stages may have predatory effects on schistosome sporocysts, amphistomes may have an unappreciated detrimental effect on schistosome transmission in such streams. We have examined amphistome-schistosome interactions to learn if amphistomes are dominant when present in co-infections, and if amphistomes can be manipulated to achieve an even greater effect on schistosome transmission. Thus far we have identified 19 distinct lineages of amphistomes in Kenya, at least four of which rely on *B. pfeifferi* as their snail host. Field-collected *B. pfeifferi* with patent amphistome infections can rarely be superinfected with *S. mansoni*. Attempts to establish experimental infections with amphistomes in *B. pfeifferi* have been unsuccessful, but further study has revealed that if snails are first exposed to amphistomes, and later exposed to *S. mansoni* infection, that the snails subsequently shed amphistome cercariae, or in some cases, amphistomes and schistosomes. We have since confirmed that field-derived snails not shedding any kind of cercariae when exposed to *S. mansoni* surprisingly often shed amphistome cercariae. We interpret the results to mean that amphistome miracidia may frequently penetrate *B. pfeifferi*, but then require a later facilitating effect from *S. mansoni* to achieve a patent infection. Once facilitated though, they largely dominate the schistosome infection. Widespread exposure of snails to amphistomes, at least with the isolates we are working with now, may have two distinct effects: 1) preventing subsequent production of *S. mansoni* cercariae by these snails; and 2) only snails exposed to *S. mansoni* (or possibly other trematodes) would shed amphistome cercariae.

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DETECTION OF MIXED SCHISTOSOME INFECTIONS BY AMPLIFYING SPECIES-SPECIFIC DNA FROM URINE SEDIMENT OBTAINED BY FILTRATION

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Differential diagnosis of *Schistosoma mansoni* and *S. haematobium,* which often occur sympatrically in Africa, requires both urine and stool and the procedures are low in sensitivity. Frequently used diagnostic tests, such as Kato-Katz (KK) for *S. mansoni* eggs and presence of haematuria for *S. haematobium* both lack sensitivity, produce false-negative results and show reduced accuracy with decreasing intensity of infection. The need for a single diagnostic procedure with high sensitivity, specificity and ease of operation for both parasites is important as many African countries are implementing Mass Drug Administration (MDA) following recommendations of the World Health Organization (WHO). Our approach simplifies the collection and performance of DNA based diagnostic test and is more sensitive and specific than KK and haematuria. Importantly transport costs are considerably reduced as dried filter papers are easy to store, light weight and stable. Eighty-six samples of urine sediment obtained by filtration were collected from a group of 5 - 23 years old

people from an endemic area of southern Ghana where both parasites live sympatrically. DNA was extracted from urine sediment on filter paper from which a species-specific repeat fragment was amplified by polymerase chain reaction (PCR) with specific primers for *S. mansoni* and for *S. haematobium*. Additionally, all participants were tested by KK (stool) and dipstick for haematuria. Diagnostic parameters for all three tests were analyzed statistically. Amplification of species-specific DNA by PCR showed much higher sensitivity (99% - 100%) and specificity (100%) compared to KK and haematuria (sensitivity: 76% and 30% respectively) for both schistosome species. The same pattern was observed when the data were stratified for age group and sex specific analysis. In addition PCR amplification detected DNA from 11 individuals infected with both parasites who were negative by KK and haematuria. This approach of detecting parasite specific DNA from either or both species in a single urine specimen has practical advantages of simplicity of collection avoids the need for two specimens and is more effective than standard tests including those based on serology. This will be important for long term success of parasite control by Mass Drug Administration (MDA) intervention because of the need to detect low intensity infections that persist even after treatment has occurred.

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DIAGNOSTIC PERFORMANCE OF KATO-KATZ, MINI-PARASEP AND MINI-FLOTAC TECHNIQUES IN DETECTION OF HELMINTH INFECTIONS IN MBITA, WESTERN KENYA

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This study evaluated the diagnostic performance of Kato-Katz (the WHO recommended diagnostic technique for detecting helminth infections), Mini-Parasep (a concentration method; DiaSys, England) and Mini-FLOTAC (a floatation method based on two different floatation solutions, FS2 and FS7; University of Naples, Italy) for detection of *Schistosoma mansoni* and soil-transmitted helminths (Hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*) ova. Single stool samples were collected from 282 inhabitants from four villages along the shores of Lake Victoria, in Mbita, western Kenya. Aliquots for the Mini-Parasep and Mini-FLOTAC techniques were preserved in 10% and 5% formalin, respectively, before processing. Prevalence of *S. mansoni* was 47.2% (n = 282), 59.3% (n = 209), 3.2% (n =127) and 12.6% (n = 127) by Kato-Katz, Mini-Parasep, Mini-FLOTAC FS2 and Mini-FLOTAC FS7, respectively. Sensitivities for detection of *S. mansoni* were 76.0%, 70.9%, 2.3% and 9.1% for Kato-Katz, Mini-Parasep, Mini-FLOTAC FS2 and Mini-FLOTAC FS7, respectively, and their specificities were 73.0%, 41.7%, 60.3% and 54.4%, respectively. The prevalence of STH was too low for meaningful comparisons. Kato-Katz and Mini-Parasep had a fairly good agreement for *S. mansoni* detection (k=0.49), and a substantial agreement for *A. lumbricoides* (k=0.66). Kato-Katz and Mini FLOTAC FS7 had a fair agreement both for *S. mansoni* (k=0.28) and *T. trichiura* (k=0.35) detection. Kato-Katz diagnosed a higher number of eggs compared to Mini-Parasep (204 vs 105, p= 0.0059) and Mini-FLOTAC FS7 (204 vs 22, p= 0.0211). Kato-Katz also detected higher proportion of heavy intensity *S. mansoni* infections compared to Mini-Parasep which detected higher proportion of light intensity infections. Consistent with other studies, the saturated sodium chloride (FS2) detected more hookworm and less *S. mansoni* compared to the zinc sulphate (FS7) solution. Mini-Parasep performed better than Mini-FLOTAC and is a promising technique with comparable sensitivity and potential logistical advantages compared to the standard Kato-Katz technique.

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PHARMACOKINETICS/PHARMACODYNAMICS (PK/PD) OF 40 MG/KG VS. 60 MG/KG DOSING OF PRAZIQUANTEL IN UGANDAN CHILDREN 3-8 YEARS OLD WITH INTESTINAL SCHISTOSOMIASIS

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Whilst praziquantel (PZQ) is widely used for control of schistosomiasis by mass drug administration, there has been no pharmacokinetic/pharmacodynamic (PK/PD) study in children; treatment at either 40 mg/kg or 60 mg/kg is a direct extrapolation of common practice dosing in adults. In a time where millions of children receive PZQ in sub-Saharan Africa, there is a pressing need to optimize dosing for more effective treatment of schistosomiasis. To address this deficit, we conducted the first PZQ PK/PD study in children aged 3-8 living in a highly endemic area for *Schistosoma mansoni* around Lake Albert, Uganda. Sixty children with patent intestinal schistosomiasis, ascertained by eggs in stool and/or urine antigen test, were randomized to receive PZQ at either 40 mg/kg or 60 mg/kg dosing. Parasitological data were collected alongside and included soil-transmitted helminths eggs, a malaria rapid test as well as HIV test. Subsequent blood samples were taken at different time points (0, 1, 2, 4, 6, 12 & 24h) for later analysis of venous concentrations of PZQ. Quantification of the enantiomers of PZQ was carried out using chiral chromatography via LC-MS/MS analysis. A population methodology using the program Pmetrics was used with two structural models constructed and fitted to the observed PK data. Models were distinguished on the basis of the linear regression of the observed-predicted values before and after the Bayesian step, log-likelihood values and various measures of bias and precision. As a PD estimate, egg reduction rates at 24 days were significantly greater in older children (> 5 yo) and those with medium and heavy intensity *S. mansoni* infection receiving 60 mg/kg doses of PZQ. Although our results favour the use of higher dosing in school-age children, especially in those with moderate and heavy egg-intensity infections, ongoing PK population modelling will be used to identify optimal paediatric dosages associated with maximal anti-parasitic activity and comparable drug exposures in adults.

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HIGH INTENSITIES OF *SCHISTOSOMA MANSONI* IN UGANDAN PRIMARY SCHOOL CHILDREN AFTER TEN YEARS OF MASS DRUG ADMINISTRATION

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The Schistosomiasis Control Initiative (SCI) began mass drug administration (MDA) with praziquantel (PZQ) in Uganda in 2003. Knowledge on how parasite infections change as a result of PZQ-treatment may have important implications for the success of this and other control programmes. Baseline prevalence, intensity of infection, morbidity, host behavioural data and side-effects post-PZQ-treatment were recorded for *Schistosoma mansoni* infections, the causative agent of intestinal schistosomiasis, in children from three primary schools on the shores of Lake Victoria, in Mayuge district, Uganda. Schools were visited at twelve time points, over three years from 2004 to 2006. Children at these schools were resampled in February and March 2013, and will be revisited in April and May 2014, now over 10 years after the MDA programme began. Follow-up epidemiological and parasitological data collection pre- and post-PZQ-treatment will be repeated. Baseline field data revealed that 80.6% of children were still excreting eggs one-week-post-treatment with 40 mg/kg PZQ, and 39.9% of these had counts >100 eggs per gram (epg). Four-weeks-post-treatment 23.9% of children were still excreting

eggs with 2.9% having counts >100 epg. Hatching tests indicated that the eggs being released at both one- and four-weeks-post-treatment were viable. From 2004 to 2006, although infection intensities were lower each year, prevalence returned to baseline levels. Ten years later, infection prevalence and intensities, in 2013, were higher than at baseline, raising concerns over lower than expected treatment success and questions about the potential reasons for this. Epidemiological and parasitological data collection from 2014 will be compared with 2013 and baseline data to establish if these maintained high intensities are due to potential reduced susceptibility, poor adherence to treatment and/or a consequence of high transmission.

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MATHEMATICAL MODELLING OF INTEGRATED CONTROL OF SCHISTOSOMIASIS: TOWARDS ELIMINATION

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While the prevalence of schistosomiasis has been markedly reduced in many areas of PR China where it was previously endemic it is resistant to elimination in some areas. In other countries, such as the Philippines it remains a serious public health problem. Combination of individual control measures such as bovine and human chemotherapy, improved sanitation, and molluscicide appears to offer the best approach to further decreasing prevalence and achieving elimination. Bovine vaccination appears to offer an additional element to an integrated control strategy. While estimates of the efficacy of individual strategies are available from field trials, the overall effectiveness of integrated control can be estimated by mathematical modelling. This must incorporate realistic assumptions about such parameters as coverage and frequency of intervention and duration of efficacy, as well as knowledge of the local epidemiology and transmission of the infection. We have developed a mathematical model which incorporates a simulation of the transmission of schistosomiasis in a situation which involves multiple and heterogeneous mammalian hosts (e.g humans, water buffalos). It takes into account births, deaths and aging of such hosts, as well as the infection characteristics of each. The model is parameterised using epidemiological data collected in both PR China and the Philippines from extensive field surveillance over many years. We compare the results (in terms of infections, infection-years prevented, and probability of elimination) of various five-year programs within various epidemiological settings. In particular we estimate the minimum bovine vaccine efficacy needed to block transmission of schistosomiasis in these settings, in combination with different levels of uptake of other interventions such as human chemotherapy.

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TRANSGENIC TOOLS ALLOW THE STUDY OF P450 MIS-EXPRESSION ON INSECTICIDE RESISTANCE PHENOTYPE IN *ANOPHELES GAMBIAE*

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The recent development of molecular tools for use in transgenic mosquitoes has provided the means to enable advanced function genetic analysis to be carried out in *Anopheles gambiae.* These tools include the Gal4/UAS system, PhiC31 recombination mediated cassette exchange (RCME) and enhancer trapping. Together they allow tissue specific expression in a conditional manner and permit the direct effects of gene mis-expression to be compared *in-vivo* by negating the position effects seen using transposon based integration systems. These tools are now being applied to examine metabolic and cuticular insecticide resistance regulated through P450 expression. Resistance to insecticides in the form of target site insensitivity mutations, cuticular and metabolic resistance, is threatening the success of insecticide based vector control programmes. Whilst target site insensitivity is well studied, the mechanisms underlying metabolic and cuticular resistance are poorly understood. Genome wide expression studies have revealed a number of candidate p450 genes whose overexpression is associated with insecticide resistance in *Anopheles* species. Although *in vitro* expression has indicated that several p450 enzymes metabolize insecticides, the function of other p450s, highly over expressed in resistant mosquitoes, have remained elusive, suggesting they may have other roles in resistance. A number of p450's are highly expressed in the oenocytes, which may suggest a role in lipid and hydrocarbon formation that has been linked to cuticular resistance. To investigate the *in vivo* role of these p450 genes, we utilized the Gal4/UAS system to examine the effects of both tissue specific overexpression and stable RNAi knockdown in transgenic *An. gambiae.* Transgenic UAS responder lines were created by RCME. Crossing of these p450 responder lines to tissue specific Gal4 driver lines allowed the phenotypic effects of p450 overexpression and stable knockdown to be studied *in vivo*. The results of these crosses and the phenotypic effects on both insecticide resistance and mosquito fitness are discussed. To our knowledge, this is the first time that transgenic *Anopheles* have been used to study insecticide resistance and the work demonstrates that combining RCME with the Gal4/UAS system provides a useful tool for gene expression studies in the mosquito.

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EFFECTS OF THE KDR RESISTANCE MUTATION ON THE SUSCEPTIBILITY OF WILD *ANOPHELES GAMBIAE* POPULATIONS TO *PLASMODIUM FALCIPARUM*: A HINDRANCE FOR VECTOR CONTROL

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In the context of generalization of insecticide resistance, we hypothesized that insecticide resistance has a positive impact on the capacity of mosquitoes to transmit malaria. We therefore investigated populations of *Anopheles coluzzii* and *An. gambiae* S molecular form to assess whether different genotypes at the kdr locus are responsible for different susceptibilities. F3 progeny of *An. gambiae* s.l. collected in Dielmo were infected by direct membrane feeding with *Plasmodium falciparum* gametocyte-containing blood sampled from volunteer patients. The presence of oocysts was determined by light microscopy after 7 days, and the presence of sporozoites by ELISA after 14 days. Mosquito species and molecular forms were identified by PCR. Generalized linear models were performed using the R software to test the effect of explanatory variables on infection rate and density. The oocyst rate was 12.6 times greater in RS than in SS mosquitoes, and all RR individuals were infected. The prevalence of sporozoites was 38.6 greater in the RS group and 420 times greater in the RR group than in SS genotypes. In the model of oocyst number and sporozoite density, genotype interactions were not significant, but the effects of strain and genotype were significant. The number of oocysts infecting a mosquito when infection occurred was 9.3 times greater in the S form strain than in the RS group and 42.7 times greater in the RR group than in the SS genotype. In *An. coluzzii*, the number of oocysts was greater in the RS group and 32 times greater in the RR group than in SS. The sporozoite absorbance density was higher in RR and RS than in the SS group and higher in the S molecular form. The presence of the resistance allele at the kdr locus increases susceptibility to *Plasmodium* not only at the oocyst stage but also at the sporozoite stage in non-genetically modified wild mosquitoes. These results have significant implications and should be taken into account in the development of strategies for malaria control.

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GENETIC DIVERSITY PATTERNS IN THE MAJOR MALARIA VECTOR, *ANOPHELES FUNESTUS*, REVEAL A GENOMIC FOOTPRINT OF SELECTION ASSOCIATED WITH CONTROL INTERVENTIONS IN SOUTHERN AFRICA

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Pyrethroid resistance observed in *Anopheles funestus* is hindering efforts to control this major malaria vector throughout Southern Africa. Despite some similarities, significant differences in the resistance and gene expression profiles have recently been observed between populations of *An. funestus* in this region. The existence of barriers to gene flow and their potential impact on the spread of insecticide resistance genes between populations remains uncharacterized. Here, we resolved the population structure of *An. funestus* in Southern Africa by genotyping 17 microsatellites in 40-48 samples from ten collection sites. This genome-wide analysis revealed the presence of three population clusters across Southern Africa (pairwise Fst score p≤0.05) with northern Malawi populations genetically closer to Zambia than that of southern Malawi and Mozambique. These results parallel previous gene expression and resistance profiles data. Additionally, we observed a temporal shift in population structure associated with LLINs and IRS intervention in Southern Malawi. Fine-scale analysis of polymorphism of the 120kb rp1pyrethroid resistance QTL including the two main resistance cytochrome P450 genes, CYP6P9a and CYP6P9b, revealed a significant loss of genetic diversity in samples collected post intervention while pre-intervention mosquitoes were highly diverse. This reduced diversity spans a region of 70kb around the two genes, supporting the presence of a selective sweep in a genetic area linked to pyrethroid resistance. This study suggests that gene flow is not uniformed across Southern African populations of *An. funestus* and could impact the spread of pyrethroid resistance. More importantly, by detecting a genomic selection footprint associated with control interventions, this work reveals that control interventions may be the main driving force of pyrethroid resistance in this region.

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A SIGNIFICANT ASSOCIATION BETWEEN THE *VGSC*-L1014S KDR MUTATION AND INFECTION WITH *PLASMODIUM FALCIPARUM* IN *ANOPHELES GAMBIAE* FROM EASTERN TANZANIA

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The success of malaria vector control is threatened by widespread pyrethroid resistance. However, the extent to which insecticide resistance mechanisms impact upon the development of the malaria parasite in its vector remains unknown. The objective of this study was therefore, to investigate the association between the presence of the pyrethroid resistance *vgsc*-L1014S allele and infection with *Plasmodium falciparum* sporozoites in *Anopheles gambiae*. WHO standard methods were used to characterise susceptibility of wild female anopheles mosquitoes to 0.05% deltamethrin. PCR based molecular diagnostics were used to identify mosquitoes by species and to detect *vgsc*-L1014S alleles. The presence of *P. falciparum* sporozoites was detected using a CSP-ELISA. *Anopheles gambiae s.l.* were resistant to deltamethrin with mortality rates of 78.6% [95% CI: 74.9-81.9%]) in the dry season and 81.2% [95% CI: 76.8-84.9%]) in the rainy season. Of 545 mosquitoes genotyped, 96.5% were *An. gambiae s.s.* and 3.5% were *An. arabiensis*. The *vgsc*-L1014S mutation was detected in both *An. gambiae s.s.* and *An. arabiensis* at the allelic frequency of 0.45 (95% CI: 0.41-0.48) and 0.32 (95% CI: 0.19-0.47) respectively. In *An. gambiae* s.s., *vgsc*-L1014S was significantly associated with deltamethrin resistance (χ^2= 11.19; p=0.0008). The *P. falciparum* sporozoite infection rate in *An. gambiae s.s.* was 4.11%. There was a significant association between the presence of sporozoites and *vgsc*-L1014S mutation in *An. gambiae s.s.* (χ^2 = 6.89; p=0.009). The presence of this association suggests that *vgsc*-L1014S carrying mosquitoes are more likely to survive sufficiently long to transmit malaria infection. These findings are of importance for the epidemiology of malaria considering the widespread nature of this resistance mutation in Africa.

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EFFECTS OF AN EPIGENETIC DRUG ON MALARIA MOSQUITOES

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Malarial mosquitoes adapt to a wide range of changes in environment quickly, making malaria control an omnipresent problem in tropical countries. Epigenetic regulation of gene expression may be an important factor for mosquito development and survival at various conditions. Pharmacological studies of drug effects on cell lines and animal models have established the use of epigenetic drugs as a useful tool for modulating the genetics and physiology of cells and organisms. Epigenetic drugs are well established in cancer research, however not much is known about their effects on insects. We designed a study for examining the biochemical effects of 3-Deazaneplanocin A (DZNep), an experimental epigenetic drug for cancer therapy, on the malaria vector, *Anopheles gambiae*. Our aim was to test if DZNep may be used as an effective tool to study effect of epigenetic changes in malaria mosquitoes. A concentration-dependent increase in mortality and decrease in size was observed in larval mosquitoes exposed to DZNep whereas the drug reduced the fecundity of adult female mosquitoes relative to the control treatments. In addition, there was a concentration-dependent decrease in *S*-adenosylhomocysteine (SAH) hydrolase activity in mosquitoes following exposure to DZNep relative to control treatments. Effect of DZNep on the chromatin structure of polytene chromosomes obtained from ovarian nurse cells of exposed females was evaluated. Our results reveal that epigenetic changes in mosquitoes affect the life span and fecundity of mosquitoes. We were able to demonstrate a unique multi-prong approach for exploring the toxicological effects of water-soluble, epigenetic drugs against vector mosquitoes and other insects. The emergence of insecticide resistance warrants the exploration of novel targets for vector mosquito control. Therefore, future directions would involve identifying potential leads for targeting epigenetics of vectors.

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INSENSITIVITY TO SPATIAL REPELLENTS: A HERITABLE TRAIT?

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Novel, broadly applicable vector control strategies are needed to augment the currently available options of indoor residual spray and insecticide treated nets. Evidence has shown that the spatial repellent actions of some insecticides can reduce pathogen transmission. For this reason, efforts are underway to outline requirements and challenges for an expanded public health role for spatial repellent products. It is clear much remains to be learned about the underlying mechanisms of action that result in repellency behaviors and the impact of these active ingredients on vector populations over time. The current study was designed to investigate the heritability of spatial repellent responses in the dengue virus vector *Aedes aegypti* using an *in vitro* repellency bioassay and the active ingredient transfluthrin. Specifically, selective breeding of behavioral responder and non-responder cohorts was conducted for nine generations. Results

show the responder cohorts exhibited consistent repellent responses - no positive selection was observed. The selective breeding of non-responders resulted in a mosquito cohort that was not repelled after four generations. It is important to note that the selective breeding scheme used here is likely to produce greater selective pressures than the real world application of spatial repellents. Ongoing studies include back-cross breeding experiments to assess whether the non-responder phenotype is recessive or dominant and evaluations of transfluthrin toxicity susceptibility in both the responder and non-responder cohorts. Data collection from these additional studies will be completed by July, 2014.

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PHENOTYPIC EFFECTS OF CONCOMITANT INSENSITIVE ACETYLCHOLINESTERASE (ACE-1R) AND KNOCKDOWN RESISTANCE (KDRR) IN *ANOPHELES GAMBIAE*: A HINDRANCE FOR INSECTICIDE RESISTANCE MANAGEMENT FOR MALARIA VECTOR CONTROL

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Malaria is endemic in sub-Saharan Africa with considerable burden for human health. Major insecticide resistance mechanisms such as kdrR and ace-1R alleles constitute a hindrance to malaria vector control programs. *Anopheles gambiae* bearing both kdr and ace-1 resistant alleles become increasingly recorded in wild populations. In order to maintain the efficacy of vector control strategies, the characterization of concomitant kdr and ace-1 resistance, and their pleiotropic effects on malaria vector phenotype toward insecticide are important. Larval and adult bioassays were performed with different insecticide classes used in public health following WHO standard guidelines on four laboratory *Anopheles gambiae* strains, sharing the same genetic background but harboring distinct resistance status: KISUMU without any resistance allele; ACERKIS with ace-1R allele; KISKDR with kdrR allele and ACERKDRKIS with both resistance alleles ace-1R and kdrR. In parallel, acetylcholinesterase (AChE1) activity levels were recorded for the 4 strains. Larval bioassays indicate that the homozygote resistant strain harboring both alleles (ACERKDRKIS) displayed slightly higher but significant resistance level to various insecticides tested: carbamates (bendiocarb, $p<0.001$; propoxur, $p=0.02$) and organophosphates (chlorpyriphos-methyl, $p=0.002$; fenitrothion, $p<0.001$) when compared to ACERKIS strain. However, no differences were recorded between ACERKDRKIS and KISKDR resistance level against permethrin (Pyrethroid, $p=0.7$) and DDT (Organochlorine, $p=0.24$). For adult bioassays, the percentages of mosquitoes knocked down were significantly lower for ACERKDRKIS than for KISKDR with permethrin ($p = 0.003$) but not with deltamethrin. The percentage of mortality from adult bioassays was similar between ACERKDRKIS and ACERKIS with carbamates and organophosphates, or between ACERKDRKIS and KISKDR with pyrethroid and DDT. Concerning acetylcholinesterase enzyme, ACERKDRKIS strain showed similar AChE1 activity than ACERKIS. I conclusion, the presence of both kdrR and ace-1R alleles increase the resistance level to carbamate and organophosphate insecticides and may represent an important threat to malaria vector control in West Africa.

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INFLAMMATORY GENES ASSOCIATED WITH SEIZURES OF NEUROCYSTICERCOSIS

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Seizures are a common and early symptom of a wide spectrum of brain pathologies from infections to malignancies. They are often not treated in developing countries due to stigmatization, resulting in a high burden to the people affected and their families. In countries where pigs are raised traditionally and sanitation is poor, almost one third of epilepsies are associated with neurocysticercosis (NCC), which occurs when the larval form of *Taenia solium* infects the human brain. A reliable diagnostic test for NCC-associated seizures not requiring expensive brain imaging is not available. Hence, people with epilepsy in developing countries who seek treatment are prescribed "traditional" anti-epileptic drugs that aim at reducing brain hyperactivity rather than targeting its cause. Identifying blood markers specific to NCC-associated seizures could contribute to developing diagnostics and more targeted treatments. This study examines inflammatory genes associated with NCC in peripheral blood monocytes. In a study at the Christian Medical College Hospital, Vellore, India, inflammatory genes associated with NCC-associated seizures were determined by comparison of microarray data (Agilent platform) of peripheral blood monocyte RNA of 12 NCC patients with seizures, 10 patients with idiopathic seizures and 10 patients without seizures and normal brain images. Data analysis and normalization by percentile shift were done using GeneSpring GX software 12.0. Overall there was greater similarity in gene expression between the two seizure groups than either seizure group compared to controls. The two seizure groups shared 452 upregulated and 378 downregulated genes with innate immune responses being a significant functional annotation. Importantly, NCC patients had 151 upregulated and 27 downregulated genes that were not changed in patients with idiopathic seizures. These data suggest NCC seizures display a unique mRNA signature and share a common inflammatory background with idiopathic seizures.

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BIO-GUIDED IDENTIFICATION OF PROTEINS FOR THE DIAGNOSTIC OF CYSTICERCOSIS IN SWINE

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Cysticercosis is caused by *Taenia solium* and affects both humans and pigs. Human is the definitive host of the adult tapeworm. Pig is infected by eggs emitted in human feces and develops cysticercosis due to larval stage, in the muscles, brain, and eyes. Human develop disease either through self-contamination by eggs emitted from his tapeworm or by ingestion of contaminated food by human feces. Neurocysticercosis (NCC) is one of the most prevalent parasitic infection of the brain and the most common cause of seizures in adults in tropical countries. In Madagascar, a high prevalence of cysticercosis in swine was reported

especially in rural area (up to 30%) and consumption of pig infected meat by villagers is very common propagating taeniasis. The economic value of cysticercosis-infected meat are degraded from 20% to 50%, which is a major cause of income loss for poor farmers. Therefore, villagers are aware of cysticercosis and ask for rapid diagnostic associated with a treatment of pigs. However currently available diagnostic tests need laboratory facilities. The production of a diagnostic test usable at the farm-gate could thus be sustained by the pork-meat market itself. To design an immunochromatographic test usable at the farm gate, we started a bio-guided identification of new target proteins in the liquid of the cyst. This liquid contains water-soluble parasite proteins and is released in the host during the lysis of the dying cyst. Identification of the proteins of interest was done using ion exchange chromatography plus 2D separation of the proteins followed by western blot analysis using serums from infected pigs. Spots from the coomassie-stained gel corresponding to these proteins were then analyzed by mass spectroscopy and identified using a bank of ESTs of *T. solium*. Eighteen new proteins of interest were identified, expressed in E.coli and tested against serums

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COMPARISON OF SERUM MASS PROFILING OF PATIENTS WITH NEUROCYSTICERCOSIS EPILEPSY, IDIOPATHIC EPILEPSY AND NO NEUROLOGICAL DISEASE

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Seizures are symptomatic of brain pathologies like infections or malignancies, but also can be idiopathic. In areas where pigs are a food source and sanitation is poor, about one third of seizures are from neurocysticercosis (NCC), a *Taenia solium* larval infection of the brain. Diagnostic tests for NCC-associated seizures, other than imaging, are not readily available. A serological test to identify NCC-related seizures among individuals with seizures of unknown origin is needed to improve treatment and understanding of seizures induced by NCC. In this study, we used serum mass profiling (SMP) to distinguish patients with seizures due to NCC states from idiopathic seizures and from seizure-free control subjects. SMP is based on the hypothesis that a body's serum profile will reflect specific disease phenotypes and physiologies. Sera from patients with solitary cysticercus NCC (SNCC, N=11), multiple cysts NCC (MNCC, N=10), idiopathic seizures (S, N=5), and controls (C, N=9) were analyzed by electrospray ionization ion-trap mass spectrometry. Leave one out cross validation was used to identify m/Z (mass/charge) peaks which differed significantly ($p<0.05$) when comparing two groups at a time (e.g., multiple NCC vs control or solitary cysticercus NCC vs control). Results showed that each patient group was distinguishable from controls (SNCC v C, MNCC v C, S v C) with p-values < 4x10-8. In addition, the idiopathic seizure group was distinguished from the SNCC group or the MNCC group (S v SNCC, S v MNCC) with p-values < 4x10-8. When patient groups were randomized with their respective controls, p-values for each comparison increased to >0.2 suggesting that physiologic processes accounted for the observed differences. Moreover, the sera samples from idiopathic seizures identified with neither control nor NCC patient groups,suggesting that NCC-associated seizures and idiopathic seizures represent different serum physiological states. These data suggest serum mass profiling is useful for development of a sero-diagnostic tool for identifying NCC-induced seizures.

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SPATIAL CLUSTERING OF PORCINE AND HUMAN CYSTICERCOSIS AT VILLAGE LEVEL OF NAYALA, BOULKIEMDE, AND SANGUIE PROVINCES IN BURKINA FASO

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Taenia solium is a parasitic zoonosis transmitted between humans and pigs. In the intermediate (pigs) and accidental (humans) hosts, the larval form of the parasite causes cysticercosis when establishing in tissues. In humans, larvae may migrate to the brain, causing severe neurological disorders such as epilepsy, severe chronic headaches, and sometimes death. To interrupt the life cycle of *T. solium*, it is essential to identify clusters of porcine and human infection. This study aims at identifying village-level spatial clusters of porcine and human cysticercosis. A total of 60 randomly-selected eligible pig-raising villages located in 30 departments were sampled in three provinces of Burkina Faso. In each village, concessions (a group of households) raising sows and piglets were identified, 10 sow-raising and 30 piglet-raising concessions were randomly selected, and one sow and one piglet was randomly sampled in each selected concession. Another 40 concessions were randomly selected, and the first 60 humans living in one of the 80 selected concessions consenting to a 4 year serological follow-up provided a blood sample. Longitude and latitude coordinates of each concession were measured using PDAs. An ELISA was used to detect the presence of current cysticercosis infection in sera. Spatial analyses were conducted with ArcGIS 10.1 The GI* statistic was used to identify clusters of villages with high prevalence (Hot Spots) and low prevalence (Cold Spots). Twelve spatial clusters of porcine and seven of human cysticercosis were found when using a threshold of 11 and 12 kilometers for porcine and human cysticercosis respectively between neighboring villages. More analyses are required to assess if some of this clustering may be partly explained by socio-demographic factors in humans or management factors in pigs as well as environmental factors such as village-level sanitation indicators, or factors that may affect the survival of the eggs such as temperature, humidity, and land cover.

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EFFECT OF ALBENDAZOLE ON SEIZURES IN PATIENTS WITH SYMPTOMATIC NEUROCYSTICERCOSIS

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Neurocysticercosis is a major cause of neurological morbidity and mortality in endemic countries; however, it is unclear if antihelminthic treatment reduces the burden of neurological symptoms, particularly seizures. No trial has reported a benefit of albendazole in improving seizure outcomes, with the exception of one trial that reported that treatment with albendazole was associated with fewer "seizures with generalization." Our objective was to examine the effect of albendazole on seizures in more detail, including by seizure type, post-treatment. Data come from a trial conducted in Ecuador in which patients with symptomatic neurocysticercosis were randomized to receive albendazole 400 mg or placebo twice daily for 8 days, both with prednisone. 178 patients were randomized, with 88 in the albendazole group and 90 in the placebo group. 88% of patients in the albendazole group and 86% of patients

in the placebo group completed 12-months of follow-up. Overall, fewer patients in the albendazole group had at least one seizure during follow-up (24% in albendazole group vs. 34% in placebo group), but this difference was not statistically significant (*P*=0.14). A similar proportion of patients had at least one generalized seizure (11% in albendazole group vs. 12% in placebo group; *P*=0.77), and at least one focal seizure (22% in albendazole group vs. 25% in placebo group; *P*=0.65), during follow-up. On average, there was a lower total number of seizures, generalized seizures, and focal seizures in the albendazole group compared with the placebo group, but this was not statistically significant in unadjusted negative binomial models. After adjusting for potential confounders (age and history of generalized seizures at baseline), the difference in the mean number of generalized seizures was significant (rate ratio 0.24; 95% confidence interval: -2.68, -0.15; *P*=0.03). However, the difference in the mean number of total seizures and focal seizures was not statistically significant in adjusted models. In conclusion, albendazole may reduce the frequency of generalized seizures, but more research is needed to determine how albendazole modifies long-term disease course.

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RETROSPECTIVE REVIEW OF CYSTICERCOSIS IN RETURNED UNITED STATES TRAVELERS

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Neurocysticercosis (NCC) has become increasingly recognized in non-endemic countries during the past ten years due to immigration from endemic countries, but disease in travelers is rarely reported in the United States (US). A retrospective review of all travel-related cysticercosis (CC) cases seen at National Institute of Health, Bethesda (NIH) and Jacobi Medical Center's Tropical Medicine Clinic (JMC), Bronx between the years 1985 and 2013 was undertaken. Thirteen cases were identified, 10 at NIH and 3 at JMC. Age was 35.5 (range 22-61), 8 female and 3 foreign born. Most had history of travel to Latin America (69.2%), followed by the Caribbean (38.5%), India (23.1%) and Africa (23.1%). Reason for travel was most commonly tourism (38.5%) and duration of travel had a mean of 2.1 years (days-9 years) with 46.1% traveling less than 3 months. Seven (53.8%) had a history of Taeniasis during or after travel. Most common symptoms were seizures (61.5%), headaches (30.8%), visual changes (30.8%) and subcutaneous nodules (30.8%). Of the 4 with subcutaneous disease, 2 had neurologic symptoms due to NCC, 1 had neuroimaging evidence of NCC and 1 had only subcutaneous involvement. On imaging, 10 had parenchymal lesions, 2 subarachnoid disease (both had the most remote travel history, 14-20 years since last travel) and one had no lesions. Of the parenchymal cases, 3 had single cysts, 3 multiple cysts, 2 multiple enhancing lesions and 2 with multiple calcifications. Eight (61.5%) had positive enzyme-linked immunoblot transfer assay (EITB) serology. All 3 single cystic lesions were EITB negative, whereas all but 1 case with multiple lesions were EITB positive. This is the largest series of returned travelers with NCC in the US. In contrast to the literature, these patients had large burden of disease as evidenced by multiple parenchymal lesions and SANCC. Almost half of the patients had short term travel and greater than half had a history of *Taeniasis*. A high-index of suspicion should be maintained in returned travelers from endemic regions presenting with neurologic or subcutaneous symptoms.

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ASSESSING THE ECONOMIC BURDEN OF NEUROCYSTICERCOSIS HOSPITALIZATIONS IN THE UNITED STATES, 2003-2012

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Neurocysticercosis (NCC), caused by brain infection with *Taenia solium* larval cysts, is a leading cause of acquired epilepsy. This disease is of emerging public health concern in the United States, especially among immigrants from and travelers to cysticercosis-endemic regions. The complicated and chronic nature of NCC management results in significant cost; consequently, the economic burden associated with NCC in the United States could be considerable. To assess the economic burden of NCC in the United States, we reviewed the Nationwide Inpatient Sample dataset from 2003-2011 for hospitalizations with ICD-9 diagnostic code of 123.x and at least one additional supporting diagnostic code. We also evaluated hospitalizations for other major tropical diseases for comparison. Based on this stratified sample, we estimated there were 16,103 NCC hospitalizations with hospital charges between $650 - $915 million USD over the 9 years studied. The majority of hospitalized cases were Hispanic (74%), male (57%) and under 44 years of age (70%). Almost 60% of NCC hospitalizations presented with a coexisting diagnosis of seizure or epilepsy. Over 38% of hospitalizations were government pay, either Medicare or Medicaid, and 75% were admitted from the emergency department. California was most affected with 1/3 of all hospitalizations and almost half of total U.S. hospital charges (est. $442,000,000 USD), followed by Texas, New York, Florida and Illinois. There were almost twice as many hospitalizations and three times the total charges for NCC than malaria. NCC results in a greater number of hospitalizations and higher hospitalization charges in the U.S than any of the other tropical diseases we studied. NCC is an increasing public health concern in the United States, especially among Hispanics, and represents a significant economic burden to the U.S. healthcare system. As Hispanic immigration from NCC-endemic continues to increase, we expect to experience a greater number of U.S. cases resulting in increase disease burden.

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INSULIN-LIKE GROWTH FACTOR-I EXPRESSION IN THE PATHOGENESIS OF PANCYTOPAENIA IN CANINE AND HAMSTER VISCERAL LEISHMANIASIS

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Pancytopenia is an important alteration in visceral leishmaniasis (VL) which pathogenesis is poorly known. Some cytokines and growth factors are implicated in haematopoiesis in human VL among them the insulin-like growth factor (IGF)-I that likely has stimulatory effect on erythroid precursors. In the present study, we studied haematological parameters, bone marrow morphological alterations and IGF-I expression in *Leishmania (Leishmania) infantum*-naturally infected dogs and experimental VL in hamster. We examined 13 infected and 10 non-infected control dogs, 5 infected dogs presented pancytopaenia and 8 bicytopaenia. All infected dogs had normocytic normochromic anaemia, leukopenia and/or thrombocytopaenia. In myelogram, we observed dysgranulopoiesis (100%), dyserythropoiesis (100%) and dysmegakaryopoiesis (53.8%). VL dogs presented an increase in the myeloid:erythroid ratio compared with non-infected dogs and infected pancytopaenic dogs had a greater erythroid precursor:mature erythroid ratio when compared with infected bicytopaenic dogs. IGF-I expression by qPCR was lower in infected than in non-infected dogs. When we extended our study to *L. infantum* amastigote-infected hamsters, we observed significant haematological alteration such as anemia and leukopenia from 90 days post-infection. In the bone marrow biopsy we observed hipercellularity, increased macrophage proliferation area, reticulin proliferation and increased

myeloid:eritroid ratio. In the mielogram at 90 and 120 days of infection, we observed progressive alterations. IGF-I expression in bone marrow of hamster was higher at 90 days that decreased at 120 days of infection when compared with non-infected controls, coinciding respectively with normal or diminished hemoglobin concentrations. Anaemia was the main haematological alteration in dogs and hamster. Low IGF-I expression in infected dog or hamster with patent anaemia suggests possible involvement of this factor in the pathogenesis of anaemia during VL.

1201

DISCOVERY OF NOVEL SEROLOGICAL BIOMARKERS ASSOCIATED TO CHAGAS DISEASE USING A HIGHLY-MULTIPLEXED DISCOVERY PLATFORM BASED ON HIGH-DENSITY PEPTIDE MICROARRAYS

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The full set of antibody (B-cell) specificities associated with the response to a natural infection remains largely unexplored. We developed a highly-multiplexed discovery platform based on next-generation high-density peptide microarrays and demonstrate for the first time its potential to simultaneously identify and finely map hundreds of B-cell epitopes from a complex natural human infection. The platform consists of a HD tiling peptide array containing ~200K unique 15mer peptides synthesized *in situ* on a microarray slide, using a maskless photolithographic method. The peptides completely cover the full length of 468 *T. cruzi* proteins, scanning the protein sequence at maximal resolution (1 residue shift). These proteins include 59 previously described antigens, 50 proteins randomly selected from the proteome, 100 proteins selected using a recently published bioinformatics method (Carmona SJ et al 2012); and 232 surface proteins from a number large gene families. The array also contains 24K 15mers corresponding to ~50 neo-proteins of random sequence to estimate the array background baseline (negative control). Using this platform we have analyzed the B-cell immune response in humans with Chagas Disease, assaying 8 independent HD-arrays with 4 pools of purified IgGs from Chagas Disease patients and negative controls. After defining a very conservative cutoff we identified 549 positive 15mers in the set of query proteins (serologically uncharacterized), which define 80 new antigens. We also identified the location of linear epitopes for a number of known antigens, as well as a number of protein regions which are the target of non-specific antibody binding (putative cross-reactive responses). This high-density peptide array platform allows high-throughput identification and mapping of B-cell epitopes, opening the door to large scale studies of immune responses against human infectious diseases.

1202

VECTOR-TRANSMITTED *LEISHMANIA DONOVANI* IN BALB/C MICE DISPLAY DISTINCT FEATURES IN THE HOST IMMUNE RESPONSE AT THE BITE SITE AND SYSTEMICALLY COMPARED TO NEEDLE-INITIATED INFECTIONS

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Visceral leishmaniasis caused by *Leishmania donovani* is transmitted by sand flies with no available vaccine. The virulence of a sand fly-initiated infection abrogates the protection observed in vaccinated animals following needle challenge. This highlights the need to develop VL models initiated by vector-transmission. We developed such models in BALB/c mice using *L. donovani*-infected *Lutzomyia longipalpis* sand flies and compared to needle-initiated (i.d./i.v.) infections. Sand fly infection in BALB/c mice, developed *Leishmania*-specific antibodies 5-10 weeks post-infection that correlated with early dissemination of parasites into the spleen and liver; by weeks 20-30 post-infection, most mice displayed antibodies to *Leishmania*, a slight increase in spleen parasite burden and loss of liver parasites. Dissemination of parasites following intravenous (i.v.) needle injection was also seen in both spleen and liver similar to sand fly mediated transmission. In contrast, intradermal (i.d.) needle injection of parasites failed to disseminate to visceral organs. Splenic CD4+ and CD8 T+ cells displayed a silent immune response after sand fly-transmitted infections producing less IFNγ and TNFα at 5 and 30 weeks post-infection but similar levels of IL-10 when compared to i.v. infection. Further, vector-initiated infection induced persistent recruitment of leukocytes (neutrophils and monocytes) to the bite site compared to a weaker and transitory recruitment following i.d. inoculation of *L. donovani*. Infected sand fly bites also initiate an acute pro-inflammatory (IFNγ, IL1β, TNFα and IL12) response 3h post infection that subsided at 6h, followed by a sustained induction of IL-10 and MCP1(monocyte chemoattractant protein 1) up to 18h post-infection. Altogether, these results demonstrate that in contrast to needle-initiated infections, vector-transmission induces a distinct acute inflammatory response and a strong cellular infiltration at the bite site that may facilitate dissemination of *L. donovani* in an immunologically silent environment that favors parasite survival.

1203

IMMUNE RESPONSE TO *LUTZOMYIA INTERMEDIA* SALIVA IN INDIVIDUALS FROM A CUTANEOUS LEISHMANIASIS ENDEMIC AREA

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Sand fly saliva contains a variety of molecules that modulate the host's hemostatic and immune responses. Immunization with *Lu. intermedia* saliva, one of the main vectors of *Leishmania braziliensis* in Brazil, does not confer protection against *L. braziliensis* infection in mice. In addition, Cutaneous Leishmaniasis (CL) patients display higher levels of anti- *Lu. intermedia* saliva antibodies when compared with sera individuals with sub-clinical *L. braziliensis* infection. In the present work, we conducted a prospective study and characterized the immune response against *Lu. intermedia* saliva in residents of a CL endemic area (Corte de Pedra, BA), where *L. braziliensis* is prevalent. To this end, 264 participants were enrolled and were tested for their humoral immune response against *Lu. intermedia* saliva: antibodies were found in 150 (56.8%) subjects and a positive serology was associated with home arrival after 4:00 pm ($p=0.01$). There was a predominance of IgG1 and IgG4 subclasses and sera from naturally exposed individuals preferentially recognized *Lu. intermedia* proteins of 31, 38, 52, 76 kDa. In a subset of individuals displaying positive serology to salivary proteins, we evaluated cytokine and chemokine production following stimulation of Peripheral Blood Mononuclear Cells (PBMCs) with *Lu. intermedia* saliva. In exposed individuals, we observed higher ($p<0.01$) concentrations of IL-10, IL-13, IFN-γ, CXCL9 and CCL2 compared to non-exposed controls. The main sources of IL-10-secreting cells were $CD4^+CD25^+Foxp3^+$ expressing-cells. The co-culture of *L. braziliensis*-infected macrophages with saliva-stimulated autologous lymphocytes increased the number of intracellular amastigotes. This effect was reversed in the presence of anti-IL-10. Lastly, we observed an association between presence of an immune response to *Lu. intermedia* saliva and CL development, after a 3 year follow-up. We conclude that natural exposure to *Lu. intermedia* saliva polarizes the immune response to a regulatory phenotype, predisposing development of CL caused by *L. braziliensis*.

1204

THE INTERFACE OF THE ANTIVIRAL CELL RESPONSE IN THE INFECTION BY *LEISHMANIA AMAZONENSIS*: ROLE OF THE RNA SENSORS MOLECULES TLR3 AND PKR

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The parasite *Leishmania amazonensis* infects and replicates inside host macrophages leading to persisting cutaneous infections in humans. Although devoid of viral particles, it has been demonstrated that the dsRNA induced kinase, PKR, is activated and plays an important role in the infection by this Leishmania species. The present study was designed to test the role of TLR3, an endosomal dsRNA receptor, classically engaged in cellular antiviral response, in the infection. We demonstrated that TLR3 and the adaptor molecule TRIF (Toll/IL-1R domain-containing adaptor inducing IFN) are colocalized to the parasitophorous vacuole (PV). We also showed the TLR3 was proteolytic processed, a step required for TLR3 signaling, during the infection. Supporting the notion that TLR3 is engaged in the infection, we also demonstrated that the inhibition of TLR3 cleavage impaired the intracellular parasite growth and reduced the expression of the cytokines Interferon beta (IFN1β) and IL-10, while it induced high levels of IL-12. Moreover, the nuclear translocation of the transcription factor IRF3 was impaired when TLR3 cleavage was inhibited in infected macrophages. Accordingly, TLR3-/- macrophages infected by *L. amazonensis* restricted the intracellular parasite infection and expressed reduced levels of IFN1β, IL-10 and exhibited increased levels of IL-12. The *in vivo* infection of TLR3-/- mice by *L. amazonensis* revealed a significant reduction of lesions in the foot pad. Furthermore, we showed that the RNA sensor PKR (dsRNA activated protein kinase) cooperates with TLR3 signaling to potentiate the expression of IL-10, IFN1β and enhanced the parasite survival. Altogether, our results showed that *L. amazonensis* although devoid of viral particles does engage TLR3 signaling during the infection and utilizes this component of the innate immunity to evade the host cell response in conjunction with the PKR pathway.

1205

ROLE OF PD1/PDL1 IN THE INDUCTION OF REGULATORY T CELLS DURING *LEISHMANIA AMAZONENSIS* INFECTION

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Leishmania amazonensis is the etiological agent of diffuse cutaneous leishmaniasis in South America. In murine models of this infection, dysregulated expansion of effector T cells is related to pathogenesis, while the induction of regulatory T cells (Treg) promotes lesion resolution. The most important co-stimulator/receptor pairs for Treg induction are PD1/PDL1, ICOS/ICOSL, OX40/OX40L and GITR/GITR. In this study, we examined the roles of these molecules in *L. amazonensis*-infected C57BL/6 mice. We found that infected foot tissues had a 10- and 5-fold increase in PD1 and PDL1 expression levels, respectively, with minimal changes for other receptor/ligand pairs. In skin-draining lymph nodes of infected mice, there were an increase in the percentage of CD11c+PDL1+ dendritic cells (DC) and PD1+CD4+ T cells. To evaluate PDL1 expression on DC, we performed *in vitro* infection with promastigotes and amastigotes. *L. amazonensis* infection resulted in an increased PDL1, but decreased PD-L2, expression on DC surface. This induction-triggered PDL1 expression was partially dependent on STAT3, PI3K, mTOR and MYD88 with minor participation of MAPK/ERK, but not on JKI, JKII, JKIII and STAT5. Infected DCs were more competent in inducing CD25+FoxP3+ Treg *in vitro* than the control cells, and this Treg-promoting effect was dependent on PDL1 expression but not on TGF-beta production. Together, these data suggest a role for PD1/PDL1 in the regulation of local immune responses during *L. amazonensis* infection. This study provides new insights on immune regulation of cutaneous leishmaniasis.

1206

IL-33 DECREASES INFLAMMATORY RESPONSE IN CUTANEOUS LEISHMANIASIS PATIENTS

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Inflammatory response in cutaneous leishmaniasis (CL) patients leads to tissue damage and skin ulcer development. Lesion infiltrate is composed mostly by lymphocytes and monocytes, and few parasites are observed. Monocytes from these patients infiltrate lesion and produce high amounts of TNF, contributing to parasite destruction and tissue damage. Conversely, subjects with L. braziliensis subclinical infection do not develop disease and produce lower levels of TNF. We believe that the less inflammatory environment in these individuals controls parasitemia and prevent tissue damage. In recent studies, it was reported a protective role of IL-33 in atherosclerosis, type 2 diabetes, cardiac remodeling and toxoplasmosis through the Th1/Th2 balance. The aim of this study was to determine the role of IL-33 in immune response from CL patients. IL-33 was not detected in supernatants from biopsy cultures or peripheral blood mononuclear cells (PBMC) stimulated or not with soluble Leishmania antigen (SLA), assessed by ELISA. To evaluate the effect of IL-33 on PBMC and cells from biopsies from CL patients we stimulated these cells with SLA in presence or absence of recombinant IL-33 and after 72 hours the levels of IL-5, IL-13, IFN-γ, TNF, IL-6 and IL-1β were determined by ELISA. As expected, SLA induced high levels of IFN-γ, TNF, IL-6 and IL-1β. The addition of exogenous IL-33 to cultures of PBMC stimulated with SLA and biopsies decreased the levels of IL-1β and IL-6 and increased the production of IL-5 and IL-13. IL-1β is produced upon inflammasome activation. To test the pathway by which L. braziliensis triggers IL-1β production we infected C57BL/6 mouse macrophages lacking NLRP3, AIM2, Caspase1, ASC and IL-1R. We found that L. braziliensis-induced IL-1β production is dependent on NLRP3, Caspase1 and ASC. Altogether our data show that IL-33 decrease inflammatory responses in CL patients and may have a protective role in these individuals.

1207

DOXYCYCLINE TREATMENT SHOWS A STRONG EMBRYOSTATIC EFFECT AND CLEARANCE OF PERSISTENT MICROFILARIAE IN THE SKIN OF ONCHOCERCIASIS PATIENTS IN WHOM REPEATED IVERMECTIN TREATMENT HAD FAILED TO CLEAR MICROFILARIDERMIA

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Ivermectin (IVM) has been the drug of choice for the treatment of onchocerciasis. However, there have been reports of persistent microfilariae (Mf) in the skin of some people after many rounds of IVM treatment in Ghana, consistent with the emergence of drug resistance or sub-optimal response (SOR) to IVM. To assess the effect of targeting Wolbachia endosymbionts in *Onchocerca volvulus* on onchocerciasis patients in whom repeated IVM treatment had failed to clear Mf, 167 patients were recruited in 2 districts in Ghana where IVM SOR has been reported. They were treated with either 100mg/d doxycycline or matching placebo for 6 weeks. Three and 12 months after doxycycline treatment,

the patients took part in the IVM mass treatment. Patients were snipped before, 12 and 20 months after doxycycline treatment to assess the levels of Mf. Onchocercomata were extirpated from the patients after 20 months for assessment of embryostatic as well as macrofilaricidal effects. 20 months after treatment, 76% of living female worms from the placebo group were Wolbachia-positive, whereas only 4% in the doxycycline-treated group had a few remaining bacteria. At the same time point, 49% of living females in the placebo group showed normal embryogenesis compared to only 4% in the doxycycline group. More importantly, at 20 months post therapy, none of the nodules removed from doxycycline treated patients contained Mf. This is reflected by the absence of microfilaridermia in the same patients (97% compared to 21% of the placebo patients (P<0.001)). Due to the low dose doxycycline treatment, 52% (consistent with earlier reports) of 136 worms had died compared to 38% in the placebo group. In conclusion, targeting Wolbachia in *O. volvulus* is effective in clearing Mf in the skin of onchocerciasis patients in whom repeated standard treatment has failed to clear. Thus strategies may be developed including anti-wolbachial treatment to control the re-emergence of onchocerciasis in areas where infections persist despite the frequent use of IVM.

1208

ESTABLISHING AND TESTING THE TOOLS TO SUPPORT THE TEST AND (NOT) TREAT (TNT) STRATEGY: THE KEY TO IMPLEMENT ONCHOCERCIASIS AND LYMPHATIC FILARIASIS ELIMINATION PROGRAMS IN LOIASIS ENDEMIC AREA

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Implementation of elimination programs for lymphatic filariasis and onchocerciasis in areas where loiasis is co-endemic is problematic because individuals heavily infected with *Loa loa* may develop severe adverse events (SAEs) when treated with ivermectin. A test and treat approach aims to identify individuals with high microfilarial densities at the point-of-care (POC) prior to treatment. Individuals with microfilarial densities >30,000 microfilariae (mf)/mL are at risk for potentially fatal neurologic SAEs and should be excluded from treatment; those with >8,000 mf/mL present a risk for non-neurological side effects. We tested a POC smartphone-enabled portable video microscope, CellScope, that provides automated counting of *Loa* mf in peripheral blood. During a pilot community study conducted in two villages of Cameroon, CellScope results were compared to a calibrated blood smear (CBS) method and quantitative PCR (qPCR). Out of 205 participants, using either qPCR (from DNA extracted from dried blood spots) or CBS, 66 (32.2%), 16 (7.8%) and 1 (0.5%) individuals were found with mf density >0, >8,000 and >30,000 mf/mL, respectively. CellScope outcomes were missing from 37 participants because of initial technical problems related to movement artifact or failure to manual focus. Among the remaining 168, there were strong correlations between automated counts and counts from CBS (r= 0.96, p<0.0001) and qPCR results (r=0.88, p<0.0001). The results from the CellScope were available within 3 minutes of blood draw, while the results of qPCR and CBS required transport to a centralized laboratory and were not available for at least a week. Although the proportion of individuals with >30,000 mf/ml precludes a formal evaluation of the CellScope, the sensitivity and specificity for levels between 0, 1-8,000 mf/mL and >8,000 mf/mL were excellent. Thus, CellScope screening appears to provide a POC tool to identify individuals at risk of SAEs in community-based MDA programs where *L. loa* is endemic. Validation of the CellScope V2.0 in ~30,000 individuals is planned for late 2014.

1209

DRUG DISCOVERY AND DEVELOPMENT FOR THE TREATMENT AND CONTROL OF FILARIASIS: REPURPOSING EMODEPSIDE

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Lymphatic filariasis (lymphoedema and hydrocoele) and onchocerciasis (dermatitis and ocular inflammation) caused by the parasitic filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus* lead to severe morbidity in developing tropical countries. Mass drug administration (MDA) programmes use ivermectin or diethylcarbamazine, combined with albendazole, with the aim to eliminate filarial diseases. However, these drugs primarily only kill the first stage larvae. Co-endemicity of these diseases with loiasis impedes MDA due to the high risk of encephalopathy. Additionally, resistance to the standard MDA drugs is also a concern. Therefore, new drugs and regimes need to be in the pipeline. Here we describe research on old drug candidates such as emodepside into new treatment options. Emodepside is a registered drug for animal health, commercialized by Bayer under the name of Profender® (in combination with praziquantel) or Procox® (in combination with toltrazuril). Emodepside is extremely potent *in vitro* against various filarial nematodes (*Achatocheilonema vitae*, *Litomosoides sigmodontis*, *Brugia malayi*, *Onchocerca gutturosa, Onchocerca lienalis*). Jirds infected with *L. sigmodontis* were administered 0, 12.5, 25, 50 and 100 mg/kg orally once a day for 5 consecutive days. Tissue pathology was scored, identifiable worms from the peritoneal and pleural cavities isolated and counted. A dose-response for pathology adult worm burden was observed. Furthermore a single dose of 100 mg/kg emodepside was sufficient to clear microfilaremia. This study suggests that emodepside with its long half life warrants attention as an additional tool for drug administration strategies in filariasis.

1210

LONGTERM IMPROVEMENT OF HYDROCELE SIZE AFTER ULTRASOUND-GUIDED ASPIRATION

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An estimated 25 million men suffer from hydrocele due to lymphatic filariasis (LF). Current treatment strategies rely on mass drug administration and, in case of hydrocele, hydrocelectomy. In previous studies it could be shown that treatment with doxycycline led to improvement or halt of progression in patients suffering from lymphedema or hydrocele. The aim of this randomized placebo-controlled study was to assess the possibility of using the combination of doxycycline treatment and ultrasound-guided

aspiration of hydrocele fluid as an alternative to hydrocelectomy. After ultrasound examination (USG), 73 men with stage 2 or 3 hydrocele (stage 1 – subclinical hydrocele, stage 2 – longitudinal and transverse diameters <1.9 and <1.6 cm, stage 3 – diameters <3.8 and <3.2 cm, stage 4 – diameters >3.8 and >3.2cm (as reported previously)) were included in the study and treated with either doxycycline 200mg or matching placebo for 6 weeks. Four months after treatment onset participants were again screened by USG and underwent an ultrasound-guided aspiration in case of hydrocele stage > 1, which was successful in 47/51 men. Follow-up examinations were carried out 4 days after aspiration, and 7, 12, 24 and 44 months after treatment onset. The primary outcome analysis (Intention-to-treat) showed an improvement (reduction of hydrocele size of at least one stage at 12 months compared to pre-treatment) in 53/73 (72.2%) participants. There was no difference between doxycycline (25/37, 67.6%) or placebo treated men (28/36, 77.8%) (p = 0.433). The per protocol analysis confirmed these results. After 24 and 44 months improvement was persistent in 67.1% (doxy 59.5%, placebo 75%; p = 0.214) and 71.2% (doxy 64.9%, placebo 77.8%; p = 0.302), respectively. Thus, following aspiration a long-term improvement of hydrocele size was observed. While spontaneous improvement cannot be formally excluded given the lack of a control group in this study, this is not likely in the light of the literature. Aspiration may therefore be considered as an alternative to circumwent the throughput limits of hydrocelectomy. Treatment with doxycycline before aspiration does not lead to an additional benefit.

1211

EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF *LOA LOA* INFECTION IN PREGNANT WOMEN IN GABON: A PROSPECTIVE COHORT STUDY

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Loa loa - the African eye worm - is a filarial pathogen occurring in Central Africa. Despite its high prevalence in affected rural communities, to date there are no data on the impact of infection during pregnancy. This study aimed to describe the characteristics of pregnant women infected with L.loa in Gabon, and the impact of the infection on pregnancy outcomes. HIV-negative pregnant women participating in a randomised controlled clinical trial assessing mefloquine as an alternative drug for intermittent preventive treatment of malaria in pregnancy (MiPPAD, NCT 00811421) were invited to participate in this cohort study. *L. loa* infections were diagnosed during the study by microscopy of peripheral and cord blood and in placental biopsies. Demographic and anthropometric characteristics, clinical and laboratory measures at delivery, placental histopathological specimens were compared between women with *L. loa* infection and those without infection. 1184 pregnant women were recruited, 194 (16.4%) presented with microfilariae of *L. loa* in peripheral blood. The relative risk (RR) for L.loa infection increased with maternal age. Women older than 30 years were more frequently infected (RR: 2.31; 95% CI: 1.28-4.17; p=0.005) and maternal age and number of previous pregnancies showed some association with filarial infection as assessed by the likelihood ratio test (p=0.056 and P=0.008, respectively). Univariate analysis demonstrated that preterm birth was more prevalent in infected women (RR: 1.76; 95%CI: 0.95-3.28) and a higher proportion of low birth weight infants (RR: 1.16; 0.75-1.8) was observed - however not reaching statistical significance. Out of 186 women with microfilariae in peripheral blood, 27 (14.5%) were observed with evidence of microfilarial invasion into the intervillous space of the placenta. No signs of further pathological alterations of the placenta were observed in histological examination. These findings suggest that loiasis occurs at high prevalence in pregnant women in Gabon and that microfilariae commonly invade the placenta. Unadjusted analysis suggests an association of loiasis with adverse birth outcome. However, at this stage it remains unclear whether this association is based on a causal link or is confounded by nutritional, socio-economic or other yet unidentified factors.

1212

EFFICACY, SAFETY AND PHARMACOKINETICS OF CO-ADMINISTERED DIETHYLCARBAMAZINE, ALBENDAZOLE AND IVERMECTIN FOR THE TREATMENT OF *WUCHERERIA BANCROFTI*

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Existing drugs against lymphatic filariasis (LF) have limited ability to kill/sterilize adult worms. This study determined whether a combination of three widely used antifilarial drugs would improve activity against killing/sterilizing adult worms compared with two drug therapy. The goal was to reduce the frequency and improve compliance of mass drug administration (MDA) compared to standard therapy. We examined the efficacy, safety, and pharmacokinetics of single-dose compared to triple-drug therapy with diethylcarbamazine (DEC), ivermectin (IVM), and albendazole (ALB) in heavily infected individuals with *Wuchereria bancrofti* from Papua New Guinea. In this single-blinded trial, twenty four adults were randomized into one of two treatment arms: DEC 6mg/kg + ALB 400mg (N=12) or DEC 6mg/kg + ALB 400mg + IVM 200ug/kg (N=12) and monitored for microfilaremia, side effects and drug levels. Individuals receiving 3 drugs had >2-log reduction in mf levels at 36 and 168 hours after treatment that was associated with one or more side effects in 11 of 12 subjects. By contrast those receiving 2-drugs had ~1-log reduction in mf levels, and 5 of 12 subjects experienced side effects. These effects included fever, lymphadenitis, elevated liver enzymes, hematuria and proteinuria. Subjective symptoms included headache, nausea, pruritis, and arthralgia. Eleven of twelve individuals receiving DEC/ALB/IVM experienced one or more side effects whereas 8 of 12 receiving DEC/ALB experienced one or more side effects. All side effects were self-limiting and resolved within 48-72h after treatment. There were no significant effects of IVM on DEC or ALB drug levels. All 12 individuals receiving 3 drugs remained amicrofilaremic one year after treatment and had 49% reduction in antigen levels, whereas all but one individual in the 2 drug regimen remained microfilaremic with ~31% reduction in antigen levels (P = 0.04). Combined treatment with DEC+ALB+IVM is safe, highly efficacious and may lead to sustained reduction in microfilaria thereby requiring fewer annual treatments.

1213

TARGETING FILARIAL ABL-LIKE KINASES: REPURPOSED, ORALLY AVAILABLE, APPROVED TYROSINE KINASE INHIBITORS (TKI) ACT AS MICRO- AND MACROFILARICIDAL AGENTS AT CONCENTRATIONS EASILY ACHIEVABLE *IN VIVO*

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Global elimination of onchocerciasis and lymphatic filariasis is targeted for 2020. Given the potential for the development of drug resistance and the ongoing problem of co-incident *Loa loa* infection in Central Africa, the need for new macrofilaricides has never been greater. Thus, new orally administered drugs that are highly efficacious against the adult and microfilarial stages of the parasites are desired. Based on the genomes and transcriptomes of *L. loa*, *Wucheria bancrofti, Brugia malayi and Onchocerca volvulus*, we found that each of these filarial parasites express tyrosine kinase (TK) proteins with significant homology to the human oncogene protein product Bcr-Abl. Not only is the catalytic binding site of FDA-approved imatinib well conserved in these pathogenic filariae, but

also phylogenetically the filarial TK proteins are more closely related to human Bcr-Abl than are other parasitic helminths. To assess the antifilarial effects of imatinib and its next generation sister drugs nilotinib and dasatinib, *in vitro* killing of *B. malayi* (Bm) adult males, adult females, L3 and microfilariae (MF) were tested over a 6-day period using a wide dose range (100nM-100uM). Day 5 IC50s for Bm adult males were 58.3uM (imatinib), 10uM (dasatinib), and 49.3uM (nilotinib). IC50s for the Bm L3s were determined to be 20uM (imatinib), 17.4uM (dasatinib), and 76.4uM (nilotinib). MF IC50s were 12.1uM (imatinib), 6uM (dasatinib), and 33.5uM (nilotinib). In limited data, killing of adult females occurred within 24 hours at 75uM with complete killing by 48 hours at 10uM. Additionally, embryogenesis was markedly affected with early embryonic stages being expelled within the first 24 hours. Moreover, 3 dimensional protein modeling demonstrated how these three tyrosine kinase inhibitors (imatinib, dasatinib, nilotinib) dock at the filarial catalytic domain, thereby inhibiting its activity. Given the known safety of imatinib in humans, plans are underway to assess its efficacy in pilot clinical trials in patients infected with these pathogenic filarial parasites.

1214

THE CURIOUS CASE OF *RICKETTSIA FELIS* IN LAOS

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Rickettsia felis is a flea transmitted rickettsial pathogen, which has recently been identified in numerous studies all over the world, most notably in Africa where rickettsia pathogens have been regarded as rare. Case reports suggest that *R. felis* infections can have very severe manifestations including central nervous system involvement. In Laos, murine and scrub typhus diseases caused by related organisms *Rickettsia typhi* and *O. tsutsugamushi* have been identified as major bacterial causes of non-malarial fevers, including central nervous system infections. Over 9 years, ~3,500 blood and cerebral spinal fluid (CSF) samples (~2500 hospital patients) have been investigated for rickettsial pathogens by quantitative real-time PCR (qPCR). All positive samples were further typed by species-specific qPCR or DNA sequencing. *R. felis* was only identified in three patients, once in CSF and twice in blood. The rarity of *R. felis* infections triggered a closer investigation of the three patients and it became apparent that all had potentially impaired immune systems as well as infections with multiple pathogens, some of them vector-borne. Recently, *R. felis* was demonstrated in an afebrile patient in Kenya and other investigations identified the organism in a diversity of different vectors (chiggers, mosquitos, fleas). The low incidence and curious presentation in Lao, a country otherwise endemic for flea-borne rickettsiosis, combined with the findings from Africa, raise questions regarding alternative vectors of *R. felis* as well as its role as a real causative agent of disease.

1215

SCABIES AND BACTERIAL SUPERINFECTION AMONG CHILDREN --- AMERICAN SAMOA, 2011

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Scabies, a highly pruritic and contagious mite infestation of the skin, is endemic in tropical regions. Delayed treatment can lead to bacterial superinfection, and treatment of close contacts is necessary to prevent reinfestation. We described scabies incidence and superinfection among children in American Samoa (AS) to support scabies control recommendations. We reviewed 2011 pharmacy records from the only AS pharmacy to identify children aged ≤14 years with filled prescriptions for permethrin, the only scabicide available. Children's medical records were reviewed for physician-diagnosed scabies during January 1-December 31, 2011. We calculated scabies incidence, bacterial superinfection prevalence, and reinfestation prevalence during 14 days-12 months after first diagnosis. We used log binomial regression to calculate incidence ratios (IRs) for scabies by age. Medical record review identified 613 children with scabies (incidence: 31.6/1,000 children ≤14 years); 358 (58.4%) were male; 353 (57.6%) had a bacterial superinfection, and 94 (15.3%) had ≥1 reinfestation. Scabies incidence varied significantly among the 9 main island counties (range: 14.8-48.9/1,000). Children aged <1 year had the highest incidence (100.2/1,000). Children aged 0-4 years (incidence: 54.5/1,000; IR: 5.1; CI: 4.0-6.5) and 5-9 years (incidence: 27.7/1,000; IR: 2.4; CI: 1.9-3.2) had a significantly higher scabies incidence than children aged 10-14 years (incidence: 11.5/1,000). Investigating why certain AS counties have a lower scabies incidence can help support recommendations for improving scabies control in counties with a higher incidence. The high prevalence of bacterial superinfection and frequent reinfestations highlight the importance of diagnosing and treating patients and their close contacts at the first signs of infection. Interventions targeting infants and young children who have frequent close family contact should be considered.

1216

ZOONOTIC *BABESIA MICROTI* LINEAGES DO NOT DIFFER FROM THOSE THAT ARE LOCALLY ENZOOTIC

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Deer tick-transmitted human babesiosis due to *Babesia microti* appears to be expanding its distribution and prevalence in the northeastern United States. One hypothesis for this emergence is that "virulent" zoonotic strains have been selected in sites of longstanding transmission. We determined whether parasites infecting humans comprised a less diverse set of genetic lineages, with the alternative hypothesis being that the diversity of strains from human cases does not differ from those that are locally enzootic. We identified 9 genetic loci with tandem repeat regions that are highly variable and used these variable number tandem repeat (VNTR) markers to type parasite DNA from enzootic samples (mice and ticks) and zoonotic samples (human cases) from Martha's Vineyard and Nantucket, two sites with longstanding *B. microti* transmission. We identified 90 different VNTR genotypes from 201 samples (168 field samples and 33 human babesiosis samples); these markers appear to be variable enough to identify individual lineages. No genotypes were identified from human parasite samples more frequently than expected from the general diversity and distribution of enzootic genotypes. We conclude that humans are exposed to and become infected by any of the *B. microti* lineages that are locally enzootic and that there is no support for the hypothesis that there are specific "virulent" zoonotic lineages.

1217

HORIZONTAL TRANSMISSION OF *RICKETTSIA FELIS* BETWEEN CO-FEEDING ARTHROPODS ON VERTEBRATE HOSTS

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Rickettsia felis is the causative agent of an emerging vector-borne rickettsiosis transmitted by cat fleas, Ctenocephalides felis. Recent studies suggest that *R. felis* infections are responsible for approximately 6% of

non-malarial based fevers in hospitalized patients in Senegal and Kenya; however, transmission mechanisms required for pathogen persistence within flea populations have proved difficult to verify. Based on the variable vertical transmission efficiency associated with flea hosts, we hypothesized that maintenance of *R. felis* within the vector population is facilitated by horizontal transmission between co-feeding arthropods on a vertebrate host. In order to test this hypothesis, we developed rickettsial horizontal transmission bioassays with C3H/HeJ mice. Bioassays were conducted in three separate trials and divided into four experimental groups: bleb (intradermal inoculation of *R. felis* and uninfected fleas in feeding capsule), co-fed (*R. felis* -infected and uninfected fleas in same feeding capsule), and cross-fed (*R. felis* -infected and uninfected fleas in separate feeding capsules). Bleb and co-fed bioassays were also performed with uninfected rat fleas (Xenopsylla cheopis) and *R. felis* -infected cat fleas and uninfected rat fleas, respectively. Quantitative real-time PCR analyses (based on the rickettsial 17-kDa antigen gene) revealed that Rickettsia-uninfected cat fleas acquired the pathogen through the vertebrate host in bleb (10.0 - 20.0%), co-fed (0.0 - 40.0%), and cross-fed (0.0 - 10.0%) bioassays; uninfected rat fleas also acquired *R. felis* in bleb (0.0 - 40.0%) and co-fed (0.0 - 30.0%) bioassays. Additionally, we aimed to delineate early-phase transmission events (1, 3, 6 and 12 hrs. post infection) involved in the extrinsic incubation period by examining dissemination of *R. felis* to flea salivary glands by microscopy. Delineation of transmission mechanisms of *R. felis* is essential to fully understand the epidemiology and ecology of this emerging rickettsiosis.

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SCABIES AND ASSOCIATED BACTERIAL INFECTIONS

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Scabies is a contagious superficial skin infection caused by the parasitic mite *Sarcoptes scabiei*. Although scabies infection alone is not life-threatening, it is complicated by secondary bacterial infections. Epidemiological studies indicate co-infection by *Staphylococcus aureus* and *Streptococcus pyogenes* in mite infested skin. We hypothesised that the mites alter the healthy skin microbiota and promote the growth of opportunistic pathogens. Scabies mites produce several families of different protein classes that interfere with various host complement molecules. They are secreted into the mite gut and excreted into the epidermis with the feces. We hypothesise that scabies mite complement inhibitors create a microenvironment that promotes bacterial survival. We will present whole blood bactericidal *in vitro* assay data demonstrating that scabies mite complement inhibitors increase the growth of *S. aureus* and *S. pyogenes* in a concentration dependent manner. Deposition assays show that these proteins reduce the opsonisation of bacteria resulting in reduced phagocytosis by neutrophils. Using a porcine skin *in vivo* model we tested whether mite infestation alters the healthy skin microbiota, making way for the opportunistic pathogens. We found significant changes in the epidermal microbiome, in particular a dramatic increase in pathogenic *Staphylococcus* species correlating with the onset of mite infestation persisting beyond treatment with acaricide and healing of the skin. This is a first *in vivo* study offering experimental evidence and supporting previous assumptions that scabies infection causes establishment of pathogens. Comprehending the tripartite interactions between mites, bacteria and host immune system will result in biologically important aspects of disease pathogenesis, offering avenues for alternative therapies and novel intervention strategies. This data will correct the common perception of scabies being a simple 'itch', but often the origin and driver of a complex disease involving multiple pathogens and giving rise to serious sequealae.

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EVIDENCE OF NON-SYSTEMIC *BORRELIA BURGDORFERI* TRANSMISSION IN THE NORTH AMERICAN VECTOR-HOST SYSTEM

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Identifying key modes of pathogen transmission is crucial to understanding the spread of disease, and can help us better understand how pathogen variation and persistence within hosts are maintained. Lyme disease, caused by the bacterium *Borrelia burgdorferi* and transmitted by the *Ixodes scapularis* tick, is the most prevalent vector-borne disease of humans in the United States and Europe. *B. burgdorferi* exhibits broad genetic diversity at several loci, and these strains vary in their levels of invasiveness and persistence in both *Peromyscus leucopus*, the main reservoir host, and humans. A critical unresolved question is how strains that only persist for a short period in *P. leucopus* can be maintained in nature, since *B. burgdorferi* must survive in the host from the period of infection by *I. scapularis* nymphs to when most susceptible larvae of the next cohort feed, approximately two months later. Some larvae, however, feed simultaneously with nymphs, so non-systemic transmission would be an alternative transmission mode between these synchronously feeding nymphs and larvae. This mechanism has been shown to be key for the maintenance of tick-borne encephalitis virus in Europe, which only persists in the host for a few days. We determined whether this is a viable transmission mechanism in our system by infecting groups of *Peromyscus leucopus* mice with a non-invasive *B. burgdorferi* strain and another strain of unknown invasiveness that is common in our study area and has been increasing in frequency in the region. We found that non-systemic transmission is a viable mode of transmission in the North American Lyme disease system for both strains, which has not been previously confirmed. This key finding will be incorporated into mathematical transmission models to evaluate the extent to which it can explain the persistence of non-invasive *B. burgdorferi* strains in the northeastern United States.

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ISOLATION, CULTIVATION, AND CHARACTERIZATION OF *CANDIDATUS RICKETTSIA ASEMBOENSIS* FROM KENYAN FLEAS

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Candidatus Rickettsia asemboensis was detected molecularly in fleas collected during 2009 from Asembo, Kenya. MLST utilizing the *16S rRNA (rrs)*, 17-kD antigen gene, *gltA*, *ompA*, *ompB*, and *sca4* demonstrated that *Candidatus* R. asemboensis is closely related to *R. felis* but distinct enough to be considered for separate species classification. Despite molecular characterization of *Candidatus* R. asemboensis, the successful *in vitro* cultivation of this bacterium remained to be performed. We used *Ctenocephalides canis* and *C. felis* (dog and cat fleas, respectively) removed from dogs in Kenya to initiate the *in vitro* isolation of *Candidatus* R. asemboensis. Successful cultures were obtained from pools of dog/cat fleas using the *Drosophila melanogaster* S2 and the *Aedes albopictus* C6/36 cell lines. Cytological staining and qPCR (species-specific assay for *Candidatus* R. asemboensis) were utilized to visualize/confirm the isolation of the bacteria into both cell lines. Sequencing of fragments of the 17-kD antigen gene, *gltA*, and *ompA* has been performed and confirmed the identity of our culture isolates. Independent time course infection experiments to help define the growth kinetics of *Candidatus* R. asemboensis in the two cell lines using fresh/frozen seed material have preliminarily revealed that increases in the molecular copy numbers can

be detected during 2 weeks of culture. Infected C6/36 cells prepared for TEM were found to be heavily parasitized, and the rickettsiae appeared in round/elongated forms with the presence of double membranes and electron lucent "halos". Genome sequencing of DNA prepared from *Candidatus* R. asemboensis-infected cells has been completed and assembly of the genome is in progress. To date, we have passaged *Candidatus* R. asemboensis 7 times through S2 and C6/36 cells; and active and frozen cultures are currently being maintained. This is the first time that a *R. felis*-like organism has been maintained in culture and therefore the first time that one of them, *Candidatus* R. asemboensis, has been characterized more than just by molecular typing.

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THE DYNAMICS OF *PLASMODIUM VIVAX* PRIMARY INFECTIONS AND RELAPSES IN A COHORT OF CHILDREN IN PAPUA NEW GUINEA

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The contributions of *Plasmodium vivax* infections and relapses to blood-stage infections are generally unclear in endemic areas. The dynamics are difficult to study because infections are frequently asymptomatic due to acquired immunity, individuals may harbor several different *P. vivax* infections at the same time and the density of parasites in the blood may transiently lie below the limit of detection. High-resolution genotyping allows individual infections to be distinguished. We estimate the seasonality and incidence of primary infections and relapses using data from a cohort of children in Maprik district followed up for 16 months. The children, aged one to three years at enrolment, were followed up at 2 monthly intervals. Blood samples were taken at each routine timepoint and additionally if the child was ill. Samples positive by microscopy or LDR, a molecular method for species detection, were genotyped using high-resolution capillary electrophoresis for genetic markers, *P vivax* MS16 and *P. falciparum msp2*. The number of blood-stage infections per year at risk has previously been estimated to be 15.1 (14.1,16.2) in this cohort. The data were summarized as longitudinal patterns of success or failure to detect a genotype at each routine timepoint (eg 001000001). To the frequencies of these patterns, we fit a model which included primary infection, relapse, clearance and detectibility. We assume that the seasonality of *P vivax* primary infections follows that of *P. falciparum* since they are transmitted by the same vectors. Relapses occurring during the study period can be a consequence of infections ocurring prior to the study: we assume that the seasonal pattern of *P. vivax* primary infections repeats over time. The estimated incidence of relapse decreased with time from the primary infection: 55% of relapses occurred within 4 months and 3% occurred after 12 months. Between 45% and 80% of blood stage infections arose through relapses depending on the season. The peak incidence of relapses occurred in the two month interval following the peak for primary infections. This has implications for the timing of interventions targeting different stages of the life cycle.

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PLASMODIUM VIVAX MORBIDITY AFTER RADICAL CURE: A TWO-YEAR COHORT STUDY IN CENTRAL VIETNAM

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Plasmodium vivax malaria represents a challenge for the Vietnamese Malaria Control and Elimination Program as there is little information available on the efficacy of treatment for clinical cases and for the prevention of relapses. In order to address this question, a cohort of *P. vivax* infected patients in Central Vietnam was treated with Chloroquine (25mg/kg in 3 days) and Primaquine (0.5mg/kg/day for 10 days) and followed up for 2 years. The study was conducted between 2009 and 2011 in four rural communities of Quang Nam province. *P. vivax* infected individuals were enrolled in the cohort after individual informed consent. Treatment was directly observed and participants were monitored actively to assess treatment efficacy at Day28, then visited monthly for clinical examination and blood sampling (microscopy and PCR). A total of 260 *P. vivax* patients were enrolled in the cohort and the 240 of them who completed the 10-day treatment where include in the analysis. Most of the patients (78.7%) belonged to the M'nong ethnic group, and half of them were children<10 years. One late clinical failure and seven (2.9%) late parasitological failures were observed during the first 28 days, while 10 patients (4.2%) were positive by PCR for *P. vivax* at Day28. . About half of the participants (53.3%) had *P. vivax* recurrences detected by microscopy during the whole follow-up period, while by PCR this represented more 70%. Recurrences were mostly repeated and the number per patients ranged from 2 to 13. The incidence of *P. vivax* recurrent infections by microscopy and by PCR will be analyzed using negative binomial regression; and time to event by survival analysis and cox regression. In conclusion, after a 10-day supervised treatment with high dose primaquine, study subjects experienced a substantial number of recurrent *P. vivax* infections mainly at sub-clinical and sub-microscopic level. There is a need to develop appropriate strategies to deal with such high rate of recurrences.

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PLASMODIUM SP. INFECTION PREVALENCE IN TAK PROVINCE, THAILAND IS HIGHER THAN CURRENTLY ESTIMATED - SEROLOGICAL AND MOLECULAR EVIDENCE

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Malaria has been in decline in Thailand, where the reported annual parasite incidence dropped to 0.56 in 2007. The Southeast Asia International Centers for Excellence in Malaria Research has been monitoring the prevalence of *Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*) infections of the population of several villages of Tak Province, at the Thai-Myanmar border since 2011. Both active (ACD) and passive (PCD) case detection surveys are used, relying on microscopy as diagnostic method. ACD efforts consist of weekly visits to participants' homes to record their health status, combined with quarterly mass blood surveys (MBS) of the community of a study site. PCD is carried out in malaria clinics and hospitals. We collected samples at a study site where the parasite prevalence and the rate of symptoms reported were <0.5%, and analyzed

them by qPCR and protein microarray for serology. We found a surprisingly highly level of asymptomatic infections in the community, where 11% of individuals were either *Pf*+ or *Pv*+ while experiencing no malaria symptoms for 2 months preceding and following blood sampling. Serological analysis of both infected and non-infected individuals on a microarray revealed widespread exposure to the parasite, as seropositivity rate to 458 antigens was 100% amongst community samples. Samples collected during PCD also revealed higher infection rate than estimated by microscopy, as well as serological evidence of parasite exposure in all patients, despite of infectious status at blood collection. Using the protein microarray, we identified serological markers associated with protection from malaria disease by comparing the serological profiles of disease immune and non-immune individuals, as well as markers associated with acute symptomatic infection by comparing infected and non-infected clinic patients. We conclude that *Plasmodium* sp. prevalence is higher than recently estimated in Tak, and the large number of asymptomatic infections combined with low-sensitivity of current diagnostic methods may hamper efforts to eradicate malaria in Thailand in the future.

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EPIDEMIOLOGY OF DISAPPEARING *PLASMODIUM VIVAX* MALARIA: A CASE STUDY IN RURAL AMAZONIA

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New frontier settlements across the Amazon Basin pose a major challenge for malaria elimination in Brazil. Here we describe the epidemiology of malaria during the early phases of occupation of farming settlements in Remansinho area, Brazilian Amazonia. We examine the relative contribution of low-density and asymptomatic parasitemias to the overall *Plasmodium vivax* burden over a period of declining transmission and discuss potential hurdles for malaria elimination in Remansinho and similar settings. Eight community-wide cross-sectional surveys, involving 584 subjects, complemented by active and passive surveillance of febrile illnesses between the surveys, were carried out in Remansinho over 3 years. We used quantitative PCR to detect low-density asexual parasitemias and gametocytemias missed by conventional microscopy. Mixed-effects multiple logistic regression models were used to characterize independent risk factors for *P. vivax* infection and disease. *P. vivax* prevalence decreased from 23.8% (March-April 2010) to 3.0% (April-May 2013), with no *P. falciparum* infections diagnosed after March-April 2011. Although migrants from malaria-free areas were at increased risk of malaria, their odds of having *P. vivax* infection and disease decreased by 2-3% with each year of residence in the Amazon. Several findings indicate that low-density and asymptomatic *P. vivax* parasitemias may complicate residual malaria elimination in Remansinho: (a) the proportion of subpatent infections (i.e. missed by microscopy) increased from 43.8% to 73.1% as *P. vivax* transmission declined; (b) most (56.6%) *P. vivax* infections were asymptomatic and 32.8% of them were both subpatent and asymptomatic; (c) asymptomatic parasite carriers accounted for 54.4% of the total *P. vivax* biomass in the host population; (d) over 90% of subpatent and asymptomatic *P. vivax* had PCR-detectable gametocytemias; and (e) few (17.0%) asymptomatic and subpatent *P. vivax* infections that were left untreated progressed to clinical disease over 6 weeks of follow-up or became detectable by routine malaria surveillance.

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SUBMICROSCOPIC GAMETOCYTE CARRIAGE: PREVALENCE AND PREDICTORS ACROSS TWO SEASONS AND THREE DISTRICTS IN SOUTHERN MALAWI

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As the transmissible stage of *Plasmodium*, gametocytes are critical to population-level malaria dynamics, though the epidemiology remains poorly characterized. Because gametocytes comprise a small proportion of the parasite burden in infected individuals, detection requires highly sensitive molecular methods. A *P. falciparum* stage-specific quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay was used to test for gametocytemia in samples collected through the Malawi International Center of Excellence for Malaria Research. The study involves post-rainy and post-dry season cross-sectional sampling of individuals from 900 households across urban/low, semi-rural/moderate, and rural/high transmission areas. Peripheral blood was collected as dried spots on filter paper for PCR and preserved in RNAprotect™ (Qiagen) for qRT-PCR. Samples positive for *P. falciparum* lactate dehydrogenase by PCR were subsequently tested using qRT-PCR to detect gametocyte carriage. *P. falciparum* infection prevalence was 8.1% in 617 samples from dry season 2012 and 18.8% in 795 samples from rainy season 2013. Gametocytes were found by qRT-PCR in 3.4% and 9.4% of the population, respectively. The prevalence of gametocytemia among school-aged children (6-15 yrs) was 8.3% after the dry season and 16.4% after the rainy season, compared with only 2.1% and 6.0% among young children (≤5 yrs) and 0.7% and 5.5% among adults (>15 yrs). The proportion of infections that were gametocytemic was slightly higher in areas with lower asexual parasite prevalence, and in school-aged children relative to adults and young children. Specifically, gametocytes were detected in 56.4% of infections in school-aged children, 46.7% of those in young children, and 35.1% of those in adults (X^2, p=0.03). Additional predictors of gametocytemia will be presented, with comparisons to gametocyte detection by microscopy. School-aged children are a key reservoir for *P. falciparum* transmission, and may represent a strategic target for intervention.

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THE PREVALENCE OF MALARIA AT FIRST ANTENATAL VISIT IN PREGNANT WOMEN IN BLANTYRE, MALAWI FROM 2009-2013

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Preventing malaria during pregnancy is important for the health of mothers and newborns. Interventions which include distribution of bed nets and administration of intermittent preventive treatment (IPT) typically occur at the first antenatal visit, usually in the second or third trimester of pregnancy. During the course of our ongoing studies of malaria among pregnant women in Malawi, a universal bed net campaign occurred in the middle of 2012. We hypothesized that this intervention would decrease the prevalence of malaria among pregnant women at their first antenatal visit. We conducted quantitative PCR (qPCR) from dried blood spots collected at the first antenatal care visit (prior to administration of IPT) from women who were in their first or second pregnancy and less

than 28 weeks gestation by clinical assessment. Overall, 145/753 (19.2%) women tested for malaria at their first antenatal visit were infected. By year, the malaria infection rates were 22.0% in 2009, 23.2% in 2010, 15.7% in 2012 and 8.5% in 2013. While declines between other years did not reach statistical significance, the odds of malaria infection at the time of first antenatal visit in 2013 as compared to 2009 were 0.3 (95% CI: 0.1 - 0.9). We will continue to analyze samples from the ongoing clinical trial. Rates of malaria at first antenatal visit declined from 2009 to 2013 with the most pronounced decline in 2013 after completion of the bed net campaign. Nevertheless, infection in this cohort is still common. These first and second trimester infections may cause maternal anemia and placental malaria resulting in adverse maternal and fetal outcomes. Infection early in pregnancy may also contribute to malaria transmission as pregnant women represent a significant untreated reservoir of parasites. Universal bed nets appear to have moderate success in preventing malaria early in pregnancy and our findings support continued efforts to target women early in pregnancy and possibly all women of childbearing age.

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A COMPARISON OF HEMOLYTIC POTENTIAL OF THREE ANTI-MALARIALS ON NORMAL AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE-DEFICIENT INFANTS

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Chlorproguanil-dapsone (CD) has been linked to hemolysis in G6PD-deficient individuals but there are no data for children <1 year. Therefore, the present analysis sought to assess incidence of hemolysis in G6PD-deficient and normal infants treated with CD, sulfadoxine-pyrimethamine (SP), and mefloquine (MQ), using data from a double-blind, placebo-controlled trial of Intermittent Preventive Treatment in infants (IPTi). Hemoglobin (Hb) measurements were made at IPTi doses, regular follow-up and emergency visits. G6PD genotype was determined at 9 months looking for SNPs for the A- genotype at coding position 202. Hemolysis was defined as absolute change in Hb, ≥15% decrease in Hb, or post-dose Hb measurement <8 g/dL. These outcomes were assessed using - 1) a single follow-up Hb on day 7 after an IPTi dose; 2) Hb obtained 1 to 14 or 28 days after each IPTi dose; and 3) all follow-up Hb. A total of 1557 (64%) children had valid G6PD results - 1324 were G6PD normal, 114 homo/hemi (HH), and 119 heterozygous (HET). Of these, 329 children in the active arms had Hb measured on day 7. A strong association of HH genotype with Hb < 8 g/dL on day 7 was seen (OR = 6.65, 95% CI 1.67-26.6, p = 0.007). Similarly, an adjusted linear model for absolute changes in Hb between days 0 and 7 among children in the three active arms showed a statistically greater decline in HH children (-0.56 g/dL, 95% CI 0.01-1.12, p=0.06). However, using all follow-up measurements, Hb declined less in HH infants (0.24 g/dL, 95% CI 0.02-0.46, p=0.03). Likewise, we found lower prevalence of ≥15% declines in HH infants. There was no evidence for an effect of G6PD status on absolute declines or declines of >=15% for changes within 14 or 28 days of the IPTi dose. Finally, while we did find greater declines in Hb in the CD arm compared to both the SP and MQ arms, there was no evidence of a drug-genotype interaction. These results could be explained by low statistical power, genotypes other than A- being common in the study area or that CD did not result in acute hemolysis in G6PD-deficient children. Our ability to assess drug-induced G6PD deficiency-related risk of hemolysis remains challenging and will require creative approaches for eliciting, assessing and recording safety data in this important patient population as drugs with hemolytic potential, such as the 8-aminoquinolines, primaquine and tafenoquine are more commonly used.

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NOVEL SERO- EPIDEMIOLOGICAL TOOL FOR ASYMPTOMATIC *LEISHMANIA DONOVANI* INFECTION

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Visceral leishmaniasis (VL) is a serious disease that threatens 200 million people living in endemic areas across the Indian subcontinent, Mediterranean basin, East Africa and South America. Though diagnosis of VL disease has been made simple and cost- effective through the availability of standard serological tests, similar tools do not exist for asymptomatic infection detection, which is 4-20 times more likely than symptomatic VL disease in endemic areas. A serological test for asymptomatic infection is necessary for designing surveillance and elimination programs, already underway in the Indian sub- continent. We identified 12 novel VL- specific tandem repeat antigens by bioinformatics and one by mass spectrometry of VL patient samples. All 13 antigens were expressed recombinantly and down selected by ELISA based on their agreement and complementation of with DAT on a panel of sera from a hyperendemic district of Bangladesh, indicating those at highest risk of being asymptomatically infected. A conserved protein rKR95 and a tandem repeat antigen rTR18 together agreed at 92% with DAT on asymptomatic sera with robust signals and low background. The antigens were also able to detect 26% of DAT negative sera, proving that they are highly sensitive for asymptomatic infection. rKR95 and rTR18 have utility in a sero-epidemiological tool for asymptomatic infection in endemic regions distinct from one used to confirm disease, individually or as fusion antigens. Such a test for asymptomatic infection will have high impact on achieving active detection, a primary goal of larger surveillance and elimination programs.

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RISK FACTORS FOR VISCERAL LEISHMANIASIS IN GEDAREF STATE, SUDAN

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Risk factors for visceral leishmaniasis (VL) in areas where P. orientalis is the main vector remain unclear, limiting the development of relevant control strategies. We conducted a case-control study to identify determinants of clinical VL in 24 villages of Tabarak Allah hospital's catchment area, Gedaref State, Sudan. From September 2012 to July 2013, we recruited 198 patients newly diagnosed with probable or confirmed primary VL. Using spatial sampling, we randomly recruited 801 controls with a distribution of age, sex and village of residence proportionate to the distribution of the target population. Controls were free of VL symptoms, previous VL treatment and had a negative VL rapid test. A questionnaire was used to collect information on the demographic and socio-economic characteristics of the participants, their travel history, day and evening activities, and the characteristics of their house, yard and surroundings. In a multivariate logistic regression model, VL risk increased with household size, sleep location (outside the yard, not in the farm), evening outdoor activities in the rainy season (playing, watching TV, radio listening), use of ground nut oil as animals' repellent, presence of dogs in the yard at night, Acacia nilotica in the yard's immediate surroundings and of a forest at eye

range. VL risk appeared to decrease with the use of drinking water sources other than the village water tank, increasing distance from the adjacent house yard, and with the presence of animals other than dogs in the yard at night. Children and men were at higher risk of VL as well as individuals reporting VL patient(s) in their household in the previous year. In contrast with previous studies, housing factors, mosquito-net use, black cotton soil, ethnicity, socioeconomic index, presence of Balanites aegyptica and Azadirachta indica in the yard were not independent VL determinants. Although our results do not provide evidence of causality, they provide useful suggestions for the development of relevant VL preventive measures as well as for guiding further studies.

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PREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION AMONG BOLIVIAN IMMIGRANTS IN THE CITY OF SAO PAULO, BRAZIL, PRELIMINARY REPORT

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With the urbanization of the population of developing countries and the process of globalization, Chagas disease has become an emerging disease in urban areas of endemic and non-endemic countries. The aim of the present study was to determine the prevalence of *Trypanosoma cruzi* infection in Bolivian immigrants living in Sao Paulo Metropolitan Area. Approximately 300,000 Bolivian immigrants live in it. In 2006, PAHO estimated the prevalence of Chagas disease in Bolivian general population and among candidate blood donors in 6.8% and 8.0% respectively. Vectorial transmission of Chagas disease has been interrupted in Brazil since 2006. This prevalence survey was undertaken in a sample of 633 volunteers (being 111 children below 10 years of age), randomly selected from the clientele of primary care units located in the central districts of the City of Sao Paulo, Brazil. Inclusion criteria were the agreement to respond to a semi-structured questionnaire and to collect blood for serology after signing the informed consent form. Infection was detected by two different ELISA assays with epimastigote antigens (Bioschile and Biomeriéux), followed by immunoblot with trypomastigote antigens as confirmatory test. The prevalence of infection in 598 individuals so far analyzed was 4.68%. Among children less than 10 year old the prevalence was 2.76% and among the older than 10 years, 5.13%. This is, to our knowledge, the first information on the prevalence of infection among the Bolivian immigrant community in the City of Sao Paulo and represents a challenge to primary care clinics to manage chronic Chagas disease, its vertical transmission, as well as to investigate parasite reactivation in patients under immunossupression in tertiary health care. Additionally, these data will be useful in planning and evaluation of the surveillance and control program of Chagas disease transmission by blood derivatives and organ transplants.

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HUMAN MIGRATION DRIVES THE DISPERSAL OF EPIZOOTIC CHAGAS DISEASE: THE CASE OF HIGHLAND BOLIVIA

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An improved understanding of the interactions between natural parasite populations and their environment is crucial to establish the epidemiological risk associated with epidemic pathogenic genotypes. *Trypanosoma cruzi*, the aetiological agent of Chagas disease, is an ancient and widespread zoonosis distributed throughout the Americas. TcI is the most abundant genetic lineage; it is the principal cause of human chagasic cardiomyopathy and is ubiquitous among sylvatic transmission cycles. Multiple molecular markers consistently identify high levels of diversity within sylvatic TcI populations, and divergent, but genetically homogeneous, strains isolated from human infections. However, current understanding of the genetic determinants that drive natural *T. cruzi* diversification is incomplete. We performed high resolution nuclear and mitochondrial genotyping of contemporaneous sylvatic TcI (n=199 biological clones), isolated from a range of triatomine and mammalian hosts across Bolivia. We detected two distinct sylvatic transmission cycles in adjacent highland and lowland areas. Highland Bolivian strains were characterized by reduced genetic diversity and heterozygosity (Ar= 1.92-2.22, FIS=-0.241-0.026) compared to lowland areas (Ar = 3.40-3.93, FIS=0.176). We observed equivalent levels of subdivision among highland areas spanning >465 km (FST = 0.084, p=0.0032) and between lowland populations across 155 km (FST = 0.087, p<0.001). Measurements of isolation by distance detected greater parasite dispersal among geographically disparate, but heavily populated, highland areas (RXY= 0.053, p=0.142) than between proximate, sparsely populated, lowland foci (RXY= 0.209, p<0.001). Importantly, human isolates from highland Bolivia, were closely related to sylvatic strains circulating in the same area. Overall, the most parsimonious explanation for our results is a founder event in highland Bolivia with long-range anthropogenic dispersal of parasites across an ecological cline. We discuss the important role of humans as an abundant, but often neglected, vector of *T. cruzi* and consider their impact on the emergence of epizootic Chagas disease.

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TEMPORAL AND REGIONAL TRENDS OF FORCE INFECTION OF CHAGAS DISEASE IN COLOMBIA

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In the context of Chagas disease, the impact of vector control strategies in endemic zones is often measured via age-specific seroprevalence surveys, for which interpretation is complicated by secular changes in exposure, resulting in complex age profiles. Reconstruction of temporal trends in the force of infection (FOI) from prevalence data can help to improve our understanding of the transmission dynamics and to plan cost-effective national strategies for diagnosis and treatment. Using 52,712 registries, this study aimed to estimate the trends in the FOI (incidence) for Chagas disease in 14 endemic departments. Given the range of surveys from 1996 to 2011, the data accounted for exposure history between 1920 and 2011. Various catalytic models were fitted to estimate either a constant FOI as a function of exposure time, or a modified FOI at a specific (estimated) time, plus a parameter that accounts for other transmission routes. Parameters were estimated through Markov Chain Monte Carlo (MCMC) methods. Using the simple model, the FOI varied between 0.04 x10-3 and 0.03 person/year across all locations. The Sierra Nevada de Santa Marta region (northern Colombia) showed the highest FOI (0.023; 95% CI 0.02-0.026). Among high endemic zones, the FOI varied from 0.004 to 0.026 in the late 1990's, and between 0.001 and 0.004 in the 2009/2011 surveys. For two surveys, a change in FOI was evident; in Casanare (eastern Colombia), from 0.07 (0.014-0.28) before 1972 to 0.001 (0.0009-0.001) after 1972, and in Santander (east-centre), from 0.009 (0.002-0.08) before 1981 to 0.001 (0.0003-0.002) afterwards. These results provide evidence on the temporal and regional variation patterns in Chagas disease incidence in Colombia. Marked transitions around the 1970's and 1980's have likely resulted from control interventions, improved housing conditions, or migration movements.

Ongoing modelling analyses, allowing more flexible variations in the FOI through time, will be discussed to provide improved and updated predictions of Chagas disease prevalence and incidence in Colombia.

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EVALUATION OF INDOOR INSECTICIDE SPRAYING (IRS) PROGRAM, AN INTEGRATED APPROACH FOR VECTOR CONTROL IN VISCERAL LEISHMANIASIS

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Human visceral Leishmaniasis (VL) or commonly known as kala-azar is vector born infectious disease transmitted by *Phlebotomine argentipes* sandflies. The disease is highly endemic in Bihar state of India and is fatal if left untreated. VL is a neglected tropical disease and Indian government targets to eliminate it from the region by 2018. Active case surveillance and vector control by indoor residual spraying (IRS) using DDT are two ways to target the elimination. In the district of Muzaffarpur in Bihar, during our evaluation surveys we found IRS coverage was only 17% in 2010 which increased to 70% in 2013. However, in the villages with 100% coverage sand fly density did not reduce significantly. There were several ditches between the planning and monitoring of IRS program. We observed the use of low concentration solution, poor quality of insecticide and walls were not sprayed up to adequate height at places. Peri-domiciliary areas were inadequately sprayed. IRS was done only once in a year as against twice per year as recommended in the program guidelines. We did not find any trouble and discomfort in the community by the use of insecticides. However people felt IRS ineffective because it did not reduce the density of malaria mosquitoes. Some of the households did not allow spraying as it would disturb their life for at least one day or no male member was present at that time. Some households allowed spraying only a part of their house. Some households did complaint that the spray team did not visit their house even after request. We also sensed IRS as a means of fulfilling political agendas of village chief or local leaders. There are some other perceptions and barriers in the community against the acceptance of IRS program. These perceptions vary from community to community, from illiterate to educated ones, from lower to upper caste and from poor to rich. To understand more on these perceptions we are conducting a qualitative study in the selected villages of the district. In-depth interviews and focused group discussions are being conducted among villagers, community leaders, health providers and program in-charge. Participants represent wider section of the community in terms of socio-economic status, education, and caste. The study is expected to be completed by the end of 2014.

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TRENDS OF MORTALITY SECONDARY TO CHAGAS DISEASE AND IMPLICATIONS OF REPORTING ON ESTIMATES

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In Colombia is estimated that about 436. 000 people are infected with *Trypanosoma cruzi* and 140.000 have the cardiologic sequelae. This study aims to explore the historical trends of mortality secondary to Chagas disease (CD) and tries to explain the variations of this pattern through the 35 years of existence of the mortality reporting system. The national registry of deaths is based on death certificates, which are filled by Doctors when a patient dies, and then processed by the National Statistics Department, where direct cause and associated causes of death are classified. We use the method of associated causes of death, taking the total available registries (1979-2011). By using the direct method, we estimated crude and adjusted death rates do to CD at National, Departmental and Municipality levels. The patterns of death rates were estimated using joinpoint regression techniques. For the described period, a total of 5.349.628 deaths were registered and 1957 of those (proportional mortality rate of 0.03%) corresponded to CD. 6 departments registered 80% and 7 municipalities 60% of the deaths; 1 of these 6 departments (Capital District) is not an endemic zone. Death rates in men were almost twice the rates in women. Crude death rates increased from 0.03 per 100.000 inhabitants in 1979 to 0.23 in 2011. Adjusted death rates showed a similar increase. The rates increased with age, being the highest mortality in the group between 60 and 70 years. Regarding the joinpoint regression analysis, we observed a progressive increment for the period 1979-2008, and a final decrease in the period 2008-2011. The concentration of deaths in certain non-endemic regions could represent a consequence of migration from rural to urban areas. The higher rates in certain age groups could be the result of a cohort effect due to higher prevalence in previous decades. We found evidence of under-reporting of deaths due to CD by comparing known deaths in outbreaks of CD with the reported ones in the same year. Changes in the reporting system through time and its implications are discussed.

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THE PREVALENCE AND ORIGINS OF ARTEMISININ RESISTANT FALCIPARUM MALARIA IN MYANMAR

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Mutations in a kelch protein encoded on *Plasmodium falciparum* chromosome 13 (K13) have been shown to confer resistance to artemisinins in parasites in Southeast Asia. Mapping the prevalence and distribution of K13 mutations, and ascertaining whether resistance is spreading or emerging independently, is important to inform strategies to contain and eliminate resistant parasites. In this study we sequenced the K13 gene in samples from sites throughout Myanmar, including little-studied areas in southern, central and western Myanmar, and estimated the prevalence of K13 mutations at each site. Parasites from each site were genotyped at 33,716 SNPs using a DNA microarray, and haplotypes defined by single nucleotide polymorphisms in linkage disequilibrium with the K13 gene were used to determine the origins of K13 mutations identified at each site. Preliminary analyses suggest that different K13 mutations predominate at different study sites. Haplotype analysis indicates the independent emergence of K13 mutations in Myanmar, including the 580Y and the 574L mutations, which have been observed in other areas of Southeast Asia. Haplotype analysis for all sites will be presented and implications for resistance surveillance will be discussed.

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ARTEMISININ RESISTANCE AND THE EMERGENCE OF "SOFT" SWEEPS IN MALARIA PARASITE POPULATIONS

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Textbook "hard" selective sweeps, in which single resistance alleles have rapidly spread through malaria parasite populations driven by chloroquine and pyrimethamine treatment, have had a powerful influence in shaping our understanding of drug resistance evolution in malaria. These examples have guided the strategies used to search for novel resistance loci; but are they typical, atypical, or even misleading? The recent discovery of a novel artemisinin resistance gene, kelch, in SE Asia, in which resistance alleles

clearly have multiple independent origins, supports a radically different model of resistance evolution. In such "soft" selective sweeps, resistance alleles spread on multiple different genetic backgrounds, so are expected to have a more subtle impact on patterns of flanking genetic variation. We examined the emergence of artemisinin resistance in real time to empirically determine the impact of spreading resistance on patterns of flanking genetic variation. We characterized the emergence and spread of kelch mutations (from 0% in 2001 to 70% frequency in 2013), in malaria patients attending clinics run by the Shoklo Malaria Research Unit in Western Thailand over a thirteen year period. We describe 26 independent mutations within the gene's coding sequence, each associated with reduced parasite clearance rate. We then use genotyping data from 41 SNPs targeting 470kb flanking the kelch gene in 1,577 infections to characterize the emergence of a "soft selective sweep" surrounding this gene. Based on these data, and recent models of adaptation, we argue (1) that the patterns of variation surrounding the kelch gene are likely to be common in the early stages of drug resistance in general, (2) that more sophisticated strategies are needed for identifying selective sweeps that are able to detect both "soft" and "hard" selective events, and, (3) that SNP-by-SNP methods for conducting association studies are likely to be grossly underpowered for discovery of novel drug resistance genes.

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DISSECTING THE GENETIC BASIS OF EMERGING ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM*

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In recent years, the global deployment of artemisinin (ART)-based combination therapies (ACTs) has contributed substantially to reducing the burden of malaria throughout tropical regions. Thus, alarms were sounded in 2008 when efficacy studies in Western Cambodia documented the emergence of ART resistance in *Plasmodium falciparum*. *In vitro* selection of ART-resistant parasites and association-based field studies suggest that mutations in the propeller domain of the kelch gene (PF3D7_1343700) might be central to resistance. Prevalence of these mutations associates strongly with slow parasite clearance rate in patients as well as elevated survival of drug-exposed ring-stage parasites *in vitro*. To confirm the kelch propeller domain as a candidate ART resistance marker, we have tested the hypothesis that mutations in this gene constitute a major determinant of emerging ART resistance across Cambodian *P. falciparum* parasites. We genetically modified the kelch propeller domain in clinically or *in vitro* defined ART-resistant or -sensitive Cambodian Pf parasites using the highly efficient process of zinc finger nuclease (ZFN)-based gene editing. Parasites were transfected with a single plasmid that expressed a kelch-specific ZFN pair, a selectable marker and a kelch donor sequence containing propeller domain mutations observed in Western Cambodia. This approach allowed for discrete editing of the kelch locus and the generation of isogenic parasites lines. Our study has focused on the *in vitro*-selected M476I mutation and the C580Y, R539T and Y493H mutations that predominate in regions with high prevalence of ART resistance in western Cambodia. These mutations were introduced into drug-sensitive parasites or removed from drug-resistant parasites. The aim of this work is to determine whether and to which degree these mutations mediate resistance by using a ring-stage survival assay. We will show data from a comprehensive study of genetically edited laboratory lines and clinical isolates that assess the role of kelch-propeller domain polymorphisms in emerging ART resistance.

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EMERGENCE OF HIGH-LEVEL, STABLE ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM* UNDER ARTESUNATE PRESSURE *IN VIVO*, WITH QUININE CO-RESISTANCE

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Delayed parasite clearance among patients treated with artemisinins in Southeast Asia indicates that artemisinin resistance is evolving, but still limited in strength. We capitalized on the development of a laboratory model, which harbors high *Plasmodium falciparum* loads, to investigate the effect of progressive *in vivo* artesunate drug-pressure on *P. falciparum*. Single flash-dose and two-day regimens of artesunate were applied to *P. falciparum* (PAM) in a humanized NOD/SCID IL-2Rγ-/- mouse model; *in vitro* drug-sensitivities (IC50) were monitored in parallel. *P. falciparum* rapidly evolved high-level, stable artemisinin resistance against both regimens up to extreme, near-lethal, doses of artesunate (240mg/kg). The selection of artemisinin resistance was reproducible, occurring in 80% and 41 % of mice treated with single and two-day regimens, respectively. *In vitro* response proved ineffective as a marker of early resistance: IC50 remained stable while resistance increased *in vivo* to doses of 30mg/kg artesunate. Later, when *in vivo* resistance strengthened further, artesunate IC50 increased to 82.8nM (95%CI 58.2nM - 117.8nM) from a sensitive level of 10.5nM (95%CI 9.0nM - 12.3nM), and finally shifted ten-fold to 99nM. Emergence of artemisinin-resistance in this African strain was associated with selection of the MAL13-1718319(T) mutation, which is significantly associated with clinical artemisinin resistance in Southeast Asia, and found in a gene that encodes a putative DNA-repair protein (RAD5 homologue). Remarkably, despite exclusive exposure to artesunate, resistance to several quinolone antimalarials emerged; of particular concern resistance to quinine, the second-line treatment for severe malaria, was documented both *in vivo* (3 doses of 73 mg/kg IV over 24 h) and *in vitro* (IC50 = 214 nM). *P. falciparum* has the potential to evolve extreme artemisinin resistance and more complex patterns of multi-drug resistance than anticipated. If resistance in the field advances even partially along this trajectory, we could be faced with an unprecedented health crisis.

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K13 PROPELLER POLYMORPHISM IN COMMUNITIES OF THE *PLASMODIUM* DIVERSITY NETWORK: A NETWORK FOR INVESTIGATING AND USING *PLASMODIUM* GENETIC DIVERSITY TO INFORM MALARIA ELIMINATION POLICIES IN SUB-SAHARAN AFRICA

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Artemisinin resistance has been confirmed in South-East Asia. A concerted effort at surveying *Plasmodium* genetic diversity is required for tracking the emergence and potential spread of artemisinin resistance to sub-Saharan Africa. This comes with its underlying scientific, ethical, and practical challenges. The *Plasmodium* diversity network (PDN) is bringing together researchers from malaria endemic sub-Saharan African countries and making use of relevant existing consortial frameworks to address these challenges. The PDN includes scientists from biomedical research Institutions in 11 sub-Saharan African countries i.e. Cameroun, Côte d'Ivoire Democratic Republic of Congo (DRC), Ethiopia, Gabon, The Gambia, Ghana, Kenya, Madagascar, Mali and Tanzania. With the recent identification of K13 propeller as a major molecular marker for artemisinin resistance, we are investigating the presence and prevalence of its polymorphisms at the PDN study sites. Samples, either in the form of dried blood spots or extracted DNA, have been collected from falciparum malaria infections in PDN sites in each of the above listed countries. Capillary sequencing was performed at the Wellcome Trust Sanger Institute and SNPs called using 3D7 as the reference genome. This primary African K13 SNP survey includes 100 samples from each PDN site. Data analysis on a total of 1200 processed samples is underway and preliminary results will be presented at the meeting. This study will provide a sub-Saharan Africa-wide map of K13 propeller polymorphisms and assess the presence of mutations associated with artemisinin resistance in South-East Asia

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IDENTIFICATION OF DRUG RESISTANCE LOCI USING GENOME-WIDE SCANS

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Through rapid genetic adaptation, the *Plasmodium falciparum* parasite is able to develop resistance to antimalarial drugs, thwarting global health efforts. In recent years, genome-wide scans such as Quantitative Trait Locus (QTL) mapping and Genome Sequencing Association Studies (GSAS) have provided critical hypothesis-generating tools in the effort to understand the mechanisms by which this occurs. We have applied these tools in a population genetics approach to identify the targets of highly potent bioactives that can be powerful probes of parasite-specific biological processes. Here we present studies of a benzothiazepine amide chemotype, IDI-3783, discovered to have nanomolar activity in phenotypic whole cell assays against the chloroquine resistant (CQ^R) parasite line Dd2. Strikingly, dose-response to the compound is significantly reduced in chloroquine sensitive (CQ^S) parasites. To study the genetic basis of the inverse relationship between CQ and IDI-3783 response, the parents and progeny from the Dd2 x HB3 cross were used to genetically map the locus responsible for the phenotypic difference. QTL mapping identified a significant peak on chromosome seven. Further refinement of this signal with GSAS analysis of 40 culture adapted clinical isolates indicated a role for PfCRT haplotypes in dose response to these compounds. Independent experiments lead to the generation of *in vitro* selected Dd2 parasites that were 500-fold less sensitive to IDI-3783. Whole genome sequencing identified target mutations in PfCRT, further validating the role of this locus in IDI-3783 mode of action. Interestingly, the IDI-3783 resistant mutants also demonstrated a marked reduction in CQ EC_{50} rendering them susceptible to the drug despite retaining an otherwise CQ^R PfCRT haplotype. A ZFN-edited Dd2 cell line was then generated confirming the role of these PfCRT mutations in IDI-3783 and CQ dose response. These studies demonstrate the power of genome-wide scans in understanding the underlying genetic basis for drug resistance. As novel antimalarial chemotherapies are developed, understanding their resistance mechanisms will be critical in extending the useful lifetime of new drugs and combating this devastating disease.

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REVERSAL OF CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM* IN FRENCH GUIANA: AN ORIGINAL EVOLUTIONARY PATHWAY FROM LOW ENDEMIC SETTINGS AND IMPLICATIONS FOR RESISTANCE SURVEILLANCE IN SOUTH AMERICA

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In French Guiana, *Plasmodium falciparum* is endemic but transmitted at a low level. For several years the number of malaria cases has decreased in this region, which is now considered in a pre-elimination phase. This region, where drug resistance can appear de novo and *in situ*, is therefore an excellent model to study parasite evolution in low-endemicity settings. After decades of treatment with chloroquine (CQ), this drug was abandoned in 1995 due to high prevalence of *in vitro* CQ resistance (>90%). This was associated with the presence of the K76T mutation in

the pfcrt gene, encoding the typical South American haplotype SVMNT. Twenty years after CQ withdrawal, 70% of the isolates have regained *in vitro* susceptibility to CQ. This is not due to the reemergence of the wild-type pfcrt K76 allele, as the mutant K76T allele is fixed within the parasite population. This creates an apparently paradoxical genotype/phenotype association, indicating that *P. falciparum* populations circulating in French Guiana have acquired a novel mechanism of drug response unique to this region. Here we present the results of a genome-wide association study performed on parasites collected in French Guiana between 2009 and 2012 presenting contrasting CQ phenotypes. This allowed identification of a new mutation strongly associated with CQ susceptibility. The causal relationship has been established by gene editing in the 7G8 Brazilian strain which is responsible for a 20-fold decrease of CQ IC50. In order to date and evaluate the extent of this phenomenon, a retrospective analysis was conducted on 400 isolates from 1997 to 2013. Results showed that the new susceptible allele emerged in the early 2000s and rapidly spread thereafter throughout the population. The significant impact of this genotype on other antimalarial drug responses will be presented at the meeting. In conclusion, the utility of the mutation pfcrt K76T to monitor chloroquine resistance is severely reduced in French Guiana and potentially the greater Amazon basin region because of the presence of other mutations that render parasites CQ susceptible. This situation underlines the necessity to regularly validate molecular markers with *in vitro* drug assays before using it erroneously. It also illustrates how in these low-transmission settings where resistant alleles can reach fixation, parasites are able to follow original evolutionary paths.

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NEGATIVE CROSS RESISTANCE: A PRACTICABLE MEANS OF RESTORING PYRETHROID-SUSCEPTIBILITY TO VECTORS OF MALARIA

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Insecticide-treated nets and indoor residual spray programs for malaria control are almost entirely dependent on the use of pyrethroid insecticides. The ubiquitous exposure of *Anopheles* mosquitoes to this chemistry has selected for resistance in a number of populations. This threatens the sustainability of our most effective interventions but no operationally practicable way of conserving pyrethroid-susceptibility has yet been suggested. Combinations of pyrethroid nets or spray formulations with other insecticide classes are generally believed to have little impact on the frequency of pyrethroid-resistant genes if that resistance is already present in the target population. One interesting exception that we are exploring involves the co-application of a powerful chemosterilant (pyriproxyfen or PPF) to bed nets or resting surfaces that are usually treated only with pyrethroids. Resistant mosquitoes that are unaffected by the pyrethroid component of a PPF / pyrethroid co-treatment remain vulnerable to PPF. This chemosterilant has a far greater impact on pyrethroid-resistant mosquitoes than on susceptible ones because of their differential behavioural responses at co-treated surfaces. This imposes a specific fitness cost on pyrethroid-resistant phenotypes. The development of a pyrethroid / pyriproxyfen co-treated net was announced in early 2014 but its potential as a resistance management tool was not closely examined. We demonstrate the full potential of the combination, and a phenomenon called "behaviourally-mediated negative cross-resistance" using a mathematical model supported by empirical data on mosquito behaviour. The technique promises to select against pyrethroid-resistant genes and conserve pyrethroid-susceptibility and the sustainability of an insecticide class that is essential for malaria control.

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AUTO-DISSEMINATION OF PYRIPROXYFEN FOR CONTROL OF AFROTROPICAL MALARIA VECTORS

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Novel strategies for larvicide application to *Anopheles* larval habitats are required to minimize intervention costs. Recently adult *Aedes* mosquitoes have been used to transfer a persistent pupicide (pyriproxyfen) to preferred aquatic habitats to inhibit larval development. However pyriproxyfen (PPF) also sterilizes female mosquitoes exposed to the chemical. The aims of this study were to determine if: (1) PPF sterilizes *Anopheles gambiae* s.s. (2) sterilized females can transfer PPF to oviposition substrate (3) the best point in time to contaminate *An. gambiae* s.s. with PPF for use as agents to transfer the chemical to oviposition substrate. Female *An. gambiae* s.s. were exposed to jars treated with 2.6 mg PPF/m^2 at 48 hour before, 24 hour before, 0.5 hour before, 0.5 hour after, 24 hour after, 48 hour after and 72 hour (on the day of egg-laying) after bloodmeal. Control females were exposed to acetone-coated jar 0.5 hour before bloodmeal. Sterilization effects of PPF was assessed by providing individual females in cages with an oviposition cup for egg-laying 72 hours after bloodmeal. The number of eggs laid by individual females and number of larvae hatched per female were counted. Transfer of PPF to oviposition substrate was assessed by introducing late instar *An. gambiae* s.s. larvae into all oviposition substrate. Both the sterilizing effect and transfer of PPF was dependent on the time of exposure to PPF in reference to bloodmeal. Success to lay eggs was reduced by 85%-88% in females exposed to PPF between 24 hour before and after bloodmeal. Egg-production at this same time intervals was reduced by 15%-31% while larval hatching was overally reduced by 91%. Greater reductions in adult emergence of introduced larvae occurred with increasing time of exposure to PPF after bloodmeal. Sixty-five percent emergence inhibition occurred in oviposition substrate in which females exposed closer to oviposition laid eggs. This study indicates that PPF exhibits great potential for reducing the population of malaria vectors and reduce the huge labour costs for larviciding.

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EVIDENCE FOR POLYGENIC INSECTICIDE RESISTANCE IN THE MALARIA MOSQUITO, *ANOPHELES COLUZZII*

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Starting in 2006, admixed individuals were identified between the sympatric malaria mosquitoes *Anopheles gambiae* and *A. coluzzii* coincident with a major insecticide treated bed net campaign in Mali. We performed whole genome sequencing to reveal all major genomic changes that occurred in *A. coluzzii* post-2006. We confirmed the introgression of *kdr-w* on chromosome 2L from *A. gambiae* into *A. coluzzii* and documented a previously unreported selective sweep on standing variation at the candidate insecticide resistance gene *CYP9K1* (cytochrome monooxygenase; P450) on the X chromosome. The selected *CYP9K1* allele (*cyp-l*) has two regulatory SNPs and appears to have higher copy number than pre-2006 *A. coluzzii*. Although selection acted independently on *kdr-w* and *CYP9K1*, *A. coluzzii* individuals with the combination of *cyp-l* and *kdr-w* alleles have increased in relative frequency in the population from 60 to 92%, suggesting an additive fitness advantage in the presence of concerted insecticide use. Thus, adaptation to increased insecticide exposure in the malaria mosquito involves the accumulation of multiple beneficial alleles from both within and between species.

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DEFINING VECTOR CONTROL STRATEGIES FOR CONTROLLING MALARIA TRANSMISSION USING NEW TYPES OF COMBINATION LLIN AND IRS IN AREAS OF PYRETHROID RESISTANCE

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The development of pyrethroid-resistant *Anopheles gambiae* across Africa is a serious threat to malaria control. To combat the threat and to reduce malaria transmission long-lasting insecticidal nets (LLIN) are being used together with indoor residual spraying in many endemic areas. New types of non-pyrethroid, wash-resistant LLINs are urgently needed. Several LLIN products incorporating either PBO synergist or combinations of insecticide are in final stages of development and undergoing laboratory and experimental hut trials. Before adopting the new products or incorporating them into national malaria control strategies it is necessary to test them at community level, ideally in cluster randomised trials to evaluate their impact on malaria transmission and mosquito populations and for their potential to select for pyrethroid resistance. In northwest Tanzania *An. gambiae* is highly resistant to pyrethroids and malaria transmission was intractable. To meet the problem pyrethroid LLINs were distributed to all households and IRS with bendiocarb was sprayed using a cluster randomised design. The encouraging results of the trial on malaria transmission and on resistance will be described. However, to further reduce malaria transmission it is necessary to use new generation nets and IRS formulations whose properties and effects on resistant mosquitoes in hut trials will be discussed. To meet the continuing challenge a LLIN incorporating PBO and long lasting IRS formulation based on pirimiphos methyl are being trialled in northwest Tanzania using a unique factorial design which assesses their effects separately and together on transmission rates and development of resistance. This will help define the different control strategies to adopt in areas of high transmission, in epidemics, and in areas of lower transmission with and without resistance.

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BEHAVIOR MODIFYING EFFECTS DURING AND AFTER EXPOSURE OF PYRETHROID-SUSCEPTIBLE AND RESISTANT *ANOPHELES GAMBIAE* TO SPATIAL REPELLENTS: POTENTIAL FOR MALARIA PARASITE TRANSMISSION CONTROL

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Tackling malaria vectors indoor with Insecticide Treated Nets (ITNs) and Indoor residual Spraying (IRS) is leading to behavior change of vectors and build up of residual outdoor malaria. Spatial repellents (SRs) could be the solution but they would not be approved for malaria control until appropriate design and measurable entomological endpoints are established to show their potential to control malaria transmission in diverse epidemiological settings including areas with high pyrethroid resistance. In Benin, we used Semi-Field Tunnel (SFT) initially developed by Dr. Moore and colleagues, to assess the repellence range (up to 65m) and toxicity that pyrethroid-based coils (transfluthrin and metofluthrin) used by man would induce against pyrethroid-susceptible and resistant *Anopheles gambiae* bearing the knock down resistance (kdr) gene. The ability of mosquitoes to take subsequent blood meal after surviving the SFT space containing vapors of the SRs was evaluated using a bioassay cage containing a shaved animal. Metofluthrin and transfluthrin coils induced high repellence of both pyrethroid-susceptible and resistant *An. gambiae* (>70%) at close range (5-10m) to the source but on the longer term, i.e. distance, metofluthrin protected the best, offering >40% protection at 65m compared to only 10% with transfluthrin. With either product, repellence rates of pyrethroid-susceptible *An. gambiae* were similar to that of the resistant strain and the trends at all distance range were not distinguishable. Pyrethroid-resistant individuals recovered from SR exposure and blood-fed on the animal sooner than their susceptible counterpart. Forty hrs after exposure, between 40-60% of susceptible *An. gambiae* were unable to bloodfeed compared to nearly 100% feeding success with resistant *An. gambiae*. Transfluthrin but not so for metofluthrin, delivered sublethal deposit, killing no greater than 25% of both pyrethroid- susceptible and resistant *An. gambiae* at all range. The data supports the hypothesis that Metofluthrin and transfluthrin coils have potential for malaria transmission control and suggest that they would do so by creating a vector-free space, even in areas with pyrethroid resistance. The need to develop SRs with different mode of action to complement pyrethroids on nets or IRS is becoming urgent, and should be put on a par with the seeking of novel insecticides or vaccines.

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MICROENCAPSULATED DEET AS AN INDOOR RESIDUAL SPRAY TREATMENT FOR CONTROL OF *ANOPHELES ARABIENSIS*

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As scale up of LLIN continues, a shift in the sibling species composition of the *Anopheles gambiae* complex is being reported over increasingly large areas, with *An. arabiensis* predominating, and *An. arabiensis* is likely to become an increasingly important vector in sustaining transmission of residual malaria. Because of increasing resistance, it is important that alternative chemical classes are evaluated to complement the existing insecticides and that these are tested against *An. arabiensis*. Microencapsulated DEET CS formulation has been evaluated alongside other standard residual vector control chemicals - lambdacyhalothrin CS, permethrin EC, pirimiphos methyl CS and DDT WP in an experimental hut trial. The chemicals were sprayed on plywood panels attached to experimental hut walls to assess the efficacy against pyrethroid resistant, wild free-flying *Anopheles arabiensis*, in terms of chemical-induced mortality, blood-feeding inhibition and chemical-induced exit from huts while rotating the treatments between huts. A sub-sample of fed mosquitoes was analyzed by ELISA to determine their blood meal sources. The overall mortality of *An. arabiensis* collected in huts with all treatments (76-86%) was significantly greater than the mortality in unsprayed control huts (8%, P<0.001). Mortality in DEET sprayed huts (82%) was significantly higher than in lambdacyhothrin sprayed huts (76%, P=0.043) and similar to pirimiphos methyl sprayed huts (86%, P=0.204). Blood feeding was higher in unsprayed control hut (34%) than other sprayed huts (19-22%, P<0.002). DEET (44%) provided equivalent mosquito blood feeding inhibition to DDT and lambacyhalothin. Exiting rates were higher from DEET (98%), lambda-cyhalothrin (98%) and permethrin (96%) than from the unsprayed control huts (80%, P<0.01). This trial has demonstrated the potential of microencapsulated DEET to provide substantial protection as an IRS treatment against *An. arabiensis*.

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ADAPTATIONS OF *ANOPHELES GAMBIAE* TO BREEDING IN POLLUTED WATER: CHALLENGES TO URBAN MALARIA CONTROL

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Urban malaria is a major emerging health problem in sub-Saharan Africa. It is estimated that 800 million people will live in African cities by 2025. Thus, urbanization has a great impact on the composition of the vector system and malaria transmission dynamics. The main vectors of malaria in sub-Saharan Africa, the *Anopheles gambiae*, normally breed in clean water

sources. However, there is growing evidence suggesting the adaptation of Anopheline species to polluted breeding habitats in urban settings. In Ghana, *An. gambiae* has been found breeding in much polluted water bodies leading to an increase in urban malaria cases. This adaptation may pose challenges to the already underfunded malaria control programs. Thus, this study aims at understanding the molecular and genetic basis of this adaptation and evaluating the differences and expression of genes involved in insecticide detoxification in *An. gambiae* s.s. Three Cytochrome P450 genes (CYP_6P3, CYP_4H19 and CYP_4H24), one Glutathione S-transferase gene (GSTD_1-4) and one ABC Transporter gene (ABCC_11), were analysed to determine their expression levels in the larval and adult populations in 5 selected breeding sites, in urban Accra, Ghana. The results revealed that generally the fold expression of these genes was higher and significant in the larvae compared to the adults. The fold expressions, however, varied between sites. With the exception of GSTD 1-4, the expression of the other genes was significantly higher in the most polluted site compared to the other sites. Also, there was significant correlation between ABCC_11, GSTD 1-4, CYP_4H24 and most water quality parameters of the study sites. Analysis of enzyme activity of α-esterase, monooxygenases, glutathione S-transferase and acetylcholinesterase revealed higher and significantly different enzyme activity in larval and adult populations. These results suggest that detoxification enzymes could be involved in adaptation to polluted breeding sites. While the increased enzyme activities observed could be due to functional plasticity, it has been hypothesized that such an adaptive plasticity might continuously evolve to maximize the adaptation of mosquito larvae to breeding sites that are chemically changing. The results may also suggest that perhaps some other mechanisms are involved, which require further studies.

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IMPROVING SOCIOECONOMIC EQUITY IN INSECTICIDE-TREATED BEDNETS (ITNS) ACCESS, OWNERSHIP AND USE IN RWANDA FROM 2000-2010

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Enabled by significant increases in funding for malaria control, remarkable scale-up of malaria control interventions has occurred over the past decade in sub-Saharan Africa. Despite high national coverage with interventions such as insecticide-treated bednets (ITNs), distribution is not always equitable across the population, with coverage varying by households' socioeconomic status. In Rwanda, ownership of ITNs increased substantially from 15% to 82% between 2005 and 2010. Similarly, use of ITNs by children less than five years of age and by pregnant women rose from 4% to 70% and from 4% to 72%, respectively, from 2000 to 2010. The percentage of households owning at least one ITN for every two household members (access) increased from 3% to 39% from 2000 to 2010. To assess the equity of ITN access, ownership and use in Rwanda from 2000 to 2010, data on household wealth and ITNs from Demographic and Health Surveys in 2000, 2005 and 2010 were used to compute Lorenz Concentration Curves and Indices. Concentration Index (C-Index) values range between -1 and 1 with a value of 0 representing perfect equality. Results show drastic improvements in equity of ITN ownership over time (C-Index: 0.35 in 2005 compared to 0.07 in 2010), household ITN access (C-Index: 0.42, 0.12, 0.06 in 2000, 2005 and 2010, respectively), ITN use in children less than five years (C-Index: 0.66, 0.33 and 0.05 in 2000, 2005 and 2010, respectively) and ITN use in pregnant women (C-Index: 0.70, 0.25 and 0.03 in 2000, 2005 and 2010, respectively). Results suggest that ITN distribution programs in Rwanda have achieved increasing equity over time such that by 2010, levels of ITN access, ownership and use were similar across households of all wealth quintiles. This may be due, in part, to the shift in distribution from target populations to mass campaigns.

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STRUCTURED SUPERVISION VISITS USING TABLETS FOR IMPROVEMENT OF MALARIA CASE MANAGEMENT IN SENEGAL

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Improving the quality of malaria case management in public health facilities in Senegal is an ongoing challenge, with 32 hospitals, 89 health centers, and 1247 health posts. The National Malaria Control Program (NMCP) instituted health facility supervision visits to improve malaria services, supervising over 800 structures annually. During each visit, a lengthy paper form is completed, which includes rapid diagnostic test technique, consultation observation, register abstraction of case management of uncomplicated and severe malaria, and stock management. Supervisors are chosen from a cadre of health officers who have attended training in malariology conducted by the NMCP. Health personnel and district health teams receive feedback based on a synthesis of the results. However, are not entered electronically and capacity is limited to perform detailed analysis of the data collected. The NMCP piloted the use of Android tablet computers to facilitate improved data collection and analysis of supervision visits. The supervision form was programmed using Open Data Kit, which provided internal data checks, automatically calculated scores, and collected Global Positioning System (GPS) coordinates for each facility. After completion of each round of supervision, data are downloaded at the NMCP and analyzed. The test phase included 4 hospitals, 4 epidemic surveillance sites, 4 health centers and 3 health posts. While none were out of stock of all dose-packs of artemisinin-based combination therapy (ACT), stockouts of the infant dose, the 1-5 year dose , the 6-13 year dose, and the adult dose affected 7%, 40%, 7%, and 70% of facilities, respectively. All facilities had rapid diagnostic tests (RDT) in stock. Data were missing regarding RDT performance in 10% of febrile patients, but an RDT was performed in 95% of the suspect cases for which data were available. Of patients with a positive RDT, 92% were documented to have received an ACT. Of severe cases, 93% of patient < 5 years and 69% of patients ≥5 years were judged to have been managed correctly. During 2014, tablets will be used to conduct supervision visits nationwide, reducing time required to compile reports, and enabling rapid feedback, in-depth analysis of case management, mapping of indicators, and increased capacity to identify and correct deficiencies. This provides a powerful tool for monitoring malaria case management and an evidence base for continuous quality improvement.

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INCREASING HUMAN RESOURCES FOR MALARIA PROGRAM IMPLEMENTATION IN LOW RESOURCE SETTING: THE IMPACT OF A MALARIOLOGY COURSE FOR PUBLIC HEALTH PROVIDERS IN SENEGAL

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The Senegal National Malaria Control Program (NMCP) aggressively scaled up malaria control interventions during 2007-2010 and is now moving toward pre-elimination. However, the NMCP did not have the human resource capacity to implement and supervise the interventions, particularly correct case management, throughout the public health system of 32 hospitals, 76 district health centers, 1,247 health posts, 2,162

health huts, and 1992 home-based care providers. In order to address this challenge, the NMCP developed an annual malariology course to train public health personnel, with a focus on district health management teams. Each year, senior and middle management health personnel are invited to attend three and two week residential courses, respectively, in malariology, including planning, implementation, monitoring and evaluation. Since 2008, 50 senior and 65 middle managers have been trained, with technical support from WHO and the school of public health, at a cost of $153 per day for senior managers and $107 per day for middle managers. The NMCP recruits from this pool of trained managers for many activities, including training providers on guidelines for malaria prevention and case management, supervision of over 800 health facilities and their providers at all levels in biannual sessions of 21 days each, and periodic assessment of quality of case management. District health officials trained by the NMCP were crucial to the successful adoption of rapid diagnostic tests, enabling Senegal to test over 85% of suspected cases by the second year of implementation. During 2013, they supervised a total of 2,116 providers, seeing an average of 8 providers per working day. Currently, they are training personnel in the newly revised case management guidelines, including pre-referral treatment with rectal artesunate and treatment of severe disease with parenteral artesunate. The Senegal NMCP has trained a critical mass of district-level managers in malaria, who have facilitated the implementation, monitoring and supervision of malaria control activities in a context of a shortage of human resources, and whose contribution to the success of malaria control efforts in Senegal has been consistent and cannot be underestimated. This approach is recommended for other low resource malaria endemic countries struggling with lack of qualified personnel to implement malaria control efforts.

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FACILITATING HARMS DATA CAPTURE BY NON-CLINICIANS UTILIZING A NOVEL DATA COLLECTION TOOL DEVELOPED BY THE ACT CONSORTIUM - RESULTS OF TESTING IN THE FIELD

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In recent years, rapid diagnostic tests and enhanced delivery strategies have improved access to efficacious antimalarials by those who need them. As case management improves and preventative measures such as Mass Drug Administration (MDA) are considered in order to reduce both disease burden and transmission, the potential acceptable risk-benefit ratio to end users has shifted; monitoring the safety of these drugs, however, has been slow to rise on the public health agenda. Whilst novel strategies for improving access to antimalarials and disease burden surveillance are being employed, we still rely on the traditional weak, inefficient and in many places virtually non-existent pharmacovigilance system of clinician-led reporting within the context of the conventional healthcare setting. As antimalarials are increasingly being provided via non-conventional routes and by lower-level healthcare workers, the importance of equipping these workers with the appropriate tools to monitor and report on possible drug-related patient-experienced harms becomes paramount. Traditional pharmacovigilance data collection forms are complex and challenging to use by non-clinicians. The ACT Consortium developed data collection tools to allow lower-level healthcare workers to collect high quality harms data within a variety of contexts such as research studies and programmatic, real-life settings. These tools use a pictorial storyboard to convey the need for data collection to a low-literacy level population. A diary captures drug administration and event data in chronological relation to each other with minimal interpretation required by the data collector, thereby making it suitable for use by lower-level healthcare staff. These tools were used and tested by non-clinical data collectors within ACT Consortium projects and preliminary analysis of the results show that the harms data collected are comparable to those collected within the same and similar clinician-led studies.

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IMPACT OF SCHOOL-BASED PROGRAM OF MALARIA DIAGNOSIS AND TREATMENT ON SCHOOL ATTENDANCE IN SOUTHERN MALAWI

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Whilst real progress in the goal of education for all has been made in sub-Saharan Africa, evidence indicates children who suffer from ill health are less likely to attend and complete school. Malaria is an important cause of morbidity in school children and a significant contributor to school absenteeism. To address the burden of malaria in this age group, the Malawian National Malaria Control Programme, with support from Save the Children, are currently implementing a programme of school-based malaria case management in Southern Malawi. A cluster randomised trial in 58 schools in Zomba is evaluating the impact of this programme whereby malaria rapid diagnostic tests (mRDTs) and artemisinin-based combination therapies (ACTs) to diagnose and treat uncomplicated malaria have been placed in primary schools, as part of basic first aid kits [Learner Treatment Kits (LTKs)]. Head teachers and two additional teachers in the schools were trained in the use of mRDTs and the additional contents of the kits at a 7-day residential workshop. Twenty nine schools were randomly selected to receive the LTKs and a further twenty nine schools were selected to serve as the control. Baseline findings indicated 60.0% (95% CI: 56.2-63.7%) children in this region were infected with *Plasmodium falciparum*, while the prevalence of anaemia was 32% (95% CI: 29.2-35.5%). We present data from teachers' treatment registers describing uptake of the malaria diagnostic service by learners, including the number of malaria cases diagnosed by teachers. Treatment and referral practices will also be reported. Additionally, we present preliminary results on the impact of LTKs, comprising malaria diagnosis and treatment and basic first aid, on the principal outcome of school attendance. To our knowledge, this is the first instance in which school teachers have been trained to perform mRDTs and provide treatment on the basis of parasitological confirmation. Such a programme could provide a valuable complementary service to facility- and community-based roll out of mRDTs.

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THE ECONOMIC VALUE OF THE POST-TREATMENT PROPHYLACTIC EFFECT OF FIRST-LINE ANTIMALARIAL TREATMENTS ACROSS DIFFERENT MALARIA TRANSMISSION SETTINGS

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Recent trials show that dihydroartemisinin-piperaquine (DHAPQ) is as efficacious and safe as artemether-lumefantrine (AL) in treating uncomplicated childhood malaria and has a longer post-treatment prophylactic (PTP) effect compared to AL, reducing the risk of re-infection in children. In a recent cost-effectiveness analysis, we showed that DHAPQ was superior to AL from both the clinical and economic perspectives for treatment of uncomplicated childhood malaria in high transmission

settings (*in press at Plos One*). From a clinical and economic perspective, the benefits of post-treatment prophylaxis are, however, expected to become more significant with increasing transmission intensity and, conversely, less significant with decreasing transmission intensity. Our aim in this analysis is to assess the economic value of the PTP benefit conferred by DP compared to AL across different transmission settings. We base our analysis on primary clinical outcome data from a multi-centre clinical trial of ACTs that was conducted in Asian (n=998) and African children (n=1,698) in a wide range of malaria transmission settings (data provided by Sigma Tau I.F.R. SpA). Using the Markov model we developed for the previous analysis, simulating the progression of malarial disease and the risk of recurrent malaria, we estimate the mean incremental costs and health outcomes of the two treatment strategies per child over one year from the provider perspective in transmission settings stratified as low, moderate and high. We employ probabilistic sensitivity analysis to account for uncertainty in key model parameters. Our preliminary results show that the economic value of the PTP effect of DHAPQ over AL is significant in moderate to high transmission settings, using maximum manufacturer drug prices for ACTs set by the Global Fund. Our full analysis will report how the extent of this benefit varies by local malaria endemicity and antimalarial drug prices. Our findings should help inform the policy discussions on the choice of optimal malaria treatment strategy across endemic settings.

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THE 'PADDY PARADOX' REVISITED: HOW RICE FARMING IMPACTS ON HOUSEHOLD ECONOMIC STATUS AND MALARIA RISK IN EASTERN RWANDA

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Economic activities may entail negative externalities for public health, which is particularly problematic in poverty-stricken areas. The case of rice farming in eastern Rwanda fits this description, as it provides breeding sites for malaria-infested mosquitoes but at the same time generates cash income and improves nutritional standards locally. We add to the evidence base on the 'paddy paradox' by studying a case in Eastern Rwanda (Ruhuha district of Bugasera province). The study unpacks the impact of rice cultivation on malaria incidence by comparing households that differ in their involvement in rice cultivation and proximity to the marshlands that host the rice fields. To this purpose, a large-scale survey was conducted among more than 4,000 households (comprising 17,000 individuals) in the area from June to December 2013. Data on household demographics, economic status, malaria prevention efforts as well as health-seeking behavior has been collected. All household members have also been screened for malaria parasitemia and anemia, and a malnutrition assessment was carried out for under-five children. In addition, qualitative data was collected through nine focus group discussions. It is shown that rice farming is positively and significantly associated with households' wealth, food security, health insurance status, and protection against malaria. At the same time, it is confirmed that rice farming practices increase the risk of malaria transmission through expanded mosquito populations. Rice fields are the main breeding site in the area. Households located nearby the marshlands where rice is cultivated are the most affected by malaria. For those households who generate income from rice production directly, the income effect dominates, resulting in a lower disease burden from malaria. By contrast, households in communities that are located close to the rice cultivation areas but who do not participate in this economic activity, face a higher malaria burden. Rice farming leads to private benefits in the economic domain, which spills over into the health domain, but at the same time creates a public health risk. As a result, the 'paddy paradox' hypothesis is confirmed at the level of rice-producing households, but rejected at the wider community level. Hence, strategies need to be developed that are able to tap the private benefits of rice cultivation and re-direct these to fund collective action against malaria.

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SERUM 8,12-ISO-IPF2α-VI ISOPROSTANE MARKER OF OXIDATIVE DAMAGE AND COGNITION DEFICITS ASSOCIATED WITH CASSAVA CYANOGENIC POISONING

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We sought to determine whether motor (konzo) and cognitive deficits associated with cassava (food) cyanogenic poisoning were associated with high levels of F2-isoprostanes, well-established indicators of oxidative damage. Levels of serum F2-isoprostanes were quantified by LC-MS/MS and anchored to measures of motor proficiency and cognitive performance assessed through BOT-2 or KABC-II neuropsychological testing of 40 Congolese children [21 with konzo and 19 presumably healthy controls, overall mean age (SD): 9.3 (3.2) years]. Cyanogenic exposure was ascertained by levels thiocyanate (SCN) in plasma and urinary. Overall, levels of plasma SCN ranged from 91 to 325 µmol/l or 172 to 1032 µmol/l in plasma or urine, respectively. Levels of isoprostanes (ng/ml) ranged from 01 to 0.8 (Isoprostane-III), 0.8 to 8.3 (total Isoprostane-III), 0.1 to 1.5 (Isoprostane-VI), 2.0 to 9.0 (total Isoprostane-VI), or 0.2 to 1.3 ng/ml (8,12-iso-iPF2α-VI isoprostane). Children with konzo poorly performed both at the BOT-2 and KABC-II testing ($p<0.01$) Within a regression model controlling for age, gender, and other biochemical variables, 8,12-iso-iPF2α-VI isoprostane (ng/ml) was significantly related to overall cognitive performance (Mental Processing Index) on the KABC-II ($\beta = -32.36$ (-51.59 to -13.03 95% CI; $P<0.001$). A regression model including age, gender, motor proficiency impairment, serum albumin and triclyceride levels, and 8,12-iso-iPF2α-VI isoprostane in 20 konzo children explained over 85% of variation in the overall Mental Processing Index, but was not significant in explaining the overall motor proficiency impairment . These findings suggest that brain/behavior injury associated with cassava poisoining is mediated in part by oxidative stress injury. We conclude that 8,12-iso-iPF2α-VI isoprostane is a sensitive biomarker of the neuropathogenic mechanisms mediating brain injury in konzo, and can be used to monitor the impact of interventional trials to prevent or mitigate the neurotoxicity effects of cassava cyanogenic poisoning.

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LEAD AND IRON INTERACT TO INCREASE *PLASMODIUM FALCIPARUM* PARASITEMIA IN BENINESE INFANTS

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Lead poisoning is a major public health challenge in West Africa. It hinders the correct neurocognitive development of infants and it entails impaired growth and learning disorders as well as kidney damage and anaemia. In Benin over 80% of the children are anemic. Malaria, another major cause of anemia, is the first cause of infant morbidity and mortality. However, the interaction of lead poisoning, anaemia and malaria has not been investigated so far. By analyzing the effect of lead levels on *P. falciparum* parasitemia we aim at unravelling the impact of lead poisoning on malaria episodes among Beninese infants. We have followed 630 infants up to 12 months and we have assessed their health status with regard to malaria,

other parasites, as well as their haematological profile. In addition their blood lead levels have been analyzed at 12 months of age showing a high prevalence of lead poisoning (17% for blood lead level (BLL)≥10 µg/dL). Multivariate models show significant increased *Plasmodium falciparum* parasitemia associated with increased BLL irrespective of iron status. In addition there is a positive significant association of total body iron with *P. falciparum* parasitemia controlling for clinical, demographic and environmental malaria risk factors. The interaction of BLL and total body iron is also significantly associated with *P. falciparum* parasitemia. In conclusion the significant association impact of lead levels on *P. falciparum* parasitemia and the synergistic interaction of iron and lead on *P. falciparum* parasitemia bring up important research and public health questions in a region where malaria and lead poisoning overlap. To our knowledge this effect had not been shown so far and complementary cohort studies are required to confirm its significance. In any case, the synergistic interaction between lead and iron rises up the importance of analyzing further the convenience of iron supplementation for anaemia treatment and prophylaxis, especially in malaria endemic regions with high exposure to lead. The considerable prevalence of lead poisoning addresses the necessity of investigating the sources of lead pollution and analyzing its impact on the neuro-cognitive development of Beninese infants, as well as its impact on anaemia.

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QUALITATIVE AND QUANTITATIVE EVALUATION OF IMPROVED STOVE ACCEPTABILITY AND MULTIPLE STOVE USE IN RURAL WESTERN KENYA

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The majority of households in rural Western Kenya cook indoors using open biomass fires, increasing exposure to household air pollution (HAP), which has the potential to negatively impact health. Our study aimed to evaluate the impact of improved cookstove technologies (ICT), compared to the traditional 3-stone open fire, on effectiveness in reducing HAP and acceptability of use in the community. Preliminary findings showed that many households were using additional stoves, in conjunction with the ICT evaluated during the study. This analysis explores factors associated with multiple stove use among study households. We employed mixed methods in a cross-over study design to evaluate 6 different ICT (2 rocket, 1 rocket with chimney, 3 fan-assisted) in households. One ICT was placed in each household for a 2 week period and was intended to be the sole stove used for daily household tasks. Stoves were rotated until each house had used at least 5 different ICT. Households were monitored for 48-hour periods at the end of each 2 week round and quantitative questionnaires, a cooking activity log, qualitative interviews and focus groups were completed. Multiple stove use was defined as any use of additional stoves other than the ICT under evaluation during the 48-hour monitoring period. Of 43 households, 67% (n=29) indicated use of multiple stoves during the study [8 households (19%) at least 25% of the time, 11 (26%) half of the time, 7 (16%) 75% of the time and 3 (7%) all of the study]; 14 (33%) households reported single stove use. Multiple stove use occurred most often (17/38 households, 45%) when the chimney stove was present and least often (9/35, 26%) when rocket stove B was present. Qualitative findings indicate that stove type, ease of stove use, number of people cooked for and type of meal cooked are likely factors associated with multiple stove use. These findings demonstrate the difficulties of conducting field evaluations for ICT and highlight key factors to consider in developing ICT that are acceptable in meeting the daily needs of users.

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TRACHOMA CONTROL THROUGH IMPROVED ACCESS TO WATER AND SANITATION

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Facial cleanliness is a key activity to prevent blinding trachoma; a disease which has impaired the vision of more than 2 million people worldwide. The SAFE strategy (Surgery, Antibiotic, Facial cleanliness, Environmental improvement) for trachoma elimination is substantially weakened without access to water and sanitation facilities. According to leading water, sanitation, and hygiene (WASH) specialists, 12.7 million people in Mozambique (over half the population) don't have access to safe water or proper sanitation facilities. The relationship between water, sanitation, and trachoma is complex; those who have easy access to water and latrines may or may not have less active trachoma. To determine whether access to water and latrines at the household level is associated with greater access to functioning hand-washing facilities and soap, the national trachoma control program in Mozambique reviewed results of recent trachoma baseline prevalence mapping, which includes data on basic WASH indicators. Households with water close to the home (water source in yard) were more likely to have access to a hand-washing facility (P<.001). Households with access to a private latrine were more likely to have access to a hand-washing facility, P<.001, RR=1.20 (1.19-1.22), have water available at the hand-washing facility, P<.001, RR=1.15 (1.14-1.16), and soap available at the hand washing facility, P<.001, RR=1.06 (1.05-1.06) compared to households with only access to a public latrine. In households that have access to safe water and latrines, but lack access to hand-washing facilities and soap, programs should identify the barriers to adopting hand and face washing behavior and modify their Behavior Change Communication (BCC) strategies as appropriate. Promotion of private latrine construction may complement BCC strategies and should be targeted in areas with high prevalence of trachoma.

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HYDROLOGICAL DRIVERS OF TYPHOID TRANSMISSION IN KIBERA, AN URBAN SLUM IN NAIROBI, KENYA

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Typhoid fever is a systemic enteric disease caused by *Salmonella* vars *typhi* and *paratyphi*. Typhoid fever occurs primarily in densely populated urban areas with poor sanitation infrastructure. The primary route of transmission is thought to be via direct contact with individuals who shed the bacteria in their stool. However, recent evidence from Asia suggests that indirect transmission via contaminated surface water may also play a role in the spread of disease. Using data from a large population-based infectious disease surveillance system operated by the Kenya Medical Research Institute/US Centers for Disease Control (KEMRI/CDC), we mapped the spatial pattern of typhoid fever risk in Kibera, an urban slum in Nairobi Kenya, with an extremely high population density (70,000 individuals/sq km). Cases were defined as individuals with fever and positive blood culture for *S. typhi*. Controls were selected randomly from the population-based cohort to estimate the spatial distribution of the underlying population at risk, and were matched to cases on age, gender, and date of diagnosis. We used a spatial modeling framework to map the

geographic distribution of typhoid fever cases and to test whether any significant spatial patterns could be explained by variations in topography and surface-water-accumulation. The greatest risk of typhoid fever was among those living in the lowest-elevation areas where surface-water flow accumulates (p = 0.01). Our results support indirect environmental transmission of typhoid fever in resource-limited settings. Interventions targeted at reducing typhoid fever transmission (e.g., improvements in sanitation and hygiene, and typhoid vaccination) in upstream areas of typhoid-endemic regions may indirectly benefit residents in downstream areas, who are at increased risk of exposure to *S. typhi* from both immediate and upstream sources of contamination.

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CERAMIC WATER FILTERS AND REDUCING THE BURDEN OF DIARRHEAL DISEASE IN INFANTS - WESTERN KENYA, 2013

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Unsafe drinking water consumption is a risk factor for diarrhea, a leading cause of death in sub-Saharan African children. Ceramic water filters (CWFs) remove or inactivate waterborne diarrheal pathogens in drinking water through size exclusion and silver exposure. We examined the effectiveness of CWFs to improve drinking water quality and prevent infantile diarrhea in rural western Kenya. A randomized**,** controlled intervention trial was conducted among 240 households with infants 4-10 months old. Each household was randomized into an intervention or control group with or without CWFs, respectively. Trained interviewers performed a baseline survey and visited households weekly for 26 rounds to document recent onset of diarrhea, respiratory infection, and febrile illness in infants. Source and filtered water samples were tested to monitor *Escherichia coli* concentrations, measured as most probable number (MPN). Person-time incidence rates were calculated per 100 person-weeks of observation. Households reported using surface water (36.3%), public taps (29.2%), or rainwater (17.1%) as their primary drinking water sources. Self-reported filter use among intervention households was 99.6% across weeks observed. Compared with the control group, intervention households reported fewer diarrheal episodes (7.6 vs. 8.9, p=0.1) and fewer health facility visits for diarrhea (1.2 vs. 2.2, p<0.01). The incidence of respiratory infection (1.3 vs. 1.1, p=0.61) and febrile illness (4.1 vs. 4.1, p=0.9) remained similar. *E. coli* were detected in 93% of source water samples (median concentration 512 MPN/100mL; range 10 - $1.4x10^4$ MPN/100mL) and in only 29% of filtered water samples (median concentration 7.4 MPN/100mL; range <1.0 - >2420 MPN/100mL). Households using CWFs had improved water quality and reported lower incidence and significantly fewer health facility visits for diarrhea in infants.

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WATER ACCESS, QUALITY AND USE IN TEN HEALTHCARE FACILITIES IN HONDURAS AND GHANA

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In the developing world, it is estimated that 46% of health facilities have access to an improved water source. The 2015 Sustainable Development Goals include a provision to "provide universal access to safe drinking water in health centers." In order to meet this goal, the majority of health facilities in the developing world will need to gain access to an improved water source. However, once access to an improved source is obtained, there remain significant barriers to ensure sustainable water access, quality, and use. From 2012-2014 the Center for Global Safe Water at Emory conducted an assessment of water access, quality and use in 10 district-level hospitals in Honduras and Ghana. Water quality testing, observations and interviews were conducted at each site. All hospitals evaluated had access to improved water sources and on-site treatment systems. Despite this, barriers such as intermittent water and power supplies, wards without piped connections, broken taps, and limited water access points for patients and visitors reduced water access in the hospital. Hospitals increased water access through the use of cisterns and bucket taps. Hospitals spent significant funds to increase water access by purchasing water from tanker trucks and buying bottled water. Methods to improve access often resulted in decreased water quality and increased costs. In over 300 samples tested for E. coli, 77% of samples in Honduras and 61% in Ghana met international drinking water standards. Samples from piped taps were 4 times more likely to meet drinking water standards compared to samples from bucket taps (p=0.0256). Despite variable quality, tap water was used for a variety of drinking, hygiene and medical purposes. In Honduras, 24% of staff reported using tap water for drinking versus 5% of staff in Ghana. Tap water is used for reconstituting and giving medications by 23% of clinical staff in Honduras and 14% in Ghana. While 19% of staff in Ghana use tap water for wound care, no staff in Honduras reported using tap water for wound care. A common barrier to the use of safe water is lack of knowledge about the quality of water from various sources within the hospital. In conclusion, despite improved water sources at healthcare facilities, there exist persistent challenges to consistent safe water access and use. Attaining universal and sustained safe water access and use will require assessment of barriers and the development of mitigation strategies.

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EOSINOPHILS DRIVE CELLULAR INNATE IMMUNITY ACTIVATION TO CONTROL HELMINTH LARVAE MIGRATION AND PROMOTE LUNG TISSUE REMODELING BY A TNF-DEPENDENT PATHWAY DURING *ASCARIS* INFECTION

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While eosinophils have been associated with resistance to helminthic infections, the most important findings related to these cells are based on the peripheral eosinophilia observed in nematode-infected individuals and in the host intestinal mucosal response. In contrast, the role of eosinophils during hepatic-tracheal migrations of nematodes larvae (as frequently occurs in many human nematode infections) is not well understood. In this study eosinophils were evaluated during early *Ascaris* larval infections in wild-type (WT) BALB/c mice compared with an eosinophil-deficient mice model (ΔdblGATA). The absence of host eosinophils resulted in: 1) an increase in the number of *Ascaris* larvae migrating through the liver and lung; 2) a parallel reductions in the pulmonary inflammatory response, with reduced inflammatory infiltrated cells in the parenchymal lung tissue and bronchoalveolar lavage fluid; 3) a decrease in the levels of IL-6 and myeloperoxidase (MPO) produced by related-innate immunity cells during the peak of larvae migration; and 4) a decrease in the production of eosinophil-dependent TNF and eosinophil peroxidase (EPO) in the lungs, with impairments in pulmonary tissue remodeling. Taken together, this study suggest that eosinophils have key roles in both controlling the number of tissue-migrating *Ascaris* larvae and promoting associated airway inflammation through activation of host innate immunity pathways.

Simultaneously, eosinophils promote pulmonary tissue remodeling by EPO and TNF-dependent pathways during larval *Ascaris* sp. infections. These findings suggest an innovative hypothesis on the evolution of eosinophils in the mammalian host-parasite relationship.

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HUMANIZED NOD-SCID IL-2RΓNULL (NSG) MICE: RESPONSE TO INFECTION WITH *STRONGYLOIDES STERCORALIS*

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Strongyloides stercoralis is a parasitic nematode that infects humans, non-human primates and dogs. Immunocompetent mice will allow infections to persist for up to two weeks with little development beyond the larval stages. Both innate and adaptive immune responses control the infection in mice, with roles for complement component C3, eosinophils, neutrophils and macrophages in the protective innate immune response. Furthermore, CD4+ T cells, TH2 cytokines and the production of parasite-specific IgM and IgG are central to the adaptive immune response in mice. The goal of this study was to characterize *S. stercoralis* infection in the immunodeficient NOD-scid IL-2Rγnull (NSG) and humanized NSG (HIS) mice. Following exposure to the infective stage larvae of *S. stercoralis*, NSG mice supported larval and adult stages of the parasite. Naïve NSG mice had similar levels of C3 when compared to naïve C57BL/6J mice, which retained its ability to collaborate with C57BL/6J effector cells in killing the parasites *in vitro*. However, NSG mice demonstrated an absence of eosinophils and similar neutrophil numbers when compared to C57BL/6J mice. To determine if HIS mice were also susceptible to *S. stercoralis* infection, HIS mice were generated by engrafting NSG mice with human hematopoietic stem cells. HIS mice were susceptible to the infection, although these mice harbored 40% fewer adult parasites than NSG mice. Analysis of HIS mice following infection revealed the presence of human IgM and IgG demonstrating that HIS mice can establish a parasite-specific humoral response. We conclude from these studies that NSG mice are susceptible to the complete *S. stercoralis* life cycle. This may be explained by an absence of eosinophils or a deficit in the function of other effector cells. HIS mice had reduced parasite levels, which suggests that the human adaptive response is playing a role in the control of the parasite. These studies demonstrate that NSG and HIS mice are useful tools for dissecting the immune response of both mice and humans to *S. stercoralis*.

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EFFECTS OF MATERNAL GEOHELMINTH INFECTIONS ON THE DEVELOPMENT OF ATOPY, ECZEMA AND WHEEZE DURING THE FIRST THREE YEARS OF LIFE: FINDINGS FROM THE ECUAVIDA BIRTH COHORT

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To investigate the effects of maternal geohelminth infections on the development of atopy and allergic disease in early childhood, we analysed data from a birth cohort in a rural District of Esmeraldas Province. Ecuador. A total of 2,404 newborns were recruited in a public hospital serving the District of Quininde and were evaluated at 13, 24, and 36 months of age for eczema and wheeze. Skin prick test (SPT) reactivity to aero and food allergens was measured at 36 months. A single stool sample was collected from the mother in the third trimester of pregnancy and examined for the presence of geohelminth infections using a combination of microscopic methods including Kato-Katz and formol-ether concentration methods. Data was analyzed by multivariate logistic regression. We had complete follow-up data for 2,082 (86.6%) children through to 3 years of age. Geohelminth infections were detected in 46.1% of mothers and were predominantly infections with *Ascaris lumbricoides* (28.0% of mothers) and *Trichuris trichiura* (28.7%). The prevalence of outcomes in children by 3 years was: any episode of eczema (17.5%) and wheeze (26.0%), and SPT (17.2%). Maternal geohelminth infections were associated with an increased risk of eczema by 3 years of age (OR 1.28, 95% CI 1.02-1.61) but with a decreased prevalence of SPT (OR 0.82, 95% CI 0.61-1.00). No association was observed with wheeze (OR 1.01, 95% CI 0.82-1.25). Our data show that maternal geohelminths, in an Ecuadorian population where *Ascaris* and *Trichuris* are the predominant infections, are associated with an increased risk of eczema but with a reduced prevalence of SPT in early childhood.

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SYSTEMIC CYTOKINE PRODUCTION, GEOHELMINTH INFECTION AND NEURODEVELOPMENTAL OUTCOMES IN BANGLADESHI INFANTS

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An estimated one-third of children under 5 in low- and middle-income countries do not reach their full developmental potential. We recently published that systemic inflammation is linked to the neurodevelopment of children from a slum community in Dhaka, Bangladesh. An interesting finding from our study was that elevated levels of the Th2 cytokine IL-4 in 6-month sera were associated with higher cognitive scores. This finding raises the question of what was driving higher levels of IL-4 in children. Here we tested in the same cohort of Bangladeshi children for factors that could explain why some children had elevated levels of IL-4. Since environmental factors such as helminth infections drive a Th2 response in the host, we tested monthly surveillance stools for the first 6 months of life for the presence of intestinal helminths using multiplex PCR assays. We found that nearly 40% of children were infected with at least one intestinal helminth, with *Ascaris lumbricoides* and *Trichuris trichiura* being the most prevalent at 25% and 16%, respectively. *Ascaris lumbricoides* infection was associated with elevated levels of IL-4 in 6-month sera (p=0.02). Additionally, *Trichuris trichiura* infection was associated with higher cognitive, language, and motor scores on the Bayley Scales of Infant and Toddler Development III at 30 months of age (all p<0.05). We are validating our findings on systemic inflammation and neurodevelopment in a second cohort of children in Dhaka, and are testing for the impact of a SNP in the promoter region of IL-4 (C-589T) that has been shown to influence IL-4 production. The results from these additional studies will be presented. In conclusion, IL-4 and *Trichuris trichiura* infection were associated with better developmental test scores. In addition, elevated levels of IL-4 can partly be explained by helminth infection in this cohort of infants from a low-income setting. Elucidating the cause of elevated IL-4 would greatly enhance our ability to modulate levels of systemic IL-4, which may promote healthy cognitive development in at-risk children.

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THE GENOME AND TRANSCRIPTOME OF THE ZOONOTIC HOOKWORM *ANCYLOSTOMA CEYLANICUM* REVEAL INFECTION-SPECIFIC GENE FAMILIES

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Hookworms infect over 500 million people, stunting and impoverishing them. Sequencing hookworm genomes, and finding which genes they express during infection, should help devise new drugs or vaccines against hookworm. Unlike other hookworms, *Ancylostoma ceylanicum* infects both humans and other mammals, providing a laboratory model for hookworm disease. We determined an *A. ceylanicum* genome sequence of 313 Mb, with transcriptomic data throughout infection showing expression of 30,738 genes. ~900 genes were upregulated during early infection *in vivo*, including ASPRs, a cryptic subfamily of Activation-associated Secreted Proteins (ASPs). ASPR genes are also present in the related intestinal parasites *Necator americanus*, *Oesophagostomum dentatum*, and *Heligmosomoides bakeri*, but not the trichostrongylid parasite *Haemonchus contortus*. Genes downregulated during early infection include ion channels and G protein coupled receptors; this downregulation is observed in both parasitic and free-living nematodes. Another novel family of genes are upregulated as larvae develop to the L4 stage and migrate into the intestine; this family has homologs in *N. americanus*, *H. contortus*, and *Angiostrongylus cantonensis*, and its products are predicted to be nonclassically secreted. Still later in infection, as *A. ceylanicum* matures to young adulthood and begins drinking host blood, C-lectin genes are strongly upregulated, some of whose products resemble vertebrate more than nematode lectins. These findings provide new drug and vaccine targets, and should elucidate hookworm pathogenesis.

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DETECTION OF GASTROINTESTINAL PARASITES BY MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR AND ASSOCIATIONS WITH GROWTH DELAY IN EARLY CHILDHOOD: FINDINGS FROM A BIRTH COHORT IN RURAL ECUADOR

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Gastrointestinal (GI) parasites may have important influences on growth and nutrition in childhood. Previous studies investigating the effects of parasite infections on growth have tended to use poorly sensitive microscopic-based assays. To investigate the effects of single and multiple parasite infections on growth in young children we analyzed data from a birth cohort study in Ecuador, correlating GI parasite affects on anthropometric measures. Stool samples from a random sample of 400 children in the cohort were collected at 13, 24, and 36 months of age and analyzed using our rapid, high throughput multi-parallel quantitative real-time PCR (qPCR) for the 8 most common gastrointestinal parasite pathogens including the helminths, *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Trichuris trichiura* and protozoa, *Cryptosporidium parvum, Entamoeba histolytica* and *Giardia lamblia.* Each child had anthropometric data collected at the same time points including height, weight, head and abdominal circumference. The qPCR detected increased prevalence of infections for *Ascaris* at 13, 24, and 36 months (6.8%, 12.9%, and 15.5%, respectively). Similar results were seen for *Giardia* (31.5%, 44.5%, and 51.6%, respectively) and other parasites. Furthermore, children that were infected at a previous time point tended to be infected at subsequent observation times with higher concentrations of parasite DNA for *Ascaris* and *Giardia* (fg/μL, $p < 0.05$) For all parasites, qPCR was more sensitive than standard microscopic methods. GI parasite infections were associated with growth delays for all anthropometric parameters by comparison with WHO growth curves; growth of abdominal circumference was less in the infected group (1.5 cm) compared to the non-infected group (4 cm)($p = 0.0054$). In conclusion, we have deployed a high throughput, rapid, quantitative molecular based system that has improved diagnostic accuracy compared to stool microscopy. Our data also indicate that GI parasite infections may affect growth during the first years of life.

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THE EFFECT OF DEWORMING TIMING AND FREQUENCY ON GROWTH IN EARLY PRESCHOOL-AGE CHILDREN: RESULTS OF A RANDOMIZED-CONTROLLED TRIAL OF MEBENDAZOLE IN ONE TO TWO-YEAR OLD CHILDREN IN THE PERUVIAN AMAZON

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Children under two years of age are in the most critical window for growth and development. As mobility increases, this time also coincides with first exposure to soil-transmitted helminth (STH) infections in tropical environments. WHO recommends deworming as of 12 months in endemic areas; however, the optimal timing and frequency have been understudied in this age group. Many countries still exclude children 12-23 months in deworming programs. We conducted a randomized-controlled trial of deworming (500mg single-dose mebendazole) in 12 and 13 month-old children in Iquitos, an STH-endemic area of the Peruvian Amazon. A total of 1760 children were enrolled from September 2011 to June 2012 at 12 participating health centres. Children were randomly allocated to one of four groups: 1) deworming at 12 months of age and placebo at 18 months of age; 2) placebo at 12 months of age and deworming at 18 months of age; 3) deworming at 12 and 18 months of age; or 4) placebo at 12 and 18 months of age (i.e. control group). Participants were followed up to 24 months of age to assess the benefit of deworming on the main outcome of weight gain. Results were analyzed with an intention-to-treat approach. A total of 1563 children (88.8%) attended their 24 month visit. STH prevalence rose from 12.2% at 12 months to over 40% at 24 months. Mean weight gain (kg) between 12 and 24 months was: Group 1): 2.05 (±0.7); Group 2): 1.94 (±0.8); Group 3): 2.04 (±0.7); and Group 4): 2.00 (±0.7). There was a statistically significant improvement in weight gain in those receiving deworming once at 12 months, compared to those receiving deworming once at 18 months (p=0.028). No difference was detected between those receiving deworming once at 12 months vs. twice at 12 and 18 months (p=0.88). Results remained significant when adjusting for baseline characteristics. Additional analyses were performed to take into account clustering, multiple testing, missing data and compliance. Overall, our results indicate that deworming, provided once-yearly at 12 months of age, has important benefits on growth in early preschool-age children. These results contribute to the evidence-base on deworming policy in over 120 STH-endemic countries worldwide. Emphasis should be placed on translating results into practice, such that children in this vulnerable age group are targeted with the most cost-effective, integrated interventions to reduce health and nutritional burdens.

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RE-EMERGENCE OF DENGUE IN SOUTH TEXAS, 2013

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Sporadic dengue outbreaks have occurred in south Texas for over 30 years. In 2013, during a dengue epidemic in northern Mexico, 17 suspected dengue cases were identified in two border counties in south Texas during July-October. To characterize the outbreak, Texas Department of State Health Services and CDC implemented enhanced surveillance by: 1) reviewing medical records at eight hospitals for dengue-like illness; 2) performing RT-PCR on serum specimens from suspected dengue cases previously tested by anti-dengue virus (DENV) IgM ELISA at commercial laboratories during October-December; and 3) conducting interviews with laboratory-positive dengue case-patients and offering household members dengue diagnostic testing by RT-PCR and IgM ELISA. During 2013, clinicians in south Texas requested dengue diagnostic testing for 246 patients. Of these, 54 (22%) were laboratory-positive: 32 (59%) by IgM ELISA, 15 (28%) by RT-PCR, and 7 (13%) by both. Of 84 specimens that were negative by IgM ELISA at commercial laboratories and further tested by RT-PCR, 15 (18%) were positive. Of 22 cases positive by RT-PCR, DENV-1 was detected in 19 (86%) and DENV-3 was detected in 3 (14%). Of all laboratory-positive dengue case-patients, 26 (48%) had not left Texas in the 14 days before illness onset, and 20 (38%) reported recent travel to Mexico. Of 22 dengue case-patients' households investigated, 5 (23%) had at least one additional household member with evidence of recent DENV infection without travel history. During a dengue outbreak associated with an epidemic in northern Mexico, enhanced surveillance in south Texas identified the largest number of locally acquired dengue cases ever detected. Dengue diagnostic testing should include both IgM ELISA and RT-PCR as evidenced by the high rate of false negatives with anti-DENV IgM testing alone, due to the number of patients who submit specimens during the acute phase of their infection. Since the burden of dengue is expected to continue in south Texas, dengue surveillance and laboratory capacity should continue to be improved.

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LARGE BURDEN OF DENGUE AND WEST NILE VIRUS TRANSMISSION IN COASTAL KENYA

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Dengue virus (DENV) and West Nile virus (WNV) are endemic to most regions of the world, but accurate prevalence data are lacking in Sub-Saharan Africa. The objective of this study was to measure the burden of DENV and WNV exposure in coastal Kenya and link it to demographics and other risk factors. Demographic and exposure questionnaires were administered to 1,013 participants recruited from two coastal villages, Milalani (55% of total) and Nganja (45%), in 2009. Sera were screened for flavivirus exposure using a commercial DENV IgG ELISA and then confirmed with plaque reduction neutralization tests (PRNT). Chi square, Fisher exact test, t tests and logistic models were used to determine variables that were associated with seropositivity. 343 (35%; 95% CI 32-38%) participants were seropositive (aged 1-87 years, mean 37 years). Ten percent (95% CI 8-14%) of children were seropositive vs. 53% (95% CI 49%-58%) of adults. Of 297 PRNT confirmed positives, 203 samples (68% of positives, 20% of total) were DENV, 49 samples (16% of positives, 5% of total) were WNV, and 45 samples (15% of positives, 5% of total) had high PRNT titers for both DENV and WNV. Age was significantly associated with seropositivity (OR 1.07 per year, 95% C.I. 1.06-1.08). Males, adults who owned a radio or television, and those with schistosomiasis, malaria, or *Trichuris* were less likely to be seropositive ($p<0.05$). A greater proportion of DENV- and WNV-confirmed participants resided in Milalani, though the association with Village was not significant. Flavivirus exposure, particularly DENV, is very common in coastal Kenya, with more than half of adults exposed. Adults and females are more likely to be seropositive, whereas those with parasitic infections are less likely. Interepidemic transmission is suggested by many DENV and WNV seropositive children. The high flavivirus burden documented suggests that DENV and WNV are important causes of disease in coastal Kenya, but limited surveillance, clinical overlap with malaria and other viruses, and limited diagnostics contribute to under-reporting.

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VARIABILITY IN DENGUE TITER ESTIMATES FROM PLAQUE REDUCTION NEUTRALIZATION TESTS POSES A CHALLENGE TO EPIDEMIOLOGICAL STUDIES AND VACCINE DEVELOPMENT

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Accurate determination of neutralization antibody titers supports epidemiological studies of dengue virus transmission and vaccine trials. Neutralization titers measured using the plaque reduction neutralization test (PRNT) are believed to provide a key measure of immunity to dengue viruses, however, the assay's variability is poorly understood, making it difficult to interpret the significance of any assay reading. In addition, there is limited standardization of the PRNT cut-point or statistical model used to estimate titers across laboratories, with little understanding of the optimum approach. We used repeated assays on the same two pools of serum using five different viruses (2,319 assays) to characterize the variability in the technique under identical experimental conditions. We also assessed the performance of multiple statistical models to interpolate continuous values of neutralization titer from discrete measurements from serial dilutions and identified the optimal PRNT cut-point for the assay. We found that the variance in plaque reductions for individual dilutions was 0.016, equivalent to a 95% confidence interval of 0.45 - 0.95 for an observed plaque reduction of 0.7. We identified PRNT75 as the optimum cut-point with a variance of 0.025 (log10 scale), indicating a titer reading of 1:500 had 95% confidence intervals of 1:240 - 1:1000 (2.70±0.31 on a log10 scale). The choice of statistical model was not important for the calculation of relative titers, however, cloglog regression out-performed alternatives where absolute titers are of interest. Finally, we estimated that only 0.7% of assays would falsely detect a four-fold difference in titers between acute and convalescent sera where no true difference exists. Estimating and reporting assay uncertainty will aid the interpretation of individual titers. Laboratories should perform a small number of repeat assays to generate their own variability estimates. These could be used to calculate confidence intervals for all reported titers and allow benchmarking of assay performance.

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CROSS-REACTIVITY TO HETEROLOGOUS DENV TYPES INCREASES IN THE YEARS FOLLOWING PRIMARY INFECTION AND IS MAINTAINED FOLLOWING SECOND INFECTION IN A PEDIATRIC DENGUE COHORT STUDY IN NICARAGUA

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The four dengue virus (DENV) serotypes infect an estimated 390 million individuals each year. Following a first DENV infection, the neutralizing antibody response is thought to become increasingly specific to the infecting serotype, and over time, is expected to remain only to the infecting serotype. After secondary infection, neutralization of all serotypes is thought to be relatively balanced and persist over time. However, these hypotheses have not been tested rigorously in a longitudinal cohort. We used reporter viral particles (RVPs) to measure changes in the type-specificity of neutralizing antibody responses to all four DENV serotypes in healthy annual samples from a cohort of Nicaraguan children for 1-6 years after their natural first, second, and, in some cases, third infections. Post-infection neutralizing titers were ranked from highest to lowest in the year after infection, and fold-difference in neutralization between the best-neutralized serotype and each heterologous serotype was measured every year until subsequent infection. As expected, the trajectories of neutralizing responses after infection varied by individual in magnitude and degree of cross-reactivity between serotypes. However, when first-infection neutralizing antibody responses were analyzed as a group, increasing cross-reactivity was observed over time. Specifically, the difference between the best and second-best neutralized serotypes decreased over time, with a mean decline of 1.3-fold/year ($p<0.01$), from an average difference of 8.5-fold in the first year after infection ($p<0.001$). This effect remained significant when serotype, year, and infection outcome were taken into account. Indeed, while the titer to the infecting type did not change significantly over time, the second-best neutralized serotype and third-best neutralized serotype increased by 1.3 fold/year ($p<0.01$) and 1.2 fold/year ($p<0.05$), respectively. When neutralizing antibody responses were analyzed in the same subjects following subsequent second infections, cross-reactivity between serotypes did not change significantly over time, although the magnitude of titers to all four serotypes decreased over time. We find that children living in Nicaragua become more cross-reactive to heterologous DENV serotypes over time following first infection and maintain cross-reactive responses following second infection.

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RELATIVE INCIDENCE OF ADULT AND PEDIATRIC DENGUE VIRUS INFECTION IN A PROSPECTIVE LONGITUDINAL COHORT IN THE PHILIPPINES

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Dengue is the most common mosquito-borne viral infection globally. In Asia where dengue has been hyperendemic for many years, the average age of dengue has been increasing. However, dengue incidence and clinical spectrum have not been as well characterized in adults as in children. In order to determine the incidence of dengue virus (DENV) infection in adults compared to children and the relative proportion of subclinical versus symptomatic infections, a longitudinal prospective cohort of approximately 1000 subjects aged ≥6 months was initiated in Cebu, Philippines in March 2012 and underwent community-based active surveillance for febrile episodes. Acute and 3-week convalescent blood samples were obtained and tested by DENV RT-PCR/nested PCR (acute samples) and DENV IgM/IgG EIA (acute/convalescent samples). Enrollment and 12-month follow up samples were tested by DENV hemagglutination inhibition (HAI) to identify subclinical seroconversion. During one year of surveillance, the annual incidence of total and symptomatic DENV infection in the cohort was 8.5% and 1.5%, respectively. The total and symptomatic incidence in the 6 month-5 year old age group was 11.0% and 2.5%; 6-15 years was 15.3% and 4.4%; 16-30 years was 7.4% and 0.5%; 31-50 years was 4.2% and 0%; >50 years was 4.4% and 0%. DENV-1 was the predominant serotype. The total and symptomatic incidence among 139 subjects with negative DENV HAI at enrollment was 10.1% and 2.9%; among 32 subjects with one positive DENV HAI serotype was 31% and 9.4%; among 682 subjects with ≥2 positive HAI serotypes was 7.3% and 0.7%. Fifty-five percent of subjects ≤15 years old had multitypic HAI whereas 96% of those >15 years old were multitypic. Our results indicate that DENV infection is less frequent and less likely to be symptomatic in adults than children in a hyperendemic area, but much of this effect is due to preceding immune status. DENV infection in adults may become more symptomatic if force of infection decreases due, for example, to future pediatric vaccination programs.

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DENGUE VIRUS-SPECIFIC T CELL RESPONSES IN THE GENERAL POPULATION OF NICARAGUA VARY AS A FUNCTION OF THE INFECTING SEROTYPE AND ARE DOMINATED BY HLAB35-RESTRICTED EPITOPES

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Infections with any of the four dengue virus serotypes (DENV1-4) occur with high incidence in more than 100 countries around the world, accounting for as many as 390 million infections each year. All four DENV serotypes have circulated extensively in Nicaragua in recent years, and as a result, the adult population has generally been exposed to all four serotypes. To assess the T cell response against all four DENV serotypes, we tested predicted motifs in an *ex vivo* ELISPOT assay for their ability to induce an IFNγ response in HLA-matched peripheral blood mononuclear cells (PBMC) of 124 Nicaraguan Red Cross blood donors from the general adult population of Managua, Nicaragua. This proteome-wide screen identified a total of 314 CD8+ T cell epitopes across all 10 DENV proteins (C, M, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Interestingly, we observed different immunodominance patterns of targeted DENV antigens depending on the serotype. DENV3-specific responses equally targeted structural and nonstructural (NS) proteins while DENV1-, DENV2- and DENV4-specific responding epitopes were found to disproportionately (>90%) target NS proteins, especially NS3, NS4B and NS5. We found 30% of the epitopes identified were novel, while 70% were also found in a previous study of Sri Lankan blood donors. Additionally, we observed a striking dominance of HLA B-restricted responses in general and of HLA B*35 in particular, both in terms of breadth as well as magnitude of the DENV-specific CD8+ T cell responses. Interestingly, this allele has been associated with protection from disease in a different population study in Malaysia. We found that the majority of responses were produced by T cells displaying an effector memory phenotype (TEMRA and TEM). In terms of cytokine expression patterns, the majority of cells were double-positive for IFNγ and TNFα, indicating a multifunctional phenotype. Our results provide new insights into HLA-restricted T cell responses against all four DENV serotypes, which are of relevance for both vaccine design and the identification of robust correlates of protection in natural immunity.

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EXPLORING THE CELLULAR METABOLOME AS A GATEWAY TO TARGET DENGUE VIRUS REPLICATION IN THE MOSQUITO VECTOR

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The cellular metabolome plays a significant role in the life cycle of enveloped arthropod-borne viruses. For instance, in both the human host and mosquito vector, flaviviruses significantly modify lipid metabolism to enter and exit from cells, as well as to assemble membrane targeted replication factories for efficient viral RNA replication. Additionally, cellular metabolic changes assist in diverting or evading host antiviral defenses. Using technical advances in high-resolution mass spectrometry we have profiled the metabolome of dengue virus (DENV) infected mosquitoes and analyzed the metabolic changes that occur in the salivary glands and midgut tissues during the time course of infection. These studies were carried out in parallel to analysis of the human metabolome, also during infection with DENV. Our results indicated that DENV infection altered the expression of lipids that had the capacity to change the physical properties of the membrane bilayer such as curvature, permeability, and the recruitment and assembly of protein complexes in the membrane. Several of the identified molecules also functioned as bioactive messengers that controlled signaling and membrane trafficking pathways in the cells. Through these efforts we have generated a metabolomic fingerprint of DENV infection within the human host and its mosquito vector, *Aedes aegypti*. We are now exploring the mechanism of how DENV exploits these metabolic pathways for its replication and are evaluating these pathways as novel avenues for the development of antivirals that could target virus replication in both the human and vector hosts. Through these efforts we have also facilitated data linkage between two NIAID Biological Resource Centers, (the virus pathogen resource (ViPR) and VectorBase (VB) to provide these data to the greater scientific community.

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TPL2-DEPENDENT INDUCTION OF IL-10 IN HUMAN ALTERNATIVELY ACTIVATED MACROPHAGES FOLLOWING MYCOBACTERIAL INFECTION: INSIGHTS INTO THE HELMINTH/MYCOBACTERIAL INTERFACE

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Filarial and other tissue invasive helminth infections are associated with an early IL-4 driven expansion of Th2 cells that in turn drive the expansion of alternatively activated macrophages (AAMφs). In light of the geographic superimposition of tuberculosis and filarial infection we sought to understand how mycobacteria are handled in the context of helminth-induced AAMφ. Using human AAMφs generated *in vitro* from human monocytes by IL-4 and comparing them to LPS and IFN-γ generated classically macrophages (CAMφs) both infected with mycobacteria (BCG), we were first able to demonstrate that AAMφs were more susceptible to infection with BCG than were CAMφs (p=0.02) and this susceptibility was associated with increased IL-10 production, a cytokine known to enhance immune evasion of mycobacteria by impairing macrophage phagolysozome killing and antigen presentation. Not only did mycobacteria increase the production of IL-10 in AAMφs and not in CAMφs; (p=0.017) but we were also able to show that tumor progression locus 2 (TPL2), an upstream activator of extracellular signal related kinases (ERKs) acting through STAT3, itself induces IL-10 production. To explore the relationship between TPL2 and IL-10 in our *in vitro* BCG AAMφ model, we generated both CAMφs and AAMφs, exposed them to BCG at an MOI of 5 and examined TPL2 and its effects. Using qRT-PCR as well as by Western blot, we found increased baseline induction of TPL2 in CAMφs but not AAMφs (p=0.04). Post BCG infection, however, TPL2 levels were increased in AAMφs only (p=0.03). AAMφs (but not CAMφs) showed significantly diminished IL-10 production following the addition of the TPL2 kinase inhibitor ($C_{21}H_{14}ClFN_6$) (IC_{50}= $500x10^{-9}$ M) (p=0.001) BCG infection. These data show that AAMφs (commonly generated in human helminth infection) but not CAMφs activate the positive feedback loop for IL-10 regulation by induction of TPL2, suggesting a mechanism by which IL-10 production is increased in response to mycobacterial infection.

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CREATING AN EVIDENCE-BASED AND CLINICALLY-RELEVANT THRESHOLD FOR TB-ASSOCIATED CATASTROPHIC COSTS: A COHORT STUDY, PERU

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Even when tuberculosis (TB) treatment is free, hidden costs incurred by TB-affected households may worsen poverty and health. Extreme TB-associated costs are termed 'catastrophic' but are poorly defined. We studied TB-affected households' hidden costs and their association with adverse TB outcome to create a clinically-relevant definition of catastrophic costs, against which we compared existing thresholds. From 2002-2009, TB patients (n=876, 11% with multi-drug resistant TB) were recruited to a prospective cohort study in shantytowns in Lima, Peru. Patients were interviewed prior to and every 2-4 weeks throughout treatment recording TB-related costs. Costs were expressed as a proportion of that household's annual income. Adverse TB outcome was defined as: death, abandonment or treatment failure, or TB recurrence. 23% (166/725) of patients had adverse TB outcomes. Total costs ≥20% of household annual income were defined as catastrophic because this threshold was most strongly associated with adverse TB outcome. Catastrophic costs were incurred by 345 households (39%). Adverse TB outcome was independently associated with multi-drug resistant TB (OR=8.4, p<0.001), previous TB (OR=2.1, p=0.005), and catastrophic costs (OR=1.7, p=0.01). Adjusted population attributable fraction of adverse outcomes explained by catastrophic costs was 18% (95%CI=6.9-28), similar to MDR TB (20%, 95%CI=14-25). Sensitivity analyses demonstrated that existing catastrophic costs thresholds (greater or equal to 10% or 15% of household annual income) were not associated with adverse TB outcome in our setting. In conclusion, despite free TB care, having TB disease was expensive for impoverished TB patients in Peru to afford. Incurring higher relative costs was associated with adverse TB outcome. Population attributable fractions implied that MDR TB and catastrophic costs had a similar association with adverse TB outcome. As opposed to existing catastrophic costs thresholds, our novel threshold was found to be clinically-relevant in our setting. Our results show that TB is a socioeconomic as well as infectious problem. Tuberculosis control interventions should address both the economic and clinical aspects of TB and policy makers should consider this new evidence-based and clinically-relevant catastrophic costs definition.

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INCREASED TUBERCULOSIS INFECTION IN MEN IN PERUVIAN SHANTYTOWNS DESPITE A DECADE OF DECREASING TUBERCULOSIS DISEASE

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Changes in tuberculosis (TB) disease incidence rates do not reliably indicate TB control because they are prone to ascertainment bias. In contrast, tuberculin skin test (TST) surveys determine the prevalence of TB infection and allow changes in the community annual risk of infection (ARI) to be estimated objectively. We aimed to analyze changes in ARI from 2000 to 2011 in Pampas de San Juan de Miraflores, a Peruvian shantytown. We conducted 3 tuberculin surveys in different years: in 2000 (n=1056), 2005 (n=103), and lastly in 2011 (n=428). Randomly selected shantytown residents were included and had a TST if not pregnant or never received TB treatment. In 2000 and 2011, participants age ≥5 years were included but in 2005 only those age ≥15 years were available. Participants were stratified into youths (5-14 years) or adults (≥15 years). In 2000, the mean age was 18 (IQR 10-32) years and increased in 2005 and 2011 to 29 (IQR 22-38) and 31 (IQR 15-48) years ($p<0.0001$). To account for age differences we standardized the 2000 ARI rate to the 2011 study age distribution when comparing overall rates. Age-standardized ARI in 2000 was 1.9% (95% CI: 1.8, 2.2), similar to actual rates in 2005 and 2011: 2.4% (95% CI: 1.9, 3.0) and 2.2% (95% CI: 1.9, 2.9). Over time, ARI increased among adult males (2.0% [95% CI: 1.7, 2.4] in 2000; 3.1% [95% CI: 2.0, 4.7] in 2005; 3.0% [95% CI: 2.3, 3.6] in 2011) but was similar for adult females (1.5% [95% CI: 1.3, 1.8] in 2000; 2.0% [95% CI: 1.4, 2.8] in 2005; 2.1% [95% CI: 1.7, 2.6] in 2011). Among youths, there were no differences for males (1.2% [95% CI: 0.8, 1.6] in 2000; 1.4% [95% CI: 0.6, 2.3] in 2011) or females (1.4% [95% CI: 1.0, 1.8] in 2000; 1.8% [95% CI: 0.9, 2.8] in 2011) over time. Thus, despite decreasing rates of diagnosed TB disease in this shantytown, transmission causing TB infection was frequent and, from 2000 to 2011, increased significantly in adult men. Consequently, by 2011, adult males were at significantly greater risk of infection than the rest of the population and should be targeted by TB control interventions.

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HIGH PREVALENCE OF *PNEUMOCYSTIS JIROVECII* INFECTIONS AMONG MOZAMBICAN CHILDREN <5 YEARS OF AGE ADMITTED TO HOSPITAL WITH SUSPECTED PNEUMONIA

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Pneumonia remains the main cause of pediatric mortality in the world. We aimed to assess the specific prevalence of *Pneumocystis jirovecii* (PJ) infections among children <5 years of age admitted to a rural Mozambican hospital with pneumonia, in an area of high HIV prevalence. Methods: As part of an etiology of pediatric pneumonia study, we recruited during 12 months 835 pediatric patients. Collection of standardized clinical data, chest X-rays and screening of nasopharyngeal aspirate (NPA) samples with PCR for 12 respiratory viruses were routinely performed, together with tests for invasive bacterial infection (IBI), malaria, and HIV. Investigations on PJ infection were performed on all NPA samples using a tri-sequential PCR strategy, assessing two multicopy mitochondrial genes (mtLSU y mtSSU) and a third unicopy one, linked to sulfa drug resistance (DHPS). 77/835 (9.2%) of the patients tested positive for at least one of the PJ genes. 32.5% (25/77) patients showed triple (mtLSU, mtSSU and DHPS) gene positivity, while further 41.6% (32/77) showed double (any combination of the three markers) positivity. Twenty (26.0%) further cases tested solely positive for mtLSU. Median age of PCP patients was 3.9 months (IQR 3.1-12.4). Only 30/77 (39.0%) of the confirmed PCP cases had a clinical picture of probable Pneumocystis jiroveci pneumonia (PCP). HIV co-infection was confirmed in 47.8% of the patients with PCP (22/46) for whom HIV results were available. Surprisingly, 16.7% (11/66) of those patients with a valid blood culture result had a concomitant IBI (6 cases of S. pneumoniae, and 5 other bacteria). Viral co-infection was frequent (36/76; 47.4%), being rhinovirus, adenovirus and human metapneumovirus the three commonest viruses found. 5 patients (6.7%) showed also positive *P. falciparum* parasitemias. 15 PCP-infected patients died during admission, yielding a case fatality rate (CFR) of 19.5%, significantly superior to that for non-PCP infections (8.8%; p=0.003). Further 5 PCP patients died at home within the first 21 days post discharge. PCP is a highly prevalent infection among Mozambican infants admitted with severe pneumonia and carries an unacceptably high risk of death, coexisting with other common pediatric infections. The true burden of pediatric PCP in Sub-Saharan Africa needs to be recognized, particularly in the context of the HIV pandemic, and measures to prevent and adequately manage it put in place urgently.

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MYCOBACTERIUM TUBERCULOSIS INFECTION INDUCES PERSISTENT NON-RESOLVING INFLAMMATION

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Infection with *Mycobacterium tuberculosis* (MTB) is accompanied by an intense inflammatory response thought to be directly related to pathogenesis. Currently, we have little understanding of this inflammatory reaction in individuals and whether it resolves in response to curative treatment. To determine the systemic inflammatory status of individuals we compared the peripheral blood inflammatory gene expression profile of 10 patients with active MTB, latent MTB infection, and cured (for more than six months) MTB, to healthy controls by quantitative PCR in India. Patients with active MTB had dramatically different inflammatory profiles as compared to latent MTB. Patients with active MTB demonstrated greater mRNA levels of potent pro-inflammatory IL-1β and neutrophil chemotactic factors CXCL1 and IL-8, while patients with latent MTB infections had higher levels of monocyte/macrophage activation and chemotactic mediators including IP30, CD14, CXCL3, CCL2 and CCL8 suggesting a switch from a neutrophil centered response to a monocyte/macrophage tailored response. Furthermore, several of these key factors including CD14, IP30, CCL2 and CCL8 remained elevated in cured patients. Our results suggest that MTB infection induces long-term persistent inflammation in the human host in the absence of active infection. Chronic inflammation is widely recognized as a potent driver of many diseases and our data suggests that a significant portion of the population, particularly in high-burden settings such as India, may remain at risk for inflammation-mediated complications even after successful treatment for MTB infection.

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LACK OF ASSOCIATION BETWEEN PARASITIC INFECTIONS AND TUBERCULIN SKIN TEST POSITIVITY IN REFUGEES

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Mycobacterium tuberculosis (Mtb) and parasites often co-infect the same person. Immunologic responses to helminth infections may blunt control of mycobacteria, and epidemiologic data suggest an association between helminth infections and tuberculosis disease. Our objective was to determine whether there is an association between parasite infections and positive tuberculin skin tests (TSTs). We reviewed records of patients seen at the Boston Medical Center Refugee Health Assessment Program from 1999-2012. We evaluated demographic characteristics and results of TSTs, stool ova and parasite examinations, complete blood counts with differential, and serologic testing for helminths (done if eosinophilia was found) and looked for an association between TST results and parasite infections. We used multivariate logistic regression models to control for possible confounders (gender, age, WHO region of birth compared to Africa, and protozoal or helminth infection depending on the model). Among 7230 participants, 3843 (53%) were male, mean age was 25 years (range 1-88), and 3355 (46.4%) had positive TSTs. Individuals with positive TSTs were older (mean age 29.9 vs. 21.7 years; p< 0.0001) and more likely to be male (OR = 1.35; 95%CI, 1.23, 1.49). Helminth infections were found in 393 (5.4%) including *Trichuris trichiura* (132/393; 33.6%), *Strongyloides stercoralis* (89; 22.6%), and *Schistosoma* infections (79; 20%). Among 2473 (34%) with protozoal infections, *Blastocystis* spp was found in 1986 (80%). TST positivity was not associated with helminth infections (adjusted OR [aOR] = 1.14; 95%CI, 0.92, 1.41) or protozoal infection (aOR = 1.08; 95%CI, 0.97, 1.20). We found no association between parasitic infections and TST positivity. Unmeasured confounders (HIV infection, poverty, malnutrition, etc.), undetected parasites, pre-departure parasite treatment and other reasons for false negative TSTs may have obscured an effect. More sensitive methods for Mtb detection and a study of the effect of individual parasite species on TSTs are needed to confirm this lack of association.

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CHALLENGES OF DETECTING RESISTANCE TO FIRST AND SECOND LINE ANTI-TUBERCULOSIS DRUGS IN SOUTHWESTERN UGANDA

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There are limited data on the drug susceptibility of tuberculosis (TB) in southwestern Uganda. We assessed the proportion of first- and second-line drug resistance in culture-positive tuberculosis specimens from treatment-naïve suspects in southwestern Uganda to guide regional empiric recommendations on multidrug-resistant tuberculosis (MDR-TB). We collected sputum samples for smear microscopy and culture on mycobacterium growth indicator tubes (MGIT) and Lowenstein Jensen (LJ) media from tuberculosis suspects with no prior TB treatment at Mbarara Regional Referral Hospital from February 2009 to February 2013. We tested archived specimens for isoniazid and rifampicin resistance using the MTBDR*plus* assay and GeneXpert. A subsample of isolates selected randomly for geographic variability was also tested with the MTBDR*sl* assay. The resistant isolates were tested further using sequencing and MGIT. Specimens were collected from 190 TB suspects residing within 23 districts of southwestern Uganda, of whom 69% were male, the median age was 33 years (26-43), and the HIV prevalence was 80/190 (42%). No isolates (0%) were rifampicin-resistant and only 1/190 (0.5%) was isoniazid-resistant (0% overall proportion of MDR-TB). Among 92 isolates tested for second-line drug resistance, 71 (77%) had interpretable results, of which 7/71 (10%), 3/71 (4.2%) and 0 (0%) were resistant to fluoroquinolone using MTBDR*sl,* sequencing, and MGIT respectively. None of the isolates were resistant to aminoglycosides, cyclic peptides, or ethambutol. We found no MDR-TB and no resistance to ethambutol or injectables among treatment naïve TB suspects in southwestern Uganda. However, the discrepancy in the fluoroquinolone resistance results of by the three approved methods makes diagnosis difficult and requires establishment of an optimum global second line testing strategy.

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ASTMH PERU: THINKING GLOBALLY AND ACTING LOCALLY TO DISSEMINATE GLOBAL HEALTH RESEARCH RESULTS AND TRAIN YOUNG SCIENTISTS

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The Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) convenes thousands of scientists from around the world to the United States to exchange advances in global health research. Although Peru has had a major presence at the meeting with over 30 abstracts per year in the last decade, the findings are paradoxically less available to many Peruvian scientists, especially scientists in training, for whom financial and logistical barriers often prevent travel to the United States. Thus, an annual local satellite meeting, ASTMH Peru, was established in Lima, as an avenue for dissemination of scientific information and implementation of research results into health policies. Six local and international established leaders in infectious disease research launched ASTMH Peru in 2011. Only abstracts presented at the previous Annual Meeting are included in ASTMH Peru, no call for new abstracts. In addition to the oral and poster sessions, there are keynote presentations by ASTMH leaders on subjects particularly relevant to Peru, including identifying local funding sources, writing scientific manuscripts and grant proposals, and strategies to implement research findings. Primary topics covered are malaria, cysticercosis, dengue fever, leishmania and diarrhea. ASTMH Peru is 100% funded by Peruvian collaborations with registration fees of $28 for professionals and $18 for students. Remaining funds support partial scholarships for young Peruvian scientists to attend the next U.S, meeting if they have an accepted oral presentation. Between 2011 and 2014, the number of posters increased from 45 to 74; oral presentations from 11 to 17, focusing on malaria, cysticercosis, dengue, leishmania and diarrhea. The number of attendees grew from 205 to 388. An important new segment is the support to informed decision making by local authorities. All sessions were heavily attended. Peruvian members in ASTMH also increased from 68 to 101. Six Peruvian scientists have attended the US meeting supported by ASTMH Peru in the last four years. ASTMH Peru constitutes a timely, low cost, and sustainable mechanism for the exchange of high quality scientific knowledge led by local research leaders bridging the Northern and Southern hemispheres together, with a special focus on the training of young scientists.

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MECHANICAL, AUTOMATIC INTRAVENOUS VOLUME REGULATOR FOR RESOURCE-LIMITED SETTINGS

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We have developed an automatic mechanical volume regulator for IV therapy in the developing world, where 1.4 million children die annually to dehydration caused by diarrhea, malaria, and dengue hemorrhagic fever. These deaths are preventable with IV therapy; however, children who are treated with IV therapy are at risk of overhydration as due to chronic understaffing and high cost of standard volume regulators such as infusion pumps, which require consumables and electricity. Because the risks of overhydration include death, clinicians use oral rehydration for treatment; however, severe cases of dehydration require IV therapy. The IV volume regulator we designed costs less than $80 and does not require electricity. It employs a lever arm with a movable counterweight (similar to a physician's scale) to incrementally dispense IV fluid. A volume indicator slides along the lever arm and allows selection of target volumes in increments of 50 mL. The change in angle of the lever arm as the IV bag drains activates a spring clamp to kink the IV tubing, stopping fluid flow. Performance was assessed in the lab by delivering target volumes of 50 to 850 mL in increments of 50 mL, five flow rates of 20 to 4000 mL/hr, and four initial IV bag volumes between 200 and 1000 mL (n=5 each; n=170 overall). Usability was quantified with a system usability survey (SUS) by 33 nurses, doctors, and medical students at Queen Elizabeth Central Hospital in Blantyre, Malawi. For all parameters, the mean and median residuals were significantly less than 25 mL, and the maximum residual was 30 mL. After training for less than 15 min, Malawian clinicians set up the device within 79.5±31.5 sec. Participants reported a SUS score of 84.4±11.1, which is greater than the 70 threshold for an acceptable product. These promising results will guide the design of a clinical trial evaluating the field accuracy of this device in summer 2014. By enabling clinicians to provide children life-saving IV fluids, this device may potentially prevent over-hydration in resource-limited settings.

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GENOME-WIDE ANALYSIS REVEALED THAT *PLASMODIUM FALCIPARUM*-DRIVEN SELECTIVE FORCES MAY HAVE INDUCED HIGH FREQUENCY OF HLA ALLELES ASSOCIATED WITH PODOCONIOSIS

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Podoconiosis is geochemical elephantiasis of the lower legs among barefoot individuals with long-term exposure to red clay soils. Globally, more than 4 million people are affected. A genome-wide association study conducted by our group in Ethiopia showed that genetic variants in the HLA class II loci confer susceptibility to podoconiosis. Subsequent HLA typing confirmed that among others, HLA-DQB1*02 is a risk allele and HLA-DRB1*13 is a protective allele to podoconiosis. Interestingly, these alleles have been shown to be associated with protection against P.falciparum malaria. It is estimated that three-quarters of the landmass of Ethiopia is malarious predisposing over two-thirds of the population to malaria. In the present study we aimed to investigate possible selective forces that induced high frequency of the HLA alleles associated with susceptibility to podoconiosis. First, we conducted genome-wide analysis of 464,642 single nucleotide polymorphisms (SNPs) comparing ethnic Wolaita Ethiopians (n=120) with 11 population groups from Africa, Europe, and Asia with aim to identify signatures of recent positive selection. We found that HLA loci showing the strongest genome-wide association with podoconiosis were under strong selective pressure in the Ethiopian population, but not in the others. Next, using data from our own cohort and publicly available HLA database, we compared the distribution of DRB1*13 and DQB1*02 alleles in three Ethiopian population groups (Wolaita, Amhara and Oromo) that form 64% of the total population of the country with that of other Sub-Saharan African population groups. We found that the Ethiopian ethnic groups had the highest frequencies of DRB1*13 compared with other populations in Sub-Saharan Africa. Previous studies showed that the DRB1*13-DQB1*05 haplotype was protective against severe malaria in the Gambian population and DRB1*13 was protective against persistent hepatitis B infection. We also found that the Ethiopian population groups had the third highest frequency of DQB1*02 following Burkina Faso's Fulani and Central African Republic's Aka Pygmy. The Fulani closely share the distribution of HLA alleles with the Amhara and Oromo of Ethiopia, and mount stronger humoral immune response against malaria. Together, these data suggest that strong pathogen-driven selective forces induced the high frequency of the risk variants for podoconiosis.

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IMPROVING MALARIA SURVEILLANCE THROUGH USE OF MOBILE TECHNOLOGY IN MAINLAND TANZANIA: FINDINGS FROM A PILOT STUDY

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The Integrated Disease Surveillance and Response (IDSR) system, a paper based system that reports public health surveillance and response data from the health facility level in Tanzania was implemented nationwide in 2002. A mobile phone based electronic system (eIDSR) was created in 2012 to replace the paper based system (paper-IDSR) to increase efficiency and completeness of reporting. A four-week pilot of the eIDSR system took place in Temeke District in Dar es Salaam in November 2013. This study aimed to explore the change in reporting and completeness of surveillance data reporting following the change from the paper-IDSR to the eIDSR system. A total of 67 (64% out of 104 eligible) health facilities participated in the eIDSR pilot following training. For the duration of the pilot, the paper-IDSR and eIDSR system worked concurrently. Data were collected at the district level for the paper-IDSR and through an internet-based database for eIDSR. A data quality assessment was conducted in January 2014 to compare timeliness and quality of data between the two systems. Preliminary findings indicate that 70% of weekly reports were submitted on time through the eIDSR compared to 78% of timely reports via the paper-IDSR system; this is due to a discrepancy in how the paper-IDSR and eIDSR define timeliness (defined by paper-IDSR as Wednesday and eIDSR as Monday 3 pm for the previous week data). Initial analysis indicates that when the same cut off time (Monday) is used for both systems, timeliness in eIDSR is substantially faster than paper-IDSR. All health facilities reported complete data through the eIDSR system while 84% reported complete data through the paper-IDSR system. The paper-IDSR system required dedicated staff to travel from the health facility to the district medical officer to deliver weekly reports. The cost of travel and person hours lost to deliver reports is completely eliminated in the eIDSR system. This

pilot shows that eIDSR improved timeliness of weekly reporting and data completeness. Implementation of eIDSR should be considered in all regions in the Mainland Tanzania to improve surveillance of infectious diseases.

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HYBRID STUDY DESIGN FOR COMPLEX INTERNATIONAL FIELD TRIALS: CONDUCTING OBSERVATIONAL RESEARCH ON A RANDOMIZED CLINICAL TRIAL PLATFORM TO ENSURE HIGHEST QUALITY STANDARDS IN SCIENTIFIC DISCOVERY FOR GLOBAL HEALTH

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Performing interventional Randomized Clinical Trials (RCT) according to strict standards is time consuming, expensive, and often designed to answer a single question about the impact of an intervention. Infrastructure development for this work is extensive to ensure subject protection, preserve trial endpoints, minimize bias, and promote generalizability of results. Most sponsors or funding agencies are increasingly interested in enhancing the value of this time, cost and infrastructure, as well as investing in innovative research around challenging problems in global health. In large and complex field settings, innovation, the rapid integration of new data and exploratory analyses may be impeded by overly rigid research designs, including standard RCT models. A new Hybrid Study Design (HSD) that combines the strengths and ameliorates the weaknesses of RCT and observational study designs may be a more robust model for maximum translational impact. The Hybrid Study Design ensures the rigor of clinical trials to safeguard data quality and subject protection, while allowing for discovery, particularly in rapidly changing fields where new knowledge needs to be tested, confirmed and applied quickly to improve health outcomes. We report on our experience using a HSD in an urban slum setting in Bangladesh to examine the role of Environmental Enteropathy, a poorly characterized disorder of the small intestine, in oral vaccine underperformance through the PROVIDE Study. With a 2x2 factorial design and two vaccine interventions, the PROVIDE study combined the ethical, regulatory, and analytic structure of a RCT with the flexibility required to successfully undertake cutting-edge research in the developing world. The rationale for the HSD will be presented and lessons learned from applying this new model in a birth cohort of 700 infants with two years of follow-up. We propose the HSD as a useful model for research in which an interventional component or non-inferiority question is added to exploratory or descriptive work, particularly in developing world settings.

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INCREASED EQUITY IN MALARIA CONTROL INTERVENTIONS IN MALAWI FROM 2000 TO 2012

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Sustained resources since 2000 have supported considerable gains in malaria control strategies, including insecticide-treated nets (ITNs) and intermittent preventive treatment in pregnancy (IPTp). Malawi has been a pioneer in adopting effective interventions, offering subsidized ITNs nationally by 2003 and encouraging IPTp by 1993. These programs contributed to a substantial increase in household ownership of at least one ITN from 27.4% in 2004 to 55.0% in 2012. Use of ITNs also increased during this time period among the traditional target groups of children under five years of age (2.8% in 2000 to 56.0% in 2012) and pregnant women (2.6% in 2000 to 50.7% in 2012). While utilization of antenatal services is high, IPTp coverage remains low, with only 53.8% of women receiving two or more doses in 2012. To assess equity of these interventions, Lorenz concentration curves and corresponding concentration index (C-Index) values were derived from 2000, 2004, and 2010 Demographic and Health Surveys and a 2012 Malaria Indicator Survey. Values approaching perfect equity across wealth quintiles (C-Index=0) show that equity has improved for all interventions studied. Increasing ITN ownership corresponded to improved equity over time (C-Index=0.29 in 2004 to C-Index=0.06 in 2012). Even larger gains were seen in ITN use by children under five (C-Index=0.47 in 2000 to C-Index=0.03 in 2012) and pregnant women (C-Index=0.45 in 2000 to C-Index=-0.01 in 2012). Equity in IPTp was stronger from an earlier date (C-Index=0.03 in 2000) and remained similar through 2012 (C-Index=0.06), suggesting that antenatal services are accessible and used equally across all wealth quintiles. The differences between ITN ownership and use and IPTp uptake show how equity may differ for various malaria control interventions. In order to achieve greater returns as Malawi moves toward universal coverage of all interventions and malaria transmission decreases, it will be important to acknowledge equity and focus resources on economic groups with outstanding needs.

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EXPLORATORY GEOSPATIAL MODELLING OF ENVIRONMENTAL FACTORS CORRELATED WITH PODOCONIOSIS IN ETHIOPIA

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Ecological studies have linked podoconiosis (endemic, non-filarial elephantiasis) to red clay soils of volcanic origin, but the precise trigger of the disease is unknown. Histopathology investigations have demonstrated phyllosilicates, aluminium, magnesium and iron in the lower limb lymph node macrophages of both patients and non-patients living barefoot on these clays. We studied local spatial variation in disease prevalence and environmental factors with the aim of increasing understanding of disease pathogenesis. Fieldwork was conducted from June 2011 to February 2013 in 12 kebeles (administrative units) in northern Ethiopia. Geo-located prevalence data and soil samples were collected and analysed along with secondary geological, topographic, meteorological and elevation data. Soil data were analysed for chemical composition, mineralogy and particle size; and interpolated using regression kriging. Exploratory, univariate and multivariate regression analysis of podoconiosis prevalence in relation to primary (soil) and secondary (elevation, precipitation and geology) covariates was conducted. Following appropriate transformation to predict soil covariates, exploratory analysis indicated that podoconiosis prevalence was associated with clay minerals (smectite, kaolinite and mica), quartz (crystalline silica), iron oxide, and zirconium. The final multivariate model included smectite (OR = 2.76, 95% CI: 1.35, 5.73; p = 0.007), quartz (OR = 1.16, 95% CI: 1.06, 1.26; p = 0.001) and mica (OR = 1.09, 95% CI: 1.05, 1.13; p < 0.001). The association between podoconiosis prevalence and smectite, quartz and mica content of the soil suggests that further environmental, biomedical and toxicology studies on podoconiosis should focus on these soil characteristics.

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IS EARLY COMPLEMENTARY FOOD INTRODUCTION RELATED TO LOW INFANT WEIGHT-FOR-HEIGHT?

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Early complementary food introduction, by definition, undermines exclusive breastfeeding, as well as increases health risks for infants. A

broad array of liquids and solids are reported as introduced early in the diets of infants in many countries despite the promotion of exclusive breastfeeding for the first six months of life as optimal. Despite this pattern, there has been comparably little investigation into factors that may underlie early complementary feeding. Mothers' interpretation that their children's poor growth is a function of inadequacy of their breast-milk alone may be one factor, particularly in low resource settings of low- and middle-income countries. This study investigated whether more extensive early complementary feeding is related to lower child weight-for-height using data from the 2007 Demographic and Health Survey of the Dominican Republic. Of the 763 children under six-months of age with complete complementary feeding data, only 10.7% were classified as exclusive breast-feeders, although 79.6% were breastfeeding at the time of the survey. Among breast feeders who were non-exclusive, plain water and other milk types were the most common complementary products used. Baby cereal and items from the bread/noodles/grain group were the most common foods consumed. A summation of responses to 22 complementary food/liquid items consumed the day prior to the survey was used to index the extent of complementary food use. Inconsistent with the study hypothesis, this measure was not related to the Z scores of the children's weigh- for-height in either bivariate analysis or a multivariate model controlling for child age. Study findings are limited given the cross-sectional nature of the dataset and lack of variables on maternal perception of thinness, the latter which may provide a better index of maternal concern about child growth than the direct anthropometric measure used in this study. Nevertheless, other variables should be examined to identify key factors driving early complementary food introduction.

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QUALITY OF EMERGENCY NEWBORN CARE IN RURAL BANGLADESH, 2013

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The neonatal mortality rate in Bangladesh is 26 deaths per 1000 live births; 14% of births are preterm. Signal functions, a list of critical interventions, were recently developed for emergency newborn care (EmNC) but their availability at national level has never been assessed in any country. Our objective was to assess the availability of EmNC signal functions at hospitals predominantly serving rural populations of Bangladesh. Directors of hospitals providing inpatient care to 50 villages, selected to be representative of rural Bangladesh, were interviewed about hospital infrastructure, staffing, and provision of four EmNC signal functions within the past 3 months (neonatal resuscitation, administration of antibiotics, administration of corticosteroids and provision of kangaroo mother care [KMC] for preterm births). Hospitals providing all 4 signal functions, with ≥3 staff on call (24-hour coverage), and an ambulance and phone for referring patients, were considered to provide high quality EmNC. Hospitals with ≥3 signal functions, ≥2 staff (or no 24-hour coverage), and a phone but no ambulance were considered to provide moderate quality EmNC. Hospitals with ≥2 signal functions and ≥1 staff and a phone provided low quality EmNC; the rest were considered sub-standard. Directors of 432 hospitals in 46 of 64 districts in Bangladesh took part; 383 hospitals (89%) were located in urban areas. Newborn care was available in 98% of hospitals. The most commonly available signal function was neonatal resuscitation (90%), followed by administration of antibiotics (86%) and corticosteroids (40%), and KMC (only 8% of facilities). KMC was more common in public hospitals (16% vs. 5%, P = 0.005). Quality of EmNC care was high in 11(3%) hospitals, moderate in 30%, low in 45%, and sub-standard in 22%. Inadequate availability of KMC and corticosteroids represent substantial barriers to providing high quality of care for particularly vulnerable preterm neonates. Efforts to motivate delivery at health facilities should be matched by strengthening EmNC at those facilities.

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INTEGRATED SURVEILLANCE FOR DISEASE CONTROL: A NEW ERA IN GLOBAL HEALTH?

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Donors, ministries of health, and other global health partners share the responsibility of ensuring the availability of valid and timely health indicators. There is growing recognition for the critical role these indicators have in the strategic development of effective responses to global health issues. Unfortunately, data fragmentation across diseases, countries, funding sources, and a wide range of clinical and laboratory data sources pose a barrier to timely estimation of accurate health metrics. Such fragmentation frustrates efforts to merge, analyze, and interpret data across multiple geographical scales, and hinders attempts to use these data to develop and share transparent, scalable tools for decision-making. The purpose of this project is to develop and evaluate 'proof-of-concept' tools and technologies to support the integration and use of global health data collected across a range of diverse sources. We intend to demonstrate the value of these tools and technologies for improving decision-making related to malaria control in Uganda and The Gambia. The first phase of our work entails developing a catalog of data sources for malaria control and describing how malaria control programs, funding agencies, and partners analyze and use these data to make programmatic decisions. The second and third parts of the project involve developing and evaluating the technology and software tools that will interact with the data sources to calculate and analyze valuable health metrics. At the completion of our project, which is anticipated to be November 2014, we will have developed an open-access prototype system that will support sharing of comparable data within and across countries. The system will include tools for supporting the effective use of data, and it will provide a mechanism for facilitating convergence towards common data standards to support the control of malaria and other priority diseases.

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MONITORING THE QUALITY OF ANTIMALARIAL DRUGS IN SENEGAL: A STAKEHOLDER PERSPECTIVE

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Poor quality antimalarials may have a deleterious effect on public health in malaria endemic countries. A national drug quality surveillance system may minimise the risk of such drugs appearing in the public and private sector, but little is known about these systems in developing countries. The aim of this study was to explore the perceptions of stakeholders on their institutional roles and responsibilities for assuring the quality of drugs, and strengths and weaknesses of the drug quality surveillance system in Senegal. In-depth qualitative interviews were conducted with 27 key stakeholders including representatives of the surveillance system authorities and treatment providers in the regulated public and private health sectors. Interviews focussed on two aspects of drug quality surveillance: 1) understanding the system context including its background, challenges faced and institutional roles and responsibilities of national authorities, and 2) identifying vulnerable components of the system that, if compromised, may increase the risk of poor quality antimalarials in Senegal. A health systems viewpoint was applied, allowing for inductive expansion of emergent themes in relation to the six building blocks of health systems. Preliminary analysis indicates that all stakeholders perceived the system to operate effectively and they had confidence in

the quality of antimalarials available in Senegal. Nonetheless, coordination amongst the different national authorities involved in assuring and monitoring drug quality, and between authorities and treatment providers, was recognised as a challenge. Differences in perceived quality and efficacy of antimalarials were often assumed to be related to their cost and country of manufacture. Insufficient drug storage conditions and the existence of an informal drug sector were seen as the two main risk factors for poor quality antimalarials in Senegal.Stringent drug regulation and a secure drug supply chain were perceived to contribute to the confidence in the quality of antimalarials available Senegal. However a lack of funding, issues of governance, inadequate human resources and an absence of monitoring of the informal sector (due to concerns that acknowledgment would legitimise its existence) all threaten to impair the progress that has been made by national authorities and external partners in assuring drug quality in Senegal.

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PERCEPTIONS OF HEALTH AND VULNERABILITIES ALONG THE INTER-OCEANIC HIGHWAY IN MADRE DE DIOS, PERU: RESULTS FROM QUALITATIVE RESEARCH

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The Madre de Dios Region in the Peruvian Amazon is a biodiversity hotspot that has been highly affected by human settlement and land-use change since the construction of the inter-oceanic highway (IOH) that crosses the region. We explored societal impacts, quality of life issues, and vulnerabilities associated to the IOH, as part of a larger study focusing on transmission of rodent-borne diseases. Twelve focus group discussions with 83 community members and 21 key informant interviews with local leaders and health personnel were conducted in February 2014 in 8 communities along the IOH. Although people believe the IOH brought positive changes to their communities, they had an overall negative perception of the IOH. They attributed the increase in road accidents, crime and alcohol/drug consumption in recent years to the increase in human migration brought by the IOH. Lack of electricity and clean drinking water were common concerns. Findings suggest migrants tended to settle in the community outskirts, closer to intact rainforest, placing them at higher risk for emerging infectious diseases. For some jobs, people worked for long periods of time in remote locations in the rainforest, with its inherent risks. Several communities mentioned anemia as a significant health problem, but most communities now considered themselves healthy - dengue fever, leishmaniasis, malaria and gastrointestinal parasites were all cited as past problems. People recognized various types of rodents, and some complained about their role as pests, but did not express particular concern about diseases these may transmit. There are distinct differences between the communities north and southwest of the capital city Puerto Maldonado. While the main economic activities in all communities are logging, agriculture and Brazil nut collection, the southwest communities are often surrounded by illegal mining camps. As a result, they have much more support than northern communities from governmental and non-governmental organizations promoting diverse projects to improve the situation. Determining local peoples' perceptions of key issues and vulnerabilities in relation to health and the IOH will enable a greater understanding of how to approach current and future public health problems occurring in this region.

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INNOVATION TO ADDRESS DIAGNOSTIC NEEDS FOR RURAL POPULATIONS: INSIGHTS OF JUNIOR MEDICAL DOCTORS AT THE FRONTLINES OF RURAL CARE IN PERU

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Diagnosis of common diseases can require specific technologies and depends on a skilled workforce, which are common limitations in rural settings. Few studies have assessed health providers' perceived needs for diagnostic tools and barriers they face in diagnosis - information that can guide innovation development and implementation. The aims of this study were to examine, from the perspective of medical doctors (MDs) in rural Peru, the needs and barriers associated with the diagnostic process, and solicit ideas for innovative solutions. Three focus group discussions (12 participants) and 18 individual semi-structured interviews were conducted with recent MD graduates who had completed their medical service in rural areas of the Highlands and Amazon basin in Peru in the last two years. Data were analyzed manually to explore trends in the main themes. The main diagnostic needs for infectious diseases included point of care (POC) tests for: i) the differential diagnosis of malaria vs pneumonia, ii) dengue vs leptospirosis, iii) tuberculosis, iv) vaginal infections and cervical cancer. Ultrasound was a perceived need for obstetric and intra-abdominal conditions. In specific locations, diagnostic tools for neurocysticercosis and for heavy metals toxicity were needed. Barriers impeding the diagnostic process included: distance to and high cost of referral facilities; cultural and linguistic issues; inefficient referral and laboratory systems; and inadequate telecommunication. Innovative ideas proposed by participants included: POC equipment such as a "rural ultrasound" and telemedicine services. Our findings show there is a high demand for improved diagnostic testing in rural communities, so a system based fundamentally on referrals is inadequate: rural doctors need more tools that are technologically and socially viable in context. National strategies supporting the development and implementation of diagnostic innovations are crucial for improving health services. This process should be informed by the perspectives of health providers in the underserved areas.

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AN ESTIMATION OF THE ECONOMIC BURDEN OF TYPHOID FEVER ILLNESS IN LOW AND MIDDLE-INCOME COUNTRIES

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Background Estimation of typhoid cost of illness in low and middle income countries is important in cost-effectiveness analysis and priority setting for typhoid prevention and control activities such as vaccine introduction. In this paper, we present an estimate of total costs and cost per episode of typhoid fever illness in low and middle income countries by United Nations sub-regions. Methods A decision tree model was developed to represent the clinical outcomes of typhoid illness. The percentage of typhoid cases corresponding to each arm were estimated from a literature review. The indirect costs were measured based on a five country typhoid cost of illness study. The costs related to laboratory diagnosis, service delivery, and medicines were assessed based on literature and World Health Organization data base. Direct and indirect costs were estimated in 2010 United States dollars ($), and segregated by outpatient and inpatient status. Findings The estimated total annual typhoid fever cost of illness in low and middle income countries was $519 million (95% CI=300 million to $836 million) of which 46% came from South Asia. The average cost of typhoid illness per episode was $44 (95% CI= $25 to $69), which

comprised 28% direct costs and 78% of indirect costs. Average cost per outpatient was $31(95% CI=$16 to $52) while the cost per inpatient was about $191(95% CI=129 to 276). The predominant cost drivers were indirect costs, number of episodes in the region and hospitalization rates. Interpretation The main challenge was obtaining the probabilities of health events for the decision tree model due to a lack of data. Our cost estimates were conservative, as typhoid relapse, typhoid death, development of long-term carrier state, and gall bladder cancer were not included. South Asia and East Africa should be the priority for typhoid prevention and control activities due to their high economic burden. There is a need for collecting improved and geographical representative epidemiological and cost data for typhoid fever.

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CURRENT EFFORTS OF PHARMACEUTICAL COMPANIES TO ADDRESS THE NEED FOR PEDIATRIC PRODUCT DEVELOPMENT: AN ANALYSIS OF THE R&D PIPELINE

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The majority of medicines have primarily been developed for use by adults. Treating children with these medicines can be problematic. Children of different ages and weights need different dosages and dosing forms (such as oral liquids) to ensure efficacy, safety, and compliance. Certain age groups also metabolise medicines differently, which needs careful evaluation. In 2007, the World Health Organization (WHO) published its first Essential Medicines List for Children (EMLc). It revealed the need for more evidence on the efficacy and safety of many essential medicines for use in children (including systemic reviews and product development). The WHO repeated this call in 2013 in the update of its report 'Priority Medicines for Europe and the World'. To address such unmet needs, pharmaceutical companies can play a key role, as they have deep expertise on formulation and manufacturing, as well as intellectual property rights on adult medicines. To assess their response, we present a unique analysis of the paediatric R&D activities currently being undertaken by 21 research-based pharmaceutical companies with the highest global market capitalization (as measured by the 2014 Access to Medicine Index). The Index measures the extent to which these companies address the issue of access to medicine for 47 high-burden diseases, with significant overlap of the EMLc. As such, this analysis maps the priority paediatric R&D needs that these companies are addressing, and where gaps remain. Preliminary results show that more than 50% of these companies are involved in paediatric R&D for EMLc diseases, including developing adapted formulations and vaccines specifically for children. The vast majority are medicines target communicable diseases, including tuberculosis and malaria. There are also examples of adapted formulations for non-infectious diseases, including diabetes mellitus. Although limited, adapted formulations for neglected tropical diseases and neonatal health indicates progress in these disease areas as well. This study will be completed in September 2014.

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THE PROCESS OF CLINICAL TRIAL IMPLEMENTATION: PARTNERSHIP BETWEEN THE TRIAL RESEARCH TEAM AND LOCAL HEALTH STAFF IN BURKINA FASO, GHANA AND ZAMBIA

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Clinical trials are essential for health research, particularly for the assessment of new health interventions. In order to ensure patients' protection and research quality, these trials follow strict procedures that may not be available for routine practice in poor settings. We researched the partnership between trial teams and local health providers, more specifically whether the technical knowledge gained from the trial is beneficial for local health services in trial settings in Burkina Faso, Zambia and Ghana. We used a quantitative survey and qualitative research methods to collect data from professionals of the Ministries of Health, local health providers and clinical researchers in settings in the respective countries where trials were on-going or had recently ended and compared them to control sites where no trial research had been done. Local health services benefit from the presence of research teams but there is room for improvement. Improved communication with local health staff and their regular involvement in training opportunities provided to the research team, among other factors, could bring long-term improvements to quality of care also in local routine health care, which would be a positive added value of the research in local settings. Clinical trials are beneficial for resource-poor communities not only because of their primary objective of investigating better treatments, but also for the improved quality of care provided during the trial. Nevertheless, local health services could additionally benefit from the trial implementation process, extend its positive impact on routine health care provided to the local populations.

1300

HOSPITALIZATIONS AND DEATHS DUE TO DIARRHEAL AND RESPIRATORY DISEASES AMONG CHILDREN UNDER FIVE YEARS OF AGE-HAITI, 2011-2013

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Diarrheal and respiratory diseases are leading causes of morbidity and mortality among children aged <5 years in developing countries; however, data on the burden of these diseases in Haiti are scarce. We conducted a retrospective review of hospital admission registries for children aged <5 years during January 2011 through December 2013 in six major hospitals in Haiti. We recorded numbers of all-cause, diarrheal and respiratory disease admissions by age group, hospital, and epidemiologic week. Diarrheal diseases included diarrhea, gastroenteritis, dehydration, parasitosis, cholera, amoebiasis, dysentery, shigellosis, giardiasis, food poisoning and hypovolemic shock. Respiratory diseases included bronchitis; bronchioloitis; acute sinusitis, epiglottitis, tracheitis, viral rhinitis,

pharyngitis or respiratory illness; bronchopneumonia, asthma, influenza, influenza-like symptoms, bronchiectasis, pneumonitis, laryngotracheitis, croup, pleural effusion, respiratory failure/distress, empyema, pleurisy, apnea, shortness of breath, tachypnea, wheezing, stridor, cough, diphtheria. A total of 31,565 hospital admissions and 1763 deaths were recorded among children aged < 5 years at the six sites. Diarrheal diseases accounted for 8063 (26%) hospitalizations and 224 (13%) deaths. Diarrheal diseases accounted for 39%, and 36% of hospitalizations in children aged 6-11 months and 12-23 months, respectively. While children aged 0-5 months constituted 25% of all diarrheal disease hospitalizations, diarrheal diseases accounted for only 15% of all hospitalizations in this age group. Diarrheal disease admissions peaked in January-April before the rainy season. Respiratory diseases accounted for 9183 (29%) hospitalizations and 301 (17%) deaths. Children aged 0-23 months accounted for 76% of all respiratory disease admissions. Children aged 0-5 months had the lowest proportion of hospitalization due to respiratory diseases (19%) while children aged 6-23 months had the highest (38%). Respiratory disease hospitalizations followed a bimodal seasonal pattern, with peaks during May-June and October-December. Diarrheal and respiratory diseases constitute a significant health burden among children aged < 5 years in Haiti. Having these data before rotavirus and pneumococcal vaccine introduction will be important to monitor the impact of vaccines and other health interventions.

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MENTORSHIP FOR GLOBAL HEALTH RESEARCHERS

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Mentorship plays a critical role in the development of global health researchers and the strengthening of health research capacity in both high income countries (HIC) and low and middle income countries (LMIC). In 2012 the Canadian Coalition of Global Health Research (CCGHR) brought together colleagues from Canada, Argentina, Chile, Guatemala, Kenya, and the UK/Europe to collate current examples of mentorship practice in global health research (GHR). Using a research story and narrative approach, a set of eleven GHR mentorship research stories were developed on diverse mentorship experiences and programs. Teleconferences and an online project management platform were used to manage global communication, to guide the story development through peer review and reflection, and to promote conjoint analysis. A tabular and curatorial analytic approach highlighted the unique aspects of each of the stories but also provided insights into the challenges, benefits and commonalities that arise in GHR mentorship. The fundamental principles of mentoring were used to develop diverse programs to meet the needs and contexts of the global health researchers for which they were developed. These included mentorship programs for researchers in the field of: global mental health and substance use in Kenya, tobacco control in Argentina, malaria in Sub-Saharan Africa, chronic disease in Guatemala, Chilean led research training in Africa, and Canadian led interdisciplinary programs to create environments for mentorship, bring together mentors and mentees in GHR from HICs and LIMCs in globally hosted summer institutes, and build communities of practice at three Canadian universities. Key outcomes from the project include online access to the mentorship stories in English, French and Spanish, and the creation of an international working group of global health researchers engaged in GHR mentorship within HICs and LIMCs; and who are well situated to contribute to the emergent literature in GHR mentorship.

1302

APPLICATION OF THE UNIFIED THEORY OF ACCEPTANCE AND USE ON COMMUNITY HEALTH VOLUNTEERS' ACCEPTANCE OF MHEALTH

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Studies have been conducted to examine the potential benefits of the use of mobile health (mHealth) tools to improve the work of community health volunteers (CHVs). The use of mHealth is expected to enable CHVs take on more challenging tasks and enable them produce more timely and accurate data for their health sectors. However, little is known about CHVs level of readiness and acceptance to use mHealth. This study therefore aims to determine the factors that influence CHVs acceptance and intention to use mHealth applications to collect and report data during mass drug administration (MDA) for lymphatic filariasis control. All CHVs in two districts in Ghana (approx. 300) will complete questionnaires to determine their readiness to adopt and use mHealth for lymphatic filariasis treatment coverage data reporting. The Unified Theory of Acceptance and Use of Technology (UTAUT) model has been used to determine the probability of acceptance of new technology among specific groups to whom new technology tools are being introduced and who are potentially less inclined to accept them. Though this tool has been used to validate technology acceptance among formal health sector workers, little is known about technology acceptance by CHVs. CHVs acceptance and intention to use of mHealth will be measured by four constructs; performance expectation, effort expectation, social influences and other facilitating conditions. The UTAUT model will provide a means to assess the probability of mHealth acceptance and intention to use technology among CHVs. It will also provide theoretical and practical implications for sustaining health sector adoption of mHealth in a low resource setting. This study will be completed by June 2014.

1303

ENVIRONMENTAL PUBLIC HEALTH IMPACTS OF DUST AND SAND STORMS IN CENTRAL AND SOUTHWESTERN REGIONS OF IRAQ

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Recent severe drought and water shortages in Iraq have led to an increased incidence of devastating dust and sand storms. Drought, degradation of the environment, conflict and climate change are imposing a significant impact on public health with a spatial dimension. Our geospatial approach includes modeling the complex determinants of dust and sand storm development and propagation using remote sensing satellite imagery, geospatial information systems, and geostatistical analysis using modeling techniques with multiscale nested sampling designs. Predictions from this approach are relevant to many environmentally dependent living ecosystems (human, agricultural, water) and can be linked to socio-economic and public health consequences. We account for site specific land ecosystem conditions in Iraq by adapting a geospatial thematic mapping technique that has been successfully used in other types of semi-arid environments at a broad (i.e., "landscape") scale. We use these dynamic geospatial modeling and thematic mapping techniques as a predictive tool to improve the forecasting of dust and sand storms throughout Iraq to better understand the relationship between environment and ecosystem degradation and its impact on public health. A significant outcome of this research is geared towards the analysis of dust and sand dynamics and their effects on sustainable land use decision

making that is important for environmental public health. These geospatial models can inform communities, regional land managers, government policymakers, other constituents and diverse stakeholders regarding the potential impacts of increased dust and sand storms on public health in Iraq.

1304

PRELIMINARY RESULTS OF SENTINEL SURVEILLANCE OF UNDIFFERENTIATED FEBRILE ILLNESSES IN GEORGIA IN 2013

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This surveillance project seeks to determine the burden of infectious agents of undifferentiated febrile illnesses (UFI) and hemorrhagic fever syndrome (HFS). From June to December of 2013, patients≥4 years of age with a temperature of ≥38°C for ≥48 hours or HFS were enrolled. In addition to blood culture, serologic testing (ELISA) was conducted to detect antibodies against *Leptospira* spp., *Brucella* spp., *Coxiellaburnetii*, CCHF virus, hantavirus, Spotted Fever Group (SFGR), Scrub Typhus group (STG), and Typhus group (TG) *Rickettsiae.* Hantavirus ELISA results were confirmed by IgM/IgG IFA.There were 245 patients enrolled in the study; 30 (12%) returned for the voluntary follow-up visit. Blood culture was positive for only 7 (2.8%). Fourteen (5.7%) patients tested positive by both IgM and IgG against *Brucella* spp. and 29 (11.8%) demonstrated only IgG positivity. *Brucella melitensis* was isolated from one patient. Additionally, *Leptospira* spp. IgM, SFG IgG and *C.burnetii* IgM was positive in 23 (9%), 9 (3.6%) and 7 (2.8%) patients, respectively. Of patients positive for hantavirus, 17 (6.9%) were positive for IgM and 7 (2.8%) were positive for IgG using ELISA. Six of the IgM and 4 of the IgG hantavirus positive samples have been retested using IgM/IgG IFA and were negative. Three (1.2%) patients demonstrated both IgM/IgG and 8 (3%) only IgG positivity against CCHF virus, but none of them had a recent or present history of HFS.These initial results suggest that brucellosis is one of the leading causes of the UFI in Georgia. Additionally our findings suggest that leptospirosis, rickettsiosis and Q-fever are diseases requiring a high index of suspicion by physicians and improved laboratory capacity for correct diagnosis and treatment to take place. Initial ELISA findings on hantavirus and CCHF virus suggest that a more specific test is needed. Surveillance will continue until 2016 to improve the detection and treatment of selected diseases with an emphasis on developing capacity for diagnosis and laboratory confirmation.

1305

A PRELIMINARY ANALYSIS OF THE QUALITY OF PEDIATRIC MEDICINES SUPPLIED BY PRIVATE WHOLESALERS IN KINSHASA, DRC

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The global pharmaceutical market is characterised by multiple qualitative standards. Low and middle-income countries are particularly permeable to poor quality products: the proportion of substandard medicines in sub-Saharan Africa ranges from 12% to 48%, though accurate figures are not available, especially for paediatric medicines. In the Democratic Republic of Congo, one of the prime objective of the national Health Development Plan 2011-2015 is the reduction of infant mortality and a transversal objective is to ensure that 80% of the medicines available is of good quality. In 2013, the introduction of Minilabs® revealed the presence of substandard products but the actual prevalence of poor-quality medicines in the country is unknown. In the context of a North-South bilateral cooperation program, a cross-sectional survey on the quality of products available in the private market in Kinshasa was carried out with the national medicine regulatory authority (DPM). Paediatric formulations of amoxicillin, artemether/lumefantrine and paracetamol were selected as tracers of medicine quality, based on 8 public health criteria and on the results of informal interviews. Covert shoppers purchased a defined quantity of packs of each brand available in all the licensed wholesalers of the city. To obtain a representative subsample of the most marketed products, the inspectors of the DPM collected the yearly distribution figures from the wholesalers. From all the purchased samples, a weighted subset of 100 for each molecule was randomly selected for analysis. The DPM performed the visual inspection on all the purchased products while the subsample was sent in Belgium and tested according to the United States Pharmacopoeia (USP) analyses. The Medicine Quality Assessment Reporting Guideline was followed for reporting and the information arising from visual inspection was used for identifying lacks in the current legislation. Between 7th and 16th April 2014, 417 samples were collected: 86 paracetamol tablets, 143 amoxicillin and 188 artemether/lumefantrine, both powders for suspension. The visual inspection will be performed in May and pharmacopoeial analyses in August 2014. The overall results are expected by October 2014 and will be presented.

1306

GLOBAL IMMUNIZATION POLICY FORMATION FOR NEW VACCINES

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Stakeholder involvement in the immunization policymaking process is complex and occurs at many different levels. Similarly, the process from vaccine development to implementation and use in an immunization program has many different phases and is typically very lengthy. To ensure that vaccines are having the maximum impact, there is need for vaccine developers to incorporate public health use considerations into these early phases. Implementing vaccine policies can often prove challenging for many countries, yet vaccine developers often overlook these policy challenges In this review paper, international immunization policy is understood to be the immunization policy set by the World Health Organization (WHO) for the purpose of informing regional and national immunization practices and regional immunization policy is the immunization policy set by the six WHO regional offices. To understand

the immunization policymaking process, a review of available documents outlining the various immunization policymaking sub-processes and WHO committees involved in the immunization policymaking process was conducted in concert with a review of available articles on the details of Strategic Advisory Group of Experts (SAGE) and SAGE's working groups, as well as other advisory bodies of the WHO that contribute to the fulfillment of SAGE's mission. Recommendations for immunization policy are made, beginning with at WHO and continuing through the WHO Regional Offices and their respective Immunization Technical Advisory Groups (ITAG), with most finishing with the National Immunization Technical Advisory Groups (NITAG), although others have state or municipal level immunization advisory groups that make even more specific recommendations. Though immunization requirements and laws can only be made at the national, state, or municipal level, the WHO and its Regional Offices play a major role in formulating and influencing national immunization policies. While there is uniformity in the process across national and regional borders, there are stark differences in the actual practice of formulating and/or adopting immunization policy across municipalities, or city or town governments, and countries. This paper attempts to clearly delineate the policymaking process for immunization recommendations from beginning to end, in addition to bringing simplicity and clarity to the intricate process for the average stakeholder.

1307

THE EFFECT OF MASS AZITHROMYCIN DISTRIBUTIONS ON CHILDHOOD MORTALITY: BELIEFS AND ESTIMATES OF EFFICACY

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A single cluster-randomized trial conducted in Ethiopia found that mass distribution of azithromycin reduced childhood mortality by 50% in the first year (relative rate, 0.50; 95% confidence interval, 0.29 - 0.86). The magnitude of the observed effect was surprising given that other effective population-level interventions have resulted in more modest benefits. To further investigate, the relative risk of childhood mortality in communities given mass azithromycin distributions was estimated using two different methods: an expert survey and a Bayesian analysis of the cluster-randomized trial. Experts in public health, infectious disease, and demography were asked to estimate the true effect of oral azithromycin distributions on childhood mortality. Separately, an empirical Bayesian estimation of the efficacy was performed. This estimation was determined given the randomized trial's results and prior estimates based on the efficacy of effective non-antibiotic population-level interventions, including vitamin A supplementation and chemoprophylaxis for malaria. The surveyed experts believed mass azithromycin lowers childhood mortality (relative risk, 0.83; 95% credible interval, 0.70 - 1.00). The relative risk from the Bayesian analysis was 0.71 (95% credible interval, 0.39 - 0.93). Both expert opinion and the Bayesian analysis suggest that azithromycin may have a true mortality benefit, but that the most likely effect is smaller than that found in the single available randomized controlled trial. Survey respondents may have used prior information about other beneficial population-level interventions to inform their opinions about the efficacy of mass azithromycin. A large multi-site randomized controlled trial will be necessary to confirm a mortality benefit from mass azithromycin treatments and assess the magnitude of any such benefit.

1308

RECOMMENDATIONS FOR VALID CONSENT FOR RESEARCH WITH ADOLESCENTS IN LOW-INCOME SETTINGS

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Paediatric research is particularly relevant in the tropics where paediatric disease is such an important cause of morbidity and mortality. The current model for consent – where children provide assent (defined as "active agreement") for medical research and their parents must also consent – is not always appropriate, especially in low-income settings. We argue that assuming the research carries minimal risks and meets international ethical guidelines, more emphasis should be placed on the child's wishes. We propose that the default position should be that children who are able to provide valid consent should consent for themselves regardless of age. Many older children (adolescents) in low-income settings have adult responsibilities, may be parents themselves or may be estranged or living independently and not have parents or guardians to look after their interests. The requirements for a valid adolescent's consent should be the same as for adults: (1) the adolescent must be competent, and have the ability to reasonably understand and retain the information, weigh the options and make a decision; (2) the adolescent must be appropriately informed, meaning that the information must be presented in understandable language and illustrated by meaningful examples, and address concerns that are important to adolescents such as stigma and missing school; and (3) the consent must be voluntary and not coerced, taking into consideration that adolescents can easily take to praise and rewards, and may be afraid of adults and those in authority. Apart from these usual requirements, we propose two additional elements: (1) the adolescent must be genuinely mature, meaning he or she has had the life experiences necessary to make such decisions, is able to understand difficult concepts like research, altruism, participant responsibilities and the impact of participation on his or herself and others; and (2) the adolescent should be sufficiently independent: having his or her own accommodation, the ability to travel to attend follow-up visits, and a job or being a parent themselves.

1309

STATISTICAL UNCERTAINTY IMPOSES INHERENT LIMITS ON THE EFFICACY OF TARGETED DISEASE CONTROL

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Contributions of different individuals, groups, or geographic areas to the transmission of infectious diseases are often highly heterogeneous. One of the primary motivations for understanding transmission heterogeneity is the possibility of targeting control measures, such as vaccines, drugs, or insecticides, on individuals, on certain groups, or in areas that make greater contributions to transmission than others. Any effort to target controls must, however, be performed on the basis of some set of measurable factors presumed to be predictive of potential contributions to transmission. The success of efforts to target controls is determined then by the predictive capacity of the factors on which targeting is performed. In light of inherent limits on the capacity of any measurable factor to predict transmission potential, I extend a general and well-known mathematical framework to account for this type of uncertainty. For a given degree of transmission heterogeneity (e.g., 20% of individuals account for 80% of transmission, or the "20/80 rule"), I show how the proportion of variation in transmission potential explained by a set of predictive factors (i.e., R^2) determines the relative benefit from targeting in terms of reducing 1) the critical vaccination proportion, 2) the invasion probability of an emerging pathogen, and 3) the expected size of an outbreak. For the extent of transmission heterogeneity displayed in several well-studied disease outbreaks, I show that significant enhancement of the effectiveness of controls from targeting requires having factors to guide

targeting that explain a substantial proportion of variation in transmission potential. To conclude, I highlight diseases for which factors that could be used to guide targeting appear to be informative, as well as diseases for which predictive factors are unlikely to be found or for which the potential of such factors is not well known. These results highlight the importance of studying factors that underlie transmission heterogeneity and rigorously assessing their predictive capacity.

1310

REAPPRAISING END-OF-LIFE CARE IN THAILAND: A REVIEW OF POLICY AND PRACTICE COMPARED TO THE USA

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In Thailand it is not standard practice to ask patients and family members about code status (do not resuscitate, do not intubate or comfort-measures only) on admission to hospital or to allocate health care proxies. Many Thai patients have not considered these issues prior to the occurrence of critical illness. There are around 3 physicians per 10,000 people in Thailand compared to 24 in the USA, and few work in primary care. The cultural differences are not insurmountable and the US approach to terminal illness may be of benefit to patients in Thailand. A literature review was performed to compare and contrast current practice regarding cardiopulmonary resuscitation and end-of-life care in Thailand with that in the USA highlighting differences in knowledge, attitudes and cultural contexts. We propose that in Thailand early discussion of code status and appointment of a health care proxy should be adopted in hospitals to limit potential unnecessary discomfort and help provide appropriate care for patients with poor prognosis. This will require changes in health policy and training of healthcare providers and education of patients.

1311

MICRONUTRIENT SUPPLEMENTATION DURING PREGNANCY AND ANEMIA IN THE POST-PARTUM PERIOD AMONG WOMEN IN BOLIVIA'S ANDEAN PLATEAU

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Anemia contributes substantially to global morbidity in children and in women of reproductive age and can negatively influence maternal and neonatal outcomes if present during pregnancy. Micronutrient deficiencies (e.g., iron) underlie a substantial portion of the burden of anemia. Thus there is a need to quantify the prevalence of anemia as well as attitudes regarding and acceptability of micronutrient interventions among mothers and pregnant women. In Bolivia, the national universal health plan includes micronutrient supplementation for pregnant and post-partum women (pills containing iron, folic acid, and vitamin C) and for children age 6-24 months (multiple micronutrient powders). Our study assessed anemia status, access to and use of micronutrient supplements, and perceptions regarding the acceptability of supplementation among a predominantly indigenous population of mothers in El Alto, Bolivia, located in the Andean Plateau. Mothers (n=381) of one-month old infants recruited at well-child visits at two hospitals from May 2013 to March 2014 completed interviews on socio-demographic characteristics and prior use of micronutrient supplements. Researchers also performed Hemocue on venous samples and adjusted hemoglobin cutoff values for anemia according to altitude. Promisingly, 89% of mothers reported receiving iron pills during pregnancy, 76% reported taking iron, and only 24% were found to be anemic. However, more than a third of the women who took iron pills reported difficulty in taking these supplements. Similarly, less than a third of women reported having given their age-eligible children multiple micronutrient powder sachets, and only 47% of these women believed that other women would want to use them during pregnancy. These results suggest that coverage of multiple micronutrient supplementation in children lags behind that of iron supplementation of pregnant women. Furthermore, efforts to improve the desirability of these supplements may be necessary in order to maximize adherence among those who receive them.

1312

PREVALENCE OF EARLY-ONSET NEONATAL INFECTION AMONG NEWBORNS OF MOTHERS WITH BACTERIAL INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Although neonatal infections cause a significant proportion of deaths in the first week of life, little is known about the burden of neonatal disease originating from maternal infection or colonization globally. We estimate the prevalence of vertical transmission - the burden of neonatal infection among newborns exposed to maternal infection.We searched Pubmed, Embase, Scopus, Web of Science, Cochrane Library, and WHO Regional Databases for studies of maternal infection, vertical transmission, and neonatal infection. Studies that measured prevalence or incidence of bacterial vertical transmission were included. 122 studies met the inclusion criteria. Random effects meta-analyses were used to pool data to calculate prevalence estimates of vertical transmission. The prevalence of early onset neonatal lab-confirmed infection among newborns of mothers with lab-confirmed infection was 17.2% (95%CI 6.5-27.9). The prevalence of neonatal lab-confirmed infection among newborns of colonized mothers was 1.1% (95%CI 0.2-2.0). The prevalence of neonatal surface colonization among newborns of colonized mothers ranged from 30.9-45.5%. The prevalence of neonatal lab-confirmed infection among newborns of mothers with risk factors ranged from 2.9-19.2%. Only seven studies (5.7%) were from high neonatal mortality settings. Considerable heterogeneity existed between studies given the various definitions of infection, colonization, and risk factors of infection. The prevalence of early-onset neonatal infection is high among newborns of mothers with infection or risk factors for infection. More high quality studies are needed particularly in high neonatal mortality settings to accurately estimate the prevalence of early-onset infection among newborns at risk.

1313

TRAINING LAYPERSONS BASIC TRAUMA TECHNIQUES IN LOW-INCOME COUNTRIES

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Trauma accounts for over 300 million years of healthy life and 11% of the disability-adjusted life years (DALYs) worldwide. Reduction of DALYs and mortality are linked to adequate prehospital care and decreased transport times to definitive care. Given the financial and resource constraints in low-income countries, simple but systematic prehospital training programs for laypersons have been implemented in rural villages to stabilize patients. Most prehospital deaths are the result of airway compromise, respiratory failure or uncontrolled hemorrhage; all three of these conditions can be addressed by laypersons using basic first aid measures. The hypothesis is that basic prehospital and primary hospital interventions made by layperson first responders and healthcare personnel will decrease mortality and increase the number of capable first responders. In order to test this hypothesis, communities with hospitals that advertise surgical capacity in Mozambique were assessed. Six hospitals and communities served as the intervention group that receives training on four basic resuscitative

and stabilizing efforts in their native language in the Zambesia province of Mozambique. Community members received a four-hour seminar that taught four basic resuscitative and stabilizing interventions prior to transport by ambulance or taxi/bus. These techniques include a modified ABCD (airway, breathing, circulation, disability) noted in developed nations. A is for airway opening that allows victims to receive oxygen by simply opening their mouths and removing any foreign objects if present. B is for bleeding – participants learned how to apply compression or a tourniquet. C represents cervical spine immobilization with simple tools. D is for disability which is reduced by transporting victims with a flat, immobile, safe method. Hospital personnel received the same ABCD training as the community with two additions – vital sign monitoring and IV fluid resuscitation as they are markers of shock and injury. Pre- and post- tests were administered to participants in their native language. Results of the study suggest community members can be trained in basic resuscitative techniques. In conclusion, while laypersons and hospital personnel may receive and feel comfortable administering basic resuscitation techniques, further data must be collected to see if this intervention improves mortality.

1314

PAPER TEST CARDS FOR SCREENING PHARMACEUTICAL QUALITY IN THE FIELD

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This presentation will describe a "lab on paper" that can characterize low quality pharmaceutical dosage forms and detect active pharmaceutical ingredients in falsified "herbal" medicines. The test card carries out a dozen color tests in parallel in under 5 minutes, producing a characteristic "fingerprint" of colors in the readout area. Pharmaceutical products which contain little or no active ingredient or which include substitute ingredients give different fingerprints from authentic products, as do "herbal" medicines that are actually spiked with pharmaceutical ingredients. The test cards can be read by eye or with an image analysis program. They are portable, easy to use, and testing of dosage forms can be done in minutes on the corner of a desk. In blinded lab validation studies, the sensitivity and selectivity values for detection of very low quality antibiotics, antimalarials, and tuberculosis medicines are measured as more than 90%. In this presentation, correlations between paper test card results by naive and expert readers and between test card results and HPLC analysis of field samples will be presented. Samples include authentic and falsified drugs as well as "herbal" medications provided by collaborators in Kenya, the US Food and Drug Administration, and the Israeli Ministry of Health Division of Inspection and Enforcement. I will also discuss a new test card for quantitative analysis of beta lactam antibiotics; this card could be used to detect substandard or degraded medications even if there is no giveaway signal from an unapproved excipient. The role of inexpensive screening tests as the top of a "funnel" for monitoring very low quality drugs globally will be discussed.

1315

A COHORT STUDY ON BREASTFEEDING AND EARLY INFANT FEEDING PRACTICES IN THE FIRST SIX MONTHS OF LIFE IN FORTALEZA, CEARÁ, BRAZIL

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Nutritional transition occurs in Brazil, and contrasting trends such as the co-existence of obesity/overweight and anemia are found in our population. The determinants of these trends in children may be associated with early and poor complementary feeding in the first months of life. This study aimed to describe breastfeeding, feeding practices and nutritional status in early childhood in a community from northeast Brazil. A cohort of the 6 first months of life of 242 children was conducted from November, 2010 to February, 2013. Exclusive breastfeeding was received by 64.5% of the children in the first month of life and only 4.8% in the 6th month of life. Complementary feeding was early offered to children: 9.5% received grain derived foods, 15.3% were feed with infant formulas and 13.1% with other milks in the first month of life. We observed increases in z scores for weight-for-age, length-for-age and weight-for-length during the follow up. The prevalence of high weight-for-length and high weight-for-age was 18.9% and 14.9% in the 6th month of life. Nevertheless, at 7 months of age, 42.1% of children had hemoglobin levels under 11mg/dL. The reduction in exclusive breastfeeding during the 6 months of study was associated with the prevalence of high weight-for-length (Chi-squared, $p < 0,0001$). Data is consistent with the nutritional transition phenomena occurring in Brazil and shows the need for public policy focusing on overweight and healthy feeding practices.

1316

ROSIE THE SPRAY TEAM LEADER, EXPANDING OPPORTUNITIES FOR WOMEN IN MALARIA PREVENTION

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The President's Malaria Initiative (PMI) currently conducts indoor residual spraying (IRS) in 12 countries in Africa. Local country teams of 15-20 full time staff members in each country organize the spray campaign and hire hundreds or even thousands of seasonal employees to serve in roles ranging from spray operators to storekeepers and supervisors, all of which are essential to a successful spray campaign. Traditionally, women have been under represented in the IRS workforce and have generally been employed in lower level positions, such as washers. Four gender assessments were conducted in Ethiopia, Rwanda, Ghana, and Senegal in 2013 in order to better understand the number and type of positions that women held in IRS campaigns, identify gender-related challenges and constraints, and suggest areas for improvement. The assessments included focus group discussions, interviews, and an analysis of spray operations. The results indicate that women experience cultural, structural, and social challenges when joining the IRS workforce. Such challenges include women's lack of self-determination in regards to their participation in the spray campaign and perceptions that women are not physically fit enough to be spray operators. PMI has increased women's participation in IRS by specifically targeting them for recruitment through meetings held at the community level and adapting information, education, and communication materials to incentivize women to join the IRS workforce. As a result of these assessments and efforts to increase the number of women employed, and to ensure that they are employed in higher level positions such as spray operators and storekeepers, women currently hold 25% of IRS positions on average. Out of the thousands of workers trained for the 2012 and 2013 Rwanda spray campaigns, 26% and 31% (respectively) were women. This percentage increased to 50% of participation for women in the most recent 2014 spray campaign. This presentation will detail how IRS programs are working toward the proven and achievable goal of equal gender participation in other African countries who conduct IRS.

1317

EXPANDING HEALTH MINISTRY CAPACITY TO DELIVER MALARIA AND OTHER HEALTH COMMODITIES AT THE COMMUNITY LEVEL IN NIGERIAN STATES

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The highly participative process of community directed interventions (CDI) was first pioneered in 1996 by the African Program for Onchocerciasis Control for the delivery of ivermectin. CDI was further tested and found effective in delivering other health commodities. In 2007 Jhpiego began a proof of concept project in Akwa Ibom State, Nigeria and learned that CDI could be a useful vehicle for increasing access to and coverage of malaria in pregnancy interventions. Building on this success, Jhpiego expanded this work to include integrated community case management of malaria, diarrhoea and pneumonia. through community led efforts. The World Bank Malaria Booster Program, observing Jhpiego's efforts in Akwa Ibom State, asked the Nigeria National Malaria Control Program to enlist Jhpiego's help in building the capacity of seven State Ministries of Health (MOH) to organize CDI for what was termed the malaria plus package consisting of community case management and health promotion activities. The scale-up process started with workshops for state CDI implementation teams consisting of staff from malaria control and primary health care in the MOHs. Then these state teams developed their own intervention packages and organized workshops for local government teams, who in turn trained staff from their front line health facilities. These facility staff mobilized communities in their facility catchment areas (wards) to select volunteers for training on the CDI process and intervention package. Although technical assistance was provided to each state, challenges arose including commodity supplies and coordination among different program units within the state MOHs. In conclusion, state teams can train local government teams, ultimately cascading CDI to the community in order to scale up maternal and child health interventions.

1318

INFRASTRUCTURE INDEPENDENT POINT-OF-CARE MOLECULAR DIAGNOSTICS FOR LOW-RESOURCE SETTINGS

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In low-resource settings (LRS), limited access to centralized medical facilities presents a critical barrier to timely diagnosis, treatment, and related control and elimination of infectious diseases. Inadequate diagnostic laboratory infrastructure results in increased costs, lost test results, delays, and loss to follow-up associated with specimen transportation to health centers and subsequent response. At the same time, the most accurate diagnostic tests with low limits of detection (LODs) and high clinical sensitivity and specificity are only available through laboratory-based testing and more recently through portable nucleic acid amplification tests (NAATs). Indeed, NAATs are becoming increasingly important to identify and prevent transmission from asymptomatic infections (e.g. malaria) using active infection detection interventions in eliminating countries. NAATs are similarly important for early infection detection scenarios such as early infant or acute case detection (e.g. HIV). Unfortunately, many NAAT approaches are still tied to laboratory-style equipment and instrumentation such as heat blocks, centrifuges, optical detectors, and refrigerated cold-chain logistics, and therefore have limited reach. Additional hurdles must be overcome when considering specimen acquisition and lysis, and nucleic acid extraction and purification sufficient for subsequent amplification and detection in a NAAT. Isothermal amplification NAATs seem to address some low-resource requirements by obviating the need for thermal cycling and improved enzyme tolerance to inhibitors, reducing sample purification requirements. To date, appropriate, portable, rapid, infrastructure-independent, highly accurate NAATs that meet the WHO ASSURED guidelines remain elusive. In this presentation, we will address important considerations for the utilization of molecular diagnostics in LRS and present recent advancements from PATH's product development partnerships toward increasing access to sample-to-results NAATs in remote communities with limited resources, electricity, and infrastructure.

1319

SHARE YOUR FINDINGS: A GUIDE FOR SCIENTISTS AND MEDIA PROFESSIONALS TO GENERATE PUBLIC INTEREST IN RESEARCH

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Global health research aims to address the inequalities in health and improve the lives of populations at risk. But are research outcomes being effectively communicated to those who can put them into practice? An increasing number of funders see the value in allocating resources (budget and staff time) to the dissemination of research and results. Media professionals - from press and communications officers at research institutions to broadcast, print or online journalists - can make an important contribution in bridging the gap between academia and communities affected by global health issues. But many scientists still find themselves feeling frustrated about their work being simplified when it is communicated to wider audiences. On the other hand, the open access movement and the social media revolution are paving the way for scientific knowledge to be broadly publicised. This presentation will share lessons learned by representatives involved in the collaborative process of pitching and disseminating research, with the aim to increase collaboration and best practice. These include communications professionals from research institutions and a research funding organisation, an editorial member of a major peer-reviewed scientific journal, a journalist who has reported on and from an endemic country and a global health scientist with experience in translating research findings into policy.

1320

DE NOVO MICROSATELLITE MARKER MINING FROM SCARCE AMOUNTS OF *CULICOIDES* GENOMIC DNA: PATHWAY TO UNDERSTANDING DISPERSAL AND POPULATION OF THE VECTOR OF OROPOUCHE VIRUS

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Oropouche virus is a member of the family Bunyaviridae and the cause of an important arboviral disease in South America. Since its first isolation in 1955, the virus has caused more than 30 epidemics and half a million infections. *Culicoides paraensis* is the major vector of Oropouche virus in urban epidemics of the disease. Dispersal and isolation levels of the different vector populations are key factors for spread of the pathogen and the potential vector control tools. Populations genetic studies of *C. paraensis* will be facilitated by improved genomic resources of the vector. Microsatellites are among the most informative and frequently used genetic markers. Their novel isolation from non-colonizable organisms and with limited quantity of genomic DNA (such as *Culicoides* vectors) can be a major challenge. Identifying effective means of increasing the amount of DNA for de novo microsatellite isolation from *Culicoides* spp. will facilitate study of their genetic variability and adaptation. *C. brevitarsis* is a known vector for bluetongue virus in Australia. Its genomic size overlaps that of *C. paraensis*. DNA from two pools of 15 female *C. brevitarsis* was amplified using the multiple displacement amplification technique. This was subsequently sequenced on ¼ picotitre plate of 454 GS FLX Roche sequencer. A total of 120 005 reads was obtained,

2594 putative microsatellite repeats were isolated from the raw reads using Msatcommander 8.0.2 program. 528 primers were designed to the flanking regions of the microsatellite repeats using primer 3 software. A fraction of these primer pairs were selected for validation. Eight of the primer pairs that amplified 100% of the populations have successfully been used to genotype 96 individuals from two populations of *C. brevitarsis*. This study has been able to overcome huge technical constraint due to the very tiny size of this vector and has developed technical workflow easily translatable to *C. paraensis*, an important vector of Oropouche virus.

1321

COMMUNITY BASED INDOOR RESIDUAL SPRAYING THE TOOL FOR REDUCING COST AND COMPLEXITY OF IRS: A PILOT STUDY IN TANZANIA

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Tanzania mainland has implemented indoor residual spraying (IRS) using different operational designs, starting with highly centralized (2007-2009) and medium decentralized (2010-2013). These two approaches were perceived to be complex to manage and expensive. We report a pilot of community based IRS (CBIRS) which is less complex, relatively cheaper and more community owned. CRIRS was organized and implemented at the village level, including: recruitment of spray operators by village governments; use of bicycles by spray operators for transportation; consent by village government to implement IRS; and construction of effluent waste disposal structures using local materials. To evaluate CBIRS pilot, focus group discussion were undertaken with Regional and District IRS technical teams, site managers, sub-site supervisors and Team Leaders, Village mobilizers and Site based mobilizers, Spray operators and community leaders. The evaluation also reviewed IRS implementation guide, IRS performance report, IEC meeting minutes, supervisors report and undertook inspection of constructed sub-sites for compliance to environmental requirements. The evaluation suggests that objectives of CBIRS were attained. CBIRS reduced implementation cost; increased community participation and ownership; reduced organizational complexity of IRS; achieved acceptable quality and quantity of IRS; and maintained compliance to environmental protection requirements. The evaluation revealed aspects that need improvement: training of team leaders was inadequate to cover their important roles in CBIRS; village mobilizer and sub-site supervisor were redundant; effluent disposal sites were unnecessarily large compared to small number of spray teams in CBIRS; and installation of two soak-pits was unnecessary as one pit can accommodate the small amounts of effluent waste generated by a small team.Community based IRS is an ideal approach to reduce cost and complexity of implementing IRS in Tanzania. Some modifications need be considered which include; omitting unnecessary roles like village mobilizer and sub-site supervisor; simplify fabrication of effluent waste disposal structures; and increasing the level of team leaders' training.

1322

NOVEL DETECTION OF *CARDINIUM* ENDOSYMBIONT IN *CULICOIDES* SPECIES

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Culicoides are blood-sucking midges identified as one of the most significant genera in the family Ceratopogonidae, due to the ability to transmit a diverse range of pathogens. *Culicoides* serve as vectors of medically significant viruses, such Oropouche virus, and transmit a range of nematodes species including *Mansonella perstans* which causes perstans filariasis in Africa and South America. *Culicoides* also transmit a range of viral and filarial pathogens of veterinary importance. Global occurrence, capability of rapid and widespread dispersal and lack of effective control options makes *Culicoides* a major risk factor for the introduction and spread of these pathogens. In recent years, the characterization and use of endosymbiotic bacteria for the prevention of mosquito-transmission of pathogens has proved to have a high success rate in the laboratory. The most predominant example of this being the transinfection of *Wolbachia* into the dengue mosquito vector *Aedes aegypti* and the subsequent blocking of the mosquito's ability to transmit dengue virus. *Wolbachia* and *Cardinium* are both naturally occurring bacterial endosymbionts which infect *Culicoides*. There is currently a lack of information on the distribution and occurrence of these bacterial endosymbionts within these insects and their effects, if any, on pathogen transmission. This study has profiled the distribution of *Culicoides* species in south-eastern Australia and developed a range of screening assays to detect low level infections and explore the distribution of these endosymbionts. We have identified *Cardinium* infection in a range of *Culicoides* species including some of the most significant vector species. Sequence analysis has revealed that this is the same *Cardinium* species which is infecting multiple *Culicoides* species from a range of geographical locations including Japan, Israel, Madagascar, Australia and Africa. Experiments are currently underway to determine the potential influence that *Cardinium* infection may have on the host *Culicoides*. The identification and profiling of the endosymbiont *Cardinium,* could provide the first step towards endosymbiont-based control of these significant vectors of both medically and veterinary important pathogens.

1323

GLUTAMATE-GATED ION RECEPTORS IN THE TSETSE FLY *GLOSSINA MORSITANS MORSITANS*

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Insect glutamate-gated receptors include ionotropic receptors (IRs) that mediate detection of volatiles, ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) which mediate impulse transmission across synapses. The IRs, are expressed in antennal coeloconic sensillae neurons, while iGluRs and mGluRs are expressed on post-synaptic membranes in central nervous system. In order to identify the *Glossina morsitans morsitans* IRs, iGluRs and mGluRs, known homologs from *Drosophila melanogaster* and ab initio approach based on glutamate-gated channel specific domains were used to search *Glossina* genome assembly and transcriptome Yale strain GMOY1.1. Phylogenetic relationships among *Glossina* IRs, iGluRs and mGluRs and their drosophila and anopheles homologs were determined using Maximum Likelihood estimates, and numerical relationships with selected diverse species gleaned from Phylome database. Relative expression levels among *Glossina* IRs, iGluRs and mGluRs were established using RNA-seq data of adult female fly. Overall, 40 putative glutamate-gated receptor loci comprising 19, 15 and six IRs, iGluRs and mGluRs respectively were recovered in *Glossina*. The *Glossina* iGluRs and mGluRs had higher sequence conservation than IRs relative to drosophila homologs. The *Glossina* IRs lacked at least one glutamate interacting residues except IR8a and IR25a, which showed high sequence similarity to iGluRs. Relative to D. melanogaster, annotation of *Glossina* revealed lower numbers of IRs, but certain loci had multiple related copies. The iGluRs numbers were similar, while mGluR-like loci were more. Suggestively, *Glossina* over-invests specific IR gene lineages for odor detection, but broadens odor-space discrimination in CNS. There was no glutamate-gated homolog of IR93a recoverable in *Glossina*. Apparently, there were three species-specific divergent IRs, perhaps relating to *Glossina*'s stable host range, thus reducing the range of odors to sample unlike other diptera. Because glutamate-gated receptors mediate rapid neuronal communications, they could be perfect targets for manipulation towards improving tsetse control tools.

1324

TARGETING EDUCATIONAL CAMPAIGNS FOR PREVENTION OF VECTOR-BORNE DISEASE: AN ASSESSMENT OF RURAL VS. URBAN SETTINGS IN THAILAND

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Vector-borne diseases, such as malaria and dengue fever, are transmitted to humans by mosquitoes. Thailand, an endemic country for both of these diseases, serves as a platform to characterize the relationship between household vector control practices and individual health-seeking behavior. Such studies can guide educational campaigns for the awareness and prevention of disease as this relationship may vary between rural and urban settings. The overall goal of this study was to assess differences between knowledge, attitude, and practice (KAP) in persons presenting to health clinics with malaria and/or dengue fever manifestations in two distinct study sites in Thailand for the purposes of identifying key variables at the individual and household level that influence health behavior related to the prevention of vector-borne disease. Specific methodologies included a survey questionnaire performed at healthcare facilities followed by household mosquito collections and house structure characterization. Analyses will include whether or not the presence of mosquitoes, perception of exposure to mosquitoes and/or current acceptance and uptake of mosquito control practices at the household level differs between rural and urban study sites. Field activities will be completed July 2014 to be presented to the Ministry of Health of Thailand to serve as a guide for enhanced targeting of educational campaigns for the prevention of vector-borne diseases.

1325

EMERGING RESEARCH ON DIPTERA AS MECHANICAL VECTORS: THE CASE OF *BACILLUS ANTHRACIS*

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Flies (Order Diptera) are well known for their role as mechanical vectors for enteric pathogens. Recently, however, researchers have found that certain flies, e.g., the Bluebottle Blow Fly (*Calliphora vicina*), the common house fly (*Musca domestica*), and the stable fly (*Stomoxys caltitrans*) are efficient mechanical vectors for *Bacillus anthracis*. Moreover, investigators have concluded that flies helped to trigger *B. anthracis* outbreaks that spanned not only neighboring districts but also international borders. This presentation will examine not only recent developments regarding the role of flies in anthrax outbreaks but also recommend possible prevention strategies.

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SCIENTISTS, PUBLICS AND TRANSGENICS: INFORMATION, TRUST, COMMUNICATION AND ENGAGEMENT ON RESEARCH DEALING WITH VECTOR-BORNE DISEASES

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Infectious diseases transmitted by mosquitoes represent a burden for a variety of countries and especially for the Global South. However research aiming at better understanding them is mainly conducted by institutions from the Global North. Apart from bringing knowledge in biology, this research is obviously associated with the development of methods aiming at reducing the burden of vector-borne diseases and this includes the creation, the use and the release of transgenic mosquitoes. For many in the scientific world, this technological approach offers a promising method against diseases such as malaria or dengue. However the recent field releases of transgenic mosquitoes in The Cayman Islands, in Malaysia and in Brazil have been the source of intense debate in the specialized press as well as in the non-specialized mass media. This lack of transparency, not to say the secrecy, in the way the first trial was conducted is without much doubt the major reason for the controversy that emerged. Brushing aside years of discussion in the scientific world and a shared recognition of the importance to consider ethical, legal and social issues this first trial could be read as a fait-accompli: the cage of transgenic mosquitoes has now been opened. In the complex interactions between science and society around GM technology we cannot avoid questions around the perception of the public by scientists and the related question: How to consult, involve and engage a variety of publics in an effective manner on science and technology? With the will to better estimate the impact of geographic differences (endemic vs non endemic countries), of research topics (work on transgenic approach or not) and of perception of research (applied/ fundamental) we have conducted in 2012/ 2013 a worldwide web-based survey on more than 1800 scientists working on vector-borne diseases. This work reveals several interesting points including the reluctance in involving the public upstream, some lack of confidence in private business as well some level of distrust towards biotechnological progress and the speed at which changes occur because of science and technology. Surprisingly it also highlights a real lack of communication even inside the scientific community.

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GENETIC DIVERSITY AND POPULATION STRUCTURE IN THE *LEISHMANIA* VECTOR *LUTZOMYIA (NYSSOMYIA) ANDUZEI* (DIPTERA: PSYCHODIDAE) FROM THE BRAZILIAN AMAZON

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Lutzomyia anduzei has large geographic distribution in northern South America. This species has been implicated as a secondary vector of *Leishmania guyanensis*, the etiological agent of cutaneous leishmaniasis, in the Brazilian Amazon. In despite of possible involvement of *L. anduzei* in the leishmaniasis transmission, its biology and ecology are poorly known and none population genetics study was performed with this species. We sequenced 74 specimens of six *L. anduzei* localities from the Brazilian Amazon by analyzing 1201 base-pairs of the *COI* gene (mtDNA) to assess genetic diversity and population structure. The genetic diversity was fairly high with 58 haplotypes. Although none haplotype was shared among the localities, all haplotypes were connected in the network. The genetic diversity intra-population was fairly high for all samples (h = 0.859 to 1.000; π = 0.00601 to 0.01008). Values of pair-wise F_{ST} had a large range from 0.042 to 0.413, which were statistically significant (P<0.0001) for the most of comparisons. Similarly, the hierarchical analysis was highly significant among samples (F_{ST} = 0.166; P<0.0001), and the sequence divergence ranged from 0.75 to 1.30%. These results suggest that populations of *L. anduzei* consist of very high genetic variability; however, the gene flow was reduced among populations analyzed that resulted in moderate to large genetic structure. These findings may be implication in the transmission of *Leishmania* and in the control efforts across its range.

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DOG SEROLOGY FOR CUTANEOUS LEISHMANIASIS IS ASSOCIATED WITH SAND FLY VECTOR ABUNDANCE AND SUGGESTS ENDEMIC TRANSMISSION IN RURAL PANAMÁ

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American Cutaneous Leishmaniasis (ACL), beyond a neglected tropical disease, is a zoonosis, where several vertebrates can be infected by *Leishmania* spp parasites. The body of evidence supporting a reservoir role for dogs (*Canis familiaris*) remains contradictory, and it is unclear whether dogs have become major reservoirs in eco-epidemiological settings that have undergone major ecological transformations. Between April and June of 2010, we studied canine ACL in 52 dogs belonging to 24 households in Trinidad de Las Minas, Western Panamá. We collected information on potential ecological (domestic animal abundance, wildlife animal species diversity among others, vegetation, peridomiciliary structure and housing quality), entomological (sand fly abundance) and epidemiological (human infections) risk factors at the household level, as well as, blood samples and information on the health status of each individual dog. Blood samples were employed for *L.* spp/ *L. panamensis* PCR, ELISA and IFAT diagnostics. Bayesian evaluation of the serodiagnostics in absence of a gold standard, showed ELISA to be the most sensitive (0.79) and specific (0.84) diagnostic. ELISA based canine ACL seroprevalence was 47%. At the household level we found *Lutzomyia trapidoi* was the main risk factor for ELISA seropositive reactions (ESR) in dogs, increasing the odds ratio (OR) 2.28 by each sand fly caught inside the households/trap-night (SFA). At the individual level the OR of dog ESR increased 3.39 and 1.35 times by each SFA and year of age, respectively. Finally, the age specific ELISA based canine ACL seroprevalence curve allowed the estimation of a basic reproductive number (R_0 ± S.E.) of 1.22 ± 0.09 which indicates that canine ACL is endemically established in dogs at our study site. Our data suggest: i) that dogs are likely an incidental *Leishmania panamensis* host where LCA is endemically established and ii) that R_0 estimates from serological surveys should be interpreted cautiously, since they are only a robust indicator for endemic establishment in a focal population.

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SLEEPING HABITS AFFECT ACCESS TO HOST BY CHAGAS DISEASE VECTOR *TRIATOMA DIMIDIATA*

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In the Yucatan peninsula (Mexico), the causative agent of Chagas disease *Trypanosma cruzi* is transmitted by the bug *Triatoma dimidiata*. While *T. dimidiata* invades and colonizes houses in other regions, this species has an intrusive behavior in Yucatan, probably attracted by artificial light and potential hosts, but has limited ability to establish colonies. Bugs collected inside the homes also have a low nutritional status, suggesting that they cannot efficiently feed inside these houses. We hypothesized here that this low feeding status of *T. dimidiata* may be associated with the local practice in Mayan communities to sleep in hammocks instead of beds, as this sleeping habit could be an obstacle for triatomines to easily reach their host, particularly for nymphal instars which are unable to fly. To test this hypothesis, we used an experimental chamber of 100 cm x 50 cm x 50 cm in which we placed a miniature bed in one side and a miniature hammock on the other side. After placing a mouse enclosed in a small cage in the bed and another one in the hammock, *T. dimidiata* specimens were released in the chamber and their activity was video recorded (7 pm-7 am). Our results show that bugs were similarly attracted to both mice in the bed and in the hammock. However, they were able to reach the mouse located in the bed significantly more frequently than that located in the hammock. Adults reached the bed most frequently by walking, while they reached the hammock most frequently by flying. Interestingly, nymphs were also able, in few occasions, to reach the mouse in hammock by walking. Our conclusion is that sleeping in hammocks as in rural Yucatan makes the host less accessible to triatomines and may explain, at least in part, the low nutritional status and limited colonization of houses by *T. dimidiata* in the region.

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ONCHOCERCIASIS TRANSMISSION IN GHANA: EFFECT OF VECTOR SPECIES ON HOST-SEEKING BEHAVIOR AND ONGOING TRANSMISSION

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The World Health Organization has set goals for the control and elimination of human onchocerciasis by 2020 in selected African countries. The feasibility of achieving this depends on the initial level of onchocerciasis endemicity in the communities, the levels of geographical and therapeutic coverage and treatment compliance, and the patterns (intensity and seasonality) of transmission, including the species composition of the simuliid vectors and host-seeking behaviour. Ghana is renowned for its sibling species diversity of the *Simulium damnosum* complex, vectors of *Onchocerca volvulus*. Ghana was originally a country within the umbrella of the Onchocerciasis Control Programme in West Africa (OCP), initially a vector control programme, which operated between 1974 and 2002. We present the spatial and temporal patterns in transmission of *Onchocerca* spp larvae of host-seeking and ovipositing adult parous female flies in communities in Southern Ghana located inside and outside the prior OCP that have been treated with ivermectin for different durations. To date, results include monthly biting rates (MBR) ranging from 714 bites/person/month at Agborle Kame (100% *S. damnosum* s.str./*S. sirbanum* in the savannah region) to 8,586 bites/person/month at Pillar 83/Djodji (98.5% *S. squamosum* in the forest mosaic). MBRs were higher in the wet season. In contrast, parous rates were higher in the dry season (41.8% vs. 18.4%), resulting in higher monthly parous biting rates in the dry season. Monthly infectious biting rates ranged from zero to 79.4 infectious bites/person/month. Monthly transmission potentials ranged from zero to 794.3 infective larvae/person/month. Results will be presented in relation to density of vector and host species and the on-going transmission of *O. volvulus* having been used to parameterise EpiOncho models on the effect of vector species on transmission. Our results show that host choice varies between cytospecies, and may be affected by vector and/or host density with epidemiological relevance for vector-borne disease models.

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CIRCULATING ANTIBODY ISOTYPES IN SCABIETIC PATIENT SERA DIRECTED AT MITE ANTIGENS

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Scabies, caused by the mite *Sarcoptes scabiei*, is a worldwide neglected disease, particularly in limited-resource settings. The mite has developed resistance to the topical acaricides commonly used to treat this disease. In its early stages, scabies is difficult to diagnose. A focus of our research is to identify molecules that potentially could be used in developing a

diagnostic test for scabies and in a vaccine for its prevention in highly susceptible populations. A confounding problem is that scabies mites are the source of many antigens that cross-react with antigens of the ubiquitous allergy-causing house dust mites *Dermatophagoides farinae, D. pteronyssinus* and *Euroglyphus maynei.* We used an isotype-specific ELISA to screen serum collected from > 100 ordinary scabies patients against extracts of *S. scabiei*, *D. farinae, D. pteronyssinus* and *E. maynei.* At the time of diagnosis, most of these ordinary scabies patients exhibited circulating antibody to scabies antigens with IgG being the predominant isotype. Most patients also had circulating antibodies that bound to antigens from the *Dermatophagoides* mites. The most striking observation was that high scabies antibody titers were paralleled by high levels of antibody that recognized antigens from *E. maynei.* This was especially clear in the case of IgM, the first isotype produced in response to a foreign antigen. The results of this study further demonstrate that the cross-reactivity among mite antigens must be considered as diagnostic tests and vaccines for scabies are developed.

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ACCURATE SPECIES IDENTIFICATION AND PHYLOGENETIC RELATIONSHIPS REVEALED BY DNA BARCODING OF PERUVIAN SAND FLIES

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Phlebotomine sand flies (Diptera: Psychodidae) are the putative vectors of leishmaniasis worldwide. A reliable species identification of these minute insects constitutes the first step in the surveillance and control of leishmaniasis in endemic areas. Identifying sand fly species based on morphological characteristics is difficult and often complicated by phenotypic plasticity and cryptic species complex as well as demanding considerable taxonomic expertise. The use of DNA barcodes has been proposed recently as a tool for identification of the species in many diverse groups of animal. We assessed the utility of DNA barcoding, based on cytochrome c oxidase subunit I (COI) sequences, for identifying sand fly species from areas where leishmaniasis is endemic in Peru. A total of 89 sand fly specimens belonging to 16 morphological species and 2 genera - Lutzomyia and Warileya, including the major disease vectors were analyzed. We were able to recover and align the target COI fragment from all sand fly species we examined. Phylogenetic analysis of the sequences indicates that the observed species groupings were in confirmation with the morphological identification. The results obtained shows that the barcoding gene was useful in species discrimination in sand flies from Peru.

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DEVELOPMENT OF A NOVEL ASSAY TO MEASURE FLIGHT CAPACITY OF *ANOPHELES GAMBIAE* S.L.

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Anophelines are important vector species in sub-Saharan Africa and contribute to the continued transmission and burden of malaria worldwide. The dry-season ecology of anophelines, specifically in the arid Sahel conditions, remains unknown, but two hypotheses have been proposed to explain the repopulation phenomenon after the dry season: aestivation and migration. To investigate the migration hypothesis, we developed an activity meter to measure flight by sound accounting for environmental conditions. We found that intensity of sound can predict flight density at frequency of 400-800 Hz; however, this was only achievable at temperatures greater than 63°C in the G3 colony. A second stimulant used to induce flight was patchouli; but, due to background noise in the lab, we could not detect change in intensity by cage density although relative observed flight did increase with cage density. Further work can expand this activity meter to a flight to exhaustion assay which is currently under development. These methods may be field-adaptable, allowing us to study if is it possible that mosquitoes repopulate by migration.

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ADULT *AEDES AEGYPTI* SURVEILLANCE USING THE BG SENTINEL TRAP IN PHNOM PENH, CAMBODIA

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Adult mosquito surveillance was conducted in Phnom Penh, Cambodia using BG sentinel traps (Biogents AG) from October 2012 through October 2013. Traps were set indoors in 18 volunteers' houses around Phnom Penh (two collection sites for every district). Traps were set for a 72 hour collection period per month and mosquitoes were collected every 24 hours from the traps. Habitat variables at each collection site such as premise condition index, presence of paddy fields in the surrounding area, mosquito control effort, and house density were measured. Mosquito specimens were transferred to laboratory and identified to species level. The relationship between weather variables (rainfall, near surface temperature, and specific humidity) and *Aedes aegypti* abundance was measured to determine weather's influence on mosquito population. In total 15,536 mosquitoes, representing 20 species in 9 genera were collected. The predominant species were *Culex quinquefasciatus* (76.57%), *Ae. aegypti* (12.93%), and *Anopheles vagus* (7.03%). *Cx. quinquefasciatuis* the primary vector for *Wuchereria bancrofti* and *Ae. aegypti* for dengue and Chikungunya viruses. Weekly accumulated rainfall (mm) was positively correlated with *Ae. aegypti* abundance at three weeks time lag (P=0.004) while monthly near surface air temperature (°C) was positively correlated at one month time lag (P<0.001). However, no positive correlation was found between specific humidity and *Ae. aegypti* abundance. Monte Carlo permutation test showed that mosquito population was significantly correlated to the presence of paddy fields in the surrounding area (P=0.001), mosquito control effort (P=0.004), and house density (P=0.048). Canonical Correspondence Analysis (CCA) showed that the presence of *Ae. aegypti* was postively associated with house density, and negatively associated with paddy field presence and mosquito control effort at collection sites.

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MISUSE AND ABUSE OF ANTIMICROBIALS: COULD WE BE SUPPORTING MALARIA PARASITE DEVELOPMENT IN THE MOSQUITO HOST?

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Naturally-occurring bacteria inhabiting the guts of mosquito vectors are important determinants of vector competence; some species can effectively kill ingested parasites, thus reducing disease transmission. Treating mosquitoes with antibiotics/antimicrobials clears the bacteria in the gut allowing for enhanced development and transmission of parasites.

We hypothesize therefore, that the overuse of antibiotics/antimicrobials among human populations may inadvertently impact on the efforts to control malaria transmission. Experiments that have shown the effect of bacteria clearance of *Plasmodium* have used high concentrations of antibiotics/antimicrobials which may not reflect levels that circulate in the human serum. This study seeks to investigate the effect of human serum concentrations of commonly administered antibiotics/antimicrobials on the gut microbiota of *Anopheles gambiae* s.l, and the consequential effect on *Plasmodium falciparum* development. Preliminary results have been obtained from the initial phase of this project which involves determining the core gut microbiome of *Anopheles gambiae* s.l. sampled from Accra, Ghana. DNA from guts of 66 female adults reared from a field collection of larvae and pupae were analyzed using 454-pyrosequencing. Using the Mothur and QIIME software, preliminary results showed the gut microbial community were predominantly *Gammaproteobacteria* (98.5%); *Enterobacter* (24.8%), *Klebsiella* (21.7%), *Serratia* (39.2%) and *Stenotrophomonas* (2.1%) species. This differs from what has been reported in *Anopheles gambiae* from Kenya, which comprised of mainly *Thorsellia* (67.6%) and *Propionibacterium* (9.08%). Further studies will investigate the effects of varying levels of commonly administered antimicrobials on the *An. gambiae* gut microbiota and the consequential effects on *P. falciparum* development. The results from this study are expected to inform on possible negative effect of the unbridled use of antimicrobials on the control of malaria.

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YEAST SYMBIONTS IN ARTHROPOD VECTORS: POSSIBLE IMPLICATIONS FOR THE CONTROL OF VECTOR-BORNE DISEASES

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The increased awareness for the environmental and the public health problems caused by the use of chemical compounds to combat vector-borne diseases (VBDs) is leading to the development of alternative control strategies. Biological control of arthropod vectors and VBDs is generally based on the use of bacteria, such as *Bacillus thuringiensis*. Although bacterial symbioses in arthropod vectors are the focus of several research programs aimed at developing strategies to control VBDs, such as malaria, dengue, and trypanosomiasis, arthropod-associated yeasts have not yet been deeply investigated. Here we present the first results of a long-term project, aimed at developing strategies for the control of VBDs, exploiting yeasts associated with arthropod vectors. The first disease vectors discovered to harbor yeast symbionts are mosquitoes, from the genera *Anopheles* and *Aedes*. Yeasts isolated from these mosquitoes have been identified as *Wickerhamomyces anomalus*, a typical killer yeast characterized by a wide-spectrum antimicrobial activity, including the production of killer toxins (KTs). Several *W. anomalus* are already used as biopreservation agents in the control of post-harvest diseases of vegetables. The antimicrobial activity of the *W. anomalus* isolated from mosquitoes has been tested *in vitro* against sensitive microbes, showing that these mosquito-associated yeasts actually release an effective antimicrobial KT. Further to mosquitoes, we are currently screening different arthropod vectors such as ticks and send flies for the presence of killer yeasts. Other experiments are aimed at determining whether killer yeasts and their toxins modulate arthropod immunity. Killer yeasts could thus be exploited for a double action, a direct anti-pathogenic effect within the vector and an immune stimulation, with an indirect effect on the reduction of the vectorial capacity. We expect that our project will increase the knowledge on this different type of symbionts, and to the development of novel tools for the biological/integrated control of vector-borne tropical diseases.

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HYPOTHESIS TESTING CLARIFIES *TRIATOMA DIMIDIATA* (LATREILLE, 1811) SYSTEMATICS USING NUCLEAR ITS-2 AND MITOCHONDRIAL CYTOCHROME B GENES

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The widespread and diverse *Triatoma dimidiata* is the species most important for Chagas disease transmission in Central America and an important vector in Mexico and northern South America. Its diversity may contribute to different Chagas disease prevalence in different localities and has led to conflicting systematic hypotheses describing various populations as subspecies or cryptic species. To resolve these conflicting hypotheses, we sequenced a nuclear (ITS-2) and mitochondrial gene (cytochrome *b*) from an extensive sampling of *T. dimidiata* across its geographic range. We assessed the congruence of ITS-2 and cyt *b* phylogenies and tested the statistical support for constrained topologies representing competing systematic hypotheses. Unconstrained phylogenies inferred from ITS-2 and cyt *b* are congruent. However, hypothesis testing does not support the division of *T. dimidiata* into the previously proposed three sub-species inferred from morphology and ITS-2. Our results identify two cryptic species and indicate *T. dimidiata* sensu stricto is not subdivided into monophyletic clades that might indicate subspecies. Extensive specimen sampling, analysis of both a hypervariable mitochondrial gene and a slower evolving nuclear gene in conjunction with statistical tests of hypotheses has facilitated the clarification of evolutionary relationships among epidemiologically important populations of *T. dimidiata*.

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ANTIBACTERIAL ACTIVITY FROM EXTRACTS OF FATTY BODIES AND HEMOLYMPH OF THE BLOWFLY *SARCONESIOPSIS MAGELLANICA* (DIPTERA: CALLIPHORIDAE)

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Sarconesiopsis magellanica is a necrophagous and hemisynanthropic fly which belongs to the Calliphoridae family. Its importance for human and veterinary medicine lies in its potential for participating as mechanical vector for pathogens such as viruses, bacteria, fungi, protozoa and helminths. Its larvae could cause miasis in some vertebrates, including human beings. Moreover, this fly is used in determining the post-mortem interval. Taking into account its necrophagous habits, this fly could be considered as a potentially useful model in larval therapy. The main goal of this work was to evaluate the antibacterial activity of the extracts of fatty bodies and hemolymph from third-instar larvae of *S. magellanica*. The results were compared with the effects obtained from the same substances derived of the blowfly *Lucilia sericata*, under *in vitro* conditions. The fatty bodies of larvae were removed by dissection technique and the hemolymph via decapitation and centrifugation of larval specimens. The tested bacteria were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The methods used to evaluate the antibacterial activity were agar diffusion and colony forming units. After accurate incubation, the results showed that the antibacterial activity of fatty bodies in both *S. magellanica* and *L. sericata* were effective against *S. aureus* and *P. aeruginosa*, but there was not significant difference between the fly species. However, in the agar diffusion assay the antibacterial activity of the extracts of fatty bodies of both species was found to be more efficient against *P. aeruginosa.*

The obtained results suggest that these substances could have a similar effect against the evaluated microorganisms in the treatment of infected wounds.

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NICHE CONSERVATISM AND PHYLOGENETIC STRUCTURE IN BROAD-SCALE SPECIES RICHNESS PATTERNS OF CHAGAS DISEASE VECTORS

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A major concern in evolutionary ecology and biogeography is the study of species distribution, especially when species from the same genus have a phylogenetic structure showing non-random spatial association. This pattern can emerge from historical processes as phylogenetic relationships and niche conservatism. In species with public health importance, such as insect vectors, looking into these patterns is of great relevance since processes keeping or avoiding the phylogenetic structure can be key factors in developing control and prevention measures and to anticipate measures to mitigate global change effects. Here, we used simulation models to analyze species richness patterns in the Triatominae (Reduviidae) based on collection data points, species distribution models, climate and phylogenetic information. Patterns of simulated co-distribution and co-diversity under different hypothesis were compared with empirical models. We found that historical processes as phylogenetic relationships and niche conservatism are important causes shaping current patterns of species richness. We consider that our approach has a broad application in quantitative biogeography of vectors of other diseases.

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DEPLETION OF TICK THIOREDOXIN REDUCTASE ATTENUATES THE NATIVE TICK MICROBIOTA

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The gulf-coast tick (*Amblyomma maculatum*) is a competent vector for a variety of pathogenic microbes, including *Rickettsia parkeri*, a causative agent of Spotted Fever Rickettsiosis. Ticks experience a variety of oxidative stress condition while on and off the vertebrate host. To counter-act the deleterious effects of reactive oxygen species, ticks have numerous antioxidant molecules in their repertoire, such as the Thioredoxin-Thioredoxin Reductase (Trx-TrxR) system, as reported previously. Tick Thioredoxin Reductase has barely been investigated. Our long-term goal is to reduce or block the spreading of vector-borne pathogens by interfering with vector proteins. In this study, we tested our hypothesis that tick TrxR facilitates the colonization of microbes in tick tissues by mitigating the reactive oxygen species. Transcriptional gene expression studies examining the level of TrxR during the prolonged blood-meal in both midguts and salivary glands indicates a potential need of this system during unfed stage. In order to evaluate the functional significance of this highly conserved system, we utilized RNA interference to selectively deplete TrxR transcripts *in vivo*. Both transcriptional gene expression and enzymatic activity studies confirmed the successful depletion of TrxR transcript and activity. However, no significant effect was observed on total tick engorgement likely due to high redundancies or compensatory mechanism in ticks but, the tick salivary glands super oxide dismutase (SOD) was found similarly down regulated with the TrxR depletion. Disruption of TrxR reduces the microbial load in the salivary glands examined by using bacterial universal 16s rRNA gene primers. Our results support the potential role of TrxR in preserving bacterial communities in tick tissues by alleviating the deleterious effect of reactive oxygen species. This work opens new avenues of research in oxidative stress within tick vectors and vector-borne pathogens.

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VECTORBASE: A BIOINFORMATICS RESOURCE CENTER FOR INVERTEBRATE VECTORS OF HUMAN PATHOGENS

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The VectorBase database is updated and expanded every two moths. In the last year we have significantly updated the gene builds, the assemblies or both for *Glossina morsitans, Aedes aegypti*, *Rhodnius prolixus, Anopheles stephensi* and *An. darlingi*. We have also almost tripled the number of hosted genomes from 11 to 30. These new genomes include the two sandflies *Lutzomyia longipalpis* and *Phlebotomus papatasi*, 16 new *Anopheles* species, and the snail *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*. Based on user feedback and internal discussions, all of our tools and resources have had multiple interface and performance improvements, including the possibility to save and reuse job parameters and their results. A new tool called the Population Biology Browser (PopBio), which we had presented initially under a beta version, was also released. This new tool is part of our ongoing efforts to integrate genomic, phenotypic (including insecticide resistance) and population data, as a strategy to integrate basic and applied research. VectorBase also includes new ontologies, mitochondrial sequences, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway information, microarray experiments, single-nucleotide polymorphisms (SNPs) data, RNAseq experiments and other datasets that are available for query and analyses. Also under development is the new VectorBase Galaxy Platform, which will provide our community with a user-friendly interface to perform large scale data analysis on a public site. The data deposited in VectorBase and in the public repositories such as NCBI, are a resource that has been subject to only very limited preliminary analysis. These data are freely available for new analyses, descriptions and hypotheses testing.

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SURVEYING THE BACTERIAL COMMUNITY PROFILES IN TICKS FROM THE VILLAGES OF INDIGENOUS PEOPLE IN MALAYSIA

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Ticks are excellent vectors for disease transmission of a wide variety of zoonotic pathogens, including viruses, bacteria and protozoa. In our laboratory, we are interested to explore the microbiome in ticks collected from the villages within the semi-forested areas in Malaysia. These areas are known as the interfacial zones of inhabitants (IZI), which provide for plenty of opportunities for contact between the indigenous people occupying the villages, tick vectors and wildlife reservoir hosts harbouring an array of zoonotic pathogens. Hence, the indigenous people in the IZI are constantly at threat from tick-borne diseases due to close contact with the tick vectors. We aim to utilize the 16S ribosomal RNA metagenomic sequencing strategy as a means to investigate the bacterial community in ticks collected from IZI, in hope of identifying emerging pathogens in ticks from IZI. Our results indicate that there is prevalence of a number of tick-borne bacterial pathogens harboured by the ticks sampled from the IZI. As the knowledge and data on pathogens harboured by ticks in Malaysia is minimal, studying the bacterial community in ticks, together with clinical surveillance, will provide knowledge that may help in the early detection of emerging pathogens among the indigenous people in Malaysia.

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FINE SCALE MAPPING OF QTL ASSOCIATED WITH REPRODUCTIVE DIAPAUSE WITHIN THE *CULEX PIPIENS* COMPLEX USING A RADTAG GENOMIC APPROACH

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Culex pipiens is a broadly distributed species complex that transmits human diseases (e.g., West Nile Virus, Lymphatic Filariasis). *Cx. p. pipiens*, one member of the complex, is found across temperate zones of the world while *Cx. p. quinquefasciatus* is restricted to subtropical and tropical regions. One physiological trait that distinguishes *Cx. p. pipiens* from its sister taxon is its ability to enter reproductive diapause. Photoperiod is the primary trigger of this complex life history trait. Previous work using markers developed with traditional methods inferred four quantitative trait loci (QTL) in an F2 mapping population. The ability to generate informative Single Nucleotide Polymorphic markers (SNPs) and infer QTL has increased dramatically with the advent of massively parallel sequence technology (e.g., Illumina HiSeq2000). In addition, a published reference genome for *Cx. p. quinquefasciatus* is available. An advanced intercross line (*Cx. p. quinquefasciatus* Johannesburg x *Cx. p. pipiens* South Bend) was established. First instar larvae collected from the F6 generation were exposed to diapause inducing conditions (i.e., 8:16 light:dark cycle and 18C). Follicle size in ten-day old adult females was used to score phenotype. Only the extreme phenotypes were sampled to construct a reduced representation paired-end library. Using a RADtag approach, each of the 100 samples had a unique identifier. SNPs were generated *in silico*; a filtered subset of 2000 SNPs was used to infer linkage groups. Linkage groups with at least 15 markers were assigned to chromosomes. Marker density on the linkage map is an order of magnitude greater than on the map used in an earlier study. Presently we are mapping QTL regions on a fine scale. This has positioned us to advance our understanding of what genes and genetic pathways regulate reproductive diapause.

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PHYSICAL MAPPING REVEALS CHROMOSOME-SPECIFIC GENOMIC LANDSCAPES IN *ANOPHELES STEPHENSI*

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Anopheles stephensi type form is the key vector of malaria on the Indian subcontinent and the Middle East. Additionally, *An. stephensi* is an emerging model species for genetic and genomic studies of mosquito biology and mosquito-parasite interactions. However, success of genomic analyses will be limited if researchers deal with numerous sequencing scaffolds, rather than with a chromosome-based genome assembly. Here we report the first chromosome-based genome assembly for the Indian wild type strain of *An. stephensi*. Our physical chromosome mapping ordered 62% of the *An. stephensi* sequencing scaffolds and facilitated analysis of chromosome arm-specific genomic landscapes that is seldom feasible in next-gen genome projects. Comparative analysis between *An. stephensi* and *An. gambiae* revealed differences in genome organization and highlighted varying rates of evolution between autosomes and the sex chromosome. The genome landscape of *An. stephensi* is characterized by relatively low repeat content compared with that of *An. gambiae*. Our analysis demonstrated extremely high rate of rearrangements in the X chromosome as compared with autosomes despite the lack of polymorphic inversions in the X chromosomes in both species. Additionally, the difference between the rates of the X chromosome and autosome evolution is much more striking in *Anopheles* than in *Drosophila*. We found that the high rates of evolution in the X chromosome highly positively correlated with the density of simple repeats, suggesting their role in genomic plasticity. While, the rate of autosomal evolution and distribution of common polymorphic inversions positively correlates with the densities of microsatellites and genes, but negatively correlates with the coverage of matrix associated regions and transposable elements. Our data indicate that overall high rates of chromosomal evolution are not restricted to Drosophila, but may be a feature common to Diptera. The chromosome-based genome assembly for *An. stephensi* will provide a valuable tool for the vector biology community as we seek a better understanding of mosquito biology.

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SILENCING CASPASE DECREASES DENV-2 INFECTION OF THE MOSQUITO VECTOR *AEDES AEGYPTI*

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The mosquito *Aedes aegypti* is the primary vector for dengue virus (DENV). An understanding of host-pathogen interaction is important in understanding what factors contribute to vector competence. However, many of the molecular mechanisms for vector competence remain unknown. Our previous global transcriptional analysis has suggested the induction of apoptotic proteins in the involvement of resistance and susceptibility to DENV-2 infection. Here we analyze the possibility that programmed cell death is actively involved in the defense of *A. aegypti* host cells to DENV-2 infection. The initiator caspase, Dronc, has been previously shown to be an essential component of the core apoptotic pathway. This caspase showed higher expression *in vitro* in infected *A. aegypti* cells as well as in resistant mosquitoes following infection. However, TUNEL staining of midguts from DENV-2-resistant and -susceptible mosquitoes revealed that apoptosis is activated at near-basal levels early during infection. Interestingly, dsRNA interference of Dronc decreased virus titer and infection in resistant mosquitoes. This reveals that Dronc may be important for affecting DENV-2 infection in *A. aegypti*. Furthermore, we investigate whether silencing of Dronc effects non-apoptotic processes influencing DENV-2 infection.

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POPULATION OF *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE) FROM PANAMA

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Although many studies on vectors of cutaneous leishmiansis have been done in Panama, the perdomestic distribution of *Lutzomyia longipalpis* have been documented recently. With the purpose of estimated the divergence time and difference in genetic population between peridomestic and selvatic species of *Lu. longipalpis*, we performed this study to estimate and compare the intra- and inter-population genetic variability of wild and near-residential populations of *Lu. longipalpis*, obtained in a locality with high incidence of cutaneous leishmaniasis in Panama. Using a mitochondrial DNA sequences of cytochrome B were analyzed of *Lu. longipalpis* populations from Panama. Of the 11 haplotypes obtained, seven were present exclusively in the town of El Limón and three, exclusively in Bona Island. A single haplotype was shared between the two communities. The haplotype and nucleotide diversities were h=0.70 and π=0.0015 for the population of Bona and h=0.95 e π=0.003 for the population of El Limón. The genetic differentiation analyses between the two populations showed significant differences (Fst=0.17; $p<0.05$) between them. Significant differences ($p<0.05$) were also obtained when the Panama sequences were compared to others obtained in Genebank cytochrome B the populations of *Lu. longipalpis* from Colombia (Fst=0,98), Costa Rica (Fst=0,98), and Brazil (Fst=0,72). The existence of unique haplotypes in each community and the significant genetic differentiation reported suggest that the *Lu. longipalpis*

populations in Panama are in the middle of a speciation process due to the isolation of the two populations because of the Pacific Ocean and the events that characterized the emergence of the Isthmus of Panama. The fact that *Lu. longipalpis* was found in near residential areas in Panama is important as a risk factor and to increase epidemiological surveillance. We result indicate the need to constantly and systematically monitor of this vector species in regions with high incidence of leishmaniasis and review the symptoms produced by different cryptic species of *Lu. longipalpis*. Meanwhile, little is currently known about the distribution, occurrence, and implications of this species in the transmission of leishmaniasis in the country.

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BIONOMICS AND PHYLOGENETICS OF THE DENGUE VECTOR *AEDES AEGYPTI* FROM THE ARABIAN PENINSULA

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Aedes aegypti is the principal vector of dengue in the world, including Saudi Arabia and Yemen in the Arabian Peninsula south-western regions; where disease outbreaks have occurred since 1995. Understanding the ecology and population genetics of *Ae. aegypti* is crucial for understanding dengue virus transmission patterns and for effective disease control. We report here on the ecology and phylogenetics of *Ae. aegypti* collected from western Saudi Arabia, from Jeddah governorate, a major harbour on the Red Sea. Phylogenetics analysis was carried out using the ribosomal DNA-internal transcribed sequence 2 (ITS2) and the mitochondrial cytochrome *c* oxidase I (COI) and NADH dehydrogenase subunit 4 (ND4) genes. *Aedes aegypti* larvae and pupae collected represented 23.9% (n= 772: 712 larvae, 60 pupae) of the total culicines mosquitoes collected. Most of water sites were anthropogenic, of which plastic drinking water tanks were the most productive for larvae (av. 55.5±55.5 larva/site). The most productive sites for pupae (47.5% of total pupae) were large concrete underground tanks or plastic elevated tanks (1000-5000 L capacity). The pupal yield is much lower than those reported from other countries. Single nucleotide polymorphisms (SNPs) and Neighbour-Joining (NJ) phylogenetic trees were built using COI and ITS2 sequences obtained from *Ae. aegypti* from Saudi Arabia or retrieved from the Genbank for other populations from Africa, Asia and the Americas. NJ trees identified ten COI and 21 ITS2 haplotypes, with many haplotypes unique to Arabian *Ae. aegypti* populations. Data on ND4 analysis will be reported when appropriate. We provide novel phylogenetic information of *Ae. aegypti* populations from the Arabian Peninsula and other parts of the Oriental, Afrotropical and Palaearctic zoogeographic zones, which shows the presence of considerable genetic differentiation between them. These studies will give broader insights on the dispersal patterns of *Ae. aegypti* and transmission dynamics of dengue virus, with important implications for disease control under national and regional biogeographic conditions.

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MOLECULAR PHYLOGENETICS OF MOSQUITOES FROM THE ORIENTAL AND AFROTROPICAL ZOOGEOGRAPHIC REGIONS

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The Arabian Peninsula (PA) has peculiar position bordering the Oriental, Afrotropical and Palaearctic zoogeographic zones with diverse ecology and fauna. In Saudi Arabia (SA) (the largest country in PA) about 35 culicine mosquito species were reported including eight dominant vector species. The most important of these are *Aedes aegypti* and *Culex pipiens* complex, vectors of arboviruses, and two *Anopheles* malaria vectors, *An. stephensi* in Asia and *An. arabiensis*, the only member of *An. gambiae* complex outside Africa. We present here new information on phylogenetics of *An. stephensi* and *An. arabiensis* and other anopheline species collected from different SA regions. Neighbour-Joining (NJ) phylogenetics trees were constructed using DNA sequences of the ribosomal DNA-internal transcribed sequence 2 (ITS2) and the mitochondrial cytochrome *c* oxidase I (COI) gene. These sequences were obtained from mosquitoes field-collected from SA or from lab colonies from other countries in the Oriental or Afrotropical zones and sequences retrieved from the Genbank. Multi-locus phylogenetic analysis of COI & ITS2 sequences of all *An. stephensi* populations identified new haplotypes, including unique haplotypes to SA, and haplotypes broadly-distributed across the Oriental zone including AP. These results confirm that *An. stephensi* is a monophyletic species composed of ecotypes. However, unlike in Iran and India, we could not differentiate between *An. stephensi* type and mysorensis ecotypes, which might be due to inter-population extensive gene exchange. New *An. arabiensis* haplotypes were identified SA and related to field and lab populations from the Afrotropical region. In this report we provided new information on the phylogenetic relationships of anopheline mosquitoes from different zoogeographic regions including malaria vectors and suspected or non-vectors. Such information is important for understanding malaria transmission under broad biogeographic conditions across different zoogeographic zones. The COI or ITS2 sequences could also be used to develop species-specific molecular assays to complement pectorial keys to accurately identify species in AP and their cryptic or ecotypes if exist.

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POPULATION STRUCTURE OF THE VECTOR MOSQUITO *AEDES AEGYPTI* AND HUMAN-MEDIATED DISPERSAL IN THE PHILIPPINE ARCHIPELAGO

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Aedes aegypti is the primary vector of most of the so-called arboviruses (ARthropod-Borne viruses), like dengue fever, yellow fever or chikungunya. Massive employment of insecticides favoured the development of insecticide resistance. *Ae. aegypti* is a highly anthropophilic mosquito and it is believed that human transportation played and still plays an important role in its dispersal. The dispersal ability of a vector is connected to its ability to spread the diseases as well as the insecticide-resistance mutations. Knowledge of the genetic structure of the populations of mosquitos can help to infer its patterns of dispersal. The Philippines are endemic for dengue fever and recently a high level of insecticide resistance was found. With its 7000 islands the philippine archipelago is therefore an appropriate environment to analyze the relationships between mosquito dispersal and both land and marine human transportation. With the objective of determining the distribution and population structure of Ae aegypti, during September-October 2013 a sampling took place in 7 major islands in the northern part of the Philippines (Luzon), in 11 seaports and 7 inland areas. In each area, at least 7 breeding sites were sampled; in order to reduce the presence of sibling individuals, (1) the flight range of *Ae. aegypti* was taken into account and (2) 1 out of 6 larvae collected from each site were randomly selected for the study, yielding between 19 and 67 individuals to be analyzed in each area. All the inland areas had to be discarded because of lack of specimen. Up to now, a preliminary analysis has been conducted with 6 microsatellite markers, but more are planned to be added henceforth. Between 4 and 9 alleles were found at each locus. Generally no significant deviation from HWE was found. The total Fst value was 0.06, quite low, suggesting gene flow between the islands. Interestingly, the pairwise Fst values were, at average, lower for the biggest and busiest seaports while higher for the smallest seaports.

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GENOME-WIDE HAPLOTYPE MAP REVEALS INSECTICIDE SELECTIVE SWEEPS IN WILD *ANOPHELES GAMBIAE* POPULATIONS FROM KENYA

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Nearly a million people die from malaria annually. *Anopheles gambiae* in Africa is the major malaria vector. Examining the molecular basis of mosquito traits of interests needs the information of genetic variations and haplotype map (HapMap) in wild *A. gambiae* populations from malaria endemic areas. We sequenced the genomes of nine wild *A. gambiae* individually, and detected 2,219,918 single nucleotide polymorphisms (SNPs) with 88% novel, and 43,765 nonsynonymous. SNPs are not distributed on *A. gambiae* evenly, and the lower SNP frequency regions overlays with heterochromatin and chromosome inversion. About 785,687 SNPs that were genotyped correctly in all individual mosquitoes with 99.6% confidence were extracted from high throughput sequencing data. Based on these SNP genotypes, we for the first time constructed the genome-wide HapMap of wild *A. gambiae* mosquitoes from malaria endemic areas in Kenya, and made it available through a public web with graphic user interface. Low LD is consistently observed with average linkage disequilibrium (LD) block size less than 40 bp. Meantime, we discovered that several large LD blocks were clustered in *A. gambiae* genome. Interestedly, detailed analysis of the genomic locus of chromosome 2 (2R:57.6-2L:4.0MB) that has fewer SNPs and largest linkage disequilibrium (LD) blocks revealed para gene at the center of this region with homozygous insecticide knock-down resistance (kdr) allele 1014F in all sample mosquitoes, supporting the hypothesis of insecticides DDT and pyrethroids selective sweeps in western Kenya.

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MOLECULAR ADAPTATION OF THE OLFACTORY SYSTEM TO HUMAN HOSTS IN *ANOPHELES GAMBIAE*

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The dominant African malaria vector *Anopheles gambiae s.s* preferentially takes it blood meals from human hosts, often at rates as high as 90%. This adaptation to human hosts is expected to have a genetic basis in the olfaction system, which includes several key gene families - the olfacation receptors (ORs), ionotropic receptors (IRs), odorant binding proteins (OBPs). We previously identified six narrow QTL for human host preference on chromosomes 2 and 3, that together explain 49% of the phenotypic variance. A total of 34 ORs, 7 IRs and 21 OBPs are located inside these QTL. In addition, a comparison of antennal transcriptomes identified 11 olfaction genes that are located inside QTL and that were significantly higher expressed in *An. gambiae* vs the zoophilic *An. quadriannulatus*. The genes involved in the adaptation of *An. gambiae* to human hosts should show evidence of positive selection. Therefore, we examined the evolution of olfaction genes (spanning all three gene families) from 95 individuals comprising five member species of the *An. gambiae* complex – *An. gambiae* (M + S), *An. arabiensis*, *An. melas*, *An. merus* and *An. quadriannulatus.* We used a phylogenetic framework (PAML) to test if the *An. gambiae* lineage evolved under positive selection– based on the ratio of non-synonymous to synonymous (dN/dS) substitutions, and signatures of selective sweeps. The presence of olfaction genes that evolved under positive selection inside human host preference QTL indicates their importance for human host choice in *An. gambiae.*

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UNEXPECTED STRONG REDUCTION OF GENE-FLOW WITHIN *ANOPHELES GAMBIAE* IN AN AREA OF HYBRIDIZATION WITH *AN. COLUZZII* IN THE "FAR-WEST" OF THEIR RANGE

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The *Anopheles gambiae* complex includes mosquito species at different stages of speciation, ranging from clearly defined, although morphologically indistinguishable, bonae species to closely-related sympatric taxa such as *An. gambiae* and *An. coluzzii* (recently raised to formal species), which represent the major vectors of human malaria in sub-Saharan Africa. Extensive genetic studies have trusted these species as models of ecological speciation and highlighted the effect of this process in malaria epidemiology and control. We sampled *An. gambiae* and *An. coluzzii* populations from diverse habitats along the Gambia River (West Africa), an area characterized by higher level of inter-specific hybridization compared to most of the species range. We carried out a comparative analysis of these samples by presumably neutral nuclear microsatellite markers on chromosome-X and -3 and by presumably adaptive chromosomal paracentric inversions on chromosome-2. Both genetic markers reveal unexpected striking genetic differences, compatible with a strong reduction of gene-flow, between *An. gambiae* populations west and east of the central part of the transect, apparently exclusively colonized by *An. coluzzii*. While *An. gambiae* western populations are characterized by low chromosomal inversion diversity, a very high degree of chromosomal variation, based on a higher number of inversion polymorphisms, is observed in eastern populations. Consistent with this chromosomal divergence, high genetic differentiation at the microsatellite level, not explained by geographic distance alone, is observed between western and eastern populations. Notably, this microsatellite differentiation is higher than that observed between *An. gambiae* and *An. coluzzii*, and mostly due to loci in the centromeric region of chromosome-X. This suggests that the two *An. gambiae* populations may be at an advanced stage of reproductive isolation, likely triggered by human-made habitat fragmentation, and provides new evidence of a speciation continuum within the *Anopheles gambiae* complex.

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METAGENOMICS OF *AEDES ALBOPICTUS*: IMPACT OF LARVAL HABITAT TYPES AND MOSQUITO AGE ON THE MICROBIOME STRUCTURE OF MOSQUITO GUTS

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Recent metagenomic studies suggest microbiomes of disease vectors may have profound impacts on vector development, reproduction, immunity against pathogens and vectorial capacity. However, the relationship between vector environments and vector microbiome structure and composition is unknown. Given mosquito larvae are confined to the aquatic habitats, it is hypothesized that microbial community in the larval habitats may largely determine the contents of mosquito larval guts, but larval gut microbials may have little effects on the gut microbial community of adult mosquitoes due to constant acquisition of new

microbials in the process of sugar and blood feeding. The present study tested this hypothesis with the Asian tiger mosquitoes (*Aedes albopictus*), a most invasive species and also an important dengue vector. We examined the dynamics of gut microbial communities of *Ae. albopictus* from three types of larval habitats, mosquito larvae, pupae and adults from these habitat types. Microbial community of the larval habitats and larval and adult mosquito guts was examined by pyrosequencing of bacterial 16S rRNA gene V4 hyper-variable region. A total of 15 million 250bp paired-end sequence reads were obtained. Preliminary analysis found that the composition of the microbiomes varied significantly among larval habitat types, and varied between larvae and adults whereas microbiomes of larvae and pupae were similar and resembled to the microbiomes of the larval habitats. *Proteobacteria*, *Bacterioidetes* and *Firmicutes* were the predominant bacteria across mosquito life stages. Blood feeding showed a significant impact on mosquito gut microbiomes. The present metagenomic study established a metagenomic foundation for better understanding the impact of environmental microbials on vector development and disease transmission.

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DISSECTING GENETIC AND MICROBIAL FACTORS OF *AEDES AEGYPTI* FIELD POPULATIONS WITH DISTINCT SUSCEPTIBILITY DO DENGUE VIRUS

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Dengue is the arboviral disease of highest public health concern due to its increasing expansion in recent years worldwide. Due to the lack of a licensed anti-dengue therapy, the prevention of dengue virus (DENV) dissemination is still limited to the control of its vector, the mosquito *Aedes aegypti*. DENV propagation and transmission is determined by the mosquito's vector competence, which has been associated to both genetic factors and the gut microbiota. Here we assess both the genetic variation of DVHFs (dengue virus host factors) and the microbial diversity of three field-derived Brazilian *Ae. aegypti* populations displaying distinct susceptibilities to DENV. Mosquitoes were collected in three different locations (Botucatu-SP, Neópolis-SE and Campo Grande-MS). We assessed dengue viral susceptibility of each population through oral infection by DENV-4 and quantified the relative number of viral particles by real-time PCR. Our data suggest that mosquitoes from Botucatu are nearly 3-fold less susceptible to the virus than those from Campo Grande ($p<0.001$). Sequencing analysis of the DVHFs lola and NADH of these two populations revealed a total of 9 SNPs, with 5 of them causing amino acid changes to the predicted polypeptide sequence of such genes. In order to verify a potential association between mosquito's microbial diversity and susceptibility to the virus, we are also performing Illumina 16S rRNA surveys to analyze the gut microbiota of such mosquitoes. Surprisingly, our results revealed that the midguts of the mosquitos from Botucatu are colonized mainly by Gram-positive bacteria from the Lactobacillus genus (34% of the total number of bacteria), even though there was a higher number of Gram-negative genera than Gram-positive ones in these mosquitoes. We are now assessing the Campo Grande population microbiome in order to determine whether the microbial diversity of these highly DENV-susceptible mosquitoes is different from that of the Botucatu population. This work will shed light on our understanding of the molecular interactions of DENV-mosquitoes-microbiota and may ultimately lead to the development of new dengue control strategies.

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GENOME-WIDE ISOLATION WITHIN THE WEST-AFRICAN MALARIA VECTOR *ANOPHELES MELAS*

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Anopheles melas is a locally important malaria vector along the West-African coast where it breeds in brackish water. A recent population genetic study of this species revealed species-level genetic differentiation between two population clusters on the mainland: *An. melas* West and *An. melas* South. *An. melas* West extends from The Gambia to Tiko, Cameroon (near Mount Cameroon). The other mainland cluster, *An. melas* South, extends from the southern Cameroonian village of Ipono to Angola. Species level differentiation was also found between mainland and Bioko Island, Equatorial Guinea populations. To examine how genetic differentiation between these three forms is distributed across the genome, we pooled samples from a representative population of each of the three genetically isolated *An. melas* clusters. We performed whole genome sequencing on these pools and conducted genome-wide analyses of divergence and selection. Our analyses reveal that these three forms show high levels of genetic differentiation across the genome, including the presence of genome-wide fixed differences. Levels of genetic differentiation are particularly high on the *X* chromosome and low in heterochromatic regions. Additionally, we analyzed genome-wide differentiation between *An. gambiae* and *An. melas* West to put our results in the context of the *An. gambiae* species complex evolution. We also investigated how divergence in specific genes and genomic regions may have led to the genomic isolation of these putative species.

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MICRORNAS (MIRS): A VIABLE OPTION FOR TRANSGENIC MOSQUITO CONTROL?

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MicroRNAs (miRs) are small non-coding RNAs that can each regulate the expression of up to hundreds of genes. Therefore miRs that are active during changes in host seeking behavior, as mosquitoes age or acquire a blood meal, may be a viable target for the transgenic manipulation of mosquitoes. However, the number of known miRs in the yellow fever mosquito is small compared to other insects and little is known about which genes they regulate. Because olfaction genes are crucial for host seeking, we examined if miRs play a role in olfaction gene regulation as mosquitoes develop and blood feed. We extracted total RNA from the antennae and head+thorax from females of various ages, as well as males (12h non-host seeking females, 4 days old host seeking females, 4 days old males, and 3h, 24h, 48h and 72h after blood feeding). Poly(A)+ RNA, 3' UTR and small RNA were sequenced on the Illumina platform. Global gene expression analyses revealed 52 genes that are highly and uniquely expressed in the antennae of 4 days old females (low or absent expression in 12h old females antenna, male antenna or head+thorax of 4 days old females). Similarly, 1,150 genes are uniquely expressed in the antenna of 12h old females. While 37 olfaction genes are differentially expressed between antenna of 12h old females and 4 day old females, only five of these are significantly different 24h after blood feeding in comparison to the 4 day old unfed females. The most expressed miRs in the antenna of females is aa-miR-236a, which has 82.65 fold higher expression compared to the head+thorax sample. We are particularly interested in miRs that do not kill mosquitoes, but decrease their attraction to humans. To identify miRs important for host seeking, we are injecting its antagomir (synthetic

anti-sense miR) into late larvae, pupa and 12h old adults. Preliminary results from pupa injection are very promising; injected mosquitoes are being subjected to a dual choice assay.

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ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN IMMUNE RESPONSE PATHWAYS GENES, AND SUSCEPTIBILITY/RESISTANCE PHENOTYPES OF *ANOPHELES DARLINGI* TO *PLASMODIUM VIVAX*

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Populations of *Anopheles* malaria vector in nature are composed of both, *Plasmodium* susceptible and resistant individuals. Susceptibility and resistance to malaria parasites were, and still are, the subject of intense study. With the availability of *Anopheles darlingi* genome sequence, this new knowledge has opened doors for investigating genetic determinants for susceptibility to *P. vivax* infection of this major malaria vector in Americas. This work aims to describe the occurrence and distribution of SNPs (single nucleotide polymorphisms) in genes of the immune signaling pathways using samples taken directly from natural populations of *A. darlingi* (collected in Amazonas and Pará States in Brazil) and to investigate their association to susceptibility / refractoriness to *P. vivax* infections. We identified homologs of 172 immune genes in the *A. darlingi* genome from the data available on VectorBase. We conducted whole genome sequencing on 24 individuals both infected and uninfected groups and identified SNPs on immune genes. A SNP genotyping assay will be developed from a panel of non-synonymous SNPs in immune genes and genotyping assay will be conducted on 400 individuals of both infected and uninfected groups. Our goal is to identify SNPs associated with the susceptibility / resistance to *P. vivax* in *A. darlingi* populations of different genetic backgrounds. We will use the knowledge of the molecular genetics of *A. darlingi*, available on the Vector Base, to create and establish a database to be used in the recognition of genetic markers, which can be used as indicative of the existence of subpopulations of *A. darlingi* with distinct vector competence for transmission of human malaria in different localities of the Amazon. We intend to develop a predictive model of transmission that will point out where are the most competent mosquitoes population for the transmission of the parasite, which may help to establish strategies focused on the monitoring and control of the disease.

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IDENTIFICATION OF *ANOPHELES* (DIPTERA: CULICIDAE) FAUNA FROM COLOMBIA THROUGH DNA BARCODES

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Taxonomic determination of *Anopheles* species constitutes an essential baseline for targeted malaria vector control. Historically, morphological characters have been used for taxonomic identification; however, the existence of species complexes, closely related species and inter and intra phenotypic variation, makes this task difficult. Therefore, a DNA barcoding strategy based on a fragment of the *COI* gene has been proposed to identify specimens at the species level. In Colombia, approximately 47 *Anopheles* morphospecies have been recorded, however, molecular work has mainly focused on the main malaria vectors. The aim of this work was to provide a sequence reference library that includes DNA barcodes available for the corresponding Colombian species. In total 41 Molecular Operational Taxonomic Units (MOTUs) representing species/lineages were compiled, 30 of them were sequences obtained by our group or from GenBank. The remaining represented specimens from neighbor countries but that have also been recorded in Colombia. Neighbour-joining analysis based on Kimura's two parameter (K2P) showed non-overlapping clusters for all species and lineages with high bootstrap support, whereas similarity methods, Best Match, Best Close Match and All Species Barcode used with the typical 3% threshold proposed for barcode, correctly assigned 95.59% , 91.82% and 67.5% of the sequences respectively, to its original species. These results demonstrate that barcode constitutes an important tool for taxonomy in *Anopheles*; however, being a single-gene method its use constitutes a baseline approach, and other biological, morphological and ecological markers should be implemented for species delimitation. Importantly, the barcode sequence library presented here can be used as a benchmark for molecular confirmation.

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ENTOMOLOGICAL INVESTIGATIONS FOR UNDERSTANDING JAPANESE ENCEPHALITIS VIRUS TRANSMISSION DYNAMICS: LESSONS FROM BANGLADESH

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Understanding pathogen transmission dynamics is imperative for identifying and implementing cost effective interventions for optimal impact. One of the first steps toward understanding transmission dynamics of mosquito-borne zoonoses is to identify the host and vector species necessary for maintaining, amplifying and bridging transmission to humans. Such investigations were first undertaken for Japanese encephalitis virus (JEV) in Japan in the 1950's. Since this time, the dominant vector species – *Culex tritaeniorhynchus* – and reservoir hosts – pigs and ardeid birds – that were identified in these studies have generally been assumed to drive JEV transmission across the whole of Asia. This transmission cycle is likely to be responsible for human risk in areas where pigs are dominant within the community of vertebrate hosts and *Cx. tritaeniorhynchus*, confirmed in field and experimental settings to feed predominantly on large mammals, is relatively more abundant than other potential vector species. Such ecological contexts are found in Thailand and Malaysia; however, the presumption that this group of species drives transmission in all regions may impede our understanding of spatiotemporal variation in transmission dynamics of JEV. Countries where transmission drivers may differ from that of Japan include India, Indonesia and Bangladesh, where dead-end hosts (cattle) are found in substantially higher density than pigs. We utilize field data obtained during a preliminary entomological survey in Bangladesh to show that the observed dominance of any mosquito species within a community can be dependent on the sampling method employed. In addition we utilize an equation for the basic reproduction number of a zoonotic mosquito-borne virus, parameterized from field data and literature surveys, to demonstrate that the vector species observed to be most abundant may not necessarily drive transmission. To conclude, we emphasize that multiple, carefully selected mosquito sampling methods should always be considered for estimation of mosquito relative abundance as well as species blood-feeding patterns, when undertaking surveys to implicate vector and host species in new geographic regions.

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EPIDEMIOLOGICAL PATTERNS OF ROSS RIVER VIRUS DISEASE IN QUEENSLAND, AUSTRALIA, 2001-2011

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Ross River virus (RRV) infection is a debilitating disease which has a significant impact on population health, economic productivity and tourism in Australia. This study examined epidemiological patterns of

the RRV disease in Queensland, Australia between January 2001 and December 2011 at a statistical local area level (Figure 1). Spatial-temporal analyses were used to identify the patterns of the disease distribution over time stratified by age, sex and space. The results show that the mean annual incidence was 54 per 100,000 people, with a male: female ratio of 1:1.1. Two space-time clusters were identified: the areas adjacent to Townsville, on the eastern coast of Queensland; and the south east areas (Figure 2). Thus, although public health intervention should be considered across all areas in which RRV occurs, it should specifically focus on these high risk regions, particularly during the summer and autumn to reduce the social and economic impacts of RRV.

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RISK EVALUATION OF THE RIFT VALLEY FEVER EMERGENCE IN EUROPE: COMPETENCE OF THE EUROPEAN MOSQUITOES AND ADAPTABILITY OF THE VIRUS

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The Rift Valley Fever virus (RVFV) first detected in Kenya in 1930 causes a zoonose with an important impact on livestock. Very recently, it has expanded its natural range of distribution outside the Sub-Saharan Africa, in Saudi Arabia, Yemen, Madagascar, the Comoros and Mayotte islands. Its current expansion questions on the risk of a RVFV emergence in Europe. RVFV is an arbovirus with an enveloped particle composed of 3 negative single-stranded RNA segments which is transmitted by more than 30 different mosquito species. It circulates among wild mammals at a low prevalence but when environmental conditions are favorable for mosquito proliferation, an epidemic can occur causing mass abortions and death of young animals. Humans are mainly contaminated by direct contacts with tissues and blood when manipulating infected animals. Thus, the economic and social impacts of a RVFV epidemic can be dramatic. The aim of our study will be to evaluate the risk of RVFV emergence in Europe and the conditions that could favor its transmission. It will be done by developing two objectives: (i) determine the distribution and the competence to RVFV of potential mosquito vectors in France, and (ii) determine if molecular changes in the viral genome can be associated to an increased transmission by European mosquitoes?

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INSECTICIDE RESISTANCE STATUS IN *ANOPHELES GAMBIAE S.L.* FOLLOWING THE SCALE UP OF MALARIA CONTROL INTERVENTIONS IN RWANDA

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The scale up of malaria vector control interventions in particular universal coverage (one net for two persons) with Long Lasting Insecticidal Nets (LLINs) achieved in February 2011 and Indoor Residual Spraying (IRS) have played a major role in reducing by 86 % malaria incidence in Rwanda. The spread of insecticide resistance that has been reported in the African region may reverse the tremendous gains made in malaria control. Since 2010, the Malaria and Other Parasitic Diseases Division (MOPDD) in Rwanda has conducted resistance monitoring of malaria vectors to detect trends and to guide vector control interventions. Since 2010, resistance monitoring of malaria vectors was conducted in eight sentinel sites located in four provinces in Rwanda with varying malaria endemicity. The collection of *Anopheles* larvae in the field was conducted as described by WHO (2002) and reared to adults in controlled field conditions. In 2010, resistance testing was carried out using the CDC bottle assay. From 2011, WHO insecticide susceptibility testing was used (WHO 1998). The susceptibility outcomes were assessd according to WHO standard procedures (WHO, 2013). In 2010, resistance of *Anopheles gambiae s.l.* was only detected to DDT 4% in two (25%) out of eight sites surveyed. In 2011, resistance to DDT 4% was confirmed in four sites (28%) and emerging resistance to Permethrin 0,75% in three (21%) out of 14 sites. In 2012, the resistance was again confirmed to DDT 4% in one site (20%) and to pyrethroids in two sites (40%) out of 5 sites. Likewise, in 2013, the resistance to DDT 4%, Pyrethroids (Lambdacyalothrin 0,05%, Permethrin 0,75%, Deltamethrin 0,05% and Etofenprox 0,5%) and Bendiocarb 0,1% was respectively found out in eight (29%), fifteen (55%) and two (7%) sites out of 27 sites monitored. During this period, all specimen of *Anopheles gambiae s.l.* tested were susceptible to organophosphates (Fenitrothion 1% and Malathion 5%) at all sites. In conclusion, the scale up of malaria vector control interventions has associated with the spread of insecticide resistance of malaria vector mainly to pyrethroids. In response to this threat, an insecticide resistance management strategy was developed by the Ministry of Health of Rwanda and has to be regularly reviewed. Therefore, further investigations have to be undertaken to elucidate the resistance mechanisms.

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POPULATION DYNAMICS OF MAJOR MALARIA VECTORS AND THE IMPACT OF INDOOR RESIDUAL SPRAYING ON ENTOMOLOGICAL INOCULATION RATE IN NASARAWA STATE, NORTH CENTRAL NIGERIA

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The President's Malaria Initiative | Africa Indoor Residual Spraying project, executed asecond year of spray operations in Nasarawa Eggon and Doma Local Government Areas of Nasarawa State, Nigeria. Molecular tools were used to identify the predominant vectors responsible for malaria transmission in the study area, and entomological inoculation rates (EIRs) were calculated pre and post intervention. Mosquitoes were identified morphologically and by molecular methods using polymerase chain reaction (PCR). The *Plasmodium falciparum* circumsporozoïte indexes were measured by ELISA and the EIRs were calculated for the 3 areas. A total of 2,539 *Anopheles* mosquitoes were caught in the intervention areas and control site. Of these, 1,653 (65.1%) were caught in Doma, 525 (20.7%) in Lafia and 361(14.2%) in N/Eggon respectively. A subsample of 1,265 *Anopheles* mosquitoes were randomly selected for PCR analysis. Morphological analysis indicated that 1,174 (92.8%) were *An. gambiae* s.l., while the remaining were *An. funestus* (3.6%), *An. pharoensis* (2.8%) and *An. squamosus* (0.8%). PCR analysis of the *Anopheles gambiae* s.l. revealed a predominance of *An. gambiae* s.s (68.5%), while 29.9% were *An. arabiensis*. ELISAs showed that *P. falciparum* sporozoite infection rates were 1.7% in *An. gambiae* s.s. and 0.6% in *An. arabiensis*. There was a significant difference between the sporozoite rate of *An. gambiae* s.s. and *An. arabiensis* (χ^2=8.696, p<0.0032, df=1). At baseline (pre-intervention), EIR was found to be 1.31infective bites/person/night (bpn) in Doma, 0.16 in N/Eggon and 0.13 in Lafia, including both indoor and outdoor collections. After the IRS intervention, EIR was reduced to 0.9 in Doma and 0.11 in N/Eggon, while it remained the same at the control area in Lafia at 0.13 bpn. There was a significant difference in EIR reduction (p<0.0001) between the intervention areas and the control site. Although ELISA tests incriminated *An. gambiae* s.s. as the predominant vector responsible for transmission of malaria in the study area, *An. arabiensis* was also found to be sporozoite positive. *An. funestus* group were not incriminated in malaria transmission. Post intervention EIRs were observed to have significantly decreased in the intervention areas. These findings provide information on the relative roles of the main malaria vectors found in the study areas and the impact of indoor residual spraying on malaria transmission.

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DYNAMIC RELATIONSHIPS BETWEEN MOSQUITO MICROBIOME AND VECTOR COMPETENCE

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The hologenome theory of evolution proposes that natural selection of an organism is also driven by its symbiotic microorganisms. Research on the insect holobiome (the host plus all associated microorganisms) has largely been descriptive and often ignored when studying influences on phenotype. For insect vectors of medical importance, pathogen transmission capability is often variable, possibly due to differences in the internal host environment. Given that, functional knowledge about the holobiome of insect vectors is key to understanding vector-borne disease distribution and anticipating possible consequences of global climate change. Several studies have described mosquito symbionts and have suggested that microbe abundance and diversity can impact malaria parasites. However, the function and utility of those microbes are virtually unknown, especially in mosquitoes that transmit viruses. We compared microbiome data of two phenotypically distinct colonies of *Culex tarsalis* mosquitoes for West Nile virus (WNV) vector competence. Our data suggests that vector competence may be influenced by the mosquito microbiome and specific candidate microbes may be responsible for these phenotypic differences. A dynamic relationship appears to exist between the mosquito holobiont and WNV vector competence in *Cx. tarsalis*.

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ANOPHELES ARABIENSIS IS NOT SUCCESSFULLY CONTROLLED BY INDOOR RESIDUAL SPRAYING IN NORTHWEST TANZANIA: IMPLICATION FOR MALARIA VECTOR CONTROL IN THE AREA

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In East Africa scale up of Insecticide Treated Net (ITN) and Indoor Residual Spraying (IRS) has been associated with a drastic reduction in the abundance of *Anopheles gambiae* s.s. the primary vector in the area. This led to an apparent shift in An. gambiae s.l. sibling species ratio toward the more zoophilic and more exophilic *An. arabiensis* which is less likely be killed by IRS and ITN. The impact of IRS with bendiocarb on the relative abundance of *An. gambiae* s.s. and *An. arabiensis* was evaluated in North West part of Tanzania during a community randomised trial. Pre intervention, *An. arabiensis* represented 18.6% (95%CI: 13.9-24.6) and *An. gambiae* s.s. 81.4% (95%CI: 75.5-86.1) of the population of *An. gambiae* s.l. collected with indoor light traps, while *An. arabiensis* accounted for 3.8% and *An. gambiae* s.s. 96.2% of the population found resting indoor. Sporozoite rate was 1.4% (95%CI: 1.1-2.0) and only *An. gambiae* s.s. were found positive. After IRS, density of *An. gambiae* s.s was reduced by 75% (p=0.046) and *An. arabiensis* by 25% (p=0.745). In the IRS villages sporozoite rate in *An. gambiae* s.s. was 1.8% and 0% for An.arabiensis. There was a significant difference in the gambiae s.s./arabiensis species ratio with *An. arabiensis* constituting 11.3% the control arm alone compared to 26.1% in the IRS arm (OR: 2.8 (95%CI: 1.1-6.8) p=0.027). Indoor Residual Spraying was more effective in controlling *An. gambiae* than *An. arabiensis* in North West Tanzania. *An. arabiensis* in this area is a secondary vector and appeared to contribute little to malaria transmission. The focus of control should remain on *An. gambiae* s.s. the main vector in this area while more specific vector control tools for *An. arabiensis* could be investigated.

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TRANSCRIPTOMICS OF DIFFERENTIAL VECTOR COMPETENCE: WNV INFECTION IN TWO POPULATIONS OF *CULEX PIPIENS QUINQUEFASCIATUS* LINKED TO OVARY DEVELOPMENT

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Understanding mechanisms that contribute to viral dissemination in mosquito vectors will contribute to our ability to interfere with the transmission of viral pathogens that impact public health. The expression of genes in two *Culex pipiens quinquefasciatus* populations from Florida with known differences in vector competence to West Nile virus (WNV) were compared using high throughput sequencing. Results A total of 15,176 transcripts were combined for comparison of expression differences between the two populations and 118 transcripts were differentially expressed ($p<0.05$). The fold change in expression of the differentially expressed genes ranged from -7.5 - 6.13. The more competent population for WNV (Gainesville) over expressed 77 genes and down regulated 44 genes, compared with the less competent population for WNV (Vero Beach). Also, splicing analysis identified 3 transcripts with significantly different splice forms between the two populations. The functional analysis showed that the largest proportion of transcripts was included in the catalytic activity and transporter activity groups except for those in the unknown group. Interestingly, the up- regulated gene set contained most of the catalytic activity function and the down- regulated gene set had a notable proportion of transcripts with transporter activity function. Immune response category was shown in only the down regulated gene set, although those represent a relatively small portion of the function. Several different vitellogenin genes were expressed differentially. Based on the RNAseq data analysis, ovary development was compared across the populations and following WNV infection. There were significant differences among the compared groups. In conclusion, this study suggests that ovary development is related to vector competence in two Culex populations in Florida. Both populations control energy allocations to reproduction as a response to WNV. This result provides novel insight into the defense mechanism used by Culex spp. mosquitoes against WNV.

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POPULATION SUBDIVISION WITHIN *ANOPHELES GAMBIAE* MAY IMPACT MALARIA TRANSMISSION IN GUINEA BISSAU

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Anopheles coluzzii and *Anopheles gambiae*, formerly *Anopheles gambiae* s.s. M and S forms, are generally characterized by low levels of hybridization along most of their west African sympatric range. However, high levels of hybridization and genetic introgression have been detected in the western African limit of their distribution, particularly in Guinea Bissau. In this study, we have characterized levels of genetic differentiation within and between *A. coluzzii* (M-form) and *A. gambiae* (S-form) samples collected from 10 localities along an east-west transect in Guinea Bissau, during the rainy season of 2010. Samples were identified to species by IGS-rDNA and SINE200X markers, genotyped for 19 microsatellites and for the insecticide resistance associated ace-1 and kdr loci. In addition, ELISA was used to determine blood meal origin and to assess sporozoite

rates in selected localities. Microsatellite data showed that hybridization between *A. coluzzii* and *A. gambiae* occurs mainly in coastal areas, with hybrid rates up to 19.4%. Moreover, Bayesian clustering analysis revealed a subdivision within *A. gambiae* into east and west/coastal populations. These populations are geographically separated by a central region where *A. coluzzii* prevails. Genetic partitioning within *A. gambiae* was also evident from the distribution of ace-1 and kdr resistance-associated alleles, that reached frequencies of 3% and 83% in east localities but were absent from west/coastal sites. West/coastal *A. gambiae* presented human blood indexes (HBI) between 26.4% and 77.8% whereas in the east population HBI was 99.3%. Sporozoite rates between 3.7% (N= 54) and 7.2% (N= 69) were recorded in east populations of *A. gambiae* but no CSP-positive mosquitoes were detected in west/coastal populations (N= 196). The differences in anthropophily and sporozoite rates found between east and west/coastal populations suggest that the genetic partitioning within *A. gambiae* is likely to have an impact on malaria transmission in the country.

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ELIZABETHKINGIA ANOPHELIS: MOLECULAR MANIPULATION AND INTERACTIONS WITH MOSQUITO HOSTS

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The microbiota of the mosquito gut can profoundly influence metabolism, fecundity, development and immunity of the mosquito host. Further, this microbiota may through natural processes or by paratransgenesis provide a promising method to constrain malaria parasite development. In this study, we used the bacterial commensal *Elizabethkingia anophelis* from *Anopheles gambiae* as a model to study its interaction with host mosquitoes. A genetic manipulation system involving plasmids, selectable markers, a reporter system, and transposons was newly developed for an *Elizabethkingia* strain isolated from our laboratory colony of *An*. *gambiae*. A replicable plasmid carrying the antibiotic resistance gene *ermF* was efficiently introduced into *Elizabethkingia* by conjugation from *E. coli*, resulting in erythromycin-resistant colonies. Plasmids from *Elizabethkingia* were successfully transferred to *Elizabethkingia* by electroporation, but transformation was at low frequency with the same plasmids and an *E. coli* donor, suggesting the presence of a restriction barrier. The transposon pMiniHimar-Em1 was conjugatively introduced into *Elizabethkingia* from *E. coli*. It transposed randomly, resulting in Em-resistant colonies; transposition efficiency was improved by modifying transposase promoter activity. A strong flavobacterial expression system based on promoter P*ompA* was engineered into pMiniHimar and adapted to *Elizabethkingia*. A GFP- and Nanoluc- tagged *E. anophelis* strain fed to larvae of *Anopheles gambiae* and *Aedes triseriatus* showed transtadial persistence and propagative growth in the *An. gambiae* gut environment but not in *Ae. triseriatus*, indicating that *Elizabethkingia* has a limited host range. Paratransgenesis potential of *Elizabethkingia anophelis* will be discussed.

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INFLUENCE OF DENGUE VIRAL TITER ON *AEDES AEGYPTI* BEHAVIORAL RESPONSE TO DEET

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Mosquito behaviors are heavily driven by odor cues within the surrounding environment. These cues are recognized by chemosensory receptors. Previous studies have shown that arboviral infections can alter mosquito behavior based on dysfunction of mosquito organs, particularly those of the nervous system.. Previously we have reported on the behavioral responses of DENV-1 infected mosquitoes exposed to DEET in the HITSS contact irritancy (CI) on 1, 4, 7, 10, 14, and 17 days post-injection (DPI). Here, we explore the association between dengue virus-1 (DENV-1) RNA copy in mosquito heads and their corresponding CI response in time series after infection. Viral RNA copy of individual head-preps of each DPI cohort are being used in reverse transcriptase RT-PCR to quantify viral RNA copy in both responders (irritated by DEET) and non-responders (no irritation upon exposure to DEET). Time to viral RNA copy plateau and, more importantly, differences in viral RNA copy between the responders and non-responders on any day post injection will be presented. Data will be used to determine the correlation between viral RNA titer with *Aedes aegypti* response against DEET. Findings will enhance our understanding of the potential attenuation in efficacy of chemical products designed to reduce the probability of human contact with infected vectors - a vital component for prevention of dengue virus transmission. Data collection will be completed by July 2014.

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EXPERIMENTAL EVIDENCE AND EMPIRICAL PROOF FOR CONTROL OF *PHLEBOTOMUS PAPATASI* SAND FLIES (DIPTERA: PSYCHODIDAE) USING A FEED-THROUGH LARVICIDAL RODENT BAIT WITH A BUILT-IN VALIDATION SCHEME

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Leishmaniasis is a neglected tropical disease for which very little can be done on an operational scale to reduce sand fly vector populations, parasite transmission, or the incidence of disease. Previously, control of sand fly larvae has not been an option because little is known about the larval ecology of most species, and because no reliable larval sampling method exists. Recently, however, stable isotope analysis of adult sand flies demonstrated that half of the sand fly larvae at a site in eastern Morocco developed to the adult stage on rodent feces. Previous lab studies have shown that rodent diets containing insecticides that pass into the feces effectively kill sand fly larvae. We report the results of a small-scale field trial on the use of feed-through larvicidal rodent baits to reduce sand fly populations. Half of the study sites received insecticidal rodent baits and half received untreated baits. Rather than solely measuring changes in the adult sand fly population, both the insecticidal and untreated rodent baits were co-formulated with a fluorescent tracer dye that passes into rodent feces, and marks both the sand fly larvae that feed on the feces and the subsequent adults. This tracer system provided a crucial entomological indicator: the proportion of adult sand flies that had fed as larvae on the feces of baited rodents. We observed significant reductions in the adult sand fly population and, through the use of the tracer, had empirical evidence for a causal link between the lower number of adult sand flies captured and the efficacy of the insecticidal rodent bait.

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A QUALITY MANAGEMENT SYSTEM FOR *ANOPHELES* INSECTARIES IN FDA-REGULATED STUDIES IN MALI

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Human feeding studies using endemic vectors for arthropod-borne diseases have become a cornerstone for evaluating novel interventions such as vaccines, drugs, and repellants. Potential barriers to evaluating these interventions in endemic settings include: ensuring the fitness and safety of the vectors, and competency of regulatory sciences by entomological staff, particularly when involving studies overseen by the U.S. Food and Drug Administration. As part of a Phase 1 trial of a *Plasmodium falciparum* transmission-blocking vaccine (Pfs25), we have created a Quality Management System for our *Anopheles* insectary in Mali. This QMS is based on the tenets of Good Laboratory Practices and Good Manufacturing Practices including: standard operating procedure improvement and harmonization, training and competency assessment, quality control & assurance, and rigorous documentation practices. Our efforts to ensure tightly regulated processes for the continuous production of high-quality, safe vectors for human studies may benefit other institutions involved in the entomological components of interventional studies.

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POPULATION DYNAMICS OF *AEDES AEGYPTI* AND *ALBOPICTUS* IN NEW ORLEANS, LA, 2009-2013

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Given the recent identification of autochthonous cases of dengue virus (DENV) in Texas and Florida and the apparent introduction of chikungunya virus (CHIKV) to the western hemisphere, it is reasonable to suspect that the viruses will eventually reach endemicity in the New Orleans, LA area, where two competent vector species, *Ae. aegypti* and *albopictus*, have established abundant populations. To investigate their population dynamics, oviposition cups were used to solicit eggs, larvae, and pupae in various areas in New Orleans, LA between 2009 and 2013. Samples are related to remotely-sensed vegetation indices and to meteorological covariates by the gamma distributed lag model of Schmidt (1974). We conclude that the two species respond systematically but differently to environmental covariates, namely temperature and precipitation, and that different weather scenarios imply predictable differences in the risk to humans of these two viruses. A paradoxical result is identified, implying that oviposition cups have a methodological bias that must be understood in practice.

1373

OCCURRENCE OF PAROUS FEMALES IN COPULA IN NATURAL SWARMS OF *ANOPHELES GAMBIAE* S.L.: EVIDENCE FOR RE-MATING BETWEEN GONOTROPHIC CYCLES

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The mating behavior of mosquito disease vectors has important implications for the implementation of novel approaches to vector control, such as the sterile insect technique (SIT) and the release of genetically-modified mosquitoes. From 2006 to 2009, the natural swarming and mating behavior of *Anopheles gambiae* s.l. was investigated in two sites near Bobo-Dioulasso, Burkina Faso. The gonotrophic status, insemination rate and parity rate of indoor resting and swarming (pairing and single) female *An. gambiae* s.l. were determined. We report the presence of parous *An. gambiae* s.l. females mating in natural swarms. The parity rates of mating females, swarming single females and indoor-resting females were, respectively, 5.0% (30/606), 4.1% (21/517) and 16.9% (239/1416). Because females lay eggs only when inseminated, these observations indicate that re-mating can occur between gonotrophic cycles in *An. gambiae* s.l., the major vector of malaria in Sub-Saharan Africa.

1374

COMPARATIVE ABILITIES OF MICROFILAREMIC VERSUS NON-MICROFILAREMIC BIRDS TO INFECT *CULEX PIPIENS* MOSQUITOES WITH WEST NILE VIRUS

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Vertebrate reservoirs of arboviruses are often infected with microfilariae (MF). Previous laboratory studies have shown that MF can enhance the infectivity of arboviruses to mosquitoes. Soon after being ingested, MF penetrate the mosquito midgut. If the blood meal also contains virus (i.e., the vertebrate reservoir is dually-infected), penetrating MF may introduce virus into the hemocoel. This can transform otherwise virus-incompetent mosquito species into virus-competent species and simultaneously accelerate viral development, allowing mosquitoes to transmit virus sooner than normal. This phenomenon is termed microfilarial enhancement of arboviral transmission. Because the prevalence of MF is very high in many passerine populations in North America, we investigated if microfilarial enhancement by microfilaremic passerines could have facilitated the spread of West Nile virus (WNV) across the USA. To do this, we injected two groups of Common Grackles with WNV; one group possessed naturally-acquired infections of Chanderella quiscali MF (n=6), and one group did not have MF infections (n=4). Different batches of *Culex pipiens* mosquitoes were allowed to feed on these birds during the next two to three days and at various time points thereafter (days 3, 4, 5, 7 and 14), mosquitoes were tested by plaque assay to determine rates of WNV infection (i.e., increased vector competence) and dissemination (i.e., decreased extrinsic incubation period). At the time of this writing (April 2014), final data are still forthcoming and will be presented at the meeting.

1375

REDUCING *AEDES ALBOPICTUS* HUMAN LANDING RATES IN ITALY THROUGH INNOVATIVE MOSQUITO TRAPS

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Since its introduction and establishment in Italy during the early 90s, the Asian tiger mosquito has spread over large parts of Italy and other Mediterranean countries. *Aedes albopictus* is not only a cause of biting nuisance but also a competent vector for various arthropod-borne diseases. Conventional attempts to control *Ae. albopictus* include source reduction, larvicides and adulticides. Although efficient traps for *Ae. albopictus* exist and are used for population monitoring, their use as a control tool has not been extensively studied. In this study, we assessed the ability of BG-Sentinel mosquito traps to control local populations of *Ae. albopictus* over a 15-week period in Cesena, Italy. Six experimental sites were matched and paired for the criteria of urbanization level, surface vegetation and mosquito density. In each pair, one site was selected as an intervention site and treated with 7-8 traps. The other site was designated as a control site and did not receive traps. Trap density ranged from one trap per 150 m^2 to one per 300 m^2. Mosquito populations in both the intervention and in control areas were monitored weekly with human landing collections and ovitraps. Results from human landing collections indicated biting rates were reduced between 60 and 90% in the treatment areas compared to the untreated control sites. These results indicate that the sustained use of efficient mosquito traps can significantly reduce the nuisance caused by *Ae. albopictus* in residential areas.

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DATA-DRIVEN MODELING FOR RECEPTIVITY AND SPREAD OF THE HIGHLY INVASIVE MOSQUITO, *AEDES ALBOPICTUS*

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The mosquito, *Aedes albopictus*, is among the world's most invasive species. Its spread has been facilitated by rapid global transport of cargo and potentially by climate warming, and it is now established on every continent except Antarctica. This species represents a "triple threat" to human health, being a day-biting pest, a competent vector of globally important dengue and chikungunya viruses, and a potential bridge vector of several zoonotic arboviruses. As a result of its importance, the biology of *Ae. albopictus* is also well-studied, but the fine-scale processes by which it becomes established in a given location are poorly understood because even intensive surveillance systems yield limited information during the early phase of invasions when densities are low, and detection often occurs after populations are relatively widespread. Fine-scale spatial models for mosquito dynamics and movement offer a way forward, marrying our understanding of *Ae. albopictus* biology with surveillance paradigms and detailed data on the real landscapes where invasions occur. Here, we consider the ongoing invasion and establishment of *Ae. albopictus* in Los Angeles since late 2011. We use hierarchical modeling with remote sensing and surveillance data from the study area to account for heterogeneities in household-level receptivity, then we model the stochastic dynamics of *Ae. albopictus* on this landscape using the suitability surface and a temperature-dependent, dynamical model for reproduction and spread. We found the probability of establishment to be much greater for introductions of eggs in containers compared to single adult females that might arrive by automobile. We also show that the rate of spread was strongly seasonal and greatest during late spring and summer, and the ability to contain the mosquitoes' spread diminished rapidly with increasing delays to detection, regardless of the control methods used.

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VILLAGE EDGE ASSOCIATION, DIEL FLIGHT ACTIVITY AND HOST SELECTION PATTERNS OF MALARIA VECTORS IN VILLAGES OF MADANG PROVINCE, PAPUA NEW GUINEA

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The diel flight and host seeking behavior of females of 5 species of *Anopheles* mosquitoes was studied in 4 malaria-endemic villages of Madang Province, Papua New Guinea, using a vertical barrier screen sampling system, during May to August, 2012. The screen consisted of shade cloth configured to posts and erected vertically to a height of 2.5 meters. It captured both host seeking and blood fed individuals throughout the night. More non-blood fed females were captured on the side of the screen facing the bush, earlier in the evening, whereas more blood fed females were captured on the village side of the screen later in the evening to early morning. These results suggest commuter behavior of host seeking females from outside to inside the village nightly, followed by village exiting behavior back to the surrounding bush. Host identification of blood meals by sequencing of the mitochondrial cytochrome B gene revealed that humans and domestic pigs were the most common and often only hosts, even though other potential vertebrate hosts were present in abundance. *An. punctulatus* and *An. koliensis* were highly anthropophagous, *An. farauti* s.s, *An. longirostris*, and *An. farauti* (species 4) relatively less so, whilst *An. bancrofti* fed mostly on pigs. The implications of these findings for malaria transmission are discussed with reference to the human blood index.

1378

PRE-CLINICAL EVALUATION OF A COMBINED LIVE ATTENUATED (LAV) AND SUBUNIT (DEN-80E) PRIME-BOOST VACCINE APPROACH AGAINST DENGUE

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Merck & Co is developing a tetravalent recombinant subunit vaccine for dengue. The current vaccine candidate (referred to as V180) consists of four truncated, soluble dengue envelope protein (DEN-80E from DENV1-4) produced in Drosophila S2 cells and administered with a saponin-based adjuvant, ISCOMATRIX™ adjuvant. In previous non-human primate studies V180 was able to (a) induce a balanced immune response across all 4 types and (b) protect against virus challenge in rhesus macaques. It is currently the subject of a Phase I trial in flavivirus naïve subjects. While the inclusion of a novel adjuvant in V180 appears necessary to induce a rapid robust response, it may also complicate the development path. In contrast, live attenuated viral (LAV) candidates typically have good immunogenicity, memory/durability, and favorable CoGs but may be complicated by interference, under/over-attenuation, and/or extended dosing schedules. The recently reported poor efficacy of the chimeric dengue vaccine against DENV2 in a Phase II trial may also suggest that the induced titers may not be sufficient to provide protective efficacy in the field. For this reason, we have conducted rhesus macaque studies in which the tetravalent DEN-80E vaccine (with or without the use of ISCOMATRIX™) is combined with a tetravalent LAV in a heterologous prime-boost immunization regimen.

The objective is to optimize the neutralizing titers across all 4 types for both magnitude and longevity. Immunological data on the heterologous and homologous prime-boost vaccinated monkeys will be presented. The combined use of live/non-live vaccine immunogens has the potential of being an effective vaccine approach against multiple dengue types.

1379

COMPARISON OF PASSIVE AND SENTINEL-ENHANCED DENGUE SURVEILLANCE SYSTEMS IN PUERTO RICO

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Dengue represents an increasingly important global and public health challenge; however, current passive surveillance systems underestimate the true burden of disease. In 2009, the World Health Organization urged countries to implement sentinel-enhanced surveillance to characterize the epidemiology of dengue to better evaluate new prevention methods. To estimate underreporting of dengue via passive surveillance in Puerto Rico, we analyzed the epidemiologic trends of suspected dengue cases reported to the long-running island-wide passive dengue surveillance system (PDSS) and compared them to those obtained from cases identified by a hospital-based Sentinel Enhanced Dengue Surveillance System (SEDSS). Dengue diagnostic testing for both PDSS and SEDSS includes RT-PCR to detect dengue virus (DENV) nucleic acid and ELISA to detect anti-DENV IgM antibody. Analyzed data were collected from PDSS and SEDSS in the Ponce health region between May 7, 2012 and May 6, 2013, the first year of operation of SEDSS. Of 3,483 suspected dengue cases reported to PDSS and 2,027 cases identified by SEDSS, 1,444 (41.5%) and 621 (30.6%) were laboratory-positive dengue cases, respectively. Compared to dengue cases reported to PDSS, those identified by SEDSS were younger (25 years vs. 19 years; $p < 0.0001$), presented for care earlier after illness onset (3 days vs. 4 days; $p < 0.0001$), were hospitalized less frequently (46% vs. 64%; $p < 0.0001$), and demonstrated higher completion of demographic and clinical variables for the case investigation form (61% vs. 27%; $p < 0.0001$). There were no significant differences in other demographic variables or DENV type distribution between SEDSS and PDSS. This evaluation demonstrated that SEDSS provides more robust clinical information and more accurately identifies non-hospitalized patients, though it may bias toward younger individuals. Enhanced dengue surveillance should be implemented in other locations of the world to complement existing passive surveillance systems to better understand the epidemiology and burden of dengue.

1380

WHAT PROPORTION OF DENGUE VIRUS INFECTIONS RESULT IN NO APPARENT DISEASE?

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The concept of the dengue iceberg is a well-known one; a large proportion of dengue virus infections result in minimal or no symptoms and are thus never reported to surveillance systems. However, there is no consensus on what this proportion is and estimates in the literature vary substantially. We define infections as either apparent or inapparent. Though the definition of an apparent infection may vary depending on the study methodology, this is generally a dengue virus infection that results in overt illness, symptoms or healthcare seeking. An inapparent infection is one in which individuals have a serological response consistent with infection, but no accompanying illness (as defined above). Estimates in the literature of the proportions of dengue virus-infected people who experience apparent or inapparent infections vary widely. Though some of this variation may be due to differential definitions, there is some evidence that these proportions depend not only on whether the infection is a first, second or post-second infection, but also on infecting serotype (and in some cases genotype) and on the age of the infected individual. Combining published data from dengue cohort studies and from outbreak situations with serological data from multiple settings over multiple years, with consideration of the definitions used in each study, we aim to estimate the proportion of dengue virus infections that are apparent and inapparent. We also aim to estimate the influence of immune history, infecting serotype and age on these proportions. By combining datasets, the similarities and differences between these settings provide increased information about the effect of each of these factors. A Bayesian framework is used, thereby allowing the inclusion of uncertainty in the data and our resulting estimates. Better estimates of the proportions of dengue virus infections that are inapparent will be of use for understanding transmission of dengue viruses in multiple settings. For example, inapparent infections may be contributing to transmission. In addition, inapparent primary infections, though not producing apparent infection and thus not contributing to disease burden, leave individuals primed for secondary infections, so will be important for understanding the future disease burden in a population.

1381

CLINICAL COURSE AND OUTCOME OF DENGUE INFECTION DURING PREGNANCY

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Dengue, a major mosquito borne infection in the tropics, is hyper-endemic in Sri Lanka since 2009 with an annual incidence of more than 25,000 cases. The maximum rate of infection is seen in the 20-39 age category, making the pregnant population a vulnerable category.A retrospective observational study was conducted on all pregnant patients with Dengue admitted during 2013 to two major hospitals in Colombo, Sri Lanka. Data including clinical & laboratory parameters, interventions made, and complications were documented for analysis.Dengue infection was confirmed in 58 patients admitted. Mean age was 28.45(SD: 5.573) yrs. 55.2% had Dengue fever (DF) while 44.8% had Dengue Haemorrhagic fever (DHF). 19%, 46.6% and 34.5% were in the first, second and third trimester respectively. 82.8% had Rh positive blood groups, with 27.6% B positive, 25.9% O positive, 20.7% A positive and 8.6% AB positive while in 3.8% the blood group was not known. All had fever and 86.2% had myalgia. Hepatic tenderness, persistent vomiting and postural dizziness were seen most commonly with DHF (81.8%, 100% & 70% respectively). Mean day of entry into critical phase was 4.5 (SD: 0.990) day of the illness. The mean lowest platelet count in DF was 90.94 while in DHF was 37.81 ($p<0.000$), which was observed on a mean day of 5.25 in DF & 6.04 in DHF ($p< 0.03$). Most of the DF patients (31.2%) had highest AST levels in the 32-100 range while most of the DHF patients (34.6%) had levels in the 501-1000 range. Most of the DF patients (34.4%) had normal ALT level whereas most of the DHF patients (38.5%) had highest ALT in the 101-300 range. 3.1% of DF and 7.7% of DHF patients had fetal distress while 3.1% of DF and 3.8% of DHF patients had intrauterine death (IUD). 52% of all patients needed HDU/ICU care. All the patients recovered completely. This study shows incidence of DHF is higher in pregnancy than in the normal population. High numbers of patients with Rh positive blood groups were among pregnant Dengue patients. Some parameters like low platelet count, high AST and ALT were significantly high in DHF, indicating these can be used to identify high risk groups for developing DHF. Careful management assures full recovery of mothers, however, adverse outcome on the fetus remains high.

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USE OF A HOUSEHOLD SEROSURVEY TO ESTIMATE THE MAGNITUDE OF A DENGUE OUTBREAK IN MOMBASA, KENYA, 2013

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Dengue is endemic in Africa where an estimated 64 million dengue virus (DENV) infections occurred in 2010. Few outbreaks have been reported from East Africa since dengue was first detected in the late 1800s, and the geographic distribution of infections is uncertain. In February 2013, several individuals with dengue-like illness and negative malaria blood smears were identified in Mombasa, Kenya. Serum samples from initial cases confirmed dengue, and an investigation was conducted to determine the incidence of DENV infection in Mombasa. A stratified multistage sample of households was selected from the Tudor community of Mombasa where the incidence of reported dengue was high. Household residents provided serum specimens and information on medical and travel history. Serum was tested for DENV nucleic acid by RT-PCR, NS1 ELISA (i.e., current DENV infection), and anti-DENV IgM antibody by ELISA (i.e., recent DENV infection). Design-based estimates incorporated probabilities for selection of households and used a finite population correction factor. Of 1,502 participants living in 701 households, 207 (14%) had evidence of current (n = 103) or recent (n = 104) DENV infection, with a design-based estimate of 13% (95% CI: 10-16). DENV-1 and -2 were detected equally. Of the 207 participants with evidence of DENV infection, 91 (44%) reported fever in the past month; three (1%) were hospitalized; and two (1%) had bleeding manifestations. Reporting a fever in the past 30 days was significantly associated with DENV infection (OR=2.8; CI 1.9-4.2). Reporting open windows at nighttime was a risk factor for infection (OR=2.3; CI 1.1-4.8). Daily use of mosquito repellent daily was protective from infection (OR=9.1; CI 3.7-20.0). This investigation revealed a high burden of dengue in this part of East Africa. Behavioral strategies to avoid mosquito bites should be advocated for individuals to avoid DENV infection. Surveillance for and clinical and public awareness of dengue should be improved in East Africa to reduce the morbidity and mortality due to this disease.

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DEVELOPMENT OF A NS1AG-ELISA FOR THE DETECTION OF ALL DENGUE 1 TO 4 TYPES

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Dengue is the most important arthropod-borne viral disease in tropical and subtropical areas of the world. It is a public health concern in the Americas, including the US, because it is endemic in Puerto Rico and caused outbreaks in Florida, Texas, Hawaii, and in other US islands. The infection is mainly transmitted by the mosquito *Aedes aegypti*. Dengue diagnosis is commonly made using MAC-ELISA which indirectly detects specific antibodies to the E protein of the Dengue virus (DENV). Direct detection by virus isolation in culture and reverse transcriptase-polymerase chain reaction (RT-PCR) is used less frequently due to time, limited availability and high cost. An attractive alternative to culture and molecular tests is the detection of NS1 in the sera of patients during the acute phase of dengue. This viral protein is produced and secreted by infected human cells in the early stages of infection. Unfortunately, NS1 assays currently available have low sensitivity and specificity, and have been reported to miss DENV-4. In order to address this issue we developed an ELISA-based NS1 antigen assay (NS1Ag-ELISA) that uses two monoclonal antibodies, selected among 10 commercially available, that recognizes all DENV1-4 NS1 proteins. Initially, we were tested: (a) 130 specimens positive for DENV RNA and/or IgM (29 DENV-1, 4 DENV-2, 7 DENV-3, 16 DENV-4 and 54 not typed), all produced positive results on the NS1Ag-ELISA. (b) 75 DENV-negative specimens (19 West Nile Virus (WNV) positive, 6 Yellow Fever Virus (YFV) positive and 50 negative). 66 tested negative while 9 (8 WNV-positive and 1 YFV-positive), tested negative for DENV by RT-PCR but produced false positive results in our assay, possibly due to cross-reactivity between Flavivirus NS1 under the current assay conditions. We are modifying the assay to enhance DENV NS1 specificity and reduce cross reactivity. We expect to have in a few months a robust diagnostic test for the acute phase of dengue infection that may help early identification of serious dengue disease and facilitate critical care.

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SPATIAL CLUSTERING OF DENGUE AT THE HOUSEHOLD LEVEL IN A HIGHLY URBAN SETTING

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In the absence of a vaccine or therapeutic, the only strategy currently available for dengue prevention is control of the mosquito vector *Aedes aegypti*. In many endemic countries, including Vietnam, this is pursued primarily by responsive insecticide spraying around homes of reported cases. Central to this approach is the assumption that most DENV infections are acquired in the home. Evidence of highly focal DENV transmission at household level has been demonstrated in a rural village setting in Thailand, but it is unclear whether this assumption is valid in highly urban, mobile populations. We conducted a community-based study to investigate clustering of dengue risk around households in Ho Chi Minh City, Vietnam. We enrolled clusters of 25-35 household members and neighbours living within 25 metres of an index case with clinically suspected dengue. Laboratory diagnosis of the index cases allowed us retrospectively to classify them as confirmed dengue cases (n=52) or non-dengue controls (n=19), and to calculate the relative risk of 1) incident DENV infection during a two-week follow up period and 2) recent DENV infection at baseline in case clusters compared to controls (representing background risk). There was no difference in the risk of incident DENV infection between case and control clusters (82 in 1341 participants (6.1%) vs 31/569 (5.4%), respectively). However participants in case clusters were significantly more likely to have had a recent DENV infection at baseline than those in control clusters (OR = 2.3; 95%CI 1.2-4.7). The prevalence of DENV-infected *Ae. aegypti* collected from index houses was low overall (1%), with no difference between cases and controls, however case houses were significantly more likely to have high adult vector densities than controls. Our findings show that although there was clustering of recent DENV infections around households, there was no excess of incident infections in the two weeks following index case detection; this suggests that responsive vector control activities in this window are unlikely to have a large impact on DENV transmission in this setting.

1384

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EPITOPE PRESERVATION, IMMUNOGENICITY AND PROTECTION OF INACTIVATED DENGUE VIRUS ANTIGENS FORMULATED WITH A NOVEL BIOLOGICAL ADJUVANT IN RHESUS MACAQUES

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Dengue viruses (DENV1-4) are considered the most important emerging, human arboviruses with worldwide distribution in the tropics, yet there are no licensed antiviral therapies or vaccines available. Although there are live attenuated virus vaccine candidates in clinical trials, there is an urgent need to accelerate the development of second-generation vaccine strategies. We developed a dengue vaccine based on a purified, inactivated virion (iDV) mixed with a novel alphavirus adjuvant (GVI3000/3A). The GVI adjuvants are disarmed viruses that derive their activity from the replication of a truncated alphavirus RNA. *In vivo*, the GVI adjuvants target DC in the DLN and mimic the earliest stages of a viral infection. The antigenic integrity of purified dengue virus antigens (iDV) was determined after inactivation by different protocols. A panel of mouse and human MAbs were used as probes to confirm the preservation of conformational epitopes in different domains of E protein, including recently characterized serotype specific, strongly neutralizing human MAbs that map to epitopes only preserved in the quaternary structure of the virion. Safety, immunogenicity and protective efficacy of GVI-adjuvanted iDV were determined in rhesus macaques, comparing 3 adjuvant doses and 2 adjuvant modalities. A tetravalent iDV mixture formulated with a GVI adjuvant demonstrated 1) significant increases in neutralizing antibody titers, 2) protection from viremia, and 3) no adverse events in any of the vaccinated animals. These results support the advancement of this new dengue vaccine candidate toward clinical trials in humans.

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THE DETECTION OF ANTI-DENGUE VIRUS IGM IN URINE AS A PUTATIVE MARKER FOR SEVERE DISEASE

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Dengue is globally the most important arbovirus disease with an estimated 300 million dengue virus (DENV) infections however, only 100 million dengue cases are reported per year. It is estimated that 5-10% of cases result in severe dengue, which may include glomerular changes associated with renal dysfunction. We looked for the presence of anti-DENV IgM antibodies in urine as an indicator for severe dengue among patients identified with acute febrile illness in our Sentinel Enhanced Dengue Surveillance System (SEDDS) site in Ponce, Puerto Rico. Between May 2012-March 2013, 1560 patients with fever or history of fever for ≤7 days were enrolled, a past medical history of chronic illnesses was obtained, and they were followed through their febrile illness. Serum and urine specimens were collected during the acute (days post onset of fever (dpo)=0-5) and convalescent phase (dpo=6-14) of their illnessAcute serum was tested for DENV RNA by RT-PCR . All urine specimens were tested for anti-DENV IgM. The results from the urine anti-DENV IgM were compared to the results in serum to determine sensitivity and specificity. The sensitivity of urine anti-IgM was 37% and specificity was 98% compared to serum. When compared to serum RT-PCR results, the sensitivity of IgM in urine was 24% and the specificity was 93%. To determine if IgM in urine might be an early indicator of disease severity, we compared this result to patient hospitalization; hospitalization being used as a surrogate for disease severity. Hospitalized dengue patients were 3.2 times more likely to test positive for IgM in urine than IgM negative (OR = 3.2 95CI 4.9-2.2).There was no correlation between the presence of IgM in urine with sex, age or pre-existing chronic diseases such as diabetes, high blood pressure, or anemia. While detection of anti-DENV IgM in urine lacked adequate diagnostic sensitivity when compared to serum, its presence may be a marker for hospitalization or disease severity.

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DEVELOPMENT OF DENGUE VIRUS PRM-REACTIVE ANTIBODIES AS TOOLS FOR MEASURING THE VIRION MATURATION STATE OF INFECTIOUS VIRIONS

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Newly formed dengue viruses (DENV) incorporate envelope (E) proteins in complex with the viral structural protein premembrane (prM) as heterotrimeric spikes. During egress from infected cells, prM is cleaved to pr and M protein by host furin-like proteases to produce infectious virions. However, we and others have shown that this process is inefficient and leads to the release of partially mature DENV that retain non-cleaved

prM and have significantly different functional properties. The extent of prM cleavage required for production of an infectious virus is unknown. In this study, we characterized a panel of murine monoclonal antibodies (mAbs) that bind prM produced by immunization with recombinant pr protein. prM-reactive mAbs were extensively cross-reactive and shown to be capable of enhancing DENV infection of Fc-receptor-expressing cells. Several prM-reactive antibodies displayed a significant capacity to neutralize infection, although this pattern was cell type-dependent. Examination of neutralization dose-response curves on Raji cells expressing DC-SIGNR revealed the presence of a fraction of virions resistant to neutralization; the size of this population could be varied by altering the efficiency of the virion maturation process. We conclude that in this context, viruses resistant to neutralization are those that display prM epitopes with a stoichiometry insufficient to satisfy the threshold requirements for neutralization. We demonstrate the potential for prM-reactive antibodies as a sensitive functional probe of the maturation state of DENV released from cells, providing a method to deconstruct the structural heterogeneity of DENV produced under a variety of conditions.

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COSTS OF DENGUE FEVER TO THE HEALTH SYSTEM AND INDIVIDUALS IN COLOMBIA IN 2010 TO 2012

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Dengue fever is an important health issue in Colombia but detailed direct information on economic costs is lacking. We estimated the average cost per case of ambulatory dengue fever (aDF), hospitalized dengue fever (hospDF) and dengue hemorrhagic fever (DHF) over the period. Tallied costs included direct and indirect medical costs, as well as non-medical costs to the healthcare system, and indirect costs to patients, using information from official databases and an extensive population-based face-to-face survey of 1,089 households with recent dengue fever patients. In 2010, the mean direct medical cost per case for the healthcare system of aDF, hospDF, and DHF were, respectively $52.8USD, $235.8, and $1,512.2. To the individuals, the mean direct non-medical costs ($29.7, $46.7 and $62.6, respectively) greater than the mean household direct medical costs ($13.3, $348 and $57.3, respectively). The average cost to the healthcare system of a case of ambulatory dengue fever in the epidemic year of 2010 was 57% that in 2011.Our results highlight the high economic burden of the disease and could be useful for assigning limited health resources..

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INNATE IMMUNITY AND TRANSCRIPTOME PROFILING AFTER ADMINISTRATION OF TAKEDA'S LIVE ATTENUATED DENGUE VACCINE CANDIDATE IN FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS: ASSOCIATION OF GENE EXPRESSION WITH DEVELOPMENT OF NEUTRALIZING ANTIBODY RESPONSES TO DENV

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We conducted a phase I, randomized, double-blind dose-escalation study of two different formulations of Takeda's live attenuated dengue vaccine candidate in 72 healthy flavivirus-naïve adults at the Saint Louis University VTEU (NCT01110551). Volunteers received 2 doses of a live attenuated tetravalent dengue vaccine candidate 90 days apart. Samples of whole blood were collected on days 0, 2, 4, 7, and 11 after vaccination to assess early responses to the vaccine by transcriptome analysis. Total RNA was isolated from whole blood and T7 transcribed-linear RNA was amplified and hybridized to Illumina Human HT12 v 4 microarrays. These microarrays detect all mRNAs expressed from the human genome. RNA expression data was exported and analyzed by genesets using the canonical pathways stored within the MSigDB database. No significant changes in geneset expression were identified that correlated with: 1) route of vaccine administration (SC vs. ID), 2) viremia after vaccination, or 3) neutralizing antibody titer (above or below the group mean on day 120 after vaccination). However, there were significant differences in geneset expression in subjects who developed a tetravalent neutralizing antibody response to DENV vs. subjects who had a mono/bivalent response. Subjects with a tetravalent response had at least a 1.5-fold increase in expression of genesets involved in integrin signaling, the complement pathway, interferon signaling, cytokine expression, and innate immune responses. In summary, increased expression of genesets which mediate the innate immune response and translation to adaptive immunity was significantly correlated with a tetravalent neutralizing antibody response to Takeda's live attenuated dengue vaccine candidate.

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IMPORTATION FOLLOWED BY LOCAL TRANSMISSION OF TWO LINEAGES OF DENGUE VIRUS TYPE 1 IN THE UNITED STATES: 2013

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Dengue, one of the most important arthropod-borne tropical diseases globally, is a significant public health concern affecting an estimated 100 million people in 2010. Dengue is caused by any of the four genetically related dengue viruses (DENV-1-4) that are maintained by transmission in large urban areas by *Aedes* mosquitoes. The proliferation of urban areas, frequent international travel and climatic changes, have been proposed to contribute to the increased dissemination of DENV-1-4. We have previously reported local transmission of a monophyletic lineage of DENV-1 in Key West, Florida in 2011-2012. In 2013, DENV-1 was identified in febrile patients residing in two Texas counties, Cameron and Hidalgo, adjacent to the border with Mexico. These patients reported no recent travel history. DENV-1 was also identified in febrile patients with no recent travel history residing in two Florida counties, Martin and St. Lucie. Identification of patients with laboratory-confirmed DENV-1 continued for time periods of 4-6 months in both locations. In this study we have conducted an in-depth envelope gene sequence analysis to characterize the emergence of DENV-1 in Texas and Florida and their relatedness with viruses from Key West, Mexico, Central America and the Caribbean. All sequences grouped within the American-African genotype of DENV-1. Bayesian phylogenetic analyses show a strong association of Texas and Northern Mexico DENV-1 with viruses from Central America, with the Texas isolates forming a monophyletic group. In contrast, the Florida isolates formed two independent subgroups: the previously reported Key West virus of Central American origin and the Martin-St. Lucie virus of Caribbean origin. The monophyletic characteristic of these lineages supports local transmission of DENV-1, and that conditions are suitable to sustain transmission with the potential to cause outbreaks.

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DENGUE QUASISPECIES COMPLEXITY ANALYSIS FOR MOSQUITO AND HUMAN SAMPLES FROM KAMPHAENG PHET, THAILAND: CLONING AND IMPLICATIONS FOR HIGH THROUGHPUT SEQUENCING METHODS

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All four serotypes of dengue virus (DENV) exist as quasispecies populations. Quasispecies are described as a spectrum of variants, genetically linked through mutation creating an interactive population where selection acts on the population rather than individual variants. While cloning provides linkage of all population mutations to single genomes; laborious methods are required to adequately sample the quasispecies population. High-throughput sequencing has become the method of choice in reconstructing quasispecies populations; though mutations within the population cannot be linked to single viral genomes. We examined the complexity and mutations discovered in clone-based quasispecies population analysis and compared results with assemblies obtained from the 454 sequencing. The E gene for 4 quasispecies populations from DENV-3 from a 2010 study in Thailand were cloned and sequenced using Sanger sequencing. Full genomes were obtained using 454 sequencing and E gene mutation comparisons with clones were conducted. The cloned populations, explored sequence space in several directions with transmission of dominant and subdominant variants (3-6 subdominant variants) and varying degrees of complexity. The percent of variants within the populations containing variable nt sites ranged from 68.9-77.6% (a.a. 46.7-57.1%) suggesting high population plasticity. Hinge region and non-functional variants were found in cloned populations however not in 454 assemblies due to low coverage. As read depth increased the probability of detecting cloned mutations increased. Full genome assemblies showed other potentially transmissible quasispecies mutations. Investigating dengue quasispecies diversity and behavior has relevance for understanding population responses to selective pressures such as innate and vaccine induced immunity. Work to investigate quasispecies population dynamics and complexity during illness and *in vivo*/vitro cycling using the MiSeq and PacBio systems to achieve high coverage and linkage of mutations to genomes within the population are underway.

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REAL-TIME FORECASTING OF THE 2014 DENGUE FEVER SEASON IN THAILAND

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Dengue is a major cause of morbidity in Thailand. Annual outbreaks of varying sizes provide a particular challenge to the public health system because treatment of severe cases requires significant resources. Advanced warning of increases in incidence could help public health authorities allocate resources more effectively and mitigate the impact of epidemics. In collaboration with the Thai Ministry of Public Health and Bureau of Epidemiology, we have developed a statistical model for infectious disease surveillance that uses data from across Thailand to give early warning of developing dengue epidemics. The model creates forecasts for each of the 77 Thai provinces. For each province, the forecast is based on (1) seasonal dynamics of dengue in the focal province, and (2) observed case counts at recent time-points from the focal province and neighbors demonstrated to be relevant through model selection using historical data. Prior to the beginning of the 2014 dengue season in Thailand, our team defined a process to generate forecasts for dengue in real-time. Beginning in April 2014, we created updated forecasts every two weeks based on the most current data from the Thai Ministry of Public Health database. We will present the results of this real-time forecasting exercise, including evaluating the performance of different forecasting models in predicting different features of the 2014 dengue season in each Thai province. Specifically, we will evaluate the ability of our models to predict the beginning, end, duration, and peak of the dengue epidemic. To our knowledge this is the first time that real-time forecasts of dengue have been attempted in Thailand based on reported case data.

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EXPLORING THE IMPACT OF INDIVIDUAL MOSQUITO SALIVARY PROTEINS ON DENV INFECTION IN THE VERTEBRATE

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Dengue virus (DENV) is transmitted during probing by an infectious mosquito concurrent with expectorated saliva. This saliva is composed of numerous proteins with anti-hemostatic and immuno-modulatory capabilities, and was shown previously to aid viral establishment within the vertebrate. IRF3/7 -/--/- (C57BL/6) mice intradermally-inoculated with DENV at sites of contemporaneous mosquito probing exhibited viremias of significantly enhanced magnitude and duration compared to mice unexposed to mosquitoes. This mosquito-driven enhancement was associated with differential regulation of immune transcripts involved in viral recognition and defense at early times post exposure. However, limited work exists on the relationship between individual *Aedes aegypti* salivary proteins and vertebrate infection with DENV. In an effort to characterize the contribution of individual salivary proteins to the enhancement of DENV infection, we have utilized recombinant salivary proteins for examination *in vivo*. One such protein was aegyptin, a known allergen and inhibitor of platelet aggregation. We intradermally-inoculated mice with and without co-inoculation of aegyptin and examined differences in viral titers and circulating leukocytes throughout viremia, along with viral titers and immune parameters at injection sites and draining lymph nodes at 48 hpi. Interestingly, co-inoculation of aegyptin resulted in decreased viral titers at inoculation sites and in circulation at 48 hpi compared to DENV alone, and these decreases were associated with alterations in cytokine concentrations in the lymph nodes of aegyptin-exposed mice. Additionally, co-inoculation of mice that had previously received multiple exposures to aegyptin resulted in further alterations to viremia titers. While co-inoculation of aegyptin did not yield universal enhancement of DENV titers, these results inform on immune pressures faced during DENV infection and support a complex system of interaction between the milieu of salivary proteins expectorated, DENV, and the vertebrate host environment.

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EXTENDING A DETAILED *AEDES AEGYPTI* MODEL TO SIMULATE SINGLE AND COMBINED DENGUE CONTROL IN IQUITOS, PERU

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Attempts to control dengue include cleaning out water containers, poisoning larvae, and spraying insecticide inside homes. Other control measures like vaccines and engineered mosquitoes are being developed. We do not know which of these new control measures might result in the fewest dengue cases, either alone or in combination. We develop a complex simulation model that can compare single and multiple control interventions. This extends a detailed, stochastic model of *Aedes aegypti* population dynamics (Skeeter Buster) to include the movement patterns and infection histories of individual humans. We use this model to study the effect of various control measures on dengue epidemics in the city of Iquitos, Peru. We show how the speed and size of an epidemic varies with the number of places people visit each day. We also show the effects of spraying insecticide in homes, releasing dengue-resistant mosquitoes, and administering vaccinations. We test for single and pairwise combinations of these interventions, but find little evidence of synergistic effects. Our results suggest that combining control measures while making similar total investments may not prevent as many dengue cases as a single control measure.

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ADJUSTING UNDERREPORTED REAL TIME CASE DATA FOR PREDICTION OF DENGUE IN THAILAND

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Symptomatic cases of dengue virus, including dengue fever and dengue hemorrhagic fever, are an important cause of morbidity in Thailand. Thailand has a comprehensive nationwide case reporting system for Dengue and efforts are currently underway to leverage this data for real-time prediction of epidemic severity. Using case data for real-time prediction across a large geographic area that has multiple distinct administrative units is fraught with challenges. A central challenge is that the administrative units that contribute to the larger reporting system as a whole have heterogeneity in reporting processes which results in a substantial and highly variable interval of time (reporting interval) between when a case record is created and when it becomes available to use for prediction. Currently, dengue prediction models must use weeks-old case counts to assure that most relevant data has been processed through the reporting system. A better understanding of the reporting process in different locations could 1) provide metrics for use in optimizing the reporting system, and 2) make it possible to use the most recent incomplete counts for prediction of dengue epidemic intensity. We have developed and applied time-to-event models to characterize the spatial and temporal variation in reporting intervals and their relationship to case load and other seasonal features. Preliminary results suggest that this problem requires the use of contaminated time-to-event distributions to characterize reporting intervals and a hierarchical approach to combining information from diverse administrative units. We will present our analysis on the reporting process over the course of the 2013 and 2014 dengue seasons in Thailand, as well as our methods for improving the usability of real-time case data.

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DENV RNA AND ANTI-DENGUE ANTIBODY INTEGRITY IN CLINICAL SAMPLES ON DRIED BLOOD STABILIZATION PRODUCTS DURING AMBIENT TEMPERATURE SHIPMENT

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Degradation of RNA and antibodies during specimen transport from collection site to diagnostic facility is a major problem affecting accurate diagnosis of RNA-based pathogens. This is particularly true when shipping may require more than a day of transit, as cold-chain is not always available in low-resource settings. In this study, we used dengue as a model RNA virus to compare the performance of three down-selected commercially available nucleic acid-stabilization products: Biomātrica DNAstāble tubes, ViveBio ViveST tubes, and Whatman FTA Micro Cards. Whole blood specimens collected from acute dengue fever patients (Days 0-4 Post Onset of Symptoms) during routine febrile surveillance in Iquitos, Peru were applied to the nucleic acid-stabilization products and dried overnight. At various time points, the stabilized specimens were shipped under ambient conditions (temperatures ranging from 9.7 to 34.3 °C and relative humidity ranging from 53.4 to 74.6% during shipment) to a diagnostic testing laboratory in Lima, Peru. Anti-dengue antibodies and dengue RNA levels were then tested via IgM ELISA and qRT-PCR, respectively, and compared to matched frozen unloaded controls. Agreements compared to each specimen's matched controls were: 97.3% IgM and 97.4% RNA (DNAstāble); 97.4% IgM and 95.0% RNA (ViveST);

and 81.6% IgM and 82.5% RNA (FTA Micro Cards).Other considerations such as cost, sample volume required, and ease-of-use were also evaluated in this study and should ultimately inform any decision to incorporate commercial sample stabilization products into a downstream diagnostic testing workflow.

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CHARACTERIZATION OF A DENGUE VIRUS TYPE 4 OUTBREAK IN SOUTH-CENTRAL MATO GROSSO, BRAZIL

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Dengue viruses (DENV) are by far the most important arboviral pathogens in the tropics globally, putting at risk of infection nearly a third of the global human population. In the current study we characterized the phylogeny and intrahost variation of 26 isolates of dengue virus type 4 (DENV-4) from acute serum samples obtained during an outbreak in South-Central Mato Grosso State (MT), Brazil, in 2012. All 26 isolates located within genotype II in two distinct lineages forming a monophyletic clade. Further confirmation of the co-circulation of two distinct lineages is obtained by analysis of the intrahost virus variation in the acute serum samples. Based on our phylogenetic analyses, there are 6 independent introductions of DENV-4 in Brazil, presumably from Venezuela, Puerto Rico, China, and Southeast Asia. The DENV-4 isolates of the 2012 outbreak in South-Central Mato Grosso State were closely related with two 2010 isolates from the geographically close regions of Amazonas and Roraima and were closely related with strains sampled from Venezuela 2007, indicating the potential origin introduction. The extent and severity of the 2012 DENV-4 outbreak is likely attributed to the lack of immunity in the population.

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CALL TO ACTION: A SCREENING TOOL FOR PREVENTION AND TREATMENT OF DENGUE IN TRAVELERS WITH CHRONIC COMORBIDITIES

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There is increased risk of Dengue Hemorrhagic Fever (DHF) in patients with comorbidities such as hypertension, diabetes, allergies and obesity. Lack of preparedness in epidemics can increase mortality rates amongst these patients when preexisting conditions are not identified and guidelines for case management not followed. In travelers, there is limited evidence for predicting clinical course of dengue in patients with chronic comorbidities. The WHO guidelines for treatment, prevention and control of dengue recommends identification of preexisting conditions and offers guidance for treating patients with obesity. Similar guidance is lacking for hypertension, diabetes and allergies. Acetylsalicylic acid (aspirin), Ibuprofen and other non-steroidal anti-inflammatory drugs (NSAID) are contraindicated for dengue; however, the prevalence of individuals on treatment regimens using these drugs is increasing. In an analysis of Behavioral Risk Factor Surveillance System (BRFSS) 2011 data, 25% of adults take aspirin daily and 31% have hypertension, of which 77% are being treated. In addition, 10%, 13% and 60% of adults had diabetes, asthma or obesity respectively. For travelers to dengue endemic regions it is important to develop and provide guidance for chronic disease management and dengue prevention. Post-travel it is important for medical professionals to have strict guidance on case management for potential dengue complications. A travel screening tool can identify high-risk travelers based on VFR status, Cultural Embeddedness, and social determinants of health in conjunction with comorbidities and current treatment. This tool can be implemented in pre-and post-travel consultation. Overall, this research recommends a call to action for researchers to 1) Develop guidelines for treating dengue patients with comorbidities; 2) Research effect of aspirin and NSAID regimens on dengue pathogenesis; and 3) Increase dengue surveillance and control in regions with high chronic disease prevalence and high population susceptibility to dengue, particularly travelers.

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INFLUENCE OF MATERNAL TOTAL IGG LEVELS ON TRANSPLACENTAL TRANSFER OF DENGUE VIRUS-SPECIFIC ANTIBODIES

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Placental transfer of maternal dengue IgG antibodies to the fetus is likely to play an essential role in immunity and pathogenesis of dengue infection in infants. In order to investigate the kinetics of dengue-specific maternal antibodies transferred to children in the first two years of life, a birth cohort of 417 children living in an area of intense circulation of dengue virus in the northeast region of Brazil has been established. Here, we carried out a preliminary analysis of 216 dengue seropositive mother-newborn pairs to investigate the transference of total and dengue-specific IgG antibodies via placenta. Maternal and umbilical cord blood samples were obtained during the time of delivery. Serotype-specific antibody profile was determined by PRNT, while in-house ELISA was used to both measure dengue-specific IgG titers and estimate the levels of total IgG in the sera. Antibody titers were log-transformed and used to evaluate the degree of dengue-specific IgG transferred from mothers to infants. The average maternal age was 23.8 years (range, 13-41 years). In maternal sera, 127 out of 216 (58.8%) showed a monotypic profile against DENV3, 25.5% to the combination of DENV3/ DENV4 and 9.8% had detectable neutralizing antibodies against three or more serotypes. Dengue-specific IgG titers were significantly higher in cord blood (4.89±0.52) than in maternal samples (4.69±0.52; p=0.0006). A consistent pattern was also observed when comparing DENV3-specific PRNT titers in infants (2.68±0.83) and mothers (2.49±0.66; p=0.0095). Maternal levels of total IgG were negatively correlated with placental transfer of dengue-specific IgG (r= -0.1818, p= 0.0074) and DENV3 neutralizing antibodies (r= -0.1289, p=0.0586), indicating that very high levels of maternal IgG increases competition among the types of IgG transferred through the placenta. These results further suggest that maternal antibody transfer is influenced by maternal total IgG levels.

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CHARACTERIZING GLOBAL AND LOCAL TRENDS IN DENGUE TRANSMISSION: INSIGHT FROM AGE-SPECIFIC SURVEILLANCE DATA

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Good characterization of global and local trends in dengue transmission has been challenging. Given that seroprevalence data, the gold standard to measure prior exposure, is very scarce, previous efforts have relied almost exclusively on total case or presence/absence data. However, while this approach has proven useful to define the distribution of dengue at a global scale, relying exclusively on counts may be misleading when looking at trends over time, or at finer spatial scales, due to the poor correlation that exists between infection and symptomatic disease. Here, we propose a framework to estimate yearly forces of infection (yearly probability of a susceptible individual being infected) and basic reproductive number

(R0) of dengue based on the age distribution of cases that are reported to surveillance systems. We use data from 4 countries where age-specific incidence data is publicly available (Thailand, Brazil, Mexico, Colombia) to estimate the force of infection and R0 over a period of 15 years at the province or, where possible, district levels. When available, we compare our estimates to those obtained from age-stratified serological surveys. Preliminary results suggest that age-specific incidence data provides a robust way to characterize dengue transmission at a global and local scale in settings of varying transmission intensity. In addition, they highlight the large heterogeneities in recent dengue epidemiology that exist within countries, provinces and probably even finer spatial scales. This is particularly true for countries such as Brazil, where dengue has recently re-emerged. Proper characterization of global and local trends in dengue epidemiology will be fundamental to target control interventions and design optimal vaccination strategies.

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MEDICAL COSTS OF DENGUE FEVER IN MEXICO

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In Mexico, dengue fever incidence has varied since its reappearance in 1970s, with peaks in 1980, 1997, and 2009 and >130 000 cases. With high incidences, accurate cost estimates of disease are needed to efficiently use finite treatment and prevention health resources (vaccination and vector control). This study assessed medical cost and cost to the infected individual using a micro-costing approach to overcome a lack of centralized data. The cost per dengue case is derived from health system direct medical costs, patient direct costs, and productivity loss-related indirect costs. Costs were calculated in the SS and the IMSS settings. To derive health system costs, an ideal protocol for dengue fever treatment was based on a review of national and international norms, guidelines, and expert consensus combined with a microcosting tool known as PAATI (program, actions, activities, tasks, inputs). For comparison to real costs, actual tasks and inputs for real dengue fever cases were derived from chart review and health personnel and hospital administrators interviews. Patient direct and indirect costs were derived from patient interviews. Indirect cost was defined as disease-associated productivity loss (to patient and carer). Of chart reviews (N=1440) foreseen in 18 Mexican states, 1293 were obtained (90%) and clinical pathways were obtained for 1168 (81%). For direct medical costs, we observed an increased cost gradient depending setting (ambulatory $92 USD, hospitalized $1644, ICU $9375). We noted a difference between ideal cost and real cost in both SS and IMSS systems. The main difference driver in ideal costs between outpatients and hospitalized patients was cost of professional services (~90% for outpatients and ~100% for hospitalized/ICU patients). Medicine accounts for a fraction of overall cost, yet real expenditure is reduced compared to ideal expenditure for drugs. Direct real medical cost of ambulatory cases of $33 for SS is lower than direct medical costs reported in Brazil ($49),Colombia ($67) whereas medical cost for IMSS system ($92) is higher and comparable to Venezuela ($118). In contrast, hospitalized patient direct medical cost, in relative terms, is higher: $490 and $1644 in SS and IMSS respectively, vs $318 for Brazil, $331 for Columbia ($864 for Venezuela). Real costs and costs associated with ideal treatment are different, particularly for outpatients, pointing to health system failings (both SS and IMSS).

1402

EARLY INDICATORS OF DENGUE AMONG CHILDREN AND ADULTS PRESENTING WITH ACUTE FEBRILE ILLNESS IN PUERTO RICO

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Early clinical diagnosis of dengue can be challenging because the initial presentation is nonspecific with signs and symptoms similar to those of other acute febrile illnesses (AFI). Rapid diagnostic testing is often not available. Early identification and timely initiation of correct treatment can reduce complications and mortality. To identify early indicators for laboratory-positive dengue, we analyzed data from a sentinel enhanced dengue surveillance system conducted at a large referral hospital in Puerto Rico. Outpatients with fever for <7 days were enrolled and followed through their illness. Serum and nasopharyngeal specimens were collected and tested by RT-PCR and immunodiagnostic methods as appropriate for dengue viruses (DENV-1-4), *Leptospira* spp., *Burkholderia pseudomallei*, 5 enteroviruses, influenza A and B viruses, and 12 other respiratory viruses. Laboratory-indeterminate cases, co-infections and infants were excluded from analysis. Among the 1,580 patients enrolled during May 7, 2012 through May 6, 2013, 570 (36.1%) were hospitalized, 805 (51.0%) were male, and the median age was 21.1 years (range: 1-91 years). There were 617 dengue-positive patients, 611 respiratory infections, 72 infections caused by other viruses or bacteria and 280 cases with no pathogen identified. Five clinical findings were found to be independently associated with a laboratory-positive dengue: retrorbital pain, leukopenia, thrombocytopenia, rash and facial erythema. Sore throat, nasal congestion and cough were less frequent on dengue-positive patients. Clinical and laboratory features that were predictive of dengue were found to vary by patient age. Dengue was associated with: leukopenia, rash and joint pain ($p<0.005$) in children aged <9 years; leukopenia and thrombocytopenia ($p < 0.005$) in individuals aged 10-19 years; and thrombocytopenia, leukopenia, rash, nausea and joint pain ($p < 0.003$) in adults aged ≥20 years. Knowledge of predictors can be used to direct anticipatory guidance.

1403

PRESENCE OF THREE DENGUE SEROTYPES IN OUAGADOUGOU, BURKINA FASO AND ITS PUBLIC HEALTH IMPLICATIONS

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The discussion about the presence of febrile non-malaria cases has increased in Burkina Faso. As other febrile diseases, dengue was considered as differential diagnosis due the presence of the vector and previous DENV reports in the country. To explore the virus presence in acute febrile non-malaria cases and *Aedes* mosquitoes in Ouagadougou, an exploratory cross sectional study was performed from December 2013 to January 2014. Five sectors and six correspondent health care centers (CSPS) were selected based on a reported presence of Flavivirus: CSPS 3 and 12 (Dapoya), 8 (Gounghin), 18 (Pissy), 25 (Somgandé) and 28 (Dassasgho). A survey about symptoms was administered to the participants and finger pricks were used to obtain the samples. Each CSPS tested every febrile non-malaria patient for dengue using dengue rapid tests (SD Bioline DengueDuo). Blood spots were obtained in filter paper from all positive results and every tenth negative for further PCR analyses. A parallel entomological survey was conducted in the CSPS's correspondent sectors. From a total of 379 patients tested, 35 (9.2%) were positive for rapid test (60% both IgM/IgG; 21% just IgG and 5% just NS1). 91% were older than 15 years old (range 0-61 years old), 60% were women and 70% came to the CSPS during the first 3 days of fever. From 60 samples tested by RT-PCR, 15 were positive (9 from positive rapid test and 6 from the subsample of negative results). The serotypes observed were DENV2 (Dassasgho and Gounghin), DENV3 (Dapoya, Pissy and Somgande) and DENV4 (Dapoya, Gounghin and Somgande). There was not DENV in the analyzed mosquitoes. The presence of dengue in acute febrile non-malaria patients in Ouagadougou was evidenced. To our knowledge, thought the presence of DENV3 and DENV4 were reported in the region, this is the first time both serotypes are evidenced in Burkina Faso. These findings have important public health implications due the need to prepare the health system and the population for dengue's presence and outbreaks prevention (Additional data will be available at the conference)

1404

EVIDENCE OF RECENT DENGUE EXPOSURE AMONG MALARIA PARASITE-POSITIVE CHILDREN IN THREE URBAN CENTERS IN GHANA

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Dengue fever is increasingly being recognized as an important neglected tropical disease in sub-Saharan Africa, with national burdens generally unknown due to misdiagnosis of cases as malaria. This study screened for evidence of dengue exposure in 222 children aged 2-14 living in Accra, Kintampo, and Navrongo, Ghana, who tested positive on a rapid diagnostic test (RDT) for malaria and were subsequently confirmed to be malaria parasite-positive via blood test. We found presence of dengue-specific IgM antibodies using indirect ELISA methods in 7 children screened across the three sites, and presence of dengue-specific IgG antibodies in 20%, 13%, and 30% for Accra, Kintampo, and Navrongo respectively. The high rates of dengue exposure among children with confirmed malaria may be just the tip of the iceberg in terms of dengue prevalence among the heavy volume of febrile illness patients who do not have confirmed malaria. We discuss demographic correlates of dengue exposure and argue that this study underscores the need for assessing Ghana's baseline dengue burden as well as general clinical knowledge of the disease.

1405

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

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Dengue is endemic in Puerto Rico with seasonal increases in incidence that often coincide with increases in other acute febrile illnesses (AFI) caused by respiratory pathogens. Consequently, co-infections are possible, which may complicate both diagnosis upon presentation and the patients' clinical course. Specifically, the presence of respiratory symptoms may reduce early diagnosis of dengue, which is important for early initiation of clinical management that can minimize medical complications and mortality. In May 2012, a sentinel enhanced dengue surveillance system (SEDSS) site was established in a tertiary care hospital in Ponce, Puerto Rico wherein patients with fever for <7 days were enrolled and followed through their illness. Serum, nasopharyngeal and oropharyngeal specimens were collected and tested by RT-PCR for multiple pathogens including: dengue virus subtypes 1-4 (DENV-1-4); influenza A and B viruses, adenovirus, respiratory syncytial virus, metapneumovirus, and parainfluenza viruses. To identify factors associated with co-infection patients with DENV and respiratory virus co-infection (cases) were age-matched to patients infected with DENV only (controls) at a ratio of 1:2. Of 715 case-patients with DENV detected in serum, 30 (4.2%) had evidence of co-infection with a respiratory virus. There were no differences by gender identified among cases and controls. Most (87%) of the co-infections were children and adolescents (<20 years). Cases were more likely than controls to report cough (odds ratio [OR] = 3.17; 95% confidence interval [CI]: 1.2-8.1) or runny nose (OR = 2.58; 95% CI: 1.02, 6.50). Cases were also more likely to have a chest x-rays ordered, although this difference was not statistically significant (OR =1.6; 95% CI: 0.61-4.0). Further analysis will include factors associated with illness severity and clinical outcome. These findings suggest that in areas with endemic dengue and respiratory pathogens, physicians should have a high index of suspicion for co-infections in children and adolescents.

1406

POSITIVE SEROLOGY FOR HANTAVIRUS IN PATIENTS WITH CLINICAL SUSPECTED DENGUE IN CEARÁ, BRAZIL

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Dengue is considered the most important arbovirus in the world in terms of morbidity and mortality, having broad and unspecific symptoms, ranging from asymptomatic to severe hemorrhagic forms. Thus, it becomes difficult to distinguish it from other febrile syndromes only by clinical and epidemiological criteria. The use of these criteria as the unique basis for diagnosis of dengue can be dangerous and may lead to false diagnoses and inappropriate treatment. Within of the spectrum of similar to dengue acute febrile diseases, some pathogens are not routinely investigated by the lack of resources and ignorance of their existence in the region. In Ceará, the hantavirus was never notified and there is only one report in the regional literature showing the probable existence of this disease in humans in the state. Thus, the aim of this study was to investigate cases of hantavirus in patients suspected of dengue in Ceará. In this study, we evaluated 95 patients, with clinical suspicion of dengue, recruited during the year 2012 in the State of Ceará. The samples were evaluated for hantavirus through ELISA-IgM and ELISA-IgG tests. One (1.05%) patient was positive for hantavirus by ELISA- IgM, detecting current or recent infection by the virus. This patient had moderate symptoms, suggesting that mild or atypical cases of hantavirus should be occurring in the State. Thirty (31.6%) patients were positive by ELISA-IgG. This result suggests that they have recently or previously infected by hantavirus, but this result does not allow to determine whether this virus was the causative agent of febrile syndrome presented by these patients. All patients in this study were questioned about the conduct of recent trips and none reported having left Ceará in recent months, probably acquired the infection locally. For a state that has only one report in the literature for this pathogen in humans, the percentage of people with prior contact with it was very high, showing the need for further investment and research on this disease in Ceará. Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

1407

DESCRIPTION OF FEBRILE ILLNESS AND DENGUE IN INFANTS LESS THAN 90 DAYS OLD

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Fever in the first 90 days of life presents a diagnostic and therapeutic challenge for pediatricians. Bacterial infections should be identified in order to provide adequate treatment, but sepsis workup is invasive and costly. Differentiation between bacterial and viral etiology is important to prevent unnecessary invasive procedures. Viruses, including dengue (DENV), are believed to be an important cause of fever in endemic countries, but they are not routinely identified and knowledge of their contribution to febrile illness in young infants is limited. This study used data obtained from the Sentinel Enhanced Dengue Surveillance System (SEDSS) established in southern Puerto Rico in May 2012. SEDSS recruits acute febrile illness (AFI) patients, collects clinical data and tests for 22 infectious agents, including DENV, respiratory pathogens and enteroviruses. Reverse transcriptase--polymerase chain reaction and ELISA are used to identify etiologic agents. Of 5,115 patients enrolled during the first year of SEDSS, 48 (0.9%) were infants less than 90 days old. Thirty (62.5%) infants were male, and 9 (18.8%) were less than 30 days old. Twenty four (50%) infants presented on the first day of fever, and most (89.4%) presented within the first 3 days. Most infants (70.8%) were admitted for cultures and treatment, including all patients less than 30 days old. The etiologic agent was identified in 13 (27%) infants: 3 (6.3%) had a bacterial infection, 8 (16.7%) had a viral infection, and 2 (4.2%) had viral/bacterial co-infection. Viruses detected included DENV (n = 2), influenza A virus (n = 4), enterovirus (n = 2), parainfluenza virus-3 (n = 1), and DENV/influenza A virus coinfection (n = 1). Viral infections were more common than bacterial infections in this pediatric cohort, and DENV infection was a rare event. These findings will assist clinicians to understand the causes of fever and the incidence of DENV infection in young infants.

1408

DENGUE VIRUS TYPE 3 CIRCULATION IN A REGION OF THE COLOMBIAN CARIBBEAN DURING AN EPIDEMIC PERIOD

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Dengue is an arthropod-borne viral disease which has become a major international public health problem in terms of economic impact, mobidity and mortality. Dengue virus 3 is a recently introduced serotype in Colombia which in addition to the simultaneous circulation of the other serotypes, could be associated with an increased transmission and appearance of severe manifestations risk; however, the local virus surveillance in areas such as the department of Sucre is not constant, leading to the lack of updated information. In the present study we describe the frequency of circulation and phylogenetic characteristics of Dengue virus type 3, present in the department of Sucre, located in the Colombian Caribbean. Clinical data and blood samples from patients with febrile syndrome were collected during the second half of 2013 and early 2014. Molecular detection of DENV was performed by a One-Step RT-PCR and IgM/IgG antibodies against the virus were determined by a capture ELISA. Two C6/36 cell passages were made with the RT-PCR positive samples, for virus isolation. Supernatants were used to amplify the complete E and NS3 genes to be subsequently sequenced in order to perform phylogenetic analysis (Bayesian Inference). 22% of the samples were positive for molecular detection of the virus, whereas 37.7 %, 11.1%, and 24% had IgM, IgG and IgM/IgG antibodies against DENV respectively. Serotypes DENV1, 2 and 3 were detected but DENV3 was the most frequent (60%). Three isolates were obtained corresponding to DENV3 (2) and DENV1 (1). The sequence analysis revealed high similarities between DENV3 isolates that were classified within the genotype III; DENV1 isolate was classified as American/African genotype closely related with Colombian and Venezuelan sequences. The results suggest that most of the Dengue reported cases during this epidemic period were caused mainly by DEN3, but other two serotypes were present. This confirm that the region is a hyperendemic area, which could potentially be a hotspot for Dengue transmission in the Colombian Caribbean.

1409

SENSITIVITY AND SPECIFICITY OF THE WORLD HEALTH ORGANIZATION DENGUE CLASSIFICATION SCHEMES FOR SEVERE DENGUE ASSESSMENT IN CHILDREN IN RIO DE JANEIRO

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The clinical definition of severe dengue fever remains a challenge for researchers in hyperendemic areas like Brazil. The ability of the traditional (1997) as well as the revised (2009) World Health Organization (WHO) dengue case classification schemes to detect severe dengue cases was

evaluated in 267 children admitted to hospital with laboratory-confirmed dengue. Using the traditional scheme, 28.5% of patients could not be assigned to any category, while the revised scheme categorized all patients. Intensive therapeutic interventions were used as the reference standard to evaluate the ability of both the traditional and revised schemes to detect severe dengue cases. Analyses of the classified cases (n = 183) demonstrated that the revised scheme had better sensitivity (86.8%, P < 0.001), while the traditional scheme had better specificity (93.4%, P < 0.001) for the detection of severe forms of dengue. This improved sensitivity of the revised scheme allows for better case capture and increased ICU admission, which may aid pediatricians in avoiding deaths due to severe dengue among children, but in turn, it may also result in the misclassification of the patients' condition as severe, reflected in the observed lower positive predictive value (61.6%, P < 0.001) when compared with the traditional scheme (82.6%, P < 0.001). The inclusion of unusual dengue manifestations in the revised scheme has not shifted the emphasis from the most important aspects of dengue disease and the major factors contributing to fatality in this study: shock with consequent organ dysfunction.

1410

EVALUATION OF TWO PARAMETERS FOR DENGUE DIAGNOSIS IN HONDURAN PATIENTS

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Dengue is an important vector borne disease in tropical and sub-tropical countries. In Honduras during year 2013, around 39,275 cases of dengue fever were registered at national level. Several approaches have been developed for laboratory diagnosis of dengue infections, nevertheless timely diagnosis is a challenge. This study was undertaken to evaluate two different tests to detect dengue virus NS1 antigen (Ag) and dengue IgM antibodies (Ab) manufactured by Standard Diagnostics (SD, South Korea) in samples from patients with dengue infections. The study was carried out in Tegucigalpa, Honduras. The study population consisted of 134 patients clinically classified with dengue hemorrhagic fever according to the WHO criteria. Out of 134 plasma samples, 61 corresponded to patients with ≤5 days of illness and 73 samples to patients with ≥6 days of illness. All samples from patients with ≤5 days of illness, characterized as dengue positive (n=48) or negative (n=13) by RT-PCR, were tested by SD dengue NS1-Ag methods (the rapid test SD Bioline NS1-Ag and SD NS1-Ag EIA); all samples from patients with ≥6 days of illness with positive (n=57) or negative (n=16) result for dengue infection by an in-house IgM-Ab capture EIA, were tested by SD dengue IgM-Ab methods: the rapid test SD Bioline IgM-Ab and SD IgM-Ab EIA. The sensitivity of SD Bioline NS1-Ag was 88% and 85% for SD NS1-Ag EIA. Regarding specificity, although it is 17% for SD Bioline NS1-Ag and 23% for SD NS1-Ag EIA this is not real, the comparison was done with RT-PCR and turn out to be false negative; because 9/10 samples are IgM-Ab positive. For SD IgM-Ab, the sensitivity and specificity was 82% and 88% for SD Bioline IgM-Ab and 88% and 69% for SD IgM-Ab EIA when were compared with the in-house EIA. These results suggest that dengue SD Bioline NS1-Ag method had slightly higher sensitivity than dengue SD NS1-Ag EIA (88% vs 85%). Higher specificity was observed for SD Bioline IgM-Ab than SD IgM-Ab EIA (88% vs 69%). In terms of sensitivity SD IgM-Ab EIA was higher than SD Bioline IgM-Ab (88% vs 82%). Early diagnosis of dengue infection by NS1 antigen could be helpful in the timely management of dengue virus infection, and it might be even superior due to the fact of the known liability of RNA used for molecular testing and confirmed in this study.

1411

SPATIOTEMPORAL CLUSTERING, CLIMATE AND SOCIAL-ECOLOGICAL RISK FACTORS FOR DENGUE IN MACHALA, ECUADOR

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Dengue fever, a mosquito-borne viral disease, is a growing public health problem in Ecuador and throughout the tropics, yet we have a limited understanding of the disease dynamics in these newly emerging regions. The aim of this study was to characterize the spatiotemporal dynamics, climate and social-ecological risk factors associated with the largest dengue outbreak on record (2010) in the coastal port city of Machala, Ecuador. Spatial analysis: Using LISA and Moran's I, we analyzed the spatial distribution of georeferenced dengue cases and found evidence of significant hotspots near the city center. We evaluated whether the presence of dengue transmission was associated with social-ecological variables at the neighborhood level by overlaying data from the 2010 national census and entomological indices. We used a multi-model selection process and found that the best-fit model to predict the presence of dengue included age and gender of the head of the household (older, female), access to piped water in the home, poor housing condition, and distance to the central hospital. Temporal analysis: Using wavelet analysis, we characterized historical patterns of weekly climate and dengue transmission (2003-2010), and we found significant climate effects associated with the outbreak. In conclusion, our findings indicate the potential to develop dengue vulnerability maps that can feed into climate-driven dengue early warning systems to inform vector control interventions. This study provides an operational methodological framework that can be broadly applied to understand local dengue risk.

1412

THE COMPLEX RELATIONSHIP BETWEEN WEATHER AND DENGUE VIRUS TRANSMISSION IN THAILAND AND PERU

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Dengue viruses cause more human morbidity and mortality than any other arthropod-borne virus. Dynamic space-time estimations of risk are needed to guide the development of more effective surveillance-intervention strategies and use of prevention resources. Weather plays an important role in regulating the location and timing of transmission due to direct effects of temperature and humidity on mosquito development cycles, life span, behavior and extrinsic incubation period. We closely examined the relationship between weather dynamics, including temperature, humidity, and rainfall, and dengue virus transmission across all of Thailand by province for 1983-2001 and all of Peru by district for 1994-2012. We quantitatively characterized the role of weather in regulating dengue transmission cycles across both countries. We observed systematic differences in the structure of seasonal transmission cycles of different magnitude, the role of weather in regulating seasonal cycles, necessary versus optimal transmission "weather-space", basis of large epidemics, and predictive indicators that estimate risk. Larger epidemics begin earlier, develop faster and are predicted at seasonal *Onset change-point* when case-counts are low. Temperature defines a viable range for transmission; humidity amplifies the potential within that range. This duality is central to transmission and epidemic magnitude. In Thailand, 80% of 1.2 million severe dengue cases occurred when mean-temperature was 27--29.5°C and mean-humidity was >75%. In Peru, with highly diverse weather

patterns spatially, broadly relevant predictors were developed using a statistical classification approach. Interventions are most effective when potentially large epidemics are identified early. Most cases occur near the local seasonal *Peak*, yet small reductions at epidemic *Onset* can substantially reduce epidemic magnitude. Monitoring the *Quiet-Phase* before *Onset* is fundamental in effectively targeting interventions pre-emptively.

1413

ESTIMATING CROSS-IMMUNITY INTERACTIONS OF DENGUE SEROTYPES USING LONGITUDINAL SEROLOGICAL DATA

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Dengue, a mosquito-borne disease whose incidence and graphic range have increased considerably in the past 50 years, is caused by any of four related but antigenically distinct virus serotypes (DENV-1, DENV-2, DENV-3, DENV-4). Following a DENV infection, in addition to lifelong immunity to the infecting serotype, an individual gains temporary immunity to infections with heterologous viruses. Although temporary cross-immunity (TCI) is a historically demonstrated phenomenon, the strength and duration of this vital component of DENV epidemiology is difficult to estimate. Critically, TCI's primary role in transmission dynamics results in the absence of heterologous infections; something that cannot be explicitly captured using hospital case data. Conversely, large longitudinal serological surveys from the same subset of the population over the course of several years can be used to estimate the risk each individual faces for infection with each serotype and observe the disproportionate decrease (or complete absence) of heterologous infections immediately following a DENV infection. Here we apply a new modeling approach that estimates TCI using a 12-year longitudinal DENV dataset from Iquitos, Peru. The dataset contained information on 14,335 individuals whose blood was assayed by PRNT every 6-9 months (38,416 total samples), and contained interval censored timing for 3,854 serotype-specific infections. We identified 455 individuals that became infected with two different serotypes during their participation in the study. Although the average time between seroconversions was 449 days (2.5 tests on average), 250 of those individuals seroconverted twice in sequential assays. Further analysis, using a spline-based approach previously designed to study the serotypes independently, is currently being leveraged to resolve when, within these testing intervals, infections likely occurred. By modeling a variety of ranges and distributions for the length of cross-immunity, we can estimate the strength of the interactions between DENV serotypes with greater accuracy than was previously possible.

1414

MECHANISMS OF TRAVELING WAVES AND PERIODIC SPATIAL SYNCHRONIZATION OF DENGUE HEMORRHAGIC FEVER INCIDENCE IN THAILAND

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Multi-annual periodicity has been observed in multiple time series of dengue including those from Thailand. These time series have been observed to have distinct spatial structuring of the timing of peaks, with spatio-temporal traveling waves and other structures observed. The mechanisms underlying this spatial dependence are not well understood. Here we describe transmission models that explore multiple hypotheses of the mechanism underlying traveling waves and periodic synchronization of dengue incidence observed in Thailand in a 40 year time series of incidence from all provinces in the country. We utilize mechanistic, meta-population models that include migration between patches to understand the incidence synchronization phenomenon. We explore scenarios with varying degrees of patch heterogeneity, migration rates, seasonal forcing and heterogeneity in birth rates to identify the main drivers behind phase structures and synchronization observed in the empirical incidence data. We discuss the potential impact of our observations for the control of dengue.

1415

DENGUE VIRUS INFECTION AMONG MEMBERS OF THE UGANDA PEOPLE'S DEFENSE FORCE

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Outbreaks of dengue have occurred in East Africa over the last several years. In May 2011, a dengue outbreak was recognized among African Union Mission in Somalia (AMISOM) peacekeepers when several Ugandans were diagnosed with an acute hemorrhagic febrile illness. In response to the outbreak, we conducted a seroincidence study to determine the risk of dengue virus (DENV) infection following Uganda Peoples Defense Force (UPDF) deployment to Somalia. Serum specimens were obtained from 337 participating UPDF soldiers to determine DENV exposure and infection rate pre- and post- deployment. Testing included anti-DENV IgG antibodies by immunoassay and neutralizing IgG antibodies by microneutralization test (MNT). A dengue case was defined as positive for IgG seroconversion and confirmed by MNT. IgG seroconversion was defined as a negative anti-DENV IgG result in the pre-deployment specimen and a positive result in the post-deployment specimen or a 4-fold titer increase. MNT positive titer to only one serotype was classified as a primary DENV infection. Reactivity to multiple DENV serotypes by MNT was classified as a secondary DENV infection. Sixty percent of the UPDF soldiers that deployed to Somalia

had seroconversion by IgG. The MNT results showed that 81% of the IgG positive specimens had neutralizing antibodies specific to DENV. Only 13.3% of the IgG positive specimens had a primary infection to either DENV1, 2 or 3. DENV-3 was the predominant serotype amongst UPDF soldiers. DENV exposure determined by the seroincidence study following UPDF deployment to Somalia matched the identified circulating serotypes in Somalia during the dengue outbreak in 2011. With dengue in the differential diagnosis for acute febrile illness for the UPDF soldiers, a quarantine recommendation should be considered for returning soldiers so as not to introduce DENV into Uganda.

1416

MOLECULAR CHARACTERIZATION OF INFLUENZA A AND B VIRUSES IN CUBA DURING 2006-2010, IMMUNOLOGICAL MARKERS RELATED TO 2009 PANDEMIC DISEASE SEVERITY

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During 2006-2010 in Cuba, Influenza and pneumonia were the fourth leading cause of death, with the detection of pandemic influenza A(H1N1) pdm09 in year 2009. Severe disease caused by pandemic virus in persons less than 55 years occurred at highest frequencies than youngest and elder groups worldwide. At that time, scientific community focused on the interaction virus-ecology-host factors. Besides, emergency of genetic variants divergent from vaccine strains and antiviral drug-resistant variants, threaten the effectiveness of prevention and control measures. In Cuba, there are non-previous studies about influenza viruses molecular characterization, we focused in genetic characterization of influenza A and B virus variants circulating during 2006-2010. In addition, the relationship between Influenza pandemic severity with host factors was determined. Study showed seasonal influenza A and B viruses into different genetic variants, some of them genetically divergent from vaccine strain. Different genetic variants of influenza virus A(H1N1) pdm09 were detected, however, they remain the genetic match with vaccine strain. High levels of RANTES and TLR-2, and the presence of CCR5Δ32 suggest their involvement in disease severity produced by the 2009 pandemic virus. M2 channel blocking drugs resistant variants were detected in seasonal influenza A(H3N2) and pandemic A(H1N1)pdm09 strains, and variants resistant to neuraminidase inhibitors emerged during 2008 in seasonal influenza A(H1N1) after permissive mutations gaining. Molecular characterization of influenza virus allowed the detection of emerging genetic variants, with potential to evade antibodies vaccine and become resistant to antiviral drugs. Moreover, results obtained provide useful laboratory criteria for control and prevention policies update by the Ministry of Public Health, and the future perspective new forms of therapy directed to virus-host interactions.

1417

THE MOLECULAR EPIDEMIOLOGY AND PHYLOGEOGRAPHY OF H3N2 INFLUENZA VIRUS IN PERU

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The evolutionary dynamics of H3N2 influenza viruses in tropical regions like Peru remain unclear, including whether lineages persist in the tropics and seed temperate areas. We aimed to test the 'source-sink' model and clarify the migration patterns of H3N2 within and between Peru and the rest of the world. Respiratory specimens from community-based influenza surveillance cohorts were collected from 2010-2012 in four ecologically diverse sites in Peru: Cusco, Tumbes, Puerto Maldonado (PM) and Lima. H3N2 positive specimens (by QIAamp Viral RNA Isolation Kit assay) were randomly selected over time and space and the complete hemagglutinin (HA) gene sequenced and compared with sequences in GenBank and GISAID databases. Alignment and DNA model selection were performed using MEGA and JmodelTest2 software, respectively. A maximum likelihood (ML) tree of all sequences was inferred using RaxML software. A maximum clade credibility (MCC) tree and time to most common recent ancestor (TMCRA) of Peruvian sequences were inferred using BEAST software, with spatial clustering robustness tested by BaTS software. Of 400 specimens selected , 389 were able to be sequenced. ML analysis of Peruvian and 2023 global comparator sequences demonstrated interseasonal extinction of Peruvian clades. Moderate clustering of Peruvian taxa and mixing with global strains were noted at all study sites. A short TMCRA of Peruvian H3N2 taxa was noted (3.8 years), consistent with rapid replenishment of the Peruvian H3N2 gene pool from international regions. The MCC tree of Peruvian taxa revealed a well-supported spatial structure at all sites ($p < 0.01$), although there was also moderate spatial mixing. Spatial clustering was weakest in Lima and PM (mean maximum clade sizes of 8.04 and 8.2, respectively). In conclusion, there is no evidence of a 'sink-source' dynamic or viral persistence in Peru. Rather, our data supporting a model of ever-migrating global metapopulations of H3N2. Peruvian H3N2 strains are replenished by a well-mixed global gene pool each season with gene flow in and out of the country at multiple locations. While spatially structured, there is evidence of H3N2 migration within Peru, particularly at the Lima and PM sites, which is consistent with high fluxes of human movement and/or larger population sizes at these two locations. These findings have implications for pandemic influenza planning in Latin America and beyond.

1418

CYTOKINE RESPONSE OF RHESUS MACAQUES EXPOSED TO LIVE EBOLA ZAIRE VIRUS CHARACTERIZED WITH MAGPIX PARAMAGNETIC BEAD TECHNOLOGY

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Viral Hemorrhagic Fevers (VHFs) are serious, frequently fatal illnesses characterized by fever and unusual susceptibility to bleeding. VHFs are caused by single-stranded RNA viruses from the families Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. Several aspects of the illnesses contribute to their importance as biological threat agents. Diagnosis is usually made at a reference lab with high risk (BSL3-4) biosafety capabilities. Early diagnosis is critical for proper management of the illness and the prevention of spread. Understanding the pathophysiology of the disease is necessary to be able to develop effective medical countermeasures. To determine if any cytokine responses might convey increased survivability, serum was analyzed from 32 Rhesus macaques that had been exposed to 1, 10, 100, or 1000 pfu of Ebola Zaire virus. Using Luminex's MAGPIX paramagnetic bead platform, the serial bleed live virus samples were analyzed in biocontainment for the development of cytokines important in immune response following infection. Using Life Technologies' Cytokine Monkey Magnetic 29-Plex Panel, time point samples were analyzed from both surviving and non-surviving animals, and has provided data on cytokine responses that correlate with the severity of Ebola virus infection.

1419

RABIES IN IRAQ: 2014 UPDATE

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Rabies control requires the combination of dependable resources, cooperation between veterinary and public health officials and accurate surveillance methods. In prior work we reported trends in human rabies cases between 2001 and 2010 and characterization of animal rabies strains from Baghdad, Iraq. Previously, there had been no systematic surveillance for rabies in animals and no laboratory confirmation of disease or virus strains. Three of 40 animal brains were positive using fluorescent antibody testing and hemi-nested RT-PCR for rabies virus (RABV). Phylogenetic analysis using partial nucleoprotein gene sequences demonstrated that the viruses belonged to a single virus variant and shared a common ancestor with viruses dating back 22 years ago from neighboring countries to the west, north and east of Iraq. These results suggested possible multiple introductions of rabies into the Middle East and regular trans-boundary movement of disease. In the present work, we discuss efforts to improve the surveillance and control of rabies in Iraq over the past several years. In 2012 there were 10 cases of rabies and 12,715 dog bites. In 2013 there were 8 cases of rabies and 15,879 reported cases of dog bites. Although 4000 years have passed since the original disease known as rabies, animals and humans are still dying of this preventable and neglected zoonosis in Iraq.

1420

VALIDATION OF A DUPLEX REAL-TIME RT-PCR ASSAY FOR SIMULTANEOUS DETECTION OF INFLUENZA A AND B VIRUSES

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Influenza viruses have been the cause of major outbreaks and pandemics with high mortality rates throughout human history. Even though vaccines are available, influenza affects around 15% of planet's population yearly. Disease surveillance is essential for rapid identification of cases, allowing implementation of treatment and control measures. Real-time PCR is a powerful diagnostic technique frequently used in influenza surveillance. We developed a duplex real-time RT-PCR to simultaneously detect influenza A and B viruses to meet the need for rapid and effective diagnostics in an respiratory disease cohort in Peru. A combined set of published primers and probes to detect a highly conserved region of the influenza A virus matrix protein (MP) gene and in-house designed primers and probes to detect the influenza B virus MP gene were optimized for single-step RT-PCR using the ABI7500 Fast real-time PCR system. Two-hundred and sixty eight clinical samples were tested using the newly designed duplex assay. Results were compared with those obtained using single-plex RT-PCR assays for influenza A and B viruses designed by the U.S. Centers for Disease Control and Prevention (CDC). Results from the duplex assay were 97% and 100% consistent with the CDC assay for influenza A and B viruses, respectively. In addition, our duplex assay was able to detect two of three influenza A and B virus co-infections. This assay provides a rapid, accurate, highly sensitive and specific diagnostic test for simultaneous detection of influenza A and B viruses.

1421

BATS IN LYSSA, CORONA AND EBOLA VIRUSES ECOLOGY IN NIGERIA; ONE HEALTH PERSPECTIVE TO INFECTIOUS DISEASE CONTROL

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The likelihood of an outbreak of emerging or re-emerging infectious disease is contingent on exposure to known or yet to be described reservoir hosts. In recent years, climate change and habitat alteration increase contact between human and animals in shared environment thereby enhancing interspecies transmission of pathogens. Previous studies have shown evidence of lyssa-viruses in fruit bats in Nigeria. These fruit bats are similar to those observed in other countries where corona and Ebola viruses have been identified. Studies on the risk of human exposure to these bats are critical in protecting public health and animal conservation in the perspective of onehealth. We carried out longitudinal survey of bats including, species identification, their habitats, habits and migration pattern in North Central Nigeria and identified human, climatic, arboreal and ecological factors that are likely to cause exposure to excretions and secretions from these animals by direct field observation. Non-invasive specimens including bat guano and urine were collected for virus detection and isolation by ELISA and culture in mammalian cell lines. Oral interviews and questionnaires survey were also carried out to assess knowledge, attitude and practices with regard to bats. Several species of fruit bats of the order pteropodidae and microchiroptera were found at the forest fringes and within parks and gardens in major cities in North central Nigeria. The choice of habitat is strongly influenced by the forest zones and the presence of forest-like parks and gardens including zoos in the North central. The pattern of migration are also influence by seasonal weather variation such that there is a pattern of movement southward during dry season and northward during rainy season to avoid wetness of the rain and or remain in the south where abundant fruits are available. Bats are reservoir of many emerging and re-emerging pathogens like lyssa-viruses. SARS/MERS corona and Ebola viruses are also considered most likely in these reservoir hosts and because of the interactions of human with bats and games in the forest, game reserve, parks and gardens, the risk of exposure to these pathogens is high. There is therefore an urgent need to design conservation friendly intervention to prevent the pandemics of the future by actions that are taken today.

1422

THE GLOBAL DISTRIBUTION OF CRIMEAN-CONGO HEMORRHAGIC FEVER

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne infection caused by a virus (CCHFV) from the Bunyaviridae family, and while it occurs primarily in animals, it also occurs in humans who work closely with these animals. Healthcare workers in endemic areas are similarly at high risk. There is no safe and effective vaccine against CCHFV which is widely available, and thus treatment for the potentially fatal disease remains primarily supportive. Therefore, an improved understanding of the distribution and level of risk for CCHF is essential for guiding improvements in disease control strategies. Here we undertake an exhaustive assembly of known records of CCHF occurrence worldwide from 1961 to the present, and use a formal modelling framework to map the global distribution of CCHF risk. We do this by first deriving a consensus on country-level presence or absence, and combine this information with the locations of known occurrences and a suite of high spatial-resolution covariates related to climate, urbanisation, agriculture,

and livestock presence to derive the probability of occurrence at a 5km x 5km resolution globally. We find CCHF to be confined to Africa, Eastern Europe, and western Asia, but with spatially heterogeneous levels of risk within these regions. Our new risk map provides novel insights into the global, regional and national threat posed by CCHF, and highlights the need for cohort studies to be carried out in high-risk zones in order to determine the public health burden posed by this neglected disease. We intend for our contemporary risk map to serve as a starting point for a wider discussion about the global impact of CCHF, and for it to help guide improvements in drug and vector-control strategies as well as evaluation of the economic burden caused by this disease.

1423

CHARACTERIZATION OF PUNTA TORO VIRUS RESPONSIBLE OF HUMAN DENGUE-LIKE CASES IN PANAMA

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The genus Phlebovirus (Bunyaviridae family) comprises over 70 antigenically distinct serotypes of viruses with a wide distribution along the tropics. Punta Toro virus (PTV) is part of the Phlebotomus fever viruses (the sand fly group). In the Americas, PTV has been isolated only in Panama from sand flies, slots, human febriles and sentinel hamsters, and viruses from Punta Toro serogroup have been described also in Colombia and Brazil. Many arboviruses, as PTV, cause in humans symptoms similar to Dengue infection; thus the true number of PTV cases could be underestimated in this Dengue endemic country. Up to a 35% seroprevalence for PTV has been reported in Panama before 1988, however there is no recent data about PTV seroprevalence and about the range of clinical illness caused by this virus. The aim of our study is to evaluate the presence of PTV in human acute sera samples referred by the Dengue surveillance program from 1998 to 2013. We inoculated Vero cells with samples that were Dengue negative, and, for now height samples from patients from Western Panama and Panama city induced cytopathic effect in Vero cells. The isolated virus was characterized as PTV by hemaglutinin inhibition assay. Fragments of the segments L, M and S of the genome of PTV were amplified to perform sanger sequencing and the obtained sequences were aligned and analyzed to compare with previous strains isolated in Panama and PTV serogroup from other regions. The phylogenetic trees show that these strains are related to previously described strain GML902878 (isolated from sentinel Sirian hamster in 1976), and are close to Balliet, a strain related to mild disease in hamster models that was isolated in 1966 also in Western Panama. Our preliminary findings suggest that PTV close to Balliet strain circulates continuously in Western and Central Panama and causes undifferentiated febrile symptoms in humans, underlining the fact that many arboviruses like PTV could be responsible of the less than 30% of Dengue-like cases that are negative for dengue in this country.

1424

COVERAGE DURING AN IMMUNIZATION CAMPAIGN PROVIDING INACTIVATED AND ORAL POLIO VACCINES IN REFUGEE CAMPS AND HOST COMMUNITIES, KENYA - DECEMBER 2013

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Poliomyelitis is a highly infectious viral disease, affecting mainly children <5 years of age. Globally, poliomyelitis cases have decreased by 99% since 1988, but outbreaks continue to occur. In May, 2013 an outbreak of wild type poliovirus with 14 cases was reported in Kenya, among them 13 cases were from Dadaab refugee camps and host communities. An immunization campaign providing inactivated poliovirus vaccine (IPV) and oral polio vaccine (OPV) was launched. We conducted a post-campaign coverage survey to assess the impact and guide future use of IPV. We selected 30 blocks in each of the five refugee camp, and 30 villages in the host communities, by probability proportional to size with replacement. We visited nine households in each block and five households in each village, plus a convenience sample of nomad settlements. Within each household we collected data on all children <5 years on IPV and/or OPV; the youngest child age 6 to 59 months was selected for questions about OPV received through routine immunization. Vaccine coverage and 95% confidence intervals were calculated accounting for clustering. We enrolled 1,084 households, including 2,173 children from refugee camps and host communities and 118 from nomad households. Coverage of OPV plus IPV in the December campaign was 92.8 %(90.2%-94.8%) in refugee camps and 95.8%(93.5%-97.3%) in host communities; OPV coverage in the November campaign was 97.2% (95.4%-98.3%) in refugee camps and 97.3%(95.0%-98.5%) in host communities. Among the 118 children <5 years of age from nomadic households, 40(34%) received IPV plus OPV in December, and 37(31%) had received OPV in November. Among caregivers, 1009(99%) reported being aware of the campaign; 766(76%) knew from megaphone announcements, 475(47%) from social mobilizer, 435(43%) from healthcare worker, and 367(36%) heard from the radio. Among 107 children >6 weeks old who missed IPV, 49(46%) caregivers cited not knowing the location of the vaccination. IPV was successfully delivered with high community acceptance both in refugee camps and host communities. Strategies are needed to improve coverage in nomadic populations.

1425

HOW UNPREPAREDNESS FOR AN EBOLA OUTBREAK LEADS TO A WIDESPREAD EPIDEMIC AND COMPLEXITY TO HALT THE EPIDEMIC; AN ANALYSIS AND DESCRIPTION OF ENCOUNTERED OPERATIONAL CHALLENGES DURING A MÉDECINS SANS FRONTIÈRES INTERVENTION IN THE REPUBLIC OF GUINEA AND LIBERIA

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Suspected cases of hemorrhagic fever were notified in different locations in the Republic of Guinea before the Ebola Zaire virus was identified and the first ever Filovirus epidemic declared in the country on the 22nd of March 2014. The epidemic rapidly spread further to the capital Conakry and cross border to Liberia. In less than 4 months a cumulative total of 163/25 suspected cases and 112/12 deaths were notified in Guinea and Liberia respectively. The attack rate and case fatality observed

are comparable with previous Ebola outbreaks; however the large geographical spread of the disease is unprecedented and leads to complex operational challenges during the interventions put in place by Médecins sans Frontières in collaboration with the Ministries of Health. The first cases were misdiagnosed because of the unfamiliarity of the disease among health staff. Disease confirmation was hampered by the lack of a reference laboratory in Guinea and challenges around sample transport. Rapid geographical spreading was achieved by the high mobility of cases unaware of their status or looking for better perceived health care. Interhuman contact was not minimized because of lack of knowledge and the non-respect of universal precautions. Corpses were moved to different locations and traditional burials greatly contributed to the spread of the epidemic. Misconceptions in the community resulted in difficulties in accepting isolation of patients and lead community members to attack and chase Médecins sans Frontières team members from an intervention site. Filoviridae can achieve an important geographical spread if countries at risk for outbreaks are not prepared. MSF advocates for awareness of viral hemorrhagic fevers, increasing the level of universal precautions at all levels, training of health staff, national laboratory testing possibilities and assuring emergency preparedness at national level. Research and development should be high on the agenda for the availability of rapid diagnostic tests for viral hemorrhagic fever viridae, active and passive immunizations for infected patients and the passive immunization of communities at risk.

1426

KINETICS OF POLIO SHEDDING FOLLOWING ORAL VACCINATION AS MEASURED BY QRT-PCR VERSUS CULTURE

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Measurements of oral polio vaccine (OPV) shedding in stool are useful to evaluate mucosal immunity, to estimate the burden of vaccine strain poliovirus, and for surveillance post-polio eradication. We developed an one-step serotype-specific real-time RT-PCR for detection of Sabin1, Sabin2, and Sabin3 strains along with an extrinsic internal control, MS2, to normalize the targets for extraction and amplification efficiency. Trivalent OPV (tOPV) was administered at week 6, 10, 14, and 52 weeks (at week 39 half of the infants received IPV and the other half tOPV) in a birth cohort study in the Mirpur region of Dhaka, Bangladesh. This assay was used to intensively study OPV shedding kinetics at weeks 14 (n=88 infants; 42 female and 26 male) and 52 (n=182 infants; 84 female and 98 male) post vaccine administration directly from stool specimens collected before the OPV administration (day 0) and on days +4, +11, +18, and +25 after administration. Of the 1350 samples examined (270 infants × 5 time points), sensitivity and specificity of qPCR was 89% and 91%, respectively, when compared to culture. Overall, the PCR detected more shedding than the standard culturing methods. A quantitative relationship was observed between culture+/qPCR+ specimens and culture-/qPCR+ specimens namely the average burden of shedding in viral copy number from the culture+/qPCR+ specimens was higher than in the culture-/qPCR+ specimens (qPCR copies $3.37\times10^{7}\pm1.22\times10^{7}$ versus $3.88\times10^{5}\pm1.45\times10^{5}$, respectively; Mann-Whitney P<0.001 two-tailed). Kinetics of shedding as revealed by qPCR and culture were generally similar at both time points. A qPCR cutoff of approximately 10^{4} viral copies on day 11 or day 18 post OPV could be used to identify the culture-positive shedders after immunization as well as their shedding duration and intensity. qPCR revealed that S3 (6.4%) was most commonly shed followed by S1 (5.1%) and then S2 (1.9%) , and mixed infections occurred in 6.5%. Our findings suggest that this one-step qRT-PCR polio assay can be used to approximate shedding both qualitatively and quantitatively, and will be useful in monitoring OPV efficacy or transmission during eradication efforts.

1427

MECHANISMS OF VIRULENCE OF MONKEYPOX VIRUS: DELETION OF GENOMIC REGIONS AND THEIR EFFECTS IN PATHOGENESIS

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Monkeypox virus (MPXV) causes a human disease similar to smallpox which is endemic in equatorial Africa. However, the emergence of MPXV in 2003 in the Western Hemisphere (USA) demonstrated the potential of these viruses for geographic expansion and worldwide transmission. Understanding the viral genetic factors associated with virulence are of vital importance for surveillance, prevention and treatment of human MPX disease. Using bioinformatics and molecular virology approaches, we identified and evaluated the effects of deletion of two genomic regions in the highly virulent MPXV-Congo strain. *In vitro* and *in vivo* studies indicated that these genomic regions play a significant role in MPXV replication, tissue spread, pathology, and mortality in susceptible CAST/EiJ mice. In this study, we demonstrated that deletion of multiple immunomodulatory (IMM) genes in MPXV is necessary to produce a pronounced attenuating effect, suggesting that targeted genomic regions contain more than one major MPXV virulence factor. More importantly, we observed marked attenuation of virus with simultaneous deletion of two regions (MPXV-ΔR1/R2) which illustrates the additive effect of genomic regions in MPXV pathogenicity. Deletions of MPXV regions in the highly virulent MPXV/Congo genome hindered cell culture growth and significantly reduced morbidity, replication, spread and mortality in infected CAST/EiJ mice. Thus, parental MPX-Congo/Luc+ caused 100% mortality while all mice infected with recombinant MPXVs/Luc+ with deletions of genomic regions survived to infection and did not show clinical signs of disease. Further, serological and histopathological evaluation confirmed that deletion of genomic regions reduced MPXV tissue pathology and elicited strong antibody responses. Our results support the hypothesis that MPXV pathogenesis is not determined by a single gene. Rather, it is the result of the combined effects and interactions of multiple viral and host factors.

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REPORT OF A PROSPECTIVE STUDY IN MENINGOENCEPHALITIS IN PERU

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Background: Meningoencephalitis (MEC) is a significant public health problem throughout the world; however, few studies have defined the etiologies outside North America and Europe. The objective of this study was to determine the etiologies of meningoencephalitis in Peru. Methods: We conducted hospital based surveillance at 12 hospitals in five Peruvian cities including Coast, Andean and Jungle geographical areas. Symptoms and medical history was obtained from patients older than 28 days with

suspected viral MEC; serum, CSF, rectal and nasopharyngeal swabs were also collected. Follow-up visits were conducted 14 days after presentation. Samples were tested for HSV-1 and 2, HIV, and 18 other viruses. HSV MEC was defined as confirmed (HSV detected by PCR in CSF) or probable (HSV detected in serum or IgG sero-conversion). Results: We enrolled 911 subjects since February 2009. 522 subjects (57.3%) were male; average age was 25.9 years (range 29 days - 86yrs). 31 patients (3.8%) died; 77 (8.5%) were co-infected with HIV. 112 subjects (12.3%)were infected with herpes simplex virus (91 confirmed, 21 probable). HSV sequence was available for 94 participants, of whom 84 (89.3%) had HSV-1 and 10 (11.7%) had HSV-2. Tuberculous meningitis was confirmed in 2 cases and suspected in 13 cases. Seven participants developed meningitis secondary to coxsackievirus. Ten cases were secondary to bacterial meningitis, seven cases were caused by Epstein-Barr virus, six cases by enterovirus, 19 cases by Cryptococcus neoformans, 2 cases by Treponema pallidum, one case by cytomegalovirus, and one by adenovirus. 26 participants exhibited co-infection. Overall, an infectious cause of MEC was identified in 296 (22.6%) participants. Conclusions: This is an updated report on the etiology of community-acquired meningoencephalitis in Peru. HSV infection remains the most common pathogen identified. Unfortunately, 77.3% of cases remain undiagnosed. Additional molecular studies are being implemented to discover the etiological cause of cases that had no pathogen detected.

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EVIDENCE OF INTRA-SUBTYPE, INTER-VACCINE CLADE REASSORTANTS OF H3N2 IN GLOBAL SURVEILLANCE SAMPLES

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Inter-species and inter-subtype genetic reassortment (antigenic shift) has played a major role in the evolution of pandemic influenza, generating viruses to which there is little to no immunity in the general population, resulting in severe disease symptoms and global spread (i.e. 1918 pandemic). Antigenic drift, on the other hand, has been suggested as being mainly responsible for the evolution of the more mild seasonal influenza, generating substitutions conferring gradual escape from previously acquired immunity and giving rise to the need of a constantly updated influenza vaccine. Recent evidence suggests, however, that the evolution of seasonal influenza is in addition substantially affected by intra-subtypic reassortment. Here we report identification of H3N2 intra-subtype reassortment variants derived from global influenza surveillance samples collected since 2009. Full genome and segment phylogenetic analyses show that the reassorted variants derive from two slightly divergent (0.1-1.2%) but well defined vaccine clades, A/Victoria/361/2011 and A/Perth/10/2010. In addition, we identified variants with segments originating from different geographical areas. Our results illustrate the ability of H3N2 to reassort segments (i.e. HA, NP, PA, NA and NS) between both geographically and antigenically defined clades. Although intra-subtypic reassortment of H3N2 occurs frequently, appearance of persistent reassorted variants originating from antigenically distinct clusters is a rare event that has previously been shown capable of producing unusually severe seasonal influenza. It thus becomes important to follow whether the A/Victoria/361/2011-A/Perth/10/2010 reassorted variants found in this study have the capacity to become fixed in the population. Intra-subtype reassortment adds yet another layer of complexity as vaccines circulate with wild type diversity potentially altering the trajectory of influenza viral evolution.

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THE EFFECT OF IMMUNIZATION ON MEASLES INCIDENCE IN THE DEMOCRATIC REPUBLIC OF CONGO

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Measles continues to be one of the largest causes of vaccine-preventable disease mortality among children under five, despite the fact that a safe and efficacious vaccine is readily available. While global vaccination coverage has improved tremendously, measles outbreaks persist through sub-Saharan Africa. Since 2010, the Democratic Republic of Congo (DRC) has seen a resurgence of measles outbreaks, mainly attributed to severe deficiencies in Routine Immunization (RI) at the Health Zone level, where only 22% of reported vaccine coverage rates reach higher than 90%. We used available data from the 2011-2012 IDSR system for measles suspected cases counts reported weekly by health zone to investigate the decline in measles incidence post-immunization (by health zone) with one dose of measles containing vaccine (MCV1) with and without the addition of Supplementary Immunization Activities (SIAs) in the provinces of Kasai-Oriental and Equateur. The impact of measles immunization by health zone was modeled using negative binomial regression. At the provincial level, in Kasai-Oriental, the mean incidence was 452.7 per 100,000 in 2011, while the mean incidence declined to 167 per 100,000 in 2012. In Equateur, the mean incidence was 15 per 100,000 in 2011, while the mean incidence increased to 148.7 per 100,000 in 2012, despite a September 2011 SIA. However, multivariate modeling at the health zone level showed that each 1% increase in MCV1 coverage was associated with a .4% decrease in incidence. Furthermore, the lack of an SIA in each health zone was associated with a 3.4% increase in incidence. While the mean yearly incidence of measles did increase in Equateur following an SIA these are provincial level estimates. Differences may be explained partially by the fact that vaccine effects are not immediate and the selective age categories of mass campaigns. Repeated occurrences of large-scale outbreaks in DRC suggest that vaccination coverage rates are grossly overestimated and signify the importance of the re-evaluation of measles virus dynamics and prevention and control strategies.

1431

DISCOVERY, CHARACTERIZATION AND ECOLOGY OF A NOVEL HEPATITIS A-LIKE VIRUS IN WILD OLIVE BABOONS (*PAPIO ANUBIS*), UGANDA

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Hepatitis A (HAV; family Picornaviridae; genus Hepatovirus) is an RNA virus that causes acute inflammatory disease of the liver in humans and nonhuman primates, and is commonly transmitted through the fecal-oral route. Most often associated with food-borne outbreaks resulting from fecal-contamination, more rarely humans have acquired HAV from the handling of infected non-human primates in captivity. Conversely, recent studies discovering high HAV antibody seroprevalence in wild non-human primates have implicated reverse zoonotic transmission in areas of sub-Saharan Africa where human-nonhuman primate contact and conflict occur frequently. We discovered and characterized by Next-Generation Sequencing (NGS) a novel Simian Hepatitis A-like virus in the blood of a wild olive baboon (*Papio anubis*) in Kibale National Park, Uganda. Furthermore, RT-PCR diagnostics detected viral RNA in the feces of 40% of

baboons sampled at the time of blood collection, suggesting the shedding of potentially infectious viral particles into the environment by wild baboons in western Uganda. Additional screening by field-deployable PCR shows non-random distribution of the virus among individuals and groups. Our results implicate this nonhuman primate as a potential zoonotic source of Hepatitis A-like viruses. This study demonstrates the value of NGS for discovering potential reservoirs of zoonotic pathogens, and supports the supposition that HAV-like viruses circulate naturally in wild nonhuman primates.

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VALIDATION OF A MULTIPLEX RT-QPCR ASSAY FOR DETECTION OF INFLUENZA A AND B IN CLINICAL SAMPLES

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Influenza viruses and their subtypes are common worldwide and produce outbreaks, often along with other respiratory viruses. In Peru, a nationwide surveillance program performs diagnosis and reports to CDC on the outbreak status and emerging influenza variants. Our aim was to develop a multiplex real-time assay for simultaneous detection of Influenza Virus A and B as well as identification of the Influenza Virus A(H3N2) and (H1N1) pdm09 variants as a way to improve the diagnosis speed. The assay consists of two one-step multiplex real-time PCR reactions (RT-qPCR). We discriminated between Influenza A and Influenza B using the matrix gene of Influenza A virus and the nucleoprotein gene of Influenza B virus. We determined subtypes H3N2 and H1N1pdm09 of Influenza A Virus using the hemagglutinin gene. The RT-qPCR reactions were standardized by amplification of serial template dilutions from isolates provided by the CDC. To validate the assay, we analyzed 109 selected clinical samples from patients collected during 2013. Samples were previously analyzed as part of a diagnosis screening and tested positive for influenza viruses. No cross-reaction was recorded with other respiratory viruses potentially present in clinical samples like Adenovirus, Parainfluenza 1, 2 and 3, Human Respiratory Syncytial Virus and Metapneumovirus. No cross-reaction was found either between samples carrying H1N1pdm09 and H3N2. The cutoff was determined at Cq ≤35 for diagnostic test on both reactions. All Influenza A(H3N2), Influenza A(H1N1)pdm09, and Influenza B clinical samples were diagnosed with 100% concordance. The assay was 100% specific for the detection of influenza subtypes in the sample analyzed. This assay is faster and more cost effective than the one reaction per tube setup previously used in the Peruvian surveillance program.

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EVALUATION OF RISK FACTORS FOR HIGH RESPONSE TO ROTAVIRUS IGA AT BASELINE

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Poor immune responses to rotavirus vaccination were observed in infants in developing countries with sero-protection of roughly 40% after vaccination. The reasons for these lower immune responses are not well understood. Therefore, it is important to measure the preexisting factors which may effects response to vaccine. We evaluated the risk factors for influencing rotavirus IgA in infants living in the urban slums of Kolkata, India. We recruited 372 infants who were 6 weeks of age, and collected their blood samples and mother's breast milk at the time of enrolment. Considering skewed distribution of rotavirus IgA titers, quantile regression that estimates conditional median or other quantiles of the response variable was used to evaluate the risk factors for rotavirus IgA response at 6 weeks. Covariates, such as mother's breastmilk at 6 weeks, mother's height, mother's BMI, and monthly household expenditure (in 1000 INR), a proxy for socio-economic status, were selected for the multivariable model. The 25th quantile regression model yielded that infant rotavirus serum IgA at baseline was significantly influenced by mother's nutritional status, which was also supported by the 50th quantile regression and the ordinary regression methods indicating the relationship is stable. Mother's BMI influenced infant rotavirus IgA titers by an average of 2.5 titers/ 10 unit of BMI in ordinary regression, and 5 titers/10 unit of BMI in 50th quintile regression analysis. However, the 25% quantile regression explained only 1.2 titers per 10 unit of mother BMI. Household socioeconomic status was found significant in lower quartile but not in higher quantile suggesting the relationship with the IgA serum level is not stable. The study identifies mother's nutritional status influence baseline serum level for rotavirus IgA in infants in India, thus this need to be considered while evaluating immunogenicity of the rotavirus vaccines. The results also suggest that the quantile regression is a useful statistical tool as it provides flexiblity to detect trends in skewed data.

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RENAL PATHOLOGY IN THE RHESUS MACAQUE/ *PLASMODIUM COATNEYI* MODEL FOR SEVERE MALARIA

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Severe falciparum malaria in adults often results in a spectrum of renal pathology ranging from minimal tubular degenerative changes to severe tubular epithelial degeneration and necrosis with hemoglobinuria, cellular casts and proteinosis, consistent with acute renal failure. The pathogenesis of disease is theoretically linked to damage to the endothelial damage within the microcirculation, inflammatory mediators, hemodynamic disturbances and hemolysis. *Plasmodium coatneyi* is one of the non-human primate malarias which serves as an animal model for *Plasmodium falciparum* induced disease in humans. We examined and described the renal pathology of 40 retrospective cases of *P. coatneyi* infection in rhesus macaques. Macroscopic evaluation of the samples was conducted by board certified veterinary and medical pathologists and were correlated with available antemortem clinical data to include terminal parasitemias. In these animals there was significant capillary sequestration within the renal interstitium as well as within the glomerular tufts as well as irregular thickening of the glomerular mesangium.. Furthermore, and closely correlating with the degree of parasitemia, there was increasing severity of vacuolar tubular epithelial degeneration and necrosis, intratubular proteinosis, hemoglobin and cellular casts, as well as parasitized erythrocytes, erythrocytic and histiocytic hemozin pigment and interstitial hemorrhage, fibrin and edema. Interestingly, there is relative absence of inflammation within the affected tissues. These findings are for the most part consistent with those described in adult humans diagnosed with malaria associated renal failure (MARF). As a result of the correlation of the antemortem symptomology, clinical and histopathologic findings in these retrospective samples, we demonstrate the utility of this animal model for specific use in the examination of acute renal failure in severe malaria.

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INCREASED LEVELS OF S-NITROSYLATION IMPROVES OUTCOMES IN A MODEL OF EXPERIMENTAL CEREBRAL MALARIA

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Decreased nitric oxide (NO) bioavailability is associated with disease severity and worse clinical outcomes in malaria infection. Traditionally NO was thought to act primarily through guanylate cyclase and the production of cGMP; however, there is now extensive evidence for NO to function via a post-translational modification, S-nitrosylation. S-nitrosylation of proteins has been shown to regulate a wide variety of cellular signaling processes and aberrant S-nitrosylation may thus contribute to many disease processes. We hypothesize that increasing bioavailable NO via S-nitrosylating strategies will improve clinical outcome in malaria. In order to test this hypothesis we examined experimental cerebral malaria (ECM) infection in S-nitrosoglutathione reductase (GSNOR) knockout C57BL/6 mice. GSNOR is an enzyme that reduces S-nitrosoglutathione and, therefore, reduces the amount of S-nitrosothiol (SNO), including S-nitrosylated proteins. The deletion of this enzyme results in increased levels of SNO, thereby increasing NO bioactivity in hematopoietic, endothelial and other host compartments. In the ECM model we infected GSNOR knockout mice or their wild type counterparts with 10^6 red blood cells infected with *Plasmodium berghei* ANKA (PbA). In ECM, mice with deletion of the GSNOR enzyme had significantly improved survival compared to wild type control mice ($p<0.0001$), despite significantly increased parasitemia ($p<0.0001$). The prolonged survival in GSNOR null animals was accompanied by improved Rapid Murine Coma and Behavioural Scores (RMCBS) compared to controls. We are currently investigating the effects of the GSNOR deletion on markers of endothelial dysfunction and blood brain barrier integrity during infection. Moreover utilizing bone marrow transplantation strategies, we are determining whether protection is dependent upon S-nitrosylation of hemoglobin, regulators of endothelial WPB exocytosis or other non-hematopoietic compartments. These experiments will help define whether interventions to increase NO bioavailability through SNOs improves outcome in severe malaria.

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CHARACTERIZATION OF *PLASMODIUM VIVAX* BLOOD TRANSMISSION STAGES

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Development of new tools for the detection of *Plasmodium vivax* sub-patent gametocytemia or asymptomatic carriage in semi-immune individuals is fundamental for the eradication agenda. However, *P. vivax* gametocytes are poorly characterized and only a few markers known. In this study we develop new tools for the characterization and field detection of *P. vivax* gametocytes. Using a down-selection based on orthology to *P. falciparum* gametocyte markers we selected a series of putative *P. vivax* gametocyte markers for antibody production and generation of qRT-PCR primers. Epitope-specific rabbit polyclonal antibodies and exon-exon spanning primer sets were tested in samples from *P. vivax* infected Aotus monkeys. Exon-Exon primers against two putative late stage gametocyte markers, PVX_117730 and PVX_117900, were successfully optimized using synthetic cDNA probes and validated in the Aotus monkey model. In these samples typical *P. vivax* gametocyte morphology, including macrogametocytes and exflagellating microgametocytes, were identified on Giemsa stained smears. Indeed, comparison with Pvs25 demonstrated stage specificity and similar sensitivity as this gold standard for the PVX_117900 primer set. Marker gene expression was detected in infected blood samples directly collected from the animals, or after prolonged *ex vivo* culture. Importantly, indirect immunofluorescence (IFA) assays with PVX_117900 antibodies, labeled *P. vivax* parasites showing gametocyte morphology in spots obtained from Percoll gradient bands, though at low frequency. qRT-PCR analysis of longitudinal sampling upon experimental *P. vivax* infection in Aotus supported previous field observations that asexual parasitemia is positively correlated with gametocytemia. Experiments are underway to apply the qRT-PCR and antibody assays to investigate P.vivax transmission during human infection and to perform histological studies in the monkey model. This work represents an excellent starting point for further characterization of *P. vivax* gametocytes *in vitro* and during infection.

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HOST CONTROL OF PARASITE GROWTH IN *PLASMODIUM BERGHEI* INFECTION

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Early infection with *Plasmodium berghei* leads to rapid parasite growth, which slows around day 5-6 of infection. Although splenic clearance is thought to be an important factor in host control of parasite growth, few studies have directly measured parasite clearance. We developed a novel protocol to study the clearance of *P. berghei* infected red blood cells (RBC) *in vivo*. Fluorescently labelled RBCs infected with GFP+ *P. berghei* ANKA (PbA-GFP+) were transfused from donor mice into recipient C57BL/6 mice, and their clearance monitored by regular sampling over the subsequent 24 hours. We compared clearance in two groups of recipient mice; one group of naïve mice, and a second group of mice that were infected with PbA-GFP- 5-days prior. Flow cytometric analysis allowed us to distinguish donor vs. host RBC, and donor vs. host parasites. We used modelling to estimate parasite growth and clearance rates in the naïve vs. 5-day-infected animals, and observed faster clearance and reduced growth of donor parasites in the 5-day-infected animals. However, the changes during infection appeared more complex than simply an increase in clearance in the infected animals. Instead, our modelling suggested that there were differences in both the life-stages of parasites recognised and cleared in infected vs. naïve animals, and in the susceptibility of RBC in these animals. We further analysed the clearance data, focusing on clearance of parasites of different life-stages. We found evidence that trophozoites were more highly targeted in 5-day-infected animals, consistent with a shift towards clearance of earlier life stages over the first 5 days of infection. We also analysed the susceptibility of recipient RBC, by comparing the donor RBC (which were the same in the two groups) parasitemia vs. recipient RBC parasitemia. This showed much higher rates of infection of RBC in naïve mice, consistent with a greatly reduced susceptibility of host RBC in 5-day-infected recipients. This approach provides novel insights into the mechanisms of innate control of parasite growth *in vivo*.

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NON-INVASIVE MEASURES OF INCREASED INTRACRANIAL PRESSURE IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

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Brain swelling seen on magnetic resonance imaging (MRI) is the best clinical predictor of mortality in Malawian children with retinopathy-positive cerebral malaria (CM). Identifying more affordable and feasible non-invasive measures of raised intracranial pressure (ICP) would facilitate recognition of this high risk group, and simplify the conduct of interventional clinical trials. During the malaria season of 2014 (January - June), we carried out serial MRI every 12-24 hours on children with retinopathy positive CM while they were in coma. Two radiologists independently assessed overall brain volume (BV) on an 8-point scale; any discrepancies were resolved by consensus. The BV scores on admission were compared to papilledema (present/absent) and opening pressure at the time of lumbar puncture (mm cerebrospinal fluid). Two non-invasive measures were assessed on admission and at intervals thereafter: optic nerve sheath diameter (ONSD) measured using ultrasound, and pupillometry (NeurOptics). When increased BV was defined as an MRI score of >6, patients with increased BV were more likely to have papilledema than those without increased BV (Fisher's exact, p<0.04). Using the same cut-off, pupillometry, ONSD and opening pressure had AUROCs of 0.35 (95%CI: 0.18-0.53), 0.66 (95%CI: 0.37-0.96), and 0.84 (95%CI: 0.61-1), respectively. Longitudinal analyses of ONSD, pupillometry and BV and case studies in which BV changed significantly over the course of the hospital stay are in progress to determine the natural history of each of the surrogate markers in relation to MRI findings. The findings to date suggest that ultrasound measures of optic nerve sheath diameter, presence of papilledema, and opening pressure are the most useful surrogates for increased BV as determined by MRI.

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VITAMIN D INSUFFICIENCY IS COMMON IN UGANDAN CHILDREN AND IS ASSOCIATED WITH SEVERE MALARIA

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Vitamin D plays a role in the immune response to infectious diseases. Activation of the vitamin D receptor in macrophages results in increased production of the anti-microbial peptides cathelidicin and beta defensin, while vitamin D supplementation reduces the inflammatory response and thus severity of influenza infection in animal models. Therefore, we hypothesized that children with severe malaria would have lower concentrations of plasma 25-hydroxy vitamin D (25[OH]D) than healthy children in a malaria-endemic region. To test this, we measured 25(OH)D in plasma by chemiluminescent immunoassay on samples collected from 40 children between the ages of 18 months and 12 years with severe malaria (20 with cerebral malaria, 20 with severe malarial anemia) and 20 healthy community children (CC) in Kampala, Uganda. We found that low plasma 25(OH)D was widespread: 95% of children with severe malaria (38 out of 40) and 80% of CC (16 out of 20) had insufficient vitamin D levels [25(OH)D < 30 ng/mL]. Of note, 20% of children with severe malaria, but no CC, had 25(OH)D levels < 15 ng/ml. Mean plasma 25(OH)D concentrations were significantly lower among children with severe malaria than among CC [mean (se): 21.2 (1.0) vs. 25.3 (1.6) ng/mL, p=0.03]. In addition, after adjusting for weight-for-age z-score (a measure of overall nutritional status), we found that the odds of having severe malaria declined by 9% [OR: 95% CI = 0.91: 0.83, 1.0] for every 1 ng/mL increase in plasma 25(OH)D. In conclusion, we describe for the first time an association between low vitamin D and severe malaria. These preliminary results suggest a possible role for vitamin D in the etiology of severe malaria. Confirmation of the findings of the present study will set the stage for a clinical trial of vitamin D treatment as a preventative or adjunctive therapeutic intervention to decrease the severity of malarial infection.

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THE REMODELLING OF NASCENT RETICULOCYTES BY *PLASMODIUM VIVAX* AND ITS PATHOLOGICAL CONSEQUENCES

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The pathobiology of *Plasmodium vivax* infections is poorly understood. As malaria parasite red cell tropism defines the course of invasion and the pathology of the resultant disease, we have focused our efforts to determine the fine specificity of *P. vivax* invasion. To do this, we used a novel field flow cytometry approach to sample the different subsets of infected reticulocytes from vivax malaria patients and a range of *ex vivo* assays. Thus, we were able to determine the fine scale tropism of *Plasmodium vivax* for nascent reticulocytes (Heilmeyer Classes I to III) .Importantly nascent reticulocytes are rare in the peripheral blood, suggesting a cryptic role for bone marrow where such target cells are abundant.Subsequent *ex vivo* culture studies of *P. vivax* (with multiple rounds of maturation and invasion) allowed us demonstrate rapid modification of membrane structure and cytoplasm of the nascent reticulocyte. The shear modulus, immunophenotype and nanostructure of the infected reticulocyte membrane were significantly altered within 3 hours of invasion. We also employed microfluidic and micropipette aspiration methods to investigate the biomechanical implications of *P. vivax* development in the reticulocytes.One key finding of these studies was that *P.vivax* rosetting (a process we recently determined is mediated by reticulocyte glycophorin C) may play a significant role in the in disappearance of vivax schizont from circulation. Interestingly, the rate of *P. vivax* rosetting is clearly affected by certain antimalarial treatments.

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RESPONSE OF *PLASMODIUM FALCIPARUM* TO OXIDATIVE STRESS

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Malaria remains a major cause of morbidity and mortality around the world. Severe malaria and malaria-related mortality are due to the human malaria parasite *Plasmodium falciparum*. Host response to the parasite involves the production of reactive oxygen species (ROS). Malaria encodes antioxidant enzymes, however a full understanding of the parasite response to ROS is lacking. ROS could also induce genetic changes in the parasite that could be beneficial for parasite survival. We first examined transcriptional change in the parasites after 4 hour ROS and identified upregulation of stress response pathways including Response to Heat (GO.

0009408), DNA Repair (GO.0006281) and (GO.0006281 DNA repair). We set out to characterize the parasite response to ROS and determine if *P. falciparum* can adapt to increased levels of ROS. To examine the effect of ROS on growth we cultivated the 3D7 strain of *P. falciparum in vitro* in human erythrocytes at 4% hematocrit in supplemented RPMI media with and without ROS. All experiments began at ~2% parasitemia. Parasite growth curves were determined by microscopy of daily smears. Oxidative stress in the form of continuous extracellular generation of hydrogen peroxide was provided by supplementing the culture with 1 mM Xanthine and increasing concentrations XO. 100 U/ml of superoxide dismutase (SOD) was added throughout to enhance formation of hydrogen peroxide from superoxide radical anions generated by the XO-catalyzed oxidation of X. We identified the lethal dose of XO and determined if the parasite could adapt to sub-lethal concentrations of XO. Parasites treated with sub-lethal concentrations of XO demonstrated a decrease in parasite growth from day 3 to day 4. On day 6 of treatment we observed that the treated parasites demonstrated similar growth as compared to untreated control. Our data suggests that parasites exposed to varying concentrations of exogenous ROS showed decrease in growth, however they were able to adapt and grow normally after a few days. We will examine these adapted parasites genetically through transcriptional analysis to characterize their ROS adaptation and examine if genetic rearrangement occurs. Taken together this work explores the impact of host physiology on the biology of the parasite to inform severe disease models of pathogenesis.

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POPULATION GENETIC STRUCTURE OF THE ZOONOTIC MALARIA PARASITE *PLASMODIUM KNOWLESI* IN MALAYSIA

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Human cases of *Plasmodium knowlesi* malaria have been seen in many parts of Southeast Asia, with the largest number in Malaysia, following the first major focus of infections reported from Malaysian Borneo 10 years ago. This parasite is recognized as being zoonotic with wild long-tailed and pig-tailed macaques incriminated as the main reservoir hosts. In order to explore the previous and current transmission of infections, and address whether it may have been adapting to humans recently, it is important to study the population genetic structure of the parasite. In this study, a set of 10 microsatellite loci with tri-nucleotide repeats in the reference *P. knowlesi* genome were developed and validated for genotyping of natural inections by hemi-nested PCR assays. Using these markers, we analysed more than 300 *P. knowlesi* isolates from patients at 9 different sampling sites in Sarawak and Sabah states of Malaysian Borneo and mainland Peninsular Malaysia. All loci were polymorphic in all sampling sites, with more than 100 alleles in total scored across all 10 microsatellite loci, but most individual human isolates had single genotype infections. The mean genetic diversity across the loci was moderate to high, with no significant difference between different sampling sites (He values between 0.65 and 0.75). Levels of multi-locus linkage disequilibrium were very low in all sampling sites, indicating that recombination commonly occurs between different parasite genotypes in mosquito vectors, many of which presumably feed on macaques with multiple genotype infections. Pairwise genetic differentiation was more marked between sites in Borneo and mainland Peninsular Malaysia (FST > 0.10) as compared to differences among sites within Borneo. The human *P. knowlesi* genotype data are compared to isolates of wild macaques in Sarawak to test if there is any restriction in gene flow between the different hosts. These results are being used to strategically plan sampling from selected sites for whole genome sequence analysis in order to scan for possible evidence of host adaptation, and to study recombination in more detail.

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DIVERSITY OF ERYTHROCYTE INVASION PATHWAYS USED BY *PLASMODIUM FALCIPARUM* IN AREAS OF CONTRASTING INFECTION ENDEMICITY IN WEST AFRICA

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Plasmodium falciparum uses a variety of alternative ligand-receptor interactions in order to invade red blood cells. The diversity of these pathways has traditionally been investigated by assessing the ability of parasite isolates to invade red blood cells that have been enzyme treated to selectively remove receptors. To date a variety of assay formats have been reported in different studies, but a standardised assay has not been applied to compare across population samples from diverse locations. Here we investigate *P. falciparum* invasion phenotypes from clinical isolates sampled in three sites on a gradient of transmission intensity in West Africa, using a single assay format. This is the first large-scale comparative analysis of erythrocyte invasion by clinical isolates from different endemic countries assayed in a single laboratory. Assays were performed on over 100 *P. falciparum* isolates from Ghana, Guinea and Senegal, that were cryopreserved at source and thawed so that the laboratory operator of the invasion assay was blinded to the sample source. These isolates were phenotyped for their ability to invade erythrocytes treated with neuraminidase, trypsin, chymotrypsin or a combination of these enzymes, in the first round of invasion following thawing but prior to adaption to culture. RNA was isolated for qRT-PCR from the schizont stage of a subset of these *ex vivo* cultured isolates in order to determine the relative expression levels of parasite invasion ligand genes. The data are analysed to explore the hypothesis that particular invasion pathways are selected in areas of high infection endemicity where there is strong acquired immunity against the parasite ligands, compared with areas of lower endemicity where immune selection is weaker.

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IRON DEFICIENCY ANEMIA AND *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS

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Iron deficiency anemia and malaria are overlapping public health concerns in large parts of the developing world. Clinical and epidemiological studies have revealed that iron deficiency is protective against malaria infection in children and pregnant women. Our comparison of *Plasmodium falciparum* growth in iron-deficient and iron-replete RBCs *in vitro* has revealed that *P. falciparum* erythrocytic stage infection is attenuated in iron-deficient RBCs. We hypothesized that the inhospitable environment of iron-deficient RBCs, which inhibits asexual erythrocytic stage *P. falciparum* propagation, may additionally impact the rate and magnitude of *P. falciparum* gametocytogenesis. Here we report the results of our study of (i) the time to and (ii) the degree of *P. falciparum* gametocytogenesis in iron-deficient as compared to iron-replete RBCs *in vitro*. Our study of iron-deficiency and *P. falciparum* provides an invaluable model for studying *P. falciparum* pathogenesis and transmission.

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EXAMINING SELECTION ON *PLASMODIUM FALCIPARUM* AT DIFFERENT ENDEMIC SITES WITHIN GHANA

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Populations of the human malaria parasite *Plasmodium falciparum* in West Africa are highly polymorphic while being closely related due to relatively unrestricted gene flow within the region. However, selection on individual local populations may vary significantly due to differences in transmission seasonality, drug pressure and levels of acquired immunity. Adaptation of populations will be a balance between local selective pressures and gene flow from neighbouring regions, which may quickly erode signatures of local selection. This will occur most extensively when populations are separated by only short distances, such as within a single country. Population specific selection has been demonstrated to differ between countries within West Africa, but the subtle differences that may exist between populations within a single country have not been investigated. In this study, the genomes of 101 *P. falciparum* clinical isolates from two different Ghanaian sites (Kintampo and Navrongo) separated by ~350km were sequenced and analysed. Transmission in Kintampo, in the forested centre of the country, is high throughout the year, while Navrongo, near the northern border with Burkina Faso, experiences high but seasonal transmission. Scans for evidence of directional selection identified several signatures apparently unique to Ghana, in that they were not seen previously in other West African countries. Patterns of balancing selection were similar in the two Ghanaian populations with high Tajima's D scores observed at loci expected to be exposed to host immune responses. Comparative analysis of the two populations indicated a very close relationship, with a mean FST of ~0.01 and only a small minority of SNPs with FST > 0.1, indicating few loci that may be under divergent selection and which will be discussed.

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DEVELOPMENT AND VALIDATION OF AN *IN VITRO*, CELL-FREE METHOD OF CULTURING MOSQUITO-STAGE *PLASMODIUM FALCIPARUM*

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Between ingestion of gametocytes by an *Anopheles* mosquito and deposition of sporozoites by that mosquito into the skin of a human host, *Plasmodium falciparum* parasites transition through multiple life-cycle stages. These developmental stages are uniquely present in the mosquito vector and present a plethora of molecular and mechanistic targets for interruption of malaria transmission. However, it can be technically difficult and/or laborious to study these developmental stages and the targets they present because they rely on the mosquito for stage progression and growth. We have developed a cell-free method for culturing mosquito-stage *P. falciparum* (NF54 strain) *in vitro*. By seeding with gametocytes from blood-stage cultures, this proprietary method is capable of producing viable ookinetes, oocysts and sporozoites that maintain GFP expression and exclude trypan blue. Immunofluorescent staining with mAb against circumsporozoite protein (CSP) indicates the sporozoites obtained through this method uniformly express CSP while a liver stage development assay indicates they are able to infect cultured human hepatocytes and progress to liver stage, still expressing GFP. Comparative gene expression and mosquito infectivity assays between culture-derived and mosquito-derived parasites are underway.

1447

COMPARABLE DEVELOPMENTAL AND MORPHOLOGIC STAGES OF *IN VITRO* CULTURED *PLASMODIUM FALCIPARUM* SUPPLEMENTED WITH TWO COMMERCIALLY AVAILABLE SERUM SUBSTITUTES

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Current culturing techniques for the *in vitro* culture of *Plasmodium falciparum* are well established. Historically, media used for the *in vitro* culture of this organism consisted of RPMI 1640, a classic well-defined basal cell culture medium, and the addition of human serum. The use of human serum as a supplement is an absolute requirement for parasite growth, but is problematic due to biosafety concerns and lot variability. For that reason, ALBUMAX© II, a lipid-rich bovine albumin serum supplement, is now used in parasite culture medium in place of human serum. It's effectiveness as a human serum substitute has been well documented, but its composition is uncharacterized. This is an issue with research investigating parasite metabolomics and proteomics, which require well-defined *in vitro* growth parameters. Recently, MP Biomedicals released a highly purified microbiological grade bovine serum albumin for use in cell culture. The objective of this study was to compare ALBUMAX© II and MP Biomedical's Microbiological Grade BSA as human serum substitutes in *P. falciparum in vitro* culture. Identical culture conditions supplemented with either ALBUMAX© II or MP Biomedical's Microbiological Grade BSA were prepared and ran simultaneously. Short-term cultures analyzed by Giemsa staining and flow cytometry revealed similar growth trends with similar proportions of parasite developmental stages. Ring-stage and trophozoite survival assays were performed to examine merozoite invasion of erythrocytes and their subsequent development. Long-term cultures with either human serum substitute were also maintained to verify similar growth trends.

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CO-LOCALIZATION OF PFCSA-L AND VAR2CSA ON SURFACE KNOBS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES THAT BIND THE PLACENTAL RECEPTOR CSA

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Placental malaria (PM) is a major cause of disease in pregnant women and their infants; it results from sequestration of *Plasmodium falciparum*-infected erythrocytes (*Pf*-IE) in the placenta via specific binding to chondroitin sulfate A (CSA). Over successive pregnancies, women become resistant to PM as they acquire antibodies against the novel protein PfCSA-L and the variant surface antigen VAR2CSA, *Pf*-IE surface proteins that bind to CSA. Here, we describe the association of PfCSA-L with VAR2CSA, and provide evidence that both protein exist in complexes at the *Pf*-IE surface. In earlier studies, we reported that PfCSA-L binds with high affinity (K_D = 6.6 x 10^{-9} M) to human placental CSPG by Surface Plasmon Resonance (SPR). We also reported that VAR2CSA and PfCSA-L interact on *Pf*-IE surface knobs (by DuoLink analysis), and that the DBL2X domain of VAR2CSA binds to PfCSA-L with subnanomolar affinity (K_D = 8.6 x 10^{-10} M). Here, we report that immune blots using PfCSA-L monoclonal antibodies detect only the PEXEL cleaved form of PfCSA-L (PfCSA-L_{PC}) in *Pf*-IE membrane preparations. Urea extraction of *Pf*-IE membranes suggests that both VAR2CSA and PfCSA-L are anchored by protein-protein (rather than protein-lipid) interactions on the IE surface, suggesting that they exist in complexes. However, VAR2CSA is resistant to alkaline sodium carbonate extraction while PfCSA-L is mostly extractible, indicative of integral and peripheral membrane proteins, respectively.

Preliminary analysis of co-immunoprecipitation and proteomics analysis confirmed direct association of PfCSA-L and VAR2CSA on the surface knobs of *Pf*-IE. These findings suggest that PfCSA-L interacts with VAR2CSA on surface knobs of *Pf*-IE, where they contribute to the CSA-binding phenotype. We are attempting to immunize rats with recombinant PfCSA-L and VAR2CSA DBL2X complexes to generate antibodies against neo-epitopes that may be capable of blocking *Pf*-IE binding to CSA. As a highly conserved protein of small size (~25 kDa), PfCSA-L appears to be a valuable component of a placental malaria vaccine.

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PLASMODIUM FALCIPARUM TOPOISOMERASE II

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Type II topoisomerases, which are well-studied drug targets for many infectious agents and cancer, remain poorly understood in the human malaria parasite *Plasmodium falciparum*. Conventional efforts to express this enzyme have been challenging, as with many important malaria proteins. Here we report expression of full-length *Plasmodium falciparum* topoisomerase II (PfTopoII) in a cell-free wheat-germ protein expression system. Electrophoresis of *in vitro* expressed, radiolabeled PfTopoII pointed to a single 169 kDa entity on an autoradiogram. Soluble PfTopoII from translated lysates displayed a magnesium-dependent, ATP-dependent, and salt-sensitive supercoiled plasmid relaxation activity, and also DNA decatenation activity. A partially truncated PfTopoII construct retained full TopoII function and was more stable. PfTopoII was purified on a DNA affinity column, and a simple and sensitive fluorescence-based screen was established to conveniently track PfTopoII decatenation reactions. Preliminary work with existing TopoII inhibitors pointed to selective inhibition of PfTopoII compared to human TopoII. The availability of pure, functional PfTopoII opens up exciting paths to discovery of new classes of antimalarial agents.

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THE EBL-1/GLYCOPHORIN B LIGAND-RECEPTOR INTERACTION DEFINES A DOMINANT *PLASMODIUM FALCIPARUM* INVASION PATHWAY

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Parasite invasion of red blood cells (RBCs) is an obligatory step in malaria pathogenesis. *Plasmodium falciparum*, the parasite that causes the most virulent form of malaria, has evolved multiple proteins known as invasion ligands that bind to specific RBC receptors to facilitate invasion of human RBCs. The EBA-175/Glycophorin A (GPA) and RH5/Basigin ligand-receptor interactions, referred to as invasion pathways, are under consideration as vaccine targets and have been the subject of intense study to the neglect of others. For this study, we chose to focus on the little-studied EBL-1/Glycophorin B (GPB) invasion pathway because polymorphisms in GPB are prevalent in malaria-endemic regions, suggesting selection from malaria pressure. Through bioinformatic analysis, we have also recently identified considerable variation in GPB transcript levels in individuals from Benin. To elucidate the relative importance of the EBL-1/GPB invasion pathway vis-à-vis the well-described EBA-175/GPA and EBA-140/Glycophorin C (GPC) invasion pathways, we used an *in vitro* RBC culture system to deplete GPA, GPB or GPC via lentiviral transduction of erythroid progenitor cells. We assessed invasion efficiency using a panel of wild type *P. falciparum* lab strains and invasion ligand knockout lines, as well as *P. falciparum* Senegalese clinical isolates and short-term culture-adapted isolates. Our results indicate that the EBL-1/GPB and EBA-175/GPA invasion pathways are of similar and greater importance than the EBA-140/GPC invasion pathway, suggesting a hierarchy of RBC receptor usage in *P. falciparum*.

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EFFECTS OF HETEROZYGOUS SICKLE HEMOGLOBIN ON *PLASMODIUM FALCIPARUM* ERYTHROCYTIC GROWTH INDICES *IN VITRO*

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Heterozygous hemoglobin S, resulting in sickle cell trait (HbAS), reduces the risk of severe *Plasmodium falciparum* infection in African children by 90%. The precise mechanisms by which HbAS confers this protection from malaria remain poorly understood. Elucidating these mechanisms may enable new strategies to neutralize the parasite therapeutically. Among other impacts of HbAS, it has been reported that parasite growth and invasion in HbAS RBCs is attenuated only at low oxygen tensions (≤5% O2), yet much of this work was done several decades ago with less detailed techniques for evaluating parasite growth and invasion. In effort to further define the phenotype of *P. falciparum* infection in patients with HbAS, we quantified parasite cellular phenotypes while cultivating *in vitro* in erythrocytes containing HbAS or normal adult hemoglobin (HbAA). Specifically, using flow cytometry-based assays, we separately examined the effect of HbAS erythrocytes on overall parasite growth, merozoite invasion of RBCs, and merozoite production (parasite erythrocyte multiplication rate). In addition, we used microscopic analyses to compare the timing of parasite maturation and development in HbAS and HbAA RBCs. With our HbAS versus HbAA RBC invasion analyses, we also report development of a simple two-color invasion assay allowing for direct comparison of parasite invasion into two cell populations labeled with the same fluorophore at differing concentrations. Finally, we investigated the impact of alpha-thalassemia upon the distinct cellular phenotypes in HbAS erythrocytes, because clinical data indicate that the co-inheritance of alpha-thalassemia attenuates the protection against severe malaria conferred by HbAS. These investigations leverage novel tools to refine our understanding of an ancient relationship between parasite and host. Further investigations of this relationship can improve our fundamental understanding of *P. falciparum* pathogenesis and enable the development of strategies to treat and prevent severe malaria.

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MICROVASCULAR TISSUE REOXYGENATION IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

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Impaired vasodilation and parasitized red blood cell adherence to blood vessel walls is thought to contribute to the pathophysiology of severe malaria, resulting in poor tissue perfusion. Decreased rates of skeletal muscle reoxygenation have been observed in Indonesian adults with severe malaria compared to healthy controls, but studies of tissue perfusion have not previously been performed on children with severe malaria. We measured rates of gastrocnemius-soleus tissue reoxygenation following a 3-minute femoral artery occlusion in Malawian children with cerebral malaria (CM) on each of 3 days following admission and at a 28-day follow-up visit. Children with uncomplicated malaria (UM) were assessed as controls. Children with cerebral malaria had lower maximum reoxygenation rates than uncomplicated malaria patients (median[IQR]; CM admission: 0.75[0.59-1.01] vs. UM: 1.22[1.12-1.34] % O2 saturation/second, p = 0.016). Maximum reoxygenation rate increased on day 3 compared to admission (CM day 2: 0.84[0.63-1.10] % O2 saturation/

second, p = 0.176 vs. CM admission; CM day 3: 1.07[0.98-1.33] % O2 saturation/second, p = 0.005 vs. CM admission). In addition, peak reoxygenation rate increased further at the 28-day follow-up visit (CM Follow-Up: 1.99[0.59-2.74] % O2 saturation/second, p = 0.039 vs. CM admission). Children with cerebral malaria appear to have an acutely diminished capacity to reoxygenate hypoxic tissue.

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RETINAL MICROCIRCULATION DYNAMICS DURING AN ACTIVE MALARIAL INFECTION

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The development of effective adjunctive therapies to treat cerebral malaria (CM) would have significant clinical impact in Africa where nearly a million children die each year due to CM infections. A better understanding of the cellular and molecular mechanisms underlying CM can lead to improved therapies and vaccines. Microcirculation in the retinal vasculature provides a window to image dynamic changes taking place in the central nervous system during CM disease progression. We have introduced a new video microscopy imaging modality using high resolution fundoscopy (HRF) and optical coherence tomography (OCT) to visualize the course of a *Plasmodium berghei* infection in a murine model of CM. Using OCT measurements the *in vivo* retinal cross-sections of infected mice do not seem to be enlarged or edematous in comparison to uninfected mice. Bright field fundoscopy reveals flowing hyper-reflective clumps that are confined to the retinal vasculature. We are actively investigating the nature of these clumps using fluorescently tagged parasites and flow cytometry to determine whether the size and behavior of the hyper-reflective bodies is correlated with disease severity and parasitemia. Infected mice are easily distinguished from uninfected controls based on the presence of hyper-reflective clumps, which suggests that HRF has diagnostic potential for establishing an individual's infection status. Using fundoscopy we detected a CM-specific increase in the number of GFP-positive immune cells (monocytes, macrophages, granulocytes) in the retina of LysM-GFP mice as the infection progressed. These preliminary data suggest that the retinal microcirculation can serve as a diagnostic window for malarial infection, and using video analysis of the microcirculation dynamics can aid in quantitatively characterizing the development of cerebral malaria under different treatments.

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AMPLICON DEEP SEQUENCING OF *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-1: FROM GENES TO STRAINS TO INDIVIDUALS TO POPULATIONS

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Malaria parasites have numerous hypervariable surface antigens. Deep sequencing of genes and the SNPs that encode this variability is a compelling new tool to answer questions about within-host diversity, between-strain competition, population genetics, and the origin of recurrences. Amplicon deep sequencing has been used to investigate these issues in malaria and other organisms. However, several questions regarding this approach remain. First, it is unclear how these results relate to other genotyping methods, such as microsatellites. Second, it is unclear how deep sequencing of individuals correlates to deep sequencing of pooled samples (PoolSeq). Here, we explore these issues while investigating the *Plasmodium vivax* population of Northern and Western Cambodia. Using ion semiconductor sequencing, we sequenced a short hypervariable fragment of the *P. vivax* merozoite surface protein-1 42-kDa domain to investigate the within-host diversity, population diversity and population structure of *P. vivax* using a cohort study (n=108 isolates) and a cross sectional survey (n=159 isolates). In these populations, we identified 67 and 35 unique haplotypes, respectively, and a total of 47 SNPs. Comparing amplicon deep sequencing to a three-locus neutral microsatellite genotyping approach on a subset of 50 isolates, we found that amplicon deep sequencing is more sensitive for resolving and following mixed infections (average MOI of 3.6 vs 2.1 variants per isolate, p<0.001). Direct comparison of PoolSeq to standard individual deep sequencing found that pooled deep sequencing of clinical isolates provides an accurate picture of parasite diversity and captured the great majority (88%) of the diversity within the population. Lastly, different population structures are seen between provinces and within provinces in Northern and Western Cambodia, suggesting that highly structured *P. vivax* populations exist within this region. This study addresses several knowledge gaps concerning the use of amplicon deep sequencing and further demonstrates its utility for studying the genetic epidemiology of malaria.

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VARYING INTERACTION BETWEEN *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN AND DUFFY-POSITIVE RED CELLS

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Plasmodium vivax invasion of erythrocytes is known to be dependent on the interaction between *Plasmodium vivax* Duffy Binding protein region II (PvDBPII) and the Duffy antigen (Fy) present on erythrocytes. However increasing evidences indicates an alternative Fy-independent invasion pathway maybe available. To better understand the mechanisms by which the PvDBP interacts with host erythrocytes we examined binding

of conformationally correct recombinant PvDBPII to Duffy-positive host erythrocytes. We found levels of PvDBPII binding to a single individual's erythrocytes highly reproducible, but we also observed considerable variability in binding among erythrocytes from different individuals of the same Duffy genotype. Some of this inter-individual variation was attributable to the Duffy polymorphism on the N-terminal region of the Fy (Fy^a/Fy^a = 2197.38 ± 608.15 vs Fy^b/Fy^b = 4713 ± 483.16). However, PvDBPII binding between two *FY*B/*B* individuals showed considerable (e.g. individual 1 vs individual 2 = 4713 ± 483.16 v/s 12924 ± 749.79). The results seem to suggest that polymorphisms in other erythrocyte membrane proteins and/or their post-translational modifications could influence interaction between PvDBP and host erythrocytes, thus modifying susceptibility to vivax malaria.

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PHARMACOVIGILANCE DURING CAMPAIGN OF SEASONAL MALARIA CHEMOPREVENTION IN SENEGAL, 2013

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In 2013, the Senegal National Malaria Control Program (NMCP) implemented seasonal malaria chemoprevention (SMC), an intervention recommended by the World Health Organization (WHO) in 2012 in areas of seasonal malaria in which at least 60 % of cases occur over a period of four months. In Senegal, SMC was implemented in a door to door campaign by community health volunteers administering a dose of sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) under directly observed therapy to children three months to ten years and leaving an additional two doses of AQ for the guardian to administer. The campaign took place in four districts with almost 60,000 children in the target age group, and was accompanied by communications, advocacy, and social mobilization activities. 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. The implementation of pharmacovigilance of antimalarials was an important step for the development of the pharmacovigilance program in Senegal and helped reorganize the national system, with the appointment of a national focal point at the Directorate of Pharmacies and Laboratories. Senegal has been able to establish a single system that takes into account all medicine programs and other products available in the country. Senegal was the 95th member of the WHO International Drug Monitoring Program and transmits notifications to the Uppsala Monitoring Center via VigiFlow software. During the 2013 SMC campaign, notices of adverse events were collected by the NMCP and processed by the Anti-Poison Center. Of the 115,547 treatments of SP+AQ administered to 59,420 children under 10 years, 20 adverse events notifications were sent to the NMCP. Adverse effects reported were mostly minor: abdominal pain, nausea, vomiting, urticaria, etc. All notifications were made by health post nurses. The Anti-Poison Center, which is responsible for determining imputability, judged that imputability was possible in 18 cases, improbable for one case, and uncategorized for one case, in which a single dose of amodiaquine was given. No severe adverse events were notified. In 2014, SMC will be implemented in 16 districts in the four regions of Kédougou, Tambacounda , Kolda and Sédhiou, targeting nearly 600,000 children. The pharmacovigilance system will be strengthened to ensure that adverse events will be notified and tracked.

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SELECTION OF ANTIRETROVIRAL TREATMENT (ART) IMPACTS ANTIMALARIAL PHARMACOKINETICS AND TREATMENT OUTCOMES IN HIV-MALARIA CO-INFECTED CHILDREN IN UGANDA

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HIV-infected children on protease-inhibitor (PI)-based ART have a lower risk for malaria compared to those on NNRTI-based ART. We evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of artemether-lumefantrine (AR-LR) in Ugandan children aged 0.5 to 8 years, providing the first intensive PK/PD data in HIV-infected children receiving lopinavir/ritonavir (LPV/r) or NNRTI [nevirapine (NVP) or efavirenz (EFV)]. HIV-uninfected children served as controls. Intensive PK (area under the concentration-time curve, AUC) and PD for 28 and 42 days, respectively was done. AR, active dihydroartemisinin (DHA), and LR in capillary plasma were measured by LCMSMS for 121 children (n=30 LPV/r; 28 NVP; 15 EFV and 48 controls). Lower AR AUC was seen with all ART groups compared with controls [geometric mean (GM) ratio; LPV/r 0.79 (ns); NVP 0.36 ($p<0.001$); EFV: 0.42, ($p=0.003$)] while DHA was reduced only in children on EFV [GM ratio 0.27, ($p<0.001$)]. For LR, AUC was 2-fold higher for children on LPV/r and 3-fold lower for children on EFV-based ART ($p<0.001$ for both). Median Day 7 LR level was 3.4-fold higher and 3.9-fold lower with LPV/r and EFV, respectively. Cumulative 28 day risk of parasitologic failure was 12%, 27%, and 33% for children on LPV/r, NVP, and EFV, respectively. Multivariate regression indicates altered malaria risk was largely due to distinctions in LR AUC ($p=0.016$). Moreover, day 7 levels were associated with 28 day risk of recurrent parasitemia (hazard ratio 0.60, $p=0.001$). Notably, 14/15 children on EFV had day 7 levels in the lowest quartile, and 13/15 had LR AUC in the lowest quartile. Use of AL in the setting of LPV/r-based ART resulted in a significant increase in LR exposure, largely explaining a reduced risk of malaria. In contrast, EFV-based ART results in significant reduction in exposure to all drugs; AR, DHA and LR; with LR reduction strongly associated with increased risk of parasitologic failure. These intensive PK/PD data demonstrate altered exposure and response supporting reevaluation of guidelines for antimalarial treatment of HIV-infected children, especially in the setting of EFV-based ART.

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ARTEMETHER-LUMEFANTRINE EXPOSURE FOLLOWING TREATMENT IN MALARIA-INFECTED CHILDREN AS COMPARED WITH ADULTS IN UGANDA

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Artemether-lumefantrine (AR-LR) is currently the most widely adopted artemisinin combination therapy world-wide. We evaluated the comparative pharmacokinetics (PK) and pharmacodynamics (PD) of AR-LR in the context of developmental changes occurring in children 6 months to 8 years and compared PK results to data from adults. All individuals were treated for malaria in a high endemic region of Uganda and enrolled for intensive PK evaluations for AR-LR with 42 day follow-up. Exposure was estimated to 21 days [area under the concentration-time curve (AUC)]. Artemether (AR), its active metabolite dihydroartemisinin (DHA), and the long-acting partner drug, LR were quantitated from capillary plasma by LC/

MS/MS. Thus far, intensive PK/PD evaluations have been completed and analyzed for 26 children 8 months to 4 years, 22 children 4 to 8 years, and 11 adults (16 to 56 years) (n=30 enrolled). As expected, parasite densities (parasites/μL) at the time of presentation were significantly different between age ranges [Geometric mean (GM) 20,615, 6232 and 604 parasites/μL in < 4 years, ≥4 to 8 years, and adults, respectively). For the artemisinins (AR and DHA), no significant changes in exposure (maximum concentration or AUC) were observed in children as compared with adults. For LR, a trend toward reduced exposure (AUC) and day 7 (D7) levels were observed for children compared to adults [AUC GM 272 vs 316 hr•ug/mL (p=0.15); D7 median 339 vs 450 ng/mL). Notably, LR levels on day 21 were significantly lower in younger children compared to adults (p=.04). These results suggest overall comparable exposure in children as compared with adults, although sample sizes are limited in the very young (n=8 less than 2 years) and adults (n=11). Enrollment is ongoing in both population and intensive PK studies, and final results will be presented.

1459

THE AFFORDABLE MEDICINES FACILITY-MALARIA (AMFM) IN GHANA: FACTORS ASSOCIATED WITH PRIVATE RETAILER'S ADHERENCE TO THE RECOMMENDED RETAIL PRICE

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The Affordable Medicines Facility-malaria (AMFm) was initiated as a pilot in 7 countries aimed at increasing availability, reducing prices, increasing market share and increasing use of co-paid quality-assured artemisinin-based combination therapies (QAACTs). As part of the AMFm supporting interventions to facilitate the high level subsidy on QAACTs reaching consumers, the AMFm green leaf-logo was widely publicized, along with a recommended retail price (RRP) in Ghana. Using data from the 2011 endline survey of the Global Fund-commissioned AMFm independent evaluation, we explored factors associated with outlets stocking some co-paid QAACTs at RRP, and those stocking all at RRP in the private-for-profit health sector in Ghana. Analyses accounted for the complex survey design. We used multivariate logistic regressions to determine the association between being aware of the RRP and correctly specifying it, and the probability of stocking some or all QAACTs at RRP. Among the 545 outlets making up our sample, and which stocked at least 1 co-paid QAACT, 1,440 co-paid QAACTs were audited, with a mean number of 2.3 per outlet (95% CI: 2.1, 2.4). Twenty-four percent of outlets stocked no co-paid QAACTs at RRP, while 68% had some, but not all their co-paid QAACTs at RRP. Almost half of all the co-paid QAACTs audited were available at RRP. Many more outlets stocked some co-paid AL over ASAQ (93 % vs. 46%). Knowledge of the RRP was associated with a much higher predicted probability of stocking some co-paid QAACTs at RRP than stocking all at RRP (83% vs. 41%), although it was a strong predictor of both outcomes (p<0.001 for both). The type of co-paid QAACT being stocked (ASAQ/AL/both) was an important predictor of an outlet stocking both some (p=0.014) and all (p=0. 005) co-paid QAACTs at RRP. Malaria prevalence was also associated with stocking some co-paid QAACTs at RRP (p=0.013). Our study shows that retailer's adherence to the RRP for co-paid QAACTs can be high when knowledge about the RRP is present. Information on the AMFm subsidy needs to be disseminated to retailers with greater focus on those areas of high malaria prevalence, such as the northern savanna zone. All recommended policy interventions should be coupled with regular monitoring of prices and other indicators in the market in order to accurately measure the trend of the effects of the interventions.

1460

EFFECTIVENESS AND TREATMENT ADHERENCE TO ARTEMETHER-LUMEFANTRINE UNIT DOSED BLISTER-PACKS VERSUS STANDARD BLISTER-PACKS IN THE TREATMENT OF UNCOMPLICATED MALARIA: A RANDOMIZED CONTROLLED EQUIVALENCE TRIAL

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Pre-packing drugs for treatment of uncomplicated malaria, into colour coded unit doses for particular age or weight groups has been shown to improve adherence; however, it creates challenges regarding procurement and administration thus reducing the benefits gained. This study sought to determine whether effectiveness and treatment adherence to standard blister-packs would be equivalent to unit dosed blister-packs. Between February and October 2010, an open label randomised controlled trial was conducted in 846 children aged 6-59 months living in a high malaria transmission setting in Uganda. Enrolled children were randomised to two study arms, receiving either unit dosed or standard blister-packs, and followed for 28 days. Outcome measures were risk of clinical and parasitological failure over 28 days' follow-up and adherence to prescribed treatment. Analyses were conducted on an intention-to-treat basis. The cure rate unadjusted by genotyping was 44.6% in the unit dosed blister-packs treatment arm compared to 41.5% for standard blister-packs (risk difference (RD) 3.1, 95% confidence interval (CI) -3.1, 9.9 p=0.375). Unadjusted risk of clinical failure was 28.7% in both treatment arms and unadjusted risk of parasitological failure was 26.7% and 29.8% in the unit dosed and standard blister-packs arms respectively (RD -3.1, CI -9.2, 3.2, p=0.330). There was no difference in adherence between the two treatment arms. Effectiveness and treatment adherence were equivalent in the two study arms. This study questions the value of unit dosed packaging given the challenges associated with ensuring uninterrupted supply, and highlights the importance of good provider-patient communication for treatment adherence. The findings suggest that standard blister-packs would improve quality of care through improved reliability of supply without compromising effectiveness and adherence to antimalarials. The implications of this study are broader than antimalarials and further research is needed to confirm and explore the potential impact of these findings.

1461

BIOLOGICAL STABILITY OF DIHYDROARTEMISININ IN PHYSIOLOGICAL CONDITIONS

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Artemisinin derivatives are the most effective antimalarials available to-date. Dihydroartemisinin (DHA) is a drug on its own and also the main metabolite of other artemisinins. These molecules, characterized by the presence of an endoperoxide pharmacophore, are highly unstable; they degrade very quickly in the presence of ferrous iron or organic solvents. Less documented is the stability of DHA, measured as antimalarial activity, when incubated *in vitro* with blood components or in different cultures conditions. We investigated this problem by incubating DHA in PBS, plasma, serum or erythrocytes lysate for different lengths of times, at different temperatures and pHs. Chloroquine, a 4-aminoquinoline antimalarial and artesunate were also used to verify if drug instability was related to the presence of the endoperoxide. Residual activity of the drugs was evaluated by determining *Plasmodium falciparum* viability with the

pLDH method. A significant reduction of the antimalarial activity of DHA was seen after incubation in plasma or serum and to a lesser extent with erythrocytes lysate or PBS: 3-hour incubation in plasma was sufficient to double the IC50 of DHA, whereas activity was almost completely lost after 24h. The serum-enriched mediums (10% human serum or 10% albumax) customarily used for *in vitro* cultures also affected DHA efficacy. DHA activity was partially preserved at 4°C or at room temperature, but was lost at 40°C. Similarly, increasing pH from 7.2 to 7.6 reduced DHA efficacy. Artesunate behaved in a similar way to DHA, whereas chloroquine was unaffected in any of the tested *in vitro* conditions. These results suggest that particular care has to be taken in conducting and interpreting *in vitro* studies, and in storing these compounds. Moreover, conditions such as fever, hemolysis or acidosis associated with malaria severity may contribute to artemisinins instability and reduce its effectiveness.

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SYNTHESIS AND BIOLOGICAL TESTING OF 2,5-SUBSTITUTED PYRIMIDINES

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Heterocyclic containing compounds have historically represented important structures in medicinal chemistry. Malaria is a debilitating and life-threatening mosquito borne disease that affects millions of people a year. From the 1600's to WWII the traditional treatment for malaria used the aromatic heterocylic compound, quinine. Vinamidinium salts are known for their ability to make aromatic heterocycles. We report on the synthesis of pyrimidines from vinamidinium salts and their biological activity against Malaria. We have prepared two vinamidinium salts and then synthesized a series of pyrimidines from each salt. These two series of pyrimidines were evaluated for anti-malarial activity.

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MALARIA PLACENTAL INFECTION AND INTERMITTENT PREVENTIVE TREATMENT IN SUBURBAN KINSHASA, DR CONGO

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Malaria is a major health threat and an obstacle in the path of economic development of individuals, communities and nations. It is the leading cause of mortality and morbidity in the DRC. Pregnant women and children under 5 years are the most vulnerable groups. For pregnant women, it may be responsible for premature abortion, fetal growth retardation and infant and maternal death. In the DRC, the NMCP recommends that all pregnant women receive two doses of IPT with sulfadoxin pyrimethamin during ANC. This study was undertaken to 1) determine the proportion of pregnant women who took the IPT, 2) Estimate the frequency of placental malaria infection at N'djili Reference Hospital, and3) Determine the frequency of chorionitis A cross sectional study was conducted among 223 women delivered at the maternity HGR N'DJILI in Kinshasa, women who accepted to participate in the study after informed consent in a period from September 2013 to March 2014. Blood sampling was performed for making a thick and a thin smear in women at childbirth, placenta prints was made and a sample was preserved in formalin for histological analysis. In addition an interview was conducted to obtain information about IPT. 223 women were included in the study. The age group most represented was 18-25 years with 45.4 %. Primiparous were 46.4 %. TPI 1 was observed in 76.9 %, while the taking of IPT 2 was observed at 23.1%. 28.7% of women took the first dose at fifth month and 44.3 % on the sixth month or after . All multiparous took the first dose of IPT, in secondiparous and primiparous group the taking was 66. 6 %. The difference between the two group was highly significant p <0.0001 For the second dose, 68.5% take up to 8th month and 16.6% have taken at nineth month 77.8% of GE examined was positive positive with *Plasmodium falciparum* 73.1% of placental prints were positive with trophozoites and 7.4% with trophozoites and schizonts of *Plasmodium falciparum*. Diiference between women with IPT and women without IPT, was significant p< 0.001 Histopathological findings will be available in late May In conclusion, the plasmodium infection in pregnant women and placental infection are very high among pregnant women in peri -urban environment Kinshasa Intermittent preventive treatment in pregnant women is not unfortunately respected Increased awareness should be held to a greater commitment to the strategy of prevention against malaria

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DIRECTLY OBSERVED THERAPY: REVIEW OF BEST PRACTICES AND THE APPLICATION TO MALARIA TREATMENT

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Directly observed therapy (DOT) is the standard of care for tuberculosis treatment and it is used for HIV/AIDS treatment in many settings. These are complex treatment regimens for which high adherence rates have been achieved. Yet, for malaria, where treatment regimens range from three to 14 days, DOT is perceived to be too difficult to implement. We are conducting a literature review of DOT best practices for tuberculosis, malaria and any other applicable disease treatment regimens. We are examining DOT for malaria across a wide variety of treatment protocols. In addition to the literature review, we will interview key informants, including community health workers and village health workers, who have implemented DOT for malaria and other diseases. The aim of the interviews is to better understand the most effective implementation strategies and any challenges encountered. We will examine factors that have promoted and hindered high treatment adherence. The standard interview questionnaire captures structured data from key informants and includes information on DOT implementation strategies, contextual information, treatment regimens, and factors leading to success and failure. It also includes questions about treatment seeking behaviors and access to health services at the community level. The National Malaria Control Program (NMCP) in Vietnam has experience implementing DOT for *Plasmodium falciparum* infection in tier 1 provinces of the containment zone. In 2014-2015, Vietnam's National Institute of Malariology, Parasitology and Entomology (NIMPE), has funding from the Global Fund to implement DOT in areas where multi-drug resistant malaria is emerging. In coordination with NIMPE, we plan to pilot the best practices identified in the literature review and key informant interviews. By June of 2014, we will have completed and analysed 20 structured interviews. The literature review and key informant data will be presented as well as the plan to pilot implementation of DOT.

1465

CHEMOGENETIC PROFILE ANALYSIS OF *PLASMODIUM FALCIPARUM* TO COMPOUNDS FROM THE MMV MALARIA BOX

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Drug resistance in *Plasmodium falciparum* requires improved use of existing drugs and enhanced methods for discovery of new drugs with unique targets and mechanisms of action. Chemogenetic profiling of *P. falciparum* mutants is a new approach to identify and prioritize drugs with novel targets and/or modes of action and is potentially a way to predict drug combination therapies with optimal synergistic anti-parasite activity. Isogenic mutants of *P. falciparum* were created by *piggyBac* transposon insertion whereby each mutant parasite carries a unique signature of affected metabolic pathways that can alter responses to drugs. An important advantage of this approach is the precise nature of the chemical-genetic profile, since single mutations are created in an identical genetic background (a clone of NF54). *piggyBac* mutant clones with insertions in identifiable links to specific GO pathways were profiled for responses to a subset of compounds from the malaria box. The wild type and *piggyBac* mutant parasites were allowed to grow for 72 hours in a range of growth inhibitors and then quantified using a DNA dye (SYBRGreen I). The different *piggyBac* mutants varied in their susceptibility to inhibitors, demonstrating unique signatures related to the specific mutation allowing us to map associations among inhibitors and mutants. Cluster and network analyses of chemicogenetic profiles to malaria box drug susceptibility profiles linked drugs with common mechanisms of action and provided potential insights into metabolic pathways targeted by the antimalarial drugs.

1466

OVERCOMING PERSISTENT BARRIERS TO THE SCALE UP OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP): PERSPECTIVES OF POLICYMAKERS, HEALTHCARE PROVIDERS AND PREGNANT WOMEN

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The World Health Organization recommends intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) for pregnant women resident in areas of moderate (stable) or high malaria transmission to prevent the adverse consequences of malaria infection during pregnancy. Despite efforts over the past decade to scale up coverage, less than one-quarter of women receive two doses. To identify persistent barriers to the scale-up of IPTp, as well as the potential to scale-up alternative regimens and/or alternative strategies, semi-structured in-depth interviews (IDIs) and focus group discussions (FGD) were conducted among healthcare providers and pregnant women in Tanzania. A total of 64 pregnant women participated in FGDs, while 28 pregnant women were included in IDIs; 14 healthcare providers participated in IDIs and, separately, 11 policymakers were interviewed. Participant responses were coded and analysed using NVivo 10.0. Content analysis was used to derive a range of themes. A major barrier to the acceptability of IPTp-SP across those interviewed was side-effects. The risk of side-effects discourages some healthcare providers from providing treatment given the ethos of 'do no harm' and the fact that most pregnant women at antenatal presentation are either not infected or have an asymptomatic infection and do not feel ill. Perceptions and experiences of side effects of SP are likely to shape whether or not replacements drugs may be brought to scale given that all current candidates involve multi-day regimens. The risk of side-effects might be more acceptable to many (but not all) policymakers, health care providers, and pregnant women if: a more efficacious therapy than SP is used in IPTp, a replacement for SP is simultaneously protective against malaria and curable sexually transmitted infections, as may be the case with azithromycin-based combination therapies, or women are screened for malaria and only women who are found to be parasitemic are then given treatment.

1467

IN VIVO EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE VS. DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED MALARIA AND ASSESSMENT OF PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE

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Antimalarial efficacy studies are recommended by the World Health Organisation to monitor the efficacy of artemisinin based combination therapy (ACT) and possibly detect evolution/emergency of tolerance/resistance to these drugs. Currently, Artemether/Lumefantrine (AL) is the only ACT which is being used in Tanzania and thus, testing of new ACTs such as dihydroartemisinin-piperaquine (DP) is important because alternative drugs are urgently required. This study will be an open-label randomized trial and aims to assess the efficacy of AL versus DP; and the role of parasite genetic/genomic factors that might be associated with treatment outcome among patients with uncomplicated malaria treated with these ACTs. The study will be conducted from May 2014 and will recruit 600 children aged 6 months to 10 years with uncomplicated falciparum malaria at Muheza Designated District Hospital and Ujiji Health Centre in Tanga and Kigoma regions respectively (150 patients per treatment arms at each site). Follow up will be done for 63 days and the primary end point will be parasitological cure on day 28 for AL and 42 for DP (non-adjusted and adjusted by PCR to correct for new infections). The secondary end points will include: parasite clearance after 72 hours, parasitological cure on day 14, extended parasitological cure on day 42 for AL and 63 for DP, improvement in haemoglobin level at day 28 compared to day 0, reduction in gametocyte carriage at day 14 and day 28 Vs 0, occurrence and severity of adverse events, and genomic profile of *P. falciparum* malaria parasite. Preliminary results will be presented and discussed, and study will provide important data to the National Malaria Control Program (NMCP) to be used in the ongoing review of treatment guidelines. The information will also support NMCP to recommend DP as the second line antimalarial drug for the treatment of uncomplicated malaria in Tanzania.

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BURIED LEGACY? AN ANALYSIS OF PSYCHIATRIC TOXICITY OF PRE-CHLOROQUINE ANTIMALARIALS

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The toxicity of synthetic anti-malarials such as primaquine, chloroquine, and mefloquine have been well documented. Their side effects range from hemolytic anemia in the case of primaquine to psychiatric disturbances in the case of chloroquine and mefloquine. The toxicity profiles of their

pre-WWII predecessors, however, have received relatively little attention. Pamaquine and mepacrine, synthetic anti-malarials developed by German industrial chemists in the 1920s and 1930s, proved invaluable for malaria control among Allied and Axis troops alike. Pamaquine and mepacrine, however, were not without their own toxicity issues. As their use expanded, first in far-flung colonial outposts and subsequently in Asian and European theatres, reports began to emerge about unexpected psychiatric toxicity. Despite these warnings, their central role as anti-malarials continued throughout the Second World War. This presentation will examine the factors that spurred their widespread use despite a growing number of contemporary reports that recommended cautious use among high-risk individuals.

1469

SEVERE MALARIA MORTALITY AND MANAGEMENT IN THREE GENERAL REFERENCE HOSPITALS IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

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Malaria remains a global problem and remains a major public health concern for the countries of Sub-Saharan Africa, particularly the Democratic Republic of Congo (DRC). It is one of the leading causes of morbidity including severe form occurs in individuals lacking premunition or those who have lost over several years without exposure , particularly children under 5 years and pregnant women . This form of malaria is based on high hospital mortality in a pediatric setting, requiring proper care, effective and consistent with national policy. This study aimed to describe the forms of severe malaria to determine the molecules used for the care and describe the evolution of children hospitalized for severe malaria. This descriptive study was conducted in the pediatric wards of General Reference Hospitals of Makala (GRHM), Kintambo (GRHK) and Universitary Clinics of Kinshasa (UCK) for the periods from 01 January 2011 to 13 July 2013 (2.5 years) by collecting information on archived records. Severe malaria cases in anemic and neurological forms were the most encountered respectively 58.59 % and 35.35 % in UCK; 62.2% and 30.8 % in GRHK; 65.5 % and 23 % in GRHM. Pulmonary and haemoglobinuric forms were also observed. Injectable quinine infusion was the most commonly used antimalarial molecule in 91.92 %; 89.7 % and 97.5% of cases respectively at UCK, GHRK and GHRM. The evolution after treatment showed a mortality of 33.3% (UCK), 23.4 % (GRHK) and 39 % (GRHM). But healing was observed in most cases at 67% (UCK), 67.1 % (GRHK) and 61 % (GRHM) without sequelae but 1.01% has sequelae In conclusion, the predominant shape was anemic form followed by neurological form, quinine antimalarial infusion was administered to support these and therapeutic evolution post cure was recovery in most cases, all times with considerable mortality. The ideal is an early treatment of malaria cases to avoid severe cases.

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ARE WE ACHIEVING SUFFICIENT POPULATION COVERAGE OF ARTEMISININ-COMBINATION THERAPY AMONG CHILDREN WITH MALARIA IN AFRICA? A SYSTEMATIC ANALYSIS OF DATA 2003-2012

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Artemisinin-based combination therapies (ACTs) are highly effective for curing uncomplicated malaria and preventing progression to severe disease. Funding for ACTs has dramatically increased and most countries in Africa have promoted ACT as first-line treatment since around 2005. Since this time, there has been a major rise in global ACT procurement. However, because of the challenges of reliably measuring the population coverage of ACTs among children with confirmed uncomplicated malaria, to date continent-wide changes in treatment coverage have not been quantified in a rigorous manner. Data from household surveys with parasite testing using antigen-detection rapid diagnostic tests (RDTs) are increasingly available, which provide a period prevalence estimate of infection that overlaps with two-week fever history. We combined data from 71 national household surveys (DHS, MIS, and MICS) to estimate the annual proportion of children with uncomplicated malaria (fever + parasite infection measured by RDT) receiving ACTs for all countries in sub-Saharan Africa 2003-2012. We used an individual-level logistic regression model including local PfPR, child age, household wealth, urban/rural, and insecticide-treated net (ITN) possession to predict RDT status for children in surveys without parasite testing. We used ACT distribution data combined with country-level covariates and temporally-correlated random effects in generalized linear regression models within a Bayesian framework to predict coverage to countries and years without data. Scale-up of treatment with ACTs among all children with uncomplicated malaria has been modest, reaching only 18% (95% Credible Interval 13%-22%) by 2011-2012, with highly variable coverage by country. The primary barriers to treatment with an ACT appear to be low treatment-seeking rates and inadequate access to health services, as coverage is much higher amongst children for whom care was sought. Additionally, children were more likely to receive an ACT in the public sector than in the private sector, but RDT+ children were only slightly more likely to have received an ACT than RDT- children, indicating a high degree of presumptive treatment. Improved access to health services and increased availability of ACTs in the private sector, coupled with increased demand for fever treatment, is critical for preventing severe disease and deaths among children with uncomplicated malaria.

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CHANGES IN THE AVAILABILITY AND AFFORDABILITY OF ACTS IN THE RURAL WEST AFRICAN PRIVATE RETAIL SECTOR: TWO AND A HALF YEARS POST AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM)

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The four main objectives of Affordable Medicines Facility - malaria (AMFm) were to: (i) to increase Artemisinin Combination Therapy (ACT) affordability; (ii) to increase ACT availability; (iii) to increase ACT use, including among vulnerable groups; and (iv) to "crowd out" oral artemisinin monotherapies. Ghana was among the nine countries which piloted the first phase of the strategy. The majority of adults and children with febrile illness, including the poorest are treated in the private retail sector. The study assessed changes in ACT availability in private retail shops 2 months before, 2 months after and 2.5 years after the arrival of the first co-paid ACTs in Ghana in August 2010. We also assessed prices of antimalarials (AM) in the shops 2.5 years after AMFm in a rural district in Ghana with an original fixed co-paid ACT price of GHC1.50. Supply, stock-out and cost issues were explored during the last survey in February 2013. Fifty-three chemical shops and 3 pharmacies out of 62 shops participated in the study. Overall, there were 398, 388 and 442 different brands of AMs in the shops during the 3 censuses. ACTs increased over the period, comprising 16.6%, 42.5% and 47.7% of AM in stock respectively. There appeared to be a slight reversal with regards to the market share of non artemesinin therapies from 34.2%, and 6.9% to 9.5% in the most recent census. Artemisinin monotherapies comprised of 9.5%, 4.6% and 3.4% AM available in the 3 time periods. Stocks of Herbal based AM preparations were relatively high forming 40-45% of all stock of AM. This did not change much over the period, constituting 39.7%, 45.9% and 39.4% of AMs respectively. For both children and adults, ACTs were the most sold AM type. Overall, 55.4% (31/56) of shops had experienced stock-outs of quality assured ACTs (QAACTs) in the preceding 2 months,

most of them (12/31; 38.7%) for a 1-2 week period. Sixteen of the 56 shops (28.6%) had no stock of QUAACTs. Buying and selling prices of QAACTs had increased by 40-100% and shopkeepers attributed this mainly to the scarcity of the commodity. In order to prevent reversal of the gains in malaria control over the last decade, consistent supply of QAACTs to the private retail sector must be assured.

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EFFECTIVENESS OF PROVIDER AND SCHOOL INTERVENTIONS ON THE TREATMENT PROVIDED TO FEBRILE PATIENTS ATTENDING PUBLIC PRIMARY HEALTH CENTRES AND MEDICINE RETAILERS IN SOUTHEASTERN NIGERIA

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A formative survey for this study found that less than 1% of patients were tested for malaria, ACTs were received by only 22.4% of all patients and 37.9% of patients received SP. There was hence the need to improve appropriate treatment malaria by designing and implementing useful interventions. The interventions were evaluated using a three-arm cluster randomized trial in a real-life setting. The three arms were: the Control; Intervention arm 1; and Intervention arm 2. In the control arm there was only normal practice with supply of rapid diagnostic tests (RDTs) with basic instruction. In arm 1 there was provider intervention with supply of RDTs. In arm 2 there was provider intervention and school-based community intervention. The interventions were evaluated using a patient exit survey, log of malaria tests conducted, provider survey and a household survey. Within each stratum and arm, a point estimate of the proportion of patients treated according to guidelines was calculated. The implementation of the interventions differed in some stratum within the same arms. There was a general increase in testing compared to formative study, but the number of patients that were tested was still low across the three different arms despite the availability of RDTs in the facilities and there were no significant differences by arm. A large proportion of patients asked for a specific medicine and 96% of those who asked for a specific medicine got what they asked for. There was also no evidence of a difference between the intervention arms and control in the proportion of test positive patients receiving an ACT. It was found that 63% of those not tested asked for a medicine compared with 19% of those tested. The interventions did not make significant improvements in the intervention arms compared to the control arm. The reasons for this may include the real life setting of the project, the non-uniform implementation of the interventions in some arms and the relative differences in the gap between implementation and evaluation of the intervention in some clusters.

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HIGH FREQUENCY OF SUBMICROSCOPIC GAMETOCYTE CARRIAGE AFTER THE TREATMENT OF UNCOMPLICATED MALARIA WITH ACTS

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Studying the parasite reservoir is a tool for monitoring the effectiveness of control strategies. The gametocyte carriage is more common in patients with asexual forms, and the occurrence of recrudescence or reinfection in patients could also be favored and contribute to the maintenance of a large reservoir of parasites. The aim of this study was to determine the prevalence of submicroscopic gametocytaemia in patients treated for uncomplicated malaria. Gametocytes carriage and density were estimated by Pfs25mRNA amplification using QT-NASBA in samples obtained at enrolment and during the follow-up (day 21 to day 42 post-treatment) in samples of children treated with either artesunate-amodiaquine (ASAQ) or artemether-lumefantrine (AL). Data were analyzed according to the study visit, the presence of asexual parasites, the type of treatment and the treatment response. Samples from 48 children were analyzed; 23 were treated with ASAQ and 25 with AL. They had 147 visits, all corresponding to treatment failure with either ASAQ or AL. None of the patients had a microscopic gametocytaemia. Overall, the frequency of SMG carriage was 51%, comparable at day 0 between the ASAQ (53%) and the AL (56%) patients (p=0.6). During the post-treatment visits, it was of 58% and 44% respectively in the ASAQ and in the AL groups respectively (p= 0.4). When pair samples of 23 children were analyzed, the gametocytaemia was positively correlated with the asexual form density the day of treatment failure (rho=0.4 in the ASAQ group and 0.5 in the AL). Logistic regression analysis showed that recrudescent infection (aOR: 12.9[1.1-14.9]) were independent risk factors for SMG carriage whereas no association was found with the type of treatment, age and number of episode. The frequency of SMG carriage is high after ACT treatment whatever the combination used. A strong association between the presence of gametocytes and a recurrent infection is also observed.

1474

LACK OF SIGNIFICANT PHARMACOKINETIC INTERACTIONS BETWEEN PIPERAQUINE AND NEVIRAPINE- OR EFAVIRENZ-CONTAINING ANTIRETROVIRAL REGIMENS IN *PLASMODIUM FALCIPARUM* NEGATIVE HIV-INFECTED MALAWIAN ADULTS STABILIZED ON HAART

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In sub-Saharan Africa (SSA), most HIV-infected (HIV+) individuals on antiretroviral therapy (ART) are exposed to malaria. Currently, Dihydroartemisinin-piperaquine (DPQ) is being rolled out in SSA but no studies have examined the pharmacokinetics (PK) and safety of DPQ in HIV+ individuals taking ART containing Nevirapine (NVP) or Efavirenz (EFV). We conducted an open label clinical trial to compare the maximum concentration (Cmax) and area under concentration-time curve (AUC) of piperaquine (PQ) in antiretroviral naive HIV+ individuals and those taking NVP and EFV-based ART. In step 1 of the trial, malaria uninfected adults (n=6/ART group) received half the standard dose of DPQ (2 tablets of 40/320mg each for participants) at times 0, 24 and 48hrs. In Step 2, another group of malaria uninfected adults (n=15/ART group) received a standard dose of DPQ (4 tablets of 40/320mg each). Data-rich PK blood sampling were performed at the following times after dosing; 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 336, 504 and 672 hrs. PQ levels were measured using HPLC-UV assays. We also assessed treatment emergent hematological and biochemistry abnormalities. The baseline demographic characteristics and CD4 cell counts were similar across the three groups. In step 1, compared with the ART-naïve group, there was a non-significant trend towards higher PQ AUC in the NVP-ART group and lower PQ AUC in the EFV-ART group. Similarly in Step 2, median PQ AUC was non-significantly lower in the EFV-ART group (15 µg/mL.hr, range: 2.6-25.6) than the ART-naïve group (22.6 µg/mL.hr: range: 11-37, p=0.052). The median PQ AUC in the NVP-ART group (29.9 µg/mL.hr, range: 14.2-80.9) was similar to the ART-naïve group (p>0.16). Cmax for PQ was similar across the three groups. In step 2, there were transient cases of grade 1 or 2 transaminitis in the ART-naïve arm and treatment-emergent grade 3 or 4 neutropenic episodes across the study arms. However, these abnormalities were not clinically significant nor persistent. Thus, there are limited PK interactions between DPQ and EFV or NVP-based ART.

1475

OVER-TREATMENT WITH ACTS AND FALSE ANTIMALARIAL DRUG HISTORY DETECTED THROUGH SERUM DRUG CONCENTRATION STUDIES

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Artemisinin Combination Therapies (ACTs) were recommended as first line therapy for patients with uncomplicated malaria fever in regions where chloroquine resistant strains of *Plasmodium falciparum* were found in the last 10 years. However, there is an emerging trend such that doctors' prescriptions contain more of chloroquine and other antimalarial agents either as monotherapy or in combination with ACTs forming triple therapies because physicians make presumptive diagnosis of resistance to ACTs. Antimalarial drug use histories of 18 adults with clinical diagnosis of uncomplicated malaria were taken in the staff clinic of the Lagos University Teaching Hospital, Nigeria. Blood samples were also tested for malaria parasitaemia and artemether/lumifantrine concentration on Day 0(pre-treatment) and Day 4 (day after completion of treatment).All patients declined the use of any antimalarial (including ACTs) during the 2 week period preceding the study and were malaria parasite negative on Days 0 and 4. Artemether was not detectable in the blood samples taken on Days 0 and 4. However, lumefantrine was detected in all blood samples taken on Days 0 and 4. The mean concentration of lumefantrine on Days 0 and 4 were 330.0µg/l±33.77 (SEM) and 349.7µg/l±18.39(SEM) respectively, these values were not significantly varied p> 0.05.This study exposed the wide spread use of artemether/lumefantrine among this group of patients before presentation at the clinic and underscores the need for confirmation of malaria parasitaemia before drug treatment. The patients' drug use history is also unreliable. Our findings may be a pointer to the fact that presumptive diagnosis of malaria resistant to ACTs should be halted even in malaria endemic regions such as Nigeria, these patients should have their blood tested for malaria parasites and blood drug concentration.

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HSP90, A POTENTIAL DRUG TARGET AGAINST *PLASMODIUM FALCIPARUM*

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The prevalence of drug resistance represents a major threat against current efforts to control malaria. Therefore, the development of new drugs or the identification of new drug targets against *Plasmodium* is a priority to reduce the impact of malaria. Towards that end, we evaluated the *in vitro* effect against *P. falciparum* of available inhibitors against its heat shock protein 90 (HSP90); this chaperone is a key component of the parasite stress response and protein folding machinery. We determined the EC50 for several chaperone inhibitors *in vitro* with a fluorescent-based assay against two *P. falciparum* reference strains 3D7 and W2. The same method was used to determine their anti-Plasmodial effect in combination with current anti-malarial drugs. Moreover, the cytocidal activities of these compounds were evaluated in long term cultures following a bolus dosage exposure. The tested compounds were highly active against the malaria parasites with EC50 values between 10-7 to 10-5 M, and some of the compound-drug combinations displayed synergistic interactions inhibiting parasite growth. In parallel, we have cloned all the four genes coding for Hsp90 family members from *P. falciparum* and generated constructs to express the protein chaperones in bacteria. The recombinant proteins have been used to assay the inhibitors specificity in biochemical assays, aimed at determine their mechanism of action. The recombinant chaperones were screened against additional compound libraries to identify new compounds with potential anti-plasmodial activity. Our preliminary results, lead us to conclude that the *P. falciparum* Hsp90 chaperones is an appealing new drug target to combat malaria.

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EVALUATION OF ARTEMETHER PLUS LUMEFANTRINE TREATMENT FAILURES IN WEST AFRICA

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Malaria caused by *Plasmodium falciparum* is a serious concern for public health and development in sub-Saharan Africa. To address these problems, a number of African countries have adopted artemisinin-based combination therapies (ACTs) as their first-line treatment for uncomplicated malaria. We have examined the effectiveness of Coartem for uncomplicated *Plasmodium falciparum* malaria in Gambissara in The Gambia, Dioro in Mali and Thiès in Senegal. These studies have enrolled participants 2-20 years of age with 2,000 to 199,999 asexual parasites per µl of blood who had no evidence of severe or complicated malaria. Primary endpoints include asexual parasite counts <25% of baseline by day 3, clearance of asexual parasites by day 7 and the absence of recurrent infection between days 8 and 42. Secondary endpoints included asexual parasite clearance times, *ex vivo* determinations of susceptibility and resistance to individual antimalarials; testing for drug resistance markers and for presumptively neutral markers (SNPs). From September 2011 to February 2013, we performed an open enrollment, multicenter study of the standard 3 day course of artemether + lumefantrine (AL) for uncomplicated *Plasmodium falciparum* malaria according to World Health Organization (WHO) guidelines. Follow-up visits were performed on days 1, 2, 3, 7, 14, 21, 28, 35 and 42 to evaluate clinical and parasitological results. These studies have now enrolled 328 subjects with uncomplicated *P. falciparum* malaria, who have been treated with arthemeter plus lumefantrine and followed for recurrent infection or other evidence of treatment failure. Of the 328 subjects enrolled, 19 have been lost to follow-up and 13 have developed recurrent infections between days 8 and 42. However, there have been no early treatment failures (on or before day 7). Twelve of the 13 subjects with recurrent infections had parasites at the time of recurrence with different genetic markers (using the SNP-based barcode). However, 1 subject had parasites with similar markers at the times of diagnosis and recurrence together with delayed parasite clearance on day 3. The isolates from this patient also had IC50s above the mean values for both artemether and lumefantrine. Apart from that subject, the results obtained thus far provide no evidence for artemisinin or Coartem resistance at the community level in The Gambia, Mali or Senegal.

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INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY WITH MEFLOQUINE IN HIV-INFECTED WOMEN RECEIVING COTRIMOXAZOLE PROPHYLAXIS: A MULTICENTER RANDOMIZED PLACEBO-CONTROLLED TRIAL

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Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended for malaria prevention in HIV-negative pregnant women, but it is contraindicated in HIV-infected women due to potential interactions with cotrimoxazole prophylaxis (CTXp). We studied the safety and efficacy of mefloquine (MQ) in women receiving CTXp and long-lasting insecticide treated nets (LLITNs). A total of 1071 HIV-infected women from Kenya, Mozambique and Tanzania were randomized to receive either three doses of IPTp-MQ (15 mg/kg) or placebo given at least one month apart; all received CTXp and a LLITN. IPTp-MQ was associated with nearly halved maternal parasitemia (RR, 0.51 [95%CI, 0.29; 0.90]; p=0.021), and placental infection (RR, 0.53 [95%CI, 0.30; 0.93]; p=0.028), and reduced incidence of all-cause and non-obstetric hospital admissions (RR, 0.65 [0.41.; 1.03]; p=0.065; and RR, 0.59 [0.37; 0.95]; p=0.031; respectively). There were no differences in the prevalence of adverse pregnancy outcomes between groups. Drug tolerability was poorer in the MQ group compared to the control group (29.6% referred dizziness and 23.9% vomiting after the first IPTp-MQ administration). HIV viral load at delivery was higher in the MQ group compared to the control group (p=0.048). The rate of perinatal mother to child transmission (MTCT) of HIV was increased in women who received MQ (RR, 1.95 [95%CI 1.12;3.39]; p=0.018). An effective antimalarial added to CTXp and LLITNs in HIV-infected pregnant women can improve malaria prevention and maternal health through reduction in hospital admissions. The translation of this information into policy actions that reduce malaria in this particularly vulnerable group should be prioritized. However, MQ was not well tolerated, limiting its potential for IPTp and indicating the need to find alternatives. MQ was associated with an increased risk of MTCT of HIV, which warrants a better understanding of the pharmacological interactions between antimalarials and antiretroviral drugs.

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SIMULATIONS TO INVESTIGATE NEW INTERMITTENT PREVENTIVE THERAPY DOSING REGIMENS FOR DIHYDROARTEMISININ-PIPERAQUINE

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A fixed-dose combination of dihydroartemisinin (DHA) and piperaquine (PQ) with monthly dosing has been suggested as a new promising alternative for Intermittent Preventive Therapy (IPT). Alternative dosing regimens for DHA-PQ was explored based on simulations with a previously developed in silico model describing the concentration-effect relationship for the malaria preventive effect. The model was developed in application to placebo controlled monthly versus bimonthly dosing regimen study of 1000 healthy male subjects in Northern Thailand. The simulations compared the clinically investigated monthly dosing regimen (120 mg DHA, 960 mg PQ dosing on three consecutive days repeated every month) to novel dosing regimens (120 mg DHA, 960 mg PQ once weekly). The usefulness of initial loading doses and robustness towards different levels of compliance was investigated for both weekly and monthly regimens. Among placebo recipient, the predicted yearly malaria incidence was 52%. In perfect compliance, the annual malaria incidence was less than 1% for weekly dosing compared to approximately 3% for the once monthly dosing regimen. Under the assumption of poor treatment compliance (60%), the weekly dosing of initial 3 day loading dose was predicted to contain the incidence below 3% compared to >15% for any monthly loading dose strategy in a year. Clinical trial simulations were applied to investigate the necessary sample size to confirm the predicted advantage with weekly compared to monthly dosing if a study was to be carried out under similar conditions as the original study. A samples size of 966 subjects (483+483) was needed to have 80% power to demonstrate a statistically significant benefit of weekly dosing over monthly dosing in a 9 months clinical trial. To have the same power to demonstrate non-inferiority (25% margin) a sample size of 684 subjects (342+342) was needed.

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PREGNANCY LOWERS THE EXPOSURE OF DIHYDROARTEMISININ: WHAT IS NEXT, INCREASE THE DOSE OR EXTEND THE TREATMENT?

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Our work, comprising paired comparisons as well as literature comparisons between pregnant and non-pregnant women, has shown that the exposure of dihydroartemisinin is decreased during pregnancy after oral administration of dihydroartemisinin, artesunate and artemether. This is worrisome as these drugs are the first line treatment during the second and third trimester of pregnancy in many countries and decreased exposures can result in therapeutic failures and an accelerated development of resistance. The aim of this study was to evaluate the pharmacokinetics and pharmacodynamics of the artemisinin drugs in the treatment of uncomplicated malaria in pregnant women and investigate different optimised dose regimens. In-silico Monte-Carlo simulations, based on the pharmacokinetic exposures and pharmacodynamic parasite reduction, were used to evaluate the effect of a dose increase and treatment extension for oral administration of dihydroartemisinin, artesunate and artemether in pregnant women with uncomplicated *Plasmodium falciparum* malaria. Simulations indicated that it was possible to achieve similar dihydroartemisinin exposures in pregnant women compared to non-pregnant patients after an increased dose. However, this would also result in higher peak concentrations which may result in toxic side effects. An extended treatment could compensate for the lower dihydroartemisinin exposures during pregnancy without this increase in peak levels, but it may also result in lower adherence. This study suggests an optimised dose regimen for pregnant women with uncomplicated *P. falciparum* malaria. New pharmacokinetic studies evaluating the suggested dose optimisations are needed to enable an evidence-based dose optimisation.

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PKPD RELATIONSHIPS BETWEEN PLASMA PIPERAQUINE LEVELS AND CARDIAC QTC PROLONGATION IN MALARIA PATIENTS ADMINISTERED DIHYDROARTEMISININ-PIPERAQUINE IN CAMBODIA SUGGEST A CONVENTIONAL 3-DAY REGIMEN IS SAFER THAN A COMPRESSED 2-DAY REGIMEN

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Dihydroartemisinin-piperaquine (DP), presently the firstline therapy for uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in Cambodia, is widely used as a standard 3-day dosing regimen (360 mg dihydroartemisinin & 2880 mg piperaquine). However, piperaquine can prolong the QTc interval, resulting in cardiotoxicity that is undetected in countries like Cambodia lacking electrocardiograms. We conducted 3 clinical studies to explore the cardiotoxicity risk of administering DP for malaria treatment and prevention in Cambodia. Comparison of the 3-day versus a compressed 2-day DP regimen (total dose equivalent to 3-day DP), the latter used by the Cambodian military for malaria treatment, revealed both regimens had similar efficacy with mild correlation of plasma piperaquine-QTc prolongation. In a follow-on randomized, double-blind, placebo-controlled study evaluating 2-day DP as a monthly malaria prevention therapy, the trial was halted after 4 out of 69 volunteers met a pre-specified safety endpoint of >500 ms QTcF prolongation. Two-day DP had moderate correlation of plasma piperaquine with QTc prolongation (spearman rho = 0.6706, p-value < 0.0001), with strong correlation in the 4 halted volunteers (spearman ρ = 0.8990, p-value < 0.0001). In an ongoing 3-day DP treatment trial, we observe greater treatment failures and piperaquine IC_{50}s relative to our treatment study conducted 3 years prior, and note mild correlation of piperaquine-QTc prolongation (spearman Rho = 0.3954, p-value < 0.0001). A significant correlation between piperaquine-QTc prolongation was observed in a larger proportion of volunteers given 2-day DP (36 out of 47, 76.6%) relative to 3-day DP (13 out of 50, 26%). Mean plasma piperaquine C_{max} at 4 hours post-1st dose of 2-day DP (633.6 ng/ml) was significantly higher than for 3-day DP (127.3 ng/ml). Mean QTcF after 4 hr post-1st dose of 2-day DP (440.9 ms) was also significantly higher than 3-day DP (405.1 ms). Our findings suggest risk for cardiotoxicity can be mitigated by using 3-day DP, rather than a compressed regimen, with additional precautions of fasting and avoiding co-administration of other QT-prolonging medications.

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DEVELOPMENT OF PEDIATRIC FORMULATION FOR TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA: COARTEM® (ARTEMETHER-LUMEFANTRINE) DISPERSIBLE

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Pediatric artemisinin-based combination therapy formulations have the potential to improve effectiveness and accuracy of dosing in young children. Coartem® (artemeter-lumefantrine; AL) Dispersible was developed in partnership with the Medicines for Malaria Venture for the treatment of uncomplicated *Plasmodium falciparum* malaria and is the first pediatric antimalarial to receive Swissmedic approval and meet WHO specifications for use in infants and children ≥5 kg. Search using PubMed, Ovid and clinical trial registry databases for AL dispersible in children revealed 6 original and 5 review articles involving 674 infants/children. In a palatability study, sweet tasting cherry was the preferred flavor by children for AL dispersible. Pharmacokinetic profile of AL dispersible was comparable to AL crushed tablets. Efficacy and safety of dispersible formulation versus crushed tablet was evaluated in a large, randomized, multicenter study in 5 sub-Saharan African countries. Efficacy and acceptability of AL dispersible were also compared to dihydroartemisinin-piperaquine (DP) pediatric in an open-label, randomized study in Kenya. A total of 674 children were randomized in both studies to receive AL dispersible with mean age 38.5 months, body temperature 38.2°C and parasite density 38,202-53,921/µl. 28- and 42-day PCR-corrected cure rates were 97.8% and 96.4%; similar to AL crushed tablets and DP in respective studies. Acceptability of AL dispersible was significantly better than DP pediatric (ease of use: p=0.007; taste of medicine: p=0.001). 28-day PCR-corrected cure rate was not related to food intake; however, consumption of milk/low fat meal increased lumefantrine bioavailability compared to no food. Efficacy of AL dispersible was comparable in children with different body weights. Median parasite and fever clearance times were 34.3 and 7.9 hours (n=447). Safety profile of AL dispersible was comparable to crushed tablets. AL dispersible was specifically tailored for the pediatric population and offers a convenient formulation with efficacy and safety similar to that of standard crushed AL tablets.

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EFFICACY, SAFETY AND POPULATION PHARMACOKINETICS OF THE ARTESUNATE MEFLOQUINE (ASMQ) FIXED DOSE COMBINATION VERSUS ARTEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN AFRICAN CHILDREN

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Artemisinin-based combination therapies (ACTs) are recommended by WHO to treat uncomplicated *Plasmodium falciparum* malaria. Artesunate

(AS) and Mefloquine (MQ), in loose or fixed-dose combination (ASMQ), was the first ACT used extensively in Asia and Latin America but there is very limited data in Africa. Our objective was to evaluate the efficacy and safety of ASMQ in comparison with the standard of care Artemether-Lumefantrine (AL), and to study the population-pharmacokinetics (PK) in African children.The clinical trial was conducted in children aged from 6 months to 5 years in Burkina Faso, Kenya and Tanzania. Febrile children with *P. falciparum* density between 2,000 and 200,000 asexual parasites/µl were randomized to receive (a) ASMQ for 3 days [6 to 11 months old: one 25mg/55 mg tablet once daily (OD); 12 to 59 months old: two tablets OD], or (b) AL for 3 days [children 5-15 Kg: one 20mg/120mg tablet BID; 15-25 Kg: two tablets BID]. All children were followed for 60 days after treatment period. The primary efficacy outcome is the cure rate based on the PCR-adjusted results by Day 63. Cure rates at 28 and 42 days are also evaluated. Patients with parasitaemia during the follow-up period were switched to the other treatment arm and followed for a further 60 days or until second recurrence. Safety was assessed during the first follow-up period (up to Day 63) and during the second one if recurrence occurred. 945 patients were randomised by June 2013. Under blinded conditions the overall safety profile did not reveal any unexpected signals. The data base lock is expected mid 2014. The population PK results of ASMQ were presented in 2013, and showed a large inter-patient variability in children; clearance and volume of distribution of MQ in children is lower than in adult patients but the terminal elimination half-life and mean absorption time are of similar magnitude.

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A PHASE III, RANDOMIZED, OPEN LABELLED, ACTIVE CONTROLLED, MULTI CENTER, SUPERIORITY TRIAL OF ARTIMIST™ VERSUS INTRAVENOUS QUININE IN CHILDREN WITH SEVERE OR COMPLICATED FALCIPARUM MALARIA, OR UNCOMPLICATED FALCIPARUM MALARIA WITH GASTROINTESTINAL COMPLICATIONS

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Malaria is a serious, infectious disease. About half the world's population (3.3bn) live in areas that have some risk of malaria transmission, 36.4% (1.2bn) of which are living in regions considered at high risk The Phase III trial was carried out in malaria endemic areas of Rwanda, Burkina Faso and Ghana over a 22-month period from November 2010 to September 2012 151 subjects were randomised and enrolled. A total of 151 subjects were analysed in the Safety Analysis Population, 141 subjects in the Modified Intention to Treat (MITT) Population, and 137 subjects in the Per Protocol (PP) Population The study's primary objective was to demonstrate that sub lingual (under the tongue) ArTiMist™ was superior to IV quinine in reduction of the parasite counts by >90% within 24 hours in children with severe or complicated falciparum malaria, or uncomplicated falciparum malaria with gastrointestinal complications. The primary objective for this study showed that ArTiMist™ demonstrated superiority over iv quinine in both efficacy populations. For the MITT population 66 of the 70 subjects (94.3%) treated with ArTiMist™ and 28 of the 71 subjects (39.4%) treated with quinine had parasitological success. The absolute difference (95% CI) between treatments, without correcting for the factor site, was 54.85 (42.25 - 67.45) % which was statistically significant ($p < 0.0001$). For the PP population 65 of the 68 subjects (95.6%) treated with ArTiMist™ and 28 of the 69 subjects (40.6%) treated with quinine had parasitological success. The absolute difference (95% CI) between treatments, without correcting for the factor site, was 55.01 (42.44 - 67.58) % which was statistically significant ($p < 0.0001$). Following sublingual administration of ArTiMist™, absorption is rapid with mean Cmax following the first dose reaching 333.2 ng/mL and 83.3 ng/mL in 1.0 h and 1.5 h for artemether and dihydroartemisinin (DHA), respectively. In conclusion, sub-lingual ArTiMist™ was superior to IV quinine, demonstrating significantly faster parasite killing and fewer early treatment failures. PK analysis demonstrated that ArTiMist™ was rapidly absorbed in children with severe or complicated falciparum malaria, or children with uncomplicated malaria with gastrointestinal complications

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INHALED NITRIC OXIDE FOR THE ADJUNCTIVE TREATMENT OF SEVERE MALARIA: A RANDOMIZED CONTROLLED TRIAL

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Severe malaria remains a major cause of childhood mortality globally. Exogenous inhaled nitric oxide (iNO) reduces endothelial activation, protects the blood-brain barrier, and improves survival in pre-clinical studies of experimental cerebral malaria. We conducted a randomized, blinded, parallel-arm, controlled trial of iNO at 80 ppm by non-rebreather mask vs room air placebo as adjunctive treatment in children (age 1 to 10 years) with severe malaria. Blinding of trial clinicians, nurses, parents, children, laboratory technicians and statistician was achieved by using room air placebo, also administered by mask by a dedicated and unblinded team that monitored dose-dependent adverse effects (methemoglobinemia) but did not participate in clinical care. The primary outcome was the rate of improvement in angiopoietin-2 levels (a biomarker of malaria severity and convalescence). 180 children were enrolled; 88 were assigned to nitric oxide and 92 to placebo (all received IV artesunate). The median [IQR] rate of change of Ang-2 over the first 72 hours of hospitalization was similar between groups: -2.2 [-3.1 to -1.2] ng/mL/day in the iNO group vs -1.9 [-3.7 to -0.56] ng/mL/day in the placebo group; p=0.68). The mortality at 48 hours was similar between groups (6/87 [6.9%] in the iNO group vs 8/92 [8.7%] in the placebo group; OR 0.78, 95% CI 0.26-2.3; p=0.65). Methemoglobinemia (>10%) was higher in the iNO group (5/88 [5.7%] vs 0/92 [0%]; p=0.026). Incidence of neurologic sequelae (<14 days), acute kidney injury, hypoglycemia, anemia and hemoglobinuria were similar between groups (p>0.05 for all comparisons). Clinical recovery times (time to eat, sit, localize pain, fever resolution, recovery of consciousness, and hospital discharge) were similar between group (p>0.05 for all comparisons). Parasites cleared quickly in both groups, with no difference in parasite clearance kinetics (p>0.05). No patient in either group had recrudescence of patent parasitemia at day 14 of follow-up. Inhaled nitric oxide at 80 ppm administered by non-rebreather mask was safe but did not accelerate endothelial stabilization, as reflected by circulating levels of Ang-2, in children with severe malaria. Alternative methods of delivering NO to the endothelium (e.g., higher dose, donor molecules, routes of administration) may be necessary to achieve a more potent biological effect and an impact on clinical outcomes.

1486

ALLOMETRIC SCALING OF PYRONARIDINE PHARMACOKINETIC PARAMETERS IN PEDIATRIC MALARIA PATIENTS

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Pyramax® is a pyronaridine/artesunate combination for the treatment of uncomplicated malaria in adult and pediatric patients. A granule formulation of this combination is being developed for treatment of

uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in pediatric patients. The population pharmacokinetics of pyronaridine (PYR) were evaluated in pediatric malaria-infected patients participating in six Pyramax® clinical trials. A total of 1085 blood PYR concentrations were available from 349 malaria patients younger than 16 years of age with mild to moderate uncomplicated malaria. Blood PYR concentrations were measured using a validated LC-MS method. Non-linear mixed effects modeling was used to obtain the pharmacokinetic and variability parameter estimates. PYR concentrations were well described by a two-compartment model with first order absorption and elimination. Allometric scaling was implemented to address the effect of body weight on clearance and volume parameters. The final parameter estimates of PYR apparent clearance (CL/F), central volume of distribution (V2/F), peripheral volume of distribution (V3/F), inter-compartmental clearance (Q/F) and absorption rate constant (Ka) were 377 L/day, 2230 L, 3230 L, 804 L/day and 17.9 day-1, respectively. The corresponding percent coefficient of variation of inter-individual variability for CL/F, V2/F, V3/F and Ka were 40.7%, 99.6%, 50.6% and 65.8%, respectively. Covariate model building conducted using forward addition ($p<0.05$) followed by backward elimination ($P<0.001$) yielded two significant covariate-parameter relationships: age on V2/F and formulation on Ka. Evaluation of bootstrapping, visual predictive check, and condition number indicated that the final model displayed satisfactory robustness, predictive power, and stability. Simulations of PYR concentration-time profiles generated from the final model show similar exposures across pediatric weight ranges, supporting the proposed labeling for weight-based dosing of Pyramax® granules.

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EFFECTS OF BREATHING NITRIC OXIDE AS AN ADJUNCTIVE TREATMENT FOR CHILDREN WITH CEREBRAL MALARIA

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Children with cerebral malaria (CM) have reduced plasma and urine levels of NO biometabolites. Two studies have reported breathing NO reduces mortality, inflammation, and CNS pathology in *Plasmodium berghei*-infected mice. Therefore, from Sept 2011 to February 2014, we completed a phase-II open-label clinical trial assessing the efficacy and safety of inhaled NO (INO) as an adjunctive treatment for cerebral malaria in pediatric patients. A total of 92 children, aged 3 months – 9 years, with CM were enrolled in the study at the Mbarara Regional Referral Hospital in Uganda. Patients were randomly assigned to receive either inhaled NO (INO), or nitrogen (N_2) via nasal cannula (INOPulse, TM, Ikaria, USA) for at least 24 hours. All patients received IV artesunate and were monitored continuously for changes in metHb% levels. The primary endpoint was the change in plasma Angiopoietin-1 (Ang-1) over 48 hours. Plasma Ang-1 levels increased over 48 hours in both treatment groups, but there was no difference between the groups. There was a decrease in plasma angiopoietin-2 and plasma cytokine levels (TNF-α, IFN-γ, IL-1β, IL-6, IL-10, and MCP-1) over 48 hours in both study arms, but no significant difference between the treatment groups. Total mortality was 12.0%. Seven (15.2%) patients died in the N_2 group, and 4 (8.7%) patients died in the INO group. Five patients in the N_2 group and 6 in the INO group had developed neurological sequelae by the time of discharge. For patients who received INO, the average hourly dose of INO delivered over the initial 48 hours was 1.13 ± 0.30 mg/kg/hr (N=39, mean $\pm$ SD). There was no difference in the baseline metHb% levels among patients in both study arms. There was an increase in metHb% in patients treated with INO, up to 4.1 ± 2.3% (N=33, mean $\pm$ SD) at 12 hours, which remained at safe elevated levels up to 72 hours. MetHb levels were unchanged throughout the treatment period in the N_2 group. This pilot trial of INO as an adjuvant therapy for CM demonstrates the safety and feasibility of delivering INO in a low-resource setting but there was no statistically significant evidence for efficacy.

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EXPERIMENTAL VIVAX TRANSMISSION TO *ANOPHELES* (EVITA), A CLINICAL TRIAL TO ASSESS MOSQUITO TRANSMISSIBILITY IN PARTICIPANTS INOCULATED WITH BLOOD STAGE *PLASMODIUM VIVAX*

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Interventions to interrupt transmission of malaria from humans to mosquitoes, including vaccines, represent appealing approaches to assist its elimination. A limitation has been the lack of a methodology to reliably test the efficacy of such interventions before proceeding to clinical efficacy trials in the field. Building on our work demonstrating the feasibility of induced blood stage *Plasmodium vivax* infection, we have undertaken a study to evaluate transmission to *Anopheles stephensi* mosquitoes. Study endpoints included the presence of the gametocyte-specific transcript *pvs25* in the blood of volunteers by qRT-PCR, and mosquito infection by midgut dissection for oocyst visualisation. The study design entailed 3 cohorts of 2 volunteers, each inoculated on Day 0 with approximately 100 viable *P. vivax*-infected human erythrocytes administered intravenously. On the three to four days up to the anticipated commencement of treatment (approximately day 11, 12, 13 and 14), transmission studies were undertaken by membrane feeding assays and direct feeds on volunteers with 30 mosquitoes per session. At the time of abstract submission, 2 of the 3 cohorts have been completed. No significant adverse events were observed. All subjects experienced mild to moderate symptoms of malaria consistent with previously published data. Direct mosquito feeding was well tolerated with all volunteers reporting mild to moderate local reactions and pruritis, easily controlled with symptomatic treatment. Elevated liver function tests were observed in 3 of the 4 volunteers in the form of asymptomatic elevations in both ALT and AST. However, this completely resolved in all subjects. Gametocytaemia detected by a positive pvs25 RT-PCR was observed in all subjects. Mosquito infection was detected by midgut dissection following both direct and indirect feeding assays. The demonstration of the feasibility of this system to test transmission-blocking interventions represents a promising development for future efficacy studies.

1489

SAFETY AND REPRODUCIBILITY OF AN INDUCED BLOOD STAGE MALARIA CHALLENGE FOR EXPEDITED TESTING OF ANTIMALARIAL TREATMENT AND VACCINES

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Despite recent progress in malaria control there is concern that momentum is slowing and new challenges are emerging, including the development of drug resistance. Thus, new interventions are required. Controlled Human Malaria Infection (CHMI) studies are assuming an increasing place in evaluation of interventions as they can lead to accrual of pivotal efficacy data in a faster and more cost-effective fashion than Phase IIa studies in endemic settings. An alternative to infection via sporozoite, either by

mosquito bite or injection of cryopreserved sporozoites, Induced Blood Stage Malaria (IBSM) infection represents a convenient approach where pre-erythrocytic stages are not being studied. Here we report on the safety and reproducibility data from 121 subjects in 12 trials from our centre, the largest report of IBSM. The majority of subjects (86%) experienced at least some symptoms of malaria infection. In total 755 adverse events were recorded however the majority (75%) were mild. No SAE's were attributed to malaria with 4 SAE's unrelated to trial protocol and 3 SAE's attributed to the investigational product. Despite the positive serostatus of the donor for CMV, and the inclusion of seronegative subjects, no CMV seroconversions were detected nor were any additional coinfections observed on extensive serological testing. Analysis of parasitaemia by sensitive qPCR demonstrates that the method is reliable and reproducible. Further analysis of the reproducibility and variability of parasite growth rates is currently underway and will be presented. These data illustrate the safety and reproducibility of an induced blood stage malaria model thus providing a valuable tool for assessing candidate drugs and vaccines for control of malaria.

1490

A PHASE IIA CLINICAL TRIAL TO CHARACTERIZE THE PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF PIPERAQUINE USING THE INDUCED BLOOD STAGE INFECTION MODEL

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Piperaquine (PQP) is a 4-aminoquinoline antimalarial structurally related to chloroquine. It was widely used for malaria control in China in the 1970's and 1980's, and more recently has undergone renewed development as component of an ACT co-formulation with dihydroartemisinin (DHA-PQP). Although knowledge of the pharmacokinetic-pharmacodynamic (PK-PD) relationship of antimalarials is essential for dose selection, there is a paucity of such data for PQP. We therefore undertook an experimental dose de-escalation clinical trial of this drug in the induced blood stage infection malaria (IBSM) system, with simultaneous measurement of drug levels and parasitemia, the latter by qPCR. The trial was designed to include 3 single dose cohorts, each of 8 volunteers. The pharmacokinetic profile of PQP following single doses of 960 and 640 mg was linear with CL/f = 89 L/hr (95%CI: 75-101 L/hr), with measurable plasma levels (>1 ng/mL) out to 672 hrs following administration of 640 mg. Recrudescent parasitemia occurred after ≥144 hours in 4 of the 7 volunteers who received 640 mg PQP; each received rescue treatment with artemether/lumefantrine. The rich dataset accrued facilitated the fitting of a PK/PD model to the PK and parasitemia data. The concentration response relationship identified by analysis of data from the 960 and 640 mg cohorts was characterized by a PRR of 3.3 (95%CI: 3.0-3.6; t1/2: 4.4 hr), an IC50 of 9.2 ng/mL (95%CI: 7.1-11.9), an MPC of 14.3 ng/mL (95%CI: 11.0-18.5 ng/mL), and an MIC of 8.1 ng/mL (95%CI: 6.3-10.5 ng/mL). The model accurately predicted the parasitemia response observed in the 480 mg PQP cohort. The IBSM system demonstrated that the PK/PD relationship of an antimalarial can be determined from data obtained from just two cohorts of 8 volunteers. The compiled PK/PD model can then be linked with safety data to forecast optimal dosing, either as a single agent or in combination, whilst accounting for effects of age, DDI and parasitemia.

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DELAYED ANEMIA ASSESSMENT IN PATIENTS TREATED WITH ORAL ARTEMISININ DERIVATIVES FOR UNCOMPLICATED MALARIA: A POOLED ANALYSIS OF CLINICAL TRIALS DATA FROM MALI

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In sub-Saharan Africa, Artemisinin-based combination therapies (ACT) and injectable artesunate are the first line treatments for uncomplicated and severe *Plasmodium falciparum* malaria, respectively. However, recent studies suggest that delayed anemia is associated with these treatments in non-immune travelers. We aimed to assess the risk factors associated with delayed anemia after falciparum malaria treatment with artemisinin-containing drugs in malaria endemic populations. Pooled, individual malaria patient data were extracted from 13 clinical trials performed from 2002 to 2011 in various settings of Mali. Treatment regimens were Artemether-Lumefantrine, Artesunate plus Amodiaquine, Artesunate plus Sulfadoxine-Pyrimethamine, Artesunate plus Sulfamethoxypyrazine-pyrimethamine, Artesunate plus Mefloquine, Artesunate-Pyronaridine, Artesunate monotherapy, Chloroquine, Sulfadoxine-pyrimethamine, Amodiaquine and Sulfadoxine-pyrimethamine plus Amodiaquine. Univariate and multivariate analyses were performed using the generalized linear and latent mixed model procedures to assess risk factors associated with hemoglobin concentration evolution and anemia during the treatment follow-up. A total of 5990 participants were recruited and followed from Day 0 to Day 28. The participants' median age was 5 years, ranging from 3 months to 70 years. There was a decrease in hemoglobin level on day 7 in all treatments arms, but the magnitude varied across treatments. There was a significant risk of hemoglobin level decrease on day 7 in the artemisinin-based therapies compared to the non-artemisinin treatments. The risk of hemoglobin concentration drop was associated with age group < 5 years old (0.61 g/dL 95% CI [0.71 to 0.51], p<0.001), baseline high parasite density (0.43 g/dL 95% CI [0.51 to 0.35], p<0.001) and treatment failure (0.40 g/dL 95% CI [0.59 to 0.20], p=0.018), while high hemoglobin level at baseline was a protective factor [0.53 to 0.59] p<0.001). No association was found between artemisinin-based therapies and severe delayed anemia. Oral artemisinin derivative treatments for uncomplicated *P. falciparum* malaria are associated with a transient and clinically moderate hemoglobin decrease by day 7 but not associated with a delayed severe anemia.

1492

ARTEMISININ PARTNER'S DRUGS DAY 7 CONCENTRATION PROFILE AND ITS EFFECT ON RECURRENT EPISODES OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Bougoula, Kolle, Sotuba are three sites in Mali participating in a large Phase III/VI trial of four ACTs (Artesunate-amodiaquine, artemether-lumefantrine, Dihydroartemisinin-piperaquine and Artesunate-pyronaridine). The role of the long half-life partner drugs is to mop up any remaining blood stage parasite biomass after treatment. Whether the long half-life may lead to accumulation when ACTs are used frequently in high transmission settings is currently not known but potentially important for drug safety and for the duration of post-treatment prophylactic. Patients with uncomplicated *Plasmodium falciparum* malaria, aged ≥6 months,

after inclusion in one of the treatment arms are followed up for two years, during which patients will receive the same treatment for any subsequent episode of malaria occurring at least 28 days after the start of the previous treatment. To date we have plasma concentrations in day 7 samples from 317 treatment episodes for desethyl-amodiaquine and 564 episodes for lumefantrine. Our first results show an increase of desethyl-amodiaquine concentrations from the first episode to consecutive episodes of malaria treatment with a median (quartile range) concentration of 70.6 ng/ml (58.8 to 89.1 ng/ml) (n=102) for the first, 90.8 ng/ml (69.0 to 111.0 ng/ml) (n=80) for the second; 80.2 ng/ml (61.8 to 100.3 ng/ml) (n=32) for the third and 97.7 ng/ml (82.2 to 1293.0 ng/ml) (n=23) for the fourth episode P<0.0001. For lumefantrine, there was no difference between the first and second episode 632.1 ng (405.7 to 948.6 ng/ml; n=343) and 697.15ng/ml (491.69 to 967.93ng/ml; n=135). There was, however, an increase between first and third episodes (789.3ng/ml; 574.3 to 1362.9 ng/ml; n=52; P = 0.002). All patients with day 7 concentration of lumefantrine below 100 ng/ml had recurrence of infections before day 42 of follow-up. These preliminary data show substantial accumulation of desethyl-amodiaquine in the study population exposed to frequent re-treatments in an area of intense seasonal malaria transmission. A larger dataset will be available at the meeting, including detailed analyses of laboratory parameters of safety and a survival analysis of time to recurrence corrected by transmission season, parasite genotypes (to distinguish recrudescent primary infections from new infections) and drug plasma concentrations.

1493

MEASURING THE EFFICACY OF FOUR ACT REGIMENS IN MALI USING QPCR-BASED ESTIMATES OF *PLASMODIUM FALCIPARUM* CLEARANCE TIME

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The efficacy of artemisinin combination therapy remains high in sub-Saharan Africa, but the prolongation of parasite clearance times of artemisinin-treated *Plasmodium falciparum* infections in Cambodia and neighboring countries is a warning that careful monitoring of efficacy is required Worldwide. As an alternative to laborious frequently-spaced blood sampling and evaluation by standard light microscopy, we have developed a qPCR-based parasite clearance assay that utilizes daily finger-prick dried blood spots for the first 72 hours of treatment, and which underwent a successful proof-of-principle trial in western Kenya. We have now applied this approach to evaluate parasite clearance in over 200 falciparum malaria patients treated with artemisinin-combination therapy in two sites in Mali: Bougoula and Kolle. All patients were participants in efficacy evaluations by the WANECAM project, and were randomised to receive either artemether-lumefantrine, dihydroartemisinin-piperaquine, amodiaquine-artesunate or artesunate-pyronaridine for all malaria episodes during two years of follow-up. Parasite clearance estimates for 209 first malaria episodes and 186 second episodes across the two sites will be presented, and estimates of the parasite reduction ratio at 48 hours derived for each episode. These data will be analysed with reference to site, regimen received, patient age and, for second episodes, time elapsed since first episode.

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STUDY ON EFFICACY OF ARTESUNATE-MEFLOQUINE COMBINATION THERAPY FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THAILAND AS PART OF A DEPARTMENT OF DEFENSE MULTI-CENTER TRIAL

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Artemisinin-resistant *Plasmodium falciparum* threatens effectiveness of all artemisinin-based combination therapies. A multi-center artesunate-mefloquine (A+M) efficacy trial is ongoing in three DoD laboratories in Peru, Kenya, and Thailand, to compare parasite clearance rates for 72 hour after artesunate initiation and to conduct standardized microscopy, in-vitro drug-sensitivity testing and molecular testing across all three sites. Only initial data from Thailand will be presented. Patients aged 5-65 years with uncomplicated *P. falciparum* malaria, with asexual parasite density between 1,000 - 200,000/µL, no signs or symptoms of severe malaria, no other cause of febrile illness were enrolled starting in September 2013. Participants received 4 mg/kg artesunate at 0, 24, and 48 h, 15 mg/kg mefloquine at 72 h, and 10 mg/kg mefloquine at 84-96 h, with 0.5 mg/kg primaquine for transmission blocking, all under direct observation therapy. We assessed parasite density on thick/thin smears every 4 h during first 12 h after first artesunate dose and every 6 h for 72 h or until two consecutive negative smears. The parasite clearance half-life will be calculated from the parasite clearance curve. Efficacy outcome for 42 days will be assessed. Between Oct 31, 2013, and Feb 3, 2014, we assessed 52 persons suspected of malaria from four malaria clinics and hospitals in Sangkhlaburi district of Kanchanaburi province in western Thailand near Thai-Myanmar border. We screened 12 and enrolled 8 patients with *P. falciparum* malaria who met inclusion criteria. Forty cases could not be screened including 11 (27%) who previously took antimalarias including artemisinin monotherapy. Five cases had parasite clearance time more than 72 h and three cleared before 72 h [GeoMean=57.7 h (95% CI + 18)]. All eight subjects met adequate clinical and parasitological response endpoint and no recurrence was reported to date. Although no resistance to A+M detected among eight subjects in Thai-Myanmar border so far, more data to include up to 59 more subjects will be presented.

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PRELIMINARY RESULTS: PHASE 2 RANDOMIZED PROOF OF CONCEPT STUDY COMPARING AN INVESTIGATIONAL AMINOQUINOLINE ANTIMALARIAL (AQ-13) TO COARTEM IN ADULT MALIAN MALES WITH UNCOMPLICATED MALARIA

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Although artemisinin-combination therapies (ACTs) are the recommended first-line treatment for uncomplicated *Plasmodium falciparum* malaria, there is increasing concern about artemisinin resistance because of prolonged parasite clearance times in southeast Asia. For this reason,

it would be helpful to have alternatives to the artemisinins that were effective against chloroquine(CQ)-resistant *P. falciparum*, were safe in human subjects and could be given orally. Our previous studies have shown aminoquinolines with modified side chains such as AQ-13 are active against CQ- and multi-resistant *P. falciparum in vitro* and in a squirrel monkey model of CQ-resistant human *P. falciparum* infection, are safe orally in human subjects and have pharmacokinetics similar to those of CQ. The preliminary results reported here are from a blinded study comparing the investigational antimalarial AQ-13 (1,750 mg over 3 days) to the current recommended first-line treatment (Coartem=artemether + lumefantrine; 480 and 2,880 mg over 3 days) for uncomplicated *P. falciparum* malaria in adult Malian males (≥18 years of age). Based on the first 33 subjects enrolled, there have been no differences in efficacy (asexual parasite clearance on or before day 7), clinical recovery (resolution of fever, chills and myalgias on or before day 3) or side effects (no serious or Grade 3 or Grade 4 adverse events) between treatment groups. Because the study is blinded, we do not know whether there are other differences between the AQ-13 and Coartem groups. However, because the second group of 33 subjects is now being enrolled, it should be possible to address those questions at the time of this presentation. The results available at this time suggest that AQ-13 alone may be as efficacious and safe as Coartem in adult Malian subjects with uncomplicated *P. falciparum* malaria.

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INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED WITH ITN USE BETWEEN 2008 AND 2013 IN A LOW MALARIA TRANSMISSION SETTING OF SOUTHERN ZAMBIA

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The prevalence of malaria has declined in parts of sub-Saharan Africa; as perceived risk decreases there are concerns that use of personal protective measures may also decrease. Determining factors that influence insecticide-treated net (ITN) ownership and use in these areas is critical to promote their continued use to achieve malaria elimination. Households in the Macha Hospital catchment area, Choma District, Southern Province, Zambia were enumerated and randomly selected using satellite imagery. Households were either visited once (cross-sectional) or every other month (longitudinal). Adults and caretakers of children were administered a survey regarding malaria-related beliefs and behaviors and a malaria rapid diagnostic test (RDT). Mosquitoes were collected in the households using light traps. Individual and household level factors associated with use among those who owned an ITN were assessed using longitudinal, multi-level regression models. Qualitative questions were tabulated to identify reasons for not owning or using an ITN. In a smaller sample of households, the association between total mosquitoes caught and ITN use was assessed to determine if culicine mosquitoes prompted use. ITN use was higher at follow-up visits (77.4%) as compared with first visits (62%) in the longitudinal cohort (p<0.0001). In the multi-level model, ITN use was 77% higher during the rainy season (OR=1.77 (95% confidence interval=1.46, 2.16)) and over twice as high after ITN distribution in June 2012 (OR=2.33 (1.21, 4.5)). Those that learned about malaria from a community health worker had 42% higher odds of using their net (OR=1.42 (1.09, 1.84)). Those that owned 3 or more nets were over twice as likely to use their ITN (OR=2.13 (1.35, 3.36)). Also, odds of ITN use was over twice as high if more than 10 culicine mosquitoes were caught in the house controlling for season and study design (OR=2.15 (1.27, 3.63)). ITN use can be sustained in low transmission settings with continued education and distributions, and may be driven in part by the presence of culicine mosquitoes.

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A PAN-AFRICAN HIGH-RESOLUTION SEASONAL MALARIA FORECASTING SYSTEM

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Malaria transmission models are useful for understanding the epidemiology of the disease, and for the development early warning systems (EWS) in epidemic-prone regions. Dynamical models explicitly model the disease dynamics through a series of differential equations. Unlike statistical models, dynamical models are not confounded by sparse or short data records. Population density is key for determining disease occurrence, and should be incorporated in the models to effectively differentiate between urban, peri-urban, and rural malaria. Weather factors such as temperature and precipitation, are key determinants of disease niche, and should also be included in the modeling framework. Accurate predictions of weather conditions could provide useful information for targeting bespoke interventions in high-risk areas one or two months in advance. Here, we coupled a state-of-the-art dynamical malaria model that can be used at a fine spatial resolution of O(10) km, and applied over a continental scale, with two operational state-of-the-art weather prediction systems to develop a pilot malaria EWS for Africa. To our knowledge, this is the first attempt to developing a pan-African malaria EWS using state-of-the-art weather forecasts and dynamical malaria models. We determined the seasons and regions in which such a forecasting system be more valuable to decision-makers, and assessed the skill of the model. The EWS provides forecasts of malaria prevalence and intensity up to four months in advance with good skill across large regions. A further evaluation of the model using sentinel-site surveillance data demonstrates that the EWS is able to predict malaria dynamics in some target regions one to four months ahead. This findings show that the EWS could significantly help public health decision makers optimising resources, and making informed decisions about the areas and periods of high risk.

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SET-UP AND VALIDATION OF POST-SCREENING TOOLS FOR A NEW MALARIA TRANSMISSION-BLOCKING APPROACH BASED ON DEFORMABILITY

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Gametocytes are the sexual forms of *Plasmodium falciparum* parasite and are essential for transmission from human to human. Unlike immature (stage I-IV) gametocytes that are stiff and sequestered, mature gametocytes (stage V) are deformable and circulate. A drug increasing their stiffness will induce their clearance by the spleen, thereby removing them from the transmission cycle. We are screening for active compounds based on their ability to induce the retention of mature gametocytes through an automated filtration process that mimics the mechanical sensing of RBC by the spleen. To validate the activity of selected compounds, we developed post-screening tools using a biomimetic microfluidic device, a simple mouse model and human spleens perfused ex-vivo. The common read-out of post-screening tools was the retention or enrichment rates of mature and immature gametocytes exposed or not to selected compounds. Typically, stage V gametocytes were pre-exposed to a

selected compound then co-infused in the microfluidic device, in mice or in the human spleens along with the same gametocyte population exposed to the solvent control. Normal RBCs, asexual ring-IRBCs and heated RBCs were used as controls. We first confirmed that unlike stage I-IV, stage V gametocytes from an *in vitro* culture were not markedly retained in microsphere-based microplate filters and in human spleen perfused ex-vivo, consistent with the hypothesis that deformability of gametocytes is a major determinant of their circulation in peripheral vessels. Using the microfluidic device, we showed that stage V exposed to a recently identified stiffening compound C were enriched to 74.9% (vs. 25.08% for unexposed controls, p=0.0001 paired t test) in narrow 2 µm-wide spaces mimicking inter-endothelial slits in the spleen. In macrophage-depleted C57 Bl/6 mice, immature gametocytes (10 mice) and heated RBCs (4 mice) were cleared by 86% or 75% in 3 hours, respectively. By contrast, a majority of mature gametocytes (5 mice) or normal RBCs (4 mice) were still circulating 3 hours after infusion (Retention rates: 44% and 30%, respectively (p=0.0058, p=0.0002). Similar results were observed in human spleens. Mature circulating gametocytes can be stiffened to induce their mechanical retention, thereby interrupting transmission. The stiffening effect can now be validated in a biomimetic microfluidic device and in a simple rodent model as a prerequisite before further development.

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THE USE OF RESPONDENT DRIVEN SAMPLING METHODS TO IDENTIFY MALARIA PREVENTION KNOWLEDGE AND BEHAVIORS BY MIGRANT AND MOBILE POPULATIONS IN WESTERN CAMBODIA

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Mobile and migrant populations (MMPs) along the Thai-Cambodian border are at high-risk for malaria infection and have been found with artermisinin resistant parasites. However, the mobile nature of this population makes it difficult to adequately measure malaria infection and risk behaviors, which is vital as we move to elimination in the region. Utilizing respondent driven sampling methods, MMPs residing within two villages in Palin province (Pang Rolim and Sala Krau) were recruited in two independent rounds of sampling (602 in 2013 and 604 in 2014). All responses were adjusted for network size and recruitment patterns allowing for calculation of population-adjusted statistics. While the prevalence of *Plasmodium vivax* is estimated to be 0.2% among the general population, this study found 2.0% and 1.3% of MMPs in these networks to be infected with *P. vivax* in 2013 and 2014 respectively, and an absence of *P. falciparum*. Most respondents from Pang Rolim, from both rounds, identified having seen malaria messages within the previous three months (99.7%, 95% CI: 97.6-100 in 2013 and 99.0%, 95% CI: 95.9-99.8 in 2014). However, in Sala Krau, the percentage of respondents answering similarly decreased from 97.0% (95% CI: 94.1-98.4) in 2013 to 59.1% (95% CI: 51.3-66.4) in 2014. While knowledge related to malaria transmission, symptoms and prevention increased noticeably in Pang Rolim, similar knowledge remained low in Sala Krau across both rounds. Furthermore, while the percentage of respondents from Pang Rolim who didn't use a net the previous night remained the same across both rounds (2.4%), there was a slight increase in non-users in Sala Krau from 6.1% (95% CI: 0.9-6.7) to 9.5% (95% CI: 5.3-16.4). These findings correlate with the fact that there were increased efforts on malaria prevention in Pang Rolim (eg. concerts and videos with prevention messaging) and not in Sala Krau; suggesting that as MMPs change frequently there is a need for sustained public health efforts to reach this population, especially within an elimination context.

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IMPLEMENTING ENHANCED HIGH-RESOLUTION SURVEILLANCE USING SPATIAL DECISION SUPPORT SYSTEMS TO GUIDE TARGETED RAPID RESPONSE IN MULTI-DRUG RESISTANT AREAS OF VIETNAM

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Emerging artemisinin resistant malaria in the Greater Mekong Subregion (GMS) has important implications for public health. A project was established to research, develop and implement enhanced surveillance and targeted appropriate intervention measures to stop the spread of multi-drug resistant malaria through elimination of the disease in the region. The aims of this project are to pilot a spatial decision support system (SDSS) approach to conduct high-resolution surveillance to guide swift and targeted responses. Pilot sites were established in selected communes in Vietnam with associated customised SDSS developed. Publically available topographic geographic information system data were uploaded into the SDSS to provide baseline information. Household and forest transmission location data were located and enumerated through field-based geographical reconnaissance using handheld computers. Passively detected malaria cases were geo-referenced to the suspected transmission location sites upon diagnosis. Using case location data in the SDSS, active transmission foci were automatically classified and response areas-of-interest (AOI) generated. Supporting data (including population, location and number of sleeping locations within the AOI) were automatically produced in the SDSS and sent to village health workers and district level units to mobilize appropriate responses. Complete pilot data for presentation are expected in September 2014. This new approach utilizing novel geo-spatial tools to support targeted, appropriate and aggressive response measures to support malaria elimination in areas of global significance will be presented.

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RESTRATIFICATION OF MALARIA EPIDEMIOLOGY IN VIETNAM FOR MORE EFFECTIVE APPLICATION OF LIMITED RESOURCES

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The National Malaria Control Program in Vietnam is updating malaria epidemiology in order to more effectively apply limited malaria diagnosis, prevention and treatment resources. The most recent prior restratification was conducted in 2009. This on-going 2014 restratificaton effort (2009-2013 data) is using the same methods of 2009 to collect all malaria case data to the commune (county) level. Indicators for classification are based on the average number of confirmed cases per 1000 population over the 5 year period, the presence of at least one of the three malaria vectors, socioeconomic disadvantaged or border commune, poor health system, drug resistant parasites, chemically resistant mosquitoes, and migratory populations. Each indicator has a score, with the sum of the scores used to define the level of endemicity and priority for interventions. This score will be used to characterize each commune into one of five zones (no