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Abstract Book

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THE IMPACT OF AN INTRODUCED HOMING ENDONUCLEASE GENE ON A REGIONAL MOSQUITO POPULATION

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Homing endonuclease genes (HEGs) exist naturally in many single-celled organisms and can show extremely strong genetic drive allowing them to spread through populations into which they are introduced. They are being investigated as tools to manipulate the populations of important vectors of human disease, in particular the mosquitoes that transmit malaria. Before HEGs can be deployed, it is important to study their spatial spread in order to design efficient release strategies. We recently analysed an individual-based simulation model which demonstrated that a HEG which acts to either male-bias the mosquito sex-ratio or reduce female fecundity could potentially eliminate local mosquito populations, as reported previously. Questions remain, however, about how readily this gene-drive mechanism will spread across larger landscapes comprised of numerous interacting local mosquito populations. In this talk I present a metapopulation model which explores how gene-drive, which facilitates the spread of a HEG, interacts with the local elimination of populations, which curtails spread. The model predicts that a HEG may persistently reduce the number of mosquito populations in a region although without causing global extinction. The load imposed by the HEG increases in a correlated landscape where mosquito population sites are clumped in space. Since the HEG effectively acts as a contagious and virulent pathogen on the mosquito population, the model can be summarised as an ‘SIER’ (Susceptible-Infected-Eliminated-Recolonised) metapopulation model. Implications of the model to other infectious diseases are discussed.

OPTIMIZING FIELD RELEASES OF ENGINEERED MALE MOSQUITOES

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Control of Aedes spp, the mosquito vectors of the dengue virus, remains the primary approach in the prevention of dengue. However, there is a lack of effective tools for controlling the vector, emphasising the importance of novel techniques. Engineering male mosquitoes that are functionally genetically sterile and releasing them to compete with wild males for a female mate is a modern alternative to the traditional sterile insect technique (SIT). Successful implementation of this approach in the field relies on efficacious release methods. One key objective of a sterile insect release is to obtain a well-balanced distribution of the released sterile insects at the required density across the targeted treatment area. Approaches that facilitate operational optimisation of releases are investigated and applied to a range of theoretical and real field scenarios. The distribution of sterile male release points in a theoretical arena is examined, leading to further investigation of the optimal placement of release points at a field site. For field releases, the primary objective of maximising the coverage of sterile males competes with secondary, limiting objectives such as the number of mosquitoes released or the number of release points that must be visited. Multiple objective particle-swarm optimisation (MOPSO) techniques are used to investigate the trade-offs between coverage and the cost of a release using a range of measures. For large-scale transgenic sterile insect programmes, releases may be conducted from moving vehicles. MOPSO is combined with an ant-colony optimisation procedure to estimate the most efficient driven route for sterile male releases at a field site.

INSIGHT ON OVIPosition CHOICE OF ANOPHELES GAMBIAE SENSU LATO PRESENTS A NEW FRONT FOR VECTOR CONTROL

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The two riskiest parts of a female mosquito’s life are taking a blood meal and laying her eggs. Whilst there is a considerable body of work that has demonstrated that Anopheles gambiae is attracted to human subjects by the volatiles produced by humans, next to nothing is known about how they locate a breeding site. Here we presented the results of a case-control study of potential breeding sites to identify the characteristics of habitats associated with the presence of Anopheles gambiae sensu lato, the principal African malaria vector. A cross-sectional study of aquatic habitats with anopheline larvae (cases) and without larvae (controls) was carried out in Rusinga Island, on Lake Victoria, Kenya. Factors evaluated include biological characteristics of the sites, zooplankton, invertebrate fauna, physical parameters, nutrients, bacteria communities and volatile chemicals released from the water. Characteristics of 120 habitats (74 cases and 46 controls) were analyzed between March and July 2012. Data were analysed using a random forest model. The presence of early instar larvae was associated with habitats located within 100 meters of the lake. Preferred habitats of An. gambiae s.l. were characterised by increasing content of phosphates, conductivity and turbidity beyond 200 NTU. Late instar culicines and small crustaceans of the orders Cyclopoidea and Cladocera were also abundant in these sites. Contrary to previous report invertebrate predators of the orders Odonata, Coleoptera and Heteroptera were common in anopheline habitats. Volatile chemicals released from the water headspace were less diverse and released in lower concentration from cases than from controls. This study demonstrates that Anopheles gambiae select breeding sites with specific characteristics These factors can potentially be used to target breeding habitats for larval control or manipulated to attract and kill gravid females.

HIGH LINKAGE DISEQUILIBRIUM IN ANOPHELES GAMBIAE FROM KILIFI, KENYA IS CONSISTENT WITH A RECENT REDUCTION IN POPULATION SIZE

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We used RADseq to genotype ~6000 SNPs in An. gambiae collected from Kilifi, Kenya and Muheza Tanzania. Linkage disequilibrium (LD) was higher but genetic diversity only slightly lower in Kilifi compared with Muheza. We investigated the relationship between rho (population recombination parameter) and theta (genetic diversity). In the Kilifi samples the ratio rho/theta departed from neutral expectations. Simulations showed that a recent, severe population reduction gives a significant reduction in rho without an equivalent change in theta. This is consistent with the rho/theta observed in Kilifi. It has been previously observed that abundance of An. gambiae has been reduced in the Kilifi district in the last 10-15 years; our results suggest that the population size change can be detected in the population genomics of the remaining mosquitoes. This result shows that monitoring linkage disequilibrium in vector populations may be an effective way of tracking recent changes in population size, due to mosquito control interventions or climate change.
UNBIASED CHARACTERIZATION OF ANOPHELES MOSQUITO BLOODMEALS BY TARGETED HIGH-THROUGHPUT SEQUENCING OF 16S RNA GENES

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Understanding mosquito blood feeding behavior is important for assessing vector competence or identifying possible reservoir hosts. To date, there is no unbiased method of evaluating mammalian host bloodmeals from mosquitoes and most current molecular assays are designed to test whether a mosquito fed on a priori selected species. Here, we describe a targeted high-throughput sequencing method that utilizes a universal primer pair, which amplifies mammalian mitochondrial 16S ribosomal genes, to identify host bloodmeals from female mosquitoes. Our assay enables analyzing 96 mosquitoes simultaneously and, with more than 1,000 sequences generated per mosquito, provides a comprehensive and quantitative perspective on each blood meal composition. We applied our approach to 529 blood-fed female Anopheles collected from five villages in Papua New Guinea. 483 mosquitoes (91%) yielded a successful PCR product and, after sequencing, showed that these Anopheles mosquitoes fed almost exclusively on humans, dogs and pigs. Interestingly, 68 mosquitoes (14%) showed clear evidence of having fed on two or more species. In one village, where similar number of mosquitoes were collected on both sides of an erected barrier screen, we observed that mosquitoes fed more often on humans on the village versus the bush side of the net (p=0.001). Our analyses also showed that An. farauti s.s. fed more often on humans in the village of Matukar than in Mirap (p=8.7e-9), revealing potential behavioral differences between these populations. Overall, our study shows that this assay enables us to objectively identify host bloodmeals from female Anopheles mosquitoes and to discover feeding behavior differences. This approach is generalizable to any insects that feed on mammalian blood and can be applied to improve our understanding of a wide variety of infectious diseases.

SOUTH AUSTRALIA’S FIRST LARGE SCALE ARBOVIRUS SURVEY: SPATIAL ANALYSIS OF SENSITIVE FTA® CARD METHOD

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Every year in Australia, over 6000 people become infected with a mosquito-borne virus (arbovirus) including Ross River virus (RRV) and Barmah Forest virus (BFV), among others. The most prevalent of these viruses is RRV which causes prolonged symptoms of fatigue, muscle soreness and polyarthritic joint pain and costs Australians tens of millions annually in diagnosis, prevention and treatment. Approximately 250 cases of human infection are reported every year in the state of South Australia. Until recently, the methods for conducting arbovirus surveillance made large scale surveys logistically and economically difficult. A modern development has capitalized on the nectar-feeding behaviour of mosquitoes to collect arboviruses on nucleic acid-preserving cards (FTA® cards), thus streamlining the virus surveillance process. We adapted this technique to an existing mosquito surveillance program and conducted the first ever large-scale arbovirus survey in South Australia. During two months of the peak virus season (January/February 2014), we set CO2-baited EVS light traps at 100 locations around South Australia with a honey-baited FTA® card inside. After collection, mosquitoes were kept in a humid environment and given one week to feed on the honey-soaked card. Cards were then tested for arboviruses using a nested PCR. We made 23 virus detections from around South Australia including 14 of RRV, 6 of BFV and 3 of Stratford virus (not previously reported from South Australia). This level of detection suggests that our low-budget method is highly sensitive at detecting infectious mosquitoes. In this presentation, we report on our methods, the resulting detections (including pending results from March) and the mosquitoes associated with the detections. We also will present a spatial analysis of how these detections correlate with human case data from the last 20 years and explore the implications of our method and findings.

CHIKUNGUNYA INCIDENCE AND CORRELATION WITH PROTECTION IN A PROSPECTIVE LONGITUDINAL COHORT IN THE PHILIPPINES

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Chikungunya is a re-emerging arboviral disease that has caused increasing epidemics and recently appeared for the first time in the Americas. However, the true incidence and clinical spectrum of chikungunya virus (CHIKV) infection have not been well established. In addition, although CHIKV antibodies have been suggested as being protective, no correlation with protection has been demonstrated in humans. In March 2012, we initiated a longitudinal prospective cohort of approximately 1000 subjects aged 26 months in Cebu, Philippines, which underwent community-based active surveillance for febrile episodes. Acute and 3-week convalescent blood samples were obtained and tested by CHIKV RT-PCR and CHIKV hemagglutination inhibition assay (HAI). Enrollment and 12-month follow-up samples were tested by CHIKV HAI to identify subclinical
seroconversion. During one year of surveillance, the annual incidence of total and symptomatic CHIKV infection in the cohort was approximately 8% and 2%, respectively. The total and symptomatic incidence in the 6 month-5 year old age group was 7% and 3%; 6-15 years was 8% and 4%; 16-30 years was 9% and 1%; 31-50 years was 9% and 1.5%; >50 years was 5% and 0.5%. The total and symptomatic incidence among 672 subjects with negative CHIKV HAI titer (<10) at enrollment was 9% and 2.5%; and among 181 subjects with positive CHIKV HAI titer (>10) was 0.5% and 0%. Subjects with negative CHIKV HAI at enrollment were more likely than those with positive HAI to have CHIKV infection (Fisher’s exact, p=0.0001) and symptomatic CHIKV infection (Fisher’s exact, p=0.019). Our results demonstrate that CHIKV infection is a common endemic infection across all age groups in the Philippines, but a greater proportion of those ≤15 years old are symptomatic. CHIKV HAI titer >10 is correlated with protection against both symptomatic and subclinical CHIKV infection. Our findings have important implications for assessment of CHIKV disease burden, understanding of virus transmission, and development of candidate vaccines.

9 HIGH RATES OF O’NYONG NYONG AND CHIKUNGUNYA VIRUS TRANSMISSION IN COASTAL KENYA

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Chikungunya virus (CHIKV) and o’nyong nyong virus (ONNV) are mosquito-borne alphaviruses endemic in Kenya that cause acute febrile illness and arthritis. The objective of this study was to measure the seroprevalence of CHIKV and ONNV in coastal Kenya and link it to demographics and other risk factors. Demographic and exposure questionnaires were administered to 1,862 participants recruited from two village clusters (Milalani-Ngana and Vuga) in 2009. Sera were tested for alphavirus exposure using standardized CHIKV IgG ELISA protocols and confirmed with plaque reduction neutralization tests (PRNT). Logistic regression models were used to determine the variables associated with seropositivity. G statistic and kernel density mapping were used to identify locations of high seroprevalence. Weighted K test for global clustering of houses with alphavirus positive participants was performed for distance ranges of 50-1,000 meters. 486 (26%) participants were seropositive (1-79 years, mean 27 years). Of 443 PRNT confirmed positives, 24 samples (5%) were CHIKV, 246 samples (56%) were ONNV, and 173 samples (39%) had equal high PRNT titers for both CHIKV and ONNV. Age was significantly associated with seropositivity (OR 1.01 per year, 95% C.I. 1.00-1.01). Males were less likely to be seropositive (p=0.05; OR 0.79, 95% C.I. 0.64-0.97). Adults who owned a bicycle (p=0.05; OR 1.37, 95% C.I. 1.00-1.85) or motor vehicle (p=0.05; OR 4.64, 95% C.I. 1.19-18.05) were more likely to be seropositive. Spatial analysis demonstrated hotspots of transmission within each village and clustering among local households in Milalani-Ngana, peaking at the 200-500m range. Alphavirus exposure is common in coastal Kenya with ongoing interepidemic transmission of both ONNV and CHIKV. Women, adults and those with higher socioeconomic status were more likely to be seropositive. Household may be one of the defining locations for the ecology of alphaviral transmission in this region, given our spatial analysis results and the fact that anophelines transmit ONNV. Human disease caused by ONNV and CHIKV in this region is being missed in clinical settings.

10 DETERMINANTS OF CHIKUNGUNYA EMERGENCE POTENTIAL IN SOUTH FLORIDA

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Arbovirus transmission is dependent on the transmission efficiency between infectious and susceptible hosts. Heterogeneity of virus acquisition from a viremic human to a susceptible mosquito is often assumed to be nearly perfect and almost always uniform across the infectious period. Chikungunya transmission models often do not account for the variation in infectiousness of a single individual, and subsequent infection of naïve mosquitoes. Understanding the contribution of human infectious heterogeneity is especially important in the context of introduction events where an infected individual carries the virus into a population(s) of competent vectors. We construct a stochastic, compartmental model to describe the heterogeneity of human viremia and calculate the probability of a successful introduction, taking into account the viremia level (and thus acquisition potential) of the index case on, and after, the day of introduction into a susceptible population. We inform our model based on the experience of dengue emergence in South Florida, 2009-2013. We further account for viral genotype and predominant vector (Ae. aegypti versus Ae. albopictus) by altering the transmission efficiency rate between the human and specific mosquito populations by exploring 1) differential vector competence between genotypes of chikungunya and 2) differential contact rates within and between the two mosquito populations. We find that the infectivity of the index case as well as the parameters affecting transmission efficiency affected the probability of emergence, but that the effects among these parameters were not equal. To predict the likelihood and magnitude of a potential chikungunya outbreak in the United States, it is critical to understand the interplay between individual human heterogeneity of infectiousness and the transmission efficiency of the virus and vector populations.

11 PRECLINICAL EVALUATION OF A LIVE ATTENUATED CHIKUNGUNYA VACCINE

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Recently, Chikungunya virus (CHIKV) has re-emerged to cause major epidemics in the Indian Ocean, India and Southeast Asia, infecting millions. Autochthonous transmission in Italy and France after CHIKV introductions via travellers and the recent outbreak in the Caribbean highlight the threat of widespread chikungunya transmission. We used a molecular attenuation approach, inserting a picornavirus internal ribosome entry site (IRES) to replace the subgenomic promoter, to generate a live attenuated vaccine strain (CHIKV/IRES). The CHIKV/IRES vaccine provides robust immunity and protection in murine models, and is incapable of infecting mosquito vectors, an important safety feature for use in nonendemic locations. The CHIK vaccine elicited a robust memory CD4+ and CD8+ T cell response. However, adoptively transferred immune T cells did not protect against wtCHIKV-LR challenge. In contrast, passive immunization with anti-CHIKV/IRES immune serum provided protection, and a correlate of a minimum protective neutralizing antibody titer was established. Vaccination of cynomolgus macaques with a single dose produced no signs of disease but was highly immunogenic. After challenge with wild-type CHIKV, the vaccine prevented the development of detectable viremia and signs of clinical CHIKV disease. We also have established processes and assays for high-liner manufacture at a large scale. The CHIKV/IRES
vaccine candidate is safe, immunogenic and efficacious in multiple animal models, supporting its potential as a human vaccine to protect against CHIKV infection and reduce transmission and further spread. Current preclinical development efforts are aiming to complete the Investigational New Drug (IND)-enabling studies necessary to begin human clinical testing.

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**CLINICAL RESULTS OF NOVEL CHIKUNGUNYA VACCINE TESTED IN PHASE 1/2 TRIAL: NEUTRALIZING ANTIBODIES AND ANTI-VECTOR IMMUNITY**

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We show here a first in man phase - 1/2 clinical trial to demonstrate the immunogenicity, safety and tolerability of a novel chikungunya virus vaccine. Chikungunya Virus is an emerging mosquito borne virus that causes a severe polyarthrits in humans. The virus is endemic in nearly 40 countries in Asia, Africa, Europe, and recently also in the Americas posing a major threat to public health. So far there is no vaccine approved for human use that prevents the disease. Nucleotide sequences encoding the Chikungunya virus capsid and envelope structural proteins were inserted into the Schwarz vaccine strain of measles virus to produce the candidate vaccine MV-CHIK. To evaluate the optimal dose of MV-CHIK in regard to immunogenicity, safety and tolerability we performed an observer blinded, block-randomized, active and placebo-controlled, dose escalation, phase 1 trial in 42 healthy volunteer subjects. All subjects received three i.m. injections (days 0, 28, 90), the first injection was a vaccine. Subjects were block-randomized to MV-CHIK or to the control-vaccine Priorix® (MV-CHIK: n=12, Priorix: n=2/ Cohort). All volunteers were additionally randomized to one of two treatment sequences, vaccination in day 0 and 28 or 0 and 90. The Immunogenicity on day 28 after vaccination was confirmed by the presence of functional antibodies as determined by the plaque reduction neutralization test (PRNT50). Preliminary findings point at excellent safety and immunogenicity profile in all three doses tested. Data are currently under final evaluation and auditing, and will be presented here for the first time.

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**A MULTIAGENT ENCEPHALITIC ALPHAVIRUS DNA VACCINE DELIVERED BY ELECTROPORATION ELICITS PROTECTIVE IMMUNITY AGAINST AEROSOL CHALLENGE WITH EASTERN AND WESTERN EQUINE ENCEPHALITIS VIRUSES IN NONHUMAN PRIMATES**

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Next-generation vaccines that can safely and effectively protect against Venezuelan, eastern, and western equine encephalitis viruses (VEEV, EEEV, and WEEV) are needed. Previously, we demonstrated that monovalent or a trivalent combination of DNA plasmids expressing codon-optimized envelope glycoprotein genes of VEEV, EEEV, or WEEV delivered by intramuscular (IM) electroporation (EP) elicited high levels of virus-neutralizing antibodies in multiple animal species and provided protective immunity against lethal aerosol homologous viral challenge in mice. Currently, we have completed studies to further assess the monovalent and trivalent DNA vaccines delivered by IM EP in nonhuman primate (NHP) models of aerosol EEEV and WEEV challenge. Although some neurological signs of disease were observed after aerosol EEEV challenge in three of four NHPs that received the monovalent EEEV DNA vaccine, these were of lesser severity than those observed in the negative control animals and all survived. Interestingly, no significant clinical signs of disease were observed after challenge in the NHPs that received the trivalent DNA vaccine. While one of four NHPs that received the monovalent WEEV DNA vaccine displayed clinical signs of disease similar to those observed in the majority of the negative control animals after aerosol WEEV challenge, the remaining animals from this group only displayed mild clinical manifestations. Similar to results observed for the EEEV NHP challenge study, no significant clinical signs of disease were observed in any of the NHPs that received the trivalent DNA vaccine. We are currently investigating the apparent synergistic protective effect achieved with the trivalent DNA vaccine in further detail. Taken together, the results of our current studies further demonstrate that IM EP delivery of a multiagent formulation of VEEV, EEEV, and WEEV DNA vaccines represents a potent means of protecting against aerosolized encephalitic alphavirus infections and support its continued development into a mature vaccine candidate suitable for future clinical testing.

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**A MOUSE MODEL FOR ACUTE AND CHRONIC, FLARING CHIKUNGUNYA VIRUS-INDUCED DISEASE**

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Chikungunya virus (CHIKV) has gained notoriety in recent years with the explosive pandemic outbreak that has affected over six million people in Africa, Indian Ocean, Asia, southern Europe, and recently the Caribbean and South American mainland. CHIKV is a mosquito-borne, enveloped, single-stranded, positive-sense RNA virus that causes fever, rash, and chronic polyarthralgia/polyarthrits in humans, often accompanied by incapacitating joint and muscle pain that can last anywhere from weeks to years. Currently there are no licensed vaccines or antiviral therapies against CHIKV. The development of a murine model that mimics both the acute and chronic, flaring phases of disease is critical to our understanding of this disease but has proven difficult. Current murine models mimic features of the acute phase but overt chronic disease has not been observed. To improve the model, we adapted to mice a wild-type strain of CHIKV by in vivo passage in musculoskeletal tissue. During the acute disease, the mouse-adapted strain of CHIKV causes swelling of both the inoculated and opposite footpad, which does not occur with the wild-type CHIKV in immunocompetent mice. Furthermore, mouse-adapted CHIKV induced chronic disease in the mice with flaring of musculoskeletal disease and swelling around three weeks post infection with chronic swelling lasting for months. Preliminary data indicate that markers of human chronic CHIKV disease are also present in our model (e.g. CCL2 and CXCL12). Recent identification of a single nucleotide change that confers this phenotype suggests possible mechanisms by which chronic disease may arise which are currently being investigated. Importantly, this model will also aid in the testing of candidate vaccines and antiviral therapeutics targeting long-term sequelae of CHIKV infection.

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**A MOUSE MODEL OF HIV AND CEREBRAL MALARIA CO-INFECTION**

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Experimental cerebral malaria (ECM) models are controversial because leukocytes and platelets accumulate in rodent brain vasculature, but sequestration of parasitized red blood cells as described in human cerebral malaria (CM) has been inconsistently observed. We have found that HIV is a risk factor for pediatric CM and that intravascular platelets and monocytes are prominent in the brains of children with fatal CM. We revisited the ECM model to study co-infection, infecting mice transgenic for a HIV-1 provirus and for the human cyclin T1 promoter with Plasmodium berghei ANKA. 56 mice (31 transgenic “HIV-JR-CSF”, 25 littermate controls) age 6 weeks-4 months were infected IV with 105
RBC. mediator of immune escape to avoid the splenic clearance of infected the mechanism of lower phagocytic clearance of infected RBC, may be burden in wt C57Bl/6 mice. Together, these results strongly suggest of CD47 neutralizing antibody caused a significantly reduction in parasite (13.6 ± 1.7 % CD47-/- vs. 3.7 ±0.7 % wt, p<0.01). Furthermore, Injection, p< 0.05) that also had a higher percentage of phagocytized infected RBC 1Food and Drug Administration, Kensington, MD, United States, 2Duke Rajdeep Banerjee

PLASMODIUM ANKA. Mice were assessed for development of CM daily using a standard behavioral scale and were sacrificed when severely impaired. Mice that did not develop CM were sacrificed when they became less active and severely anemic. Brains were harvested for histopathologic analysis. All mice developed peripheral parasitemia. During early infection HIVJR-CSF mice had higher mean parasitemia than controls (0.79% vs. 0.32% on day 4 post-infection, p=0.014), but there was no difference by day 7 when signs of CM develop. HIV viral load increased during malaria infection, from 80,000 copies/ml at baseline to 1.7 million copies/ml on day 7 (p=0.05). Evaluation of H&E stained sections on a subset of mice with CM found that all control mice and only 41% of HIVJR-CSF mice had ≥2 microhemorrhages/100 high power fields while 33% of HIVJR-CSF mice had no visible hemorrhages, similar to pathology patterns noted in our pediatric CM studies. Quantification of intravascular platelets and monocytes is ongoing. While there was no difference in incidence of CM (68% for HIVJR-CSF mice and 52% for controls, p=0.23) in young mice, older adult HIV transgenic mice continued to develop CM, unlike age-matched littermates. The mouse model may be a powerful tool to understand the pathophysiology of pediatric CM and HIV co-infection.

CD47 MEDIATED PARASITE CLEARANCE IN PLASMODIUM YOELII MALARIA

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Many Plasmodium species, including a few human malarial species that cause a relatively less severe infection have a strong preference to invade and grow inside young red blood cells (RBC). We utilized the murine GFP-Plasmodium yoelii 17XNL, which predominantly invades young RBC, to identify the host factors responsible for the preferential RBC infection and a possible mechanism for the potential cellular advantage for this preferential infection. CD47 or Integrin Associated Protein is a marker of self on most cells including RBC that, in conjugation with its receptor SIRP-α, prevents the phagocytosis of RBC. By injections of biotin in C57Bl/6 mice, we were able to differentiate between the young and old RBC by flowcytometry. On day 9 post infection (PI) with GFP-PyNL, young RBC had significantly higher parasitemia than old RBC (young RBC 45.1 ± 11.8 vs. old RBC 1.05 ± 0.8, p<0.001) and old RBC, young RBC had significantly higher CD47 (mean fluorescence intensity, young RBC 2373 ± 139 and old RBC 1156 ± 24, p<0.001). Importantly, infected RBC had higher CD47 levels than non-infected RBC throughout the course of GFP-PyNL infection and higher CD47 levels on RBC also associated with the higher parasitemia. C57B/6 CD47⁻ mice resolved their infection at an accelerated rate (wt on 17 Pl vs. CD47⁻ on 15 Pl) and developed significantly lower parasite burden (day 9, 2.3 ± 0.7 % vs. 26.48 ± 3.9 %, p<0.001). FACS analysis revealed a higher percentage of splenic F4/80 population in CD47⁻/ mice (day 7, 4.2 ± 0.4% CD47⁻ vs. 2.3 ± 0.9 % wt , p<0.05) that also had a higher percentage of phagocytized infected RBC (13.6 ± 1.7 % CD47⁻/ vs. 3.7 ±0.7 % wt, p<0.01). Furthermore, Injection of CD47 neutralizing antibody caused a significantly reduction in parasite burden in wt C57Bl/6 mice. Together, these results strongly suggest that CD47 is important for parasite growth and survival and, through the mechanism of lower phagocytic clearance of infected RBC, may be mediator of immune escape to avoid the splenic clearance of infected RBC.

GLUCOSE STARVATION REVEALS EVIDENCE OF A NOVEL FATTY ACID OXIDATION PATHWAY IN PLASMODIUM FALCIPARUM

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Plasmodium falciparum has finely tuned metabolic pathways to support growth in the various nutrient environments of its complex lifecycle. Despite its metabolic importance, relatively little is known about fatty acid (FA) metabolism in Plasmodium. Using 1H hypoxanthine incorporation assays and SYBR Green-based FACS, we show reduced growth of 3D7 in limited FA media, and titrating glucose and glutamine to 40-45% of normal levels causes additional growth restriction. Knockdown of members of the acyl CoA synthetase (ACS) gene family further reduces growth in restricted glucose and FAs, an intriguing finding, as the ACS enzymes are known to mediate FA scavenging and activation. This observation prompted us to investigate a metabolic link between the exogenous FA substrates of ACSs and carbon sources used to generate ATP in the parasite. We determined that glucose-deprived parasites exhibit FA oxidation, as measured by release of 14H₂O from parasites grown in 3H labeled FAs. Following two cycles of glucose restriction, there was an 18-24 fold increase in FA oxidation as compared to uninfected red blood cells. Parasites grown in the standard glucose concentration of RPMI1640 did not exhibit FA oxidation, suggesting that typical in vitro culture conditions mask this metabolic phenomenon. To our knowledge, this is the first reported experimental evidence for FA oxidation in Plasmodium. The canonical FA oxidation enzymes are not annotated in P. falciparum, though the pathway is believed to be present in Toxoplasma gondii. Our data suggests that glucose restriction redirects FAs into a beta-oxidation pathway to generate ATP. Furthermore, this important role for exogenous FAs offers a potential explanation for the previously reported expansion and positive selection of the ACS gene family in P. falciparum. Ongoing experiments will reveal the physiological importance of this catabolic pathway and shift in energy metabolism. Additional characterization of the molecular mechanisms may reveal novel targets for development of antimalarials.
GENOMIC ASSESSMENT OF PLASMODIUM FALCIPARUM POPULATION STRUCTURE AND DIVERSITY DURING AN ENHANCED MALARIA CONTROL PROGRAM IN WESTERN KENYA

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Malaria Control and Elimination Partnership in Africa is working toward aggressive malaria transmission reduction in Siaya County (western Kenya). The application of population genetic approaches to understand and estimate transmission dynamics in the context of malaria elimination in this setting will be crucial. To monitor changes in Plasmodium falciparum population structure in this population, we are applying estimates of parasite genetic diversity and relatedness, and assessing key biomarkers, such as those related to drug resistance that may impact efforts to reduce the malaria burden toward eradication. Additionally, we aim to provide a genetic map of parasite movement between Siaya County, and other parts of Kenya (e.g., Nairobi and central regions) and eastern Uganda where substantial movement of parasites have been spatially quantified through population movement studies.

SUSCEPTIBILITY WEIGHTED IMAGING IN PEDIATRIC CEREBRAL MALARIA AT 1.5T

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The pathogenesis of Pediatric Cerebral Malaria (PCM), a leading killer of children in sub-Saharan Africa, is unknown. The pathological hallmark of the disease is sequestration of parasitized red blood cells in brain microcirculation. Intravascular hemoglobin, a ferromagnetic breakdown product of hemoglobin metabolism and microhemorrhages in various structures, including the white matter and grey-white junction, have been reported. We imaged Zambian children with cerebral malaria on a 1.5T magnet to investigate the distribution of iron breakdown products from hemoglobin metabolism and BBB breakdown. Twelve children with strictly defined PCM peripheral blood slide positive and retinopathy positive were scanned at 1.5T. Eleven received intravenous gadolinium enhancement. SWI (susceptibility weighted imaging), T2, T1 pre and post, DWI with ADC and FLAIR imaging sequences were obtained. The children had mild PCM: none died and none had severe brain swelling. Normal intravascular gadolinium enhancement was noted but there was no parenchymal enhancement to suggest BBB breakdown. SWI imaging confirmed the present of a ferromagnetic substance in distributions similar to those of parasite sequestration and hemoglobin seen at autopsy. A ferromagnetic substance, possibly hemoglobin, the metabolic end product of hemoglobin digestion, was identified. Additional studies using R2* and SWI will assist in quantifying hemoglobin, and parasite sequestration distribution in vivo. In this small population of non-fatal PCM cases, extravasation of contrast indicative of BBB breakdown was not evident using 1.5T MRI. Additional evaluations with dynamic enhancement techniques are warranted to further assess the integrity of the BBB, as this has relevance to adjuvant treatment strategies.

SEVERE AND CEREBRAL MALARIA IN CHILDREN LEADS TO A SIGNIFICANT IMPAIRMENT OF TRANSITORY OTOACOUSTIC EMISSIONS - A MULTICENTER PROSPECTIVE STUDY

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Lately, severe and especially cerebral malaria have been suspected to influence the inner ear function. Cerebral malaria has been associated with hearing loss in 9 of 23 patients, as reported previously. However, prospective studies evaluating the inner ear function in severe and cerebral malaria are missing. An objective way to quickly evaluate the inner ear function is to measure otoacoustic emissions - a technique which is used worldwide to screen newborns for potential hearing impairment. This prospective multicenter study analyses the function of the inner ear in patients with severe and cerebral malaria up to the age of 10 years. In three study sites (Ghana, Gabon, Kenya) 144 patients with severe and cerebral malaria and 108 age-matched local control children were included. All patients were treated with artemisinin combination therapies. Of the severe malaria patients 42 % failed the initial otoacoustic emissions testing at the baseline measurement, i.e. prior to therapy, on day 28 only 27.1%. The cerebral malaria group showed a comparable number of failures with 38 % at the baseline. In contrast to the severe malaria the number of fails increased up to 66 % at day 7 after diagnosis. A slight improvement to 55% fails could be noted on day 28. Negative transient otoacoustic emissions are associated with a threshold shift of 20 dB and above. The present data shows a pathologic involvement of the inner ear in severe and cerebral malaria of around 40% prior to the initiation of schizontocidal therapy, a figure which improves in severe malaria but deteriorates over the next weeks in cerebral malaria. Therefore, especially in children with cerebral malaria, hearing screening and post-malaria audiologic work up has to be recommended after severe malaria infection.

ARTESUNATE-MEfloQUINE VERSUS CHLOROQUINE IN PATIENTS WITH ACUTE UNCOMPLICATED PLASMODIUM KNOWLESI MALARIA: AN OPEN-LABEL RANDOMIZED CONTROLLED TRIAL IN SABAH, MALAYSIA (ACT KNOW TRIAL)

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Plasmodium knowlesi is the most common cause of malaria in Malaysia, and has been reported throughout South-East Asia. There are no recommendations in the current 2010 WHO malaria treatment guidelines for the optimal treatment of uncomplicated knowlesi malaria, which replicates every 24 hours and has the potential to cause severe disease and death. P. knowlesi is commonly microscopically misreported as other Plasmodium species, including P. falciparum and P. vivax, a high proportion of which are chloroquine-resistant in Malaysia. Artemisinin combination therapy elim
REPEATED TREATMENT OF YOUNG CHILDREN 4-11 MONTHS WITH ARTEMETHER-LUMEFANTRINE AND DIHYDROARTESMISININE-PIPER AQUINE OVER A TWO-YEAR FOLLOW UP PERIOD IN REAL-LIFE SETTINGS: NEURO-O TOTOXICITY DRUG SAFETY ASSESSMENTS

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Artemisinin-combination therapies (ACTs) are widely seen as safe drugs. Early animal data of artemisinins raised the possibility of neurotoxicity with high doses of the lipophilic (oil-based) derivatives such as arte(m) ether in the form of selective damage of the brainstem involved in auditory and vestibular function, and recent animal data suggested the possibility of a cumulative effect. While the implication for human toxicity is controversial and audiology assessments in adults in Asia and single-use trials in older children over 5 years in Africa have been reassuring so far, this has never been confirmed in the most vulnerable group of young children during a phase of substantial brain maturity and high drug exposure. We conducted a cluster-randomized effectiveness trial in an area of high malaria transmission in Malawi. 814 children aged 4-11 months, recruited at community-level, were randomized at village level into either receiving artemether-lumefantrine (ArtL, lipophilic) or dihydroartesminin-piperaqueine (DHA-PPQ, hydrophilic), for any consecutive clinical malaria episodes during a 2 year follow-up period, in a ‘real-life-setting’. To explore any neuro-ototoxic adverse effect of repeated ACT exposure, two audiology safety components were conducted. Short-term and potentially reversible effects were assessed in a subset of 176 clinical malaria episodes with auditory brainstem response (ABR) tests before treatment on Day 0, and day 7 and day 42 post treatment. Long-term irreversible, cumulative effects were assessed with ABR readings at trial baseline, mid-point and end-of-study. Primary ABR endpoint was the interpeak latency between wave I and V(II)PL. Overall trial analyses are ongoing and will be finalized by September. We will present the intention-to-treat and per-protocol analyses results, adjusting for treatment exposure, artemisinin intake dose, child age, concomitant treatments, and history of exposure of known determinants of audiological changes, including relevant birth and disease history, and other reported exposure to known ototoxic drugs.

BOUTS OF MALARIA ILLNESS AS MEDIATED BY ANEMIA DIMINISHES COGNITIVE DEVELOPMENT IN VERY YOUNG UGANDAN CHILDREN

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We evaluated child development in an open-label RCT of anti-malarial chemoprevention in children living in an area of intense, year-round transmission in Eastern Uganda. Infants 4 to 5 months of age were enrolled and randomized to one of four treatment arms: no chemoprevention, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS), or monthly dihydroartesminin-piperaqueine (DP). At 2 yrs of age chemoprevention was stopped and children were followed for 1 additional year. Number of malaria episodes and anemia (hgb<10) were summarized up to cessation of chemoprevention (2 yrs of age) and again from 2 to 3 yrs of age. The Mullen Early Learning Scales (MELS) was administered to 471 children at 2 yrs and 452 children at 3 yrs. 69% were HIV-unexposed and 31% were HIV-exposed, equally distributed across treatment arms. The number of malaria episodes differed at the end of chemoprevention by trial arm (P<0.001) with DP having a high protective efficacy against malaria and anemia, TS having a moderate protective efficacy against malaria only, and SP offering no protection against malaria or anemia. Following cessation of chemoprevention, all treatment arms displayed levels of malaria (~7.5 episodes per year) and anemia comparable to that of the no chemoprevention arm. To determine the effects of the number of malaria episodes on cognitive development, linear mixed effects model related 2 repeated measures of Mullen scores (at 2 and 3 yrs) to trial arm, HIV exposure status, sex, socio-economic status, weight for age z-score, duration of breast feeding, and the number of malaria episodes in each year. Trial arm was not significantly related to any of the Mullen development outcomes. The number of bouts of malarial illness was significantly predictive of Mullen cognitive outcomes both at 2 and 3 yrs of age (P = 0.02). This relationship was mediated by the number of anemia episodes. Anemia episodes over and above those related to malaria was independently associated with poorer Mullen working memory (P = 0.02). HIV exposure was associated with lower Mullen receptive language development over and above malaria and anemia. We are the first to document within an RCT study that early malaria illness and anemia can lead to adverse developmental outcomes in very young children. Concurrent intervention for both of these risk factors in early childhood is important for enhancing developmental outcomes in at-risk children.
SEASONAL MALARIA CHEMOPREVENTION AND MICRONUTRIENT SUPPLEMENTATION IN EARLY CHILDHOOD: EFFECT ON ASYMPTOMATIC PARASITAEMIA, ANEMIA AND COGNITION

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Early childhood is a time of rapid growth and development and public health interventions during this period could yield substantial benefits across several developmental areas: physical, cognitive, and linguistic. Iron is important in brain function, and interventions that reduce iron-deficiency and anemia may improve cognitive function and learning. A randomized trial was undertaken to examine the combined impact of two newly-recommended interventions in early childhood: seasonal malaria chemoprevention and home fortification with micronutrient powders. Although each intervention has previously been shown to be associated with improvements in malaria morbidity, anemia and/or physical growth, the benefits for cognitive and linguistic development are comparatively unknown. The combined effect of these two interventions has not previously been examined. A cluster-randomized controlled trial was conducted in 60 rural communities with pre-school programs in southern Mali. Children aged less than 5 years living in the 30 intervention communities received two rounds of seasonal malaria chemoprevention in Oct and Nov 2013, followed by daily supplementation of micronutrients for four months from January-April 2014. Delivery of the two interventions at community-level was organized by pre-school management committees. The impact of the interventions will be evaluated in May 2014 through cross-sectional surveys to compare malaria infection, nutrition and cognitive performance in children aged 3 and 5 years living in intervention and control communities. Study outcomes will include asymptomatic parasitaemia, hemoglobin concentration, nutritional indices (height-for-age, weight-for-age), and cognitive foundation skills for early literacy. Findings on the combined effect of the malaria and nutrition interventions after the first year of implementation will be presented.

POPULATION PHARMACOKINETICS OF PIPERAQUINE IN YOUNG UGANDAN CHILDREN TREATED WITH DIHYDROARTEMISININ-PIPERAQUINE FOR UNCOMPlicated Malaria

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Dihydropyrrolopyrimidine (DHP) is currently recommended as first-line treatment for malaria. Doses of DHP to treat malaria in young children were initially chosen by scaling adult doses based on weight. This approach does not adequately address physiologic changes in early childhood that affect pharmacokinetics (PK). There is little information about the PK of piperaquine in children, and particularly those < 2 years of age. The objective of this study is to describe the PK of piperaquine in children 6 months to 2 years of age using a population approach that also explores covariate effects. We undertook a prospective population PK study of piperaquine (PQ) in 107 children 6 months to 2 years old who were treated with standard weight-based three-dose DP for uncomplicated malaria in Uganda. Participants underwent 5-7 finger sticks for 28 days following each treatment, providing 1282 evaluable capillary plasma samples from 218 treatments for a mixed effects model analysis with the program NONMEM®. Capillary plasma concentrations on day 7 in most patients, but especially in lower weight children 1-2 years of age, were below the value generally believed to be associated with a longer time to infection. PK data of piperaquine were well described by a 3-compartment open model with first-order absorption. Age, and not weight or other covariates, had a statistically significant effect (p<0.005) on clearance/bioavailability (CL/F) or on relative bioavailability (F) alone. This finding provides a possible contributing explanation for the low exposure in low-weight 1-2 year old children relative to higher-weight 1-2 year olds and 6 month-1 year olds, all of whom were dosed according to weight. Based on this population model we then simulated pharmacokinetic profiles of alternative treatment doses, as well as candidate PQ chemoprevention regimens, including monthly administration of standard therapeutic doses, a regimen currently being evaluated. Our results suggest that higher doses, especially in lower-weight 1-2 year olds, will increase the fraction of young children with piperaquine exposure greater than a predefined target, whether it is a previously published one or otherwise chosen. This study supports a growing body of evidence that there is a need to re-evaluate dosing of piperaquine in children.

ARTEMETHER-LUMEFANTRINE EFFICACY: POTENTIAL FOR FURTHER DOSE OPTIMIZATION

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Artemether-lumefantrine (AL) is the first line antimalarial treatment in 49 countries, administered according to four weight bands. Patients at the margins of these bands can receive significant deviation from the target dose. To assess the impact of weight adjusted (mg/kg) dose variations in therapeutic efficacy, individual patient data were shared with the WorldWide Antimalarial Resistance Network (WWARN) and collated using standardised methodology. Risk factors associated with recrudescence were evaluated using Cox’s regression model with shared frailty on study sites. Data from 14,327 patients (61 efficacy studies between 1996 and 2012 in Africa, Asia and South America) with uncomplicated P. falciparum malaria were included in the analyses. A total of 386 Polymerase Chain Reaction (PCR)-confirmed recrudescent infections were reported. The PCR adjusted cure rate was 97.6% [95% CI: 97.4-97.9%] at day 28 and 96.0% [95% CI: 95.6-96.5%] at day 42. After controlling for age and parasitemia, every unit increase in daily artemether dose reduced the risk of being parasitaemic on day 1 by 8% [95% CI:1-14%, p=0.024] and every unit increase in total artemether dose reduced the risk of gametocyte carriage by day 14 by 8% [95% CI:1-15%, p=0.037]. Overall the dose of AL did not correlate with recrudescence, however the risk of recrudescence was higher in Asian children weighing 10 to 15 kg who received a total lumefantrine dose less than 60 mg/kg (Adjusted Hazards Ratio of 2.73 [95% CI: 1.40-5.32]), accounting for 41% of all treatment failures; p=0.003. In Africa, the risk of recrudescence was greatest in malnourished children from 1 to 3 years old (PCR adjusted cure rate 94.3% [95% CI: 92.3-96.3%]). The currently recommended dose of AL provides reliable efficacy in most patients with uncomplicated malaria. However, cure rates were lowest in young children in Asia and young underweight children from Africa. A higher dose regimen should be evaluated in these groups.
ARE PUBLIC OR RETAIL SECTOR PATIENTS MORE LIKELY TO COMPLETE TREATMENT? AN ANALYSIS OF PATIENT ADHERENCE TO ARTEMETHER-LUMEFANTRINE IN SOUTHERN TANZANIA

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Artemisinin combination therapies (ACTs) are first-line treatment for malaria in the public sector of most endemic countries and increasingly available in the private sector. Most studies on ACT adherence have been conducted in the public sector, with minimal data from private retailers. We conducted parallel adherence studies in Mtwara, Tanzania, in which patients obtaining artemether-lumefantrine (AL) at 40 randomly selected public health facilities and 37 private drug shops were followed up at home and questioned about each dose taken. The effect of health sector on adherence was assessed with random effects logistic regression controlling for potential confounders. Factors associated with adherence in each sector were examined in separate models. Of 572 health facility patients and 451 drug shop patients, 75% and 70% respectively completed treatment (p=0.02), and 46% and 75% took each dose at the correct time (p=0.003). Drug shop patients were wealthier, more educated, older, sought care later in the day, and were less likely to test positive for malaria than health facility patients. Controlling for patient characteristics, the adjusted odds of drug shop patients completing treatment and taking each dose at the correct time were 0.63 and 0.67 times that of health facility patients (p=0.030 and p=0.044). Factors associated with adherence in drug shop patients were higher socioeconomic status and recalling correct dose instructions. Patients seeking care in the evening were half as likely to be adherent as those who sought care in the morning. In the public sector, having fever and recalling correct instructions were associated with completed treatment, while seeking care within two days, being tested for malaria, and taking the first dose at the facility were associated with timely completion. Patients attending drug shops differ from those at public health facilities, but when controlling for these characteristics, adherence was lower in drug shops. Better understanding is needed of which aspects of patient care are most important for adherence.

IMMUNE REGULATION BY HELMINTH PARASITES, INVOLVES MODULATION OF HUMAN DENDRITIC CELL METABOLISM INCLUDING INDUCTION OF INDOLEAMINE 2,3-DEOXGENYENASE, DOWNREGULATION OF MTOR SIGNALLING, AND INDUCTION OF AUTOPHAGY

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Helmint infection has been associated with dysregulation of professional antigen presenting cells (APC) such as Langerhans cells (LC) and dendritic cells (DC) with DC being the cell type most affected by parasite exposure, particularly when exposed to the microfilarial (mf) stage of the Brugia malayi one of the 2 major species causing lymphatic filariasis in humans. To gain further insights into nature of the mf-induced alterations in DC function and viability (previously shown), we used LC-MS/MS to elucidate the entire proteome of mf-exposed monocyte derived human DC (n=4) in comparison to mf-unexposed human DC. Our proteomics data indicated that multiple components of the Mammalian Target of Rapamycin (mTOR) signaling pathway, including mTOR, and Eukaryotic Initiation Factor 4 (eIF4) A were downregulated by mf, suggesting that mf target this pathway for immune modulation in DC. Utilizing western blot analysis we showed that similar to rapamycin (a known mTOR inhibitor), mf downregulate the phosphorylation of mTOR regulatory proteins p70S6K1 and 4E-BP1, a process essential for DC protein synthesis. As active mTOR signaling regulates autophagy by inhibiting an autophagy induction complex, we examined whether mf exposure alters autophagy-associated processes. Expression of p62, a ubiquitin-binding protein that aggregates protein in autophagosomes and is degraded upon autophagy, was reduced dramatically by mf exposure (P<0.05), suggesting that mf induce autophagy in DC. Furthermore, as amino acid deficiency is one mechanism of mTOR activation and inhibition of autophagy, upregulation of kynureninase observed in our proteomics data suggested that mf also induce tryptophan catabolism in human DC; this was corroborated by showing that the expression of Indoleamine 2,3-deoxgenase (IDO) and activity was increased dramatically by exposure to mf (p<0.05) compared to mf-unexposed cells. Together, these results suggest that Brugia malayi mf employ mechanisms of metabolic modulation in DC to influence the regulation of the host immune response by downregulating mTOR signaling resulting in increased autophagy.

HYPERREACTIVE ONCHOCERCIASIS IS CHARACTERIZED BY AN ANTIGEN INDEPENDENT BIAS TOWARDS TH17 COMBINED WITH STRONG TH2 IMMUNE RESPONSES

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Onchocerciasis or river blindness is the second leading infectious cause of blindness after trachoma. In human, the disease is caused by infections with the filarial nematode Onchocerca volvulus and presents two major polar forms: the hypereactive form or generalized onchocerciasis (GEO) and the hyperreperative form (hyperreactive onchocerciasis: HO). The latter is associated with more aggressive disease manifestations and disfiguring dermatopathology and eye lesions that can lead to blindness, whereas GEO is characterized by tolerance to the parasite and mild skin disease. The immunological determinants of this polarization of the disease are still not fully clarified. Using multicolor FACS analysis, multiplex cytokine arrays and Real Time PCR, we compared the immune profiles of a group of 16 endemic normals (EN), individuals who had no clinical or parasitological evidence of infection despite ongoing exposure to the parasite, with a group of 16 individuals with GEO and a group of 6 individuals presenting the rare but severe hyperreactive form of the disease. We could show that individuals with HO presented higher frequencies of CD4+ cells expressing Th17 related cytokines (IL-17A, IL-17C, IL-4, IL-22) and markers (RORC, STAT3), while those with GEO presented significantly higher CD25+Foxp3+ and IL-10 secreting T cells. Concordantly, TCR activation using anti-CD3/CD28 induced high amounts of IL-17 in culture supernatants of peripheral blood mononuclear cells (PBMCs) from patients with HO, whereas IL-17 was barely detectable in PBMCs from EN and GEO individuals in same conditions. Strikingly, antigen specific stimulation using Onchocerca volvulus extracts did not elicit IL-17 secretion, either in PBMCs of GEO or in those of HO individuals. In contrast, a robust Th2 response was induced upon antigen specific activation of PBMCs from HO patients, as shown by prominent expression of GATA3, IL-4, IL-5 and IL-13 in PBMCs of these individuals. These findings suggest that, strong parasite specific Th2 responses combined with robust parasite-independent Th17 polarization in the absence of adequate immune regulation is associated with HO, revealing a novel Th17/Th2 axis as potential target in the development of new therapeutical strategies to prevent HO in human.
HELMINTH INFECTIONS DURING PREGNANCY IS ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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Both animal models and human studies suggest that parasitic infections during pregnancy prime the fetal immune response and induce an immunomodulatory phenotype at birth that may affect subsequent immune responses to childhood vaccines. We investigated whether prenatal exposure to helminth infections affects the pattern of infant immune response to standard vaccination against Haemophilus influenzae (Hib), hepatitis B (Hep B), tetanus toxoid (TT) and diphtheria toxoid (DT). 450 Kenyan women were tested for LF, urogenital schistosomiasis, malaria, and intestinal helminths during pregnancy. Their newborns were followed biannually to age 36 months and tested for levels of IgG against Hib, Hep B, TT, and DT at each time point. Overall, one third of the mothers were infected with LF, urogenital schistosomiasis, malaria or hookworm. Using a generalized estimating equation analysis, the presence of multiple maternal infections was associated with lower immune response to Hib PRP-specific IgG (p=0.001, 0.002, 0.045 with one infection; p=0.028, 0.022, 0.051 with two infections at 12, 18 and 24 months of age), compared to no maternal infection. There was a significant difference in response to DT in infants of mothers with three or more infections (p=0.001 and 0.02 at 6 and 12 months) compared to no maternal infection. Response to Hib was also associated with immunophenotype; offspring putatively tolerized to filarial antigens (LF infected mothers but lacking filarial-specific Th1/Th2-type response in cord blood, N=94) compared to unexposed (no evidence of maternal LF infection or antigen responsiveness in cord blood, N=119) had a lowest vaccine-induced antibody response to Hib-specific IgG (p=0.052, 0.033 and 0.035 at 12, 18 and 24 months). Antenatal helminth infections are associated with lower immune response to Hib and DT vaccine antigens. Thus, in developing countries, eradication of chronic helminthic infections may be imperative to the success of future global vaccination efforts.

IMMUNOGENICITY AND ANTI-FECUNDITY EFFECT OF nanoparticles coated glutathione S-transferase (SjGST) DNA VACCINE AGAINST murine SCHISTOSOMA JAPONICUM INFECTION

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There is still urgent need for a vaccine against schistosomiasis, especially in Schistosoma japonicum endemic areas where even a vaccine that will interrupt zoonotic transmission will be potentially effective as an intervention tool. We had developed a novel nanoparticle gene delivery system, which has proven efficacious in gene transfection to target immune cells with complementary adjuvant effect and high protective efficacy in several diseases. Here, we have applied this nanoparticle system in combination with S. japonicum glutathione S-transferase (SjGST) DNA vaccine to show improved immunogenicity and anti-fecundity effect of the nanoparticle coated vaccine formulation against murine schistosomiasis. The nanoparticle-coated DNA vaccine formulation induced desired immune responses with significantly increased humoral response, Th-helper 1 polarized cytokine environment, higher proportion of IFN-γ-producing CD4+ T-cells and the concomitant decrease in IL-4 producing CD4+ T-cells. Although there was no effect on worm burden, the proportion of immature worms was higher in the SjGST vaccinated groups with a lower proportion of mature paired worms. We recorded an unprecedented reduction in tissue egg burden. There was 71.9% decrease in liver egg burden, 71.6% decrease in intestinal egg burden, and 54.7% reduction in the fecundity of female adult worms. In conclusion, our data showed that the combination of nanoparticle gene delivery system with SjGST DNA vaccine significantly improved the characteristic anti-fecundity effect of SjGST, thereby proving this DNA vaccine formulation as a promising candidate for anti-pathology and transmission blocking application.

BLADDER UROTHELIAL CELL CYCLE RESPONSES TO SCHISTOSOMA HAEMATOBIUM INFECTION ARE MODULATED BY IL-4 RECEPTOR-α

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The bladder urothelium, a normally quiescent epithelial organ, undergoes dynamic changes during Schistosoma haematobium infection (urogenital schistosomiasis). These alterations include hyperplasia, ulceration, dysplasia, and frank carcinogenesis, and likely involve shifts in urothelial cell cycle status. Defining the pathways underpinning these urothelial responses will contribute to a deeper understanding of how S. haematobium egg-induced expulsion, hematuria, and bladder cancer develop in humans. Akin to their responses to many different helminth infections, mammals mount an IL-4 and -13-associated type 2 immune response during S. haematobium infection. These cytokines share the IL-4 receptor-α subunit as one of their cognate receptor subunits. To determine whether IL-4 receptor-α plays a role in urothelial cell cycle alterations in urogenital schistosomiasis, we injected S. haematobium eggs or control vehicle into the bladder walls of wild type, IL-4 receptor-α-deficient, and myeloid-associated IL-4 receptor-α-deficient mice. Three weeks later, mice were sacrificed and their bladder urothelium isolated and prepared as single cell suspensions. These suspensions were stained with DAPI and antibodies to CD45 and EpCAM. CD45-EpCAM+ urothelial cells were gated and their DAPI staining analyzed to assess cell cycle status. Relative to vehicle controls, wild type mice injected with eggs demonstrated increased proportions of urothelial cells in S phase and decreased proportions in G2/M phase. Although egg-injected, macrophage-associated IL-4 receptor-α-deficient mice featured similar urothelial responses as their egg-injected wild type counterparts, egg-injected conventional IL-4 receptor-α-deficient mice exhibited fewer urothelial cells in S phase. Thus, IL-4 receptor-α signaling through non-myeloid cells indeed seems to affect urothelial cell cycle status; this effect may specifically target DNA synthesis, a crucial process in carcinogenesis. Ongoing work seeks to determine which IL-4 receptor-α-expressing cells are crucial to these phenomena, and how these cells mediate alterations in the cell cycle status of the bladder urothelium.
THE MOLECULAR CONSEQUENCES OF INTRA-PATIENT PHAGE PREDATION ON VIBRIO CHOLERAE POPULATION DIVERSITY

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Vibrio cholerae is a globally important water-borne pathogen, which is the causative agent of the severe acute diarrheal disease cholera. We analyzed genetically disparate cholera patient samples from Haiti and Bangladesh and found that ICP2-like phage can impact the evolutionary trajectory of intra-patient V. cholerae populations. Evidence of intra-patient selection of phage-resistant mutants was found in one Haitian patient sample in which >99% of V. cholerae isolates harbored mutations in the major outer membrane porin, OmpU. Mutant ompU alleles were sufficient for ICP2 resistance when moved into a clean background, indicating that ICP2 uses OmpU as a receptor to initiate infection. Phage-resistant and phage-sensitive isolates from this sample were subjected to whole genome sequencing and were found to be isogenic except for single site mutations in ompU. The mutations within ompU between phage-resistant isolates were heterogeneous, indicating that phage predation and selection of resistant mutants occurred multiple independent times during infection. High levels of ICP2 were also observed in a Bangladeshi patient sample found to harbor a mixture of toxR mutants. Similar to what was observed in the Haitian patient sample; multiple unique toxR mutants were observed within this single sample. ToxR directly activates expression of ompU and critical virulence factors. ICP2-sensitivity was restored to the clinical toxR mutants by expressing ompU in trans, demonstrating that resistance is mediated through decreased OmpU expression. The clinically isolated ompU and toxR alleles had differing impacts on V. cholerae virulence, with the toxR mutants being severely attenuated in vivo. We demonstrate that V. cholerae faces phage-mediated predatory interactions during the natural course of infection that can impose a strong selective pressure. This work highlights the notion that host-pathogen interactions are often embedded in a diverse microbial ecosystem that can impact the virulence and transmission of the pathogen.

IMMUNOGENICITY OF A KILLED ORAL BIVALENT WHOLE CELL CHOLERA VACCINE IN ADULTS WITH HIV INFECTION

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Cholera epidemics often occur in settings where human immunodeficiency virus (HIV) infection is prevalent. While some evidence suggests that individuals living with HIV are more susceptible to cholera than those that are HIV negative, little is known about how HIV alters the immune response to oral cholera vaccines. We evaluated immune responses following bivalent oral cholera vaccination (BiVWC; Shanchol) in a cohort of adults in Haiti, some of which were known to have HIV infection. Blood samples were obtained prior to immunization and 7 days after each of two doses of BiVWC vaccine were administered. A total of 25 adults with HIV infection were enrolled in the study; 23 of these individuals received both doses of BiVWC and completed the three-week observation period including all blood draws. Compared to 22 adults without known HIV infection who completed the three-week observation period, HIV-infected individuals in this cohort had significantly lower vibriocidal antibody responses against both the V. cholerae O1 Inaba and Ogawa serotypes. Among the HIV-infected vaccinees, we found an inverse relationship between CD4+ T-cell count and the subsequent vibriocidal antibody response following vaccination. However, despite lower vibriocidal antibody responses, there was still a substantial vaccine take rate in this cohort adults living with HIV infection. Both O polysaccharide antigen specific IgA and vibriocidal antibody titers increased significantly after oral cholera vaccination in HIV-infected adults. Seroconversion, defined as four-fold or greater increase in vibriocidal antibody titer, occurred at a rate of 65% against the Ogawa serotype and 74% against the Inaba serotype in adults with HIV infection. These results suggest that killed oral cholera vaccines retain substantial immunogenicity in adults with HIV infection and may provide a significant benefit in a population that is otherwise vulnerable to cholera.
EFFECTIVENESS OF AN ORAL KILLED BIVALENT WHOLE-CELL CHOLERA VACCINE IN RURAL HAITI

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Between April and June 2012, a reactive oral cholera vaccine campaign was conducted in a rural district of Haiti using the oral killed bivalent whole-cell vaccine, Shanchol®. We conducted a case-control study to estimate vaccine field effectiveness in the context of this campaign. Cases had acute watery diarrhea, sought treatment at 1 of 3 participating cholera treatment units between October 24, 2012 and March 9, 2014, and had a stool sample positive for cholera by Crystal VC® rapid test and culture. For each case, four controls (individuals who did not experience or seek treatment for acute watery diarrhea between the date of study initiation and the date of their corresponding case’s symptom onset) were matched by neighborhood, calendar time, and age. We also conducted a bias-indicator case-control study to assess the likelihood of bias in the vaccine effectiveness case-control study by examining the relationship between vaccination and non-cholera diarrhea. In the absence of bias we expected no association between vaccination and non-cholera diarrhea in the bias-indicator study. During the study period, 115 individuals presented with acute watery diarrhea. After excluding 8 individuals who lacked specimen for culture, 22 with discordant rapid test and culture results, and 1 cholera case that lacked interview data, 41 were included as cases in the vaccine effectiveness case-control study and 43 as cases in the bias-indicator study. In univariable analysis, both self-reported vaccination and verified vaccination (via vaccination card or registry) were associated with a statistically significant reduction in the risk of cholera. In adjusted analyses vaccine effectiveness was 66% [95% confidence interval: 15%-86%; p-value: 0.02] for verified vaccination. Neither self-reported nor verified vaccination (via vaccination card or registry) were associated with a statistically significant reduction in the risk of cholera. In adjusted analyses vaccine effectiveness was 66% [95% confidence interval: 15%-86%; p-value: 0.02] by self-report and 60% [95% confidence interval: 14%-82%; p-value: 0.02] for verified vaccination. Neither self-reported nor verified vaccination was significantly associated with non-cholera diarrhea. In the absence of bias we expected no association between vaccination and non-cholera diarrhea in the bias-indicator study.

SINGLE-DOSE LIVE ATTENUATED ORAL CHOLERA VACCINE (CVD 103-HgR) PROTECTS AGAINST CHOLERA AT 10 DAYS FOLLOWING VACCINATION: RESULTS OF A VIBRIO CHOLERAE O1 EL TOR INABA CHALLENGE STUDY


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No cholera vaccines are currently licensed and available in the U.S.A; two killed oral cholera vaccines licensed in other countries require 2 spaced doses. A single-dose oral cholera vaccine that rapidly elicits protection is needed for immunization of short-notice travelers to high risk areas and for use in explosive outbreaks, where practical administration and accelerated onset of protection are also desirable. Attenuated Vibrio cholerae O1 classical Inaba live oral vaccine strain CVD 103-HgR harbors a deletion of 94% of the gene encoding the cholera toxin A subunit. CVD 103-HgR, manufactured by PaxVax Inc., typically elicits vibriocidal antibody seroconversion (a correlate of protection) within 10 days of vaccination. To evaluate vaccine efficacy, we challenged healthy volunteers, 18-45 years old, with virulent V. cholerae. Consenting healthy cholera-naïve adults (n = 98, including n = 50 of blood group O, high risk for cholera gravis), were randomly allocated 1:1 to receive vaccine (10^9 CFU) or placebo. Ten days after vaccination, 68 subjects ingested 10^7 CFU wild type V. cholerae O1 El Tor Inaba strain N16961. Subjects were observed on a Research Isolation Ward for measurement of stool output and management of cholera illness. The vaccine was well tolerated: adverse events, generally mild to moderate, were reported in 13% vaccine and 14% placebo recipients. The primary endpoint, moderate (≥ 0.0 liter cumulative purge) to severe (≥ 0.5 liter cumulative purge) diarrhea occurred in 20/33 (61%) placebo and 2/35 (6%) vaccine recipients (point estimate of vaccine efficacy [VE] = 91%, p<0.0001). 13/33 (39%) placebo recipients and 1/35 (3%) vaccinees had severe diarrhea (point estimate of VE = 93%, p=0.0002). Additional subjects will be challenged at 90 days post-vaccination and overall VE will be calculated in May 2013 with pooled placebo recipients serving as the control. The encouraging preliminary analysis of VE observed 10 days after receipt of a single 10^9 CFU dose of CVD 103-HgR supports an accelerated clinical development path towards FDA licensure.

THE CASE FOR USING A SINGLE DOSE OF ORAL CHOLERA VACCINE IN EMERGENCIES

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A global stockpile of oral cholera vaccine (OCV) was recently established for emergency use, but with less than 2 million doses available worldwide officials will likely have to contend with limited vaccine. Previous work shows that speed of response is the strongest predictor of the impact of reactive vaccination campaigns. Two OCVs are internationally licensed for use as a two-dose formulation administered 1-6 weeks apart, and the delay between vaccination rounds may compromise the benefits of receiving the second dose. With evidence from clinical trials and immunological studies suggesting that a single dose may be moderately protective, there is a strong argument that more may be gained by distributing a single dose to more people quickly, than a full course to a smaller population, thereby administering as many doses of vaccine as quickly as possible. Whether this is the case will be driven by the efficacy of a single dose, the mechanism of vaccine protection, and local cholera transmission dynamics. Using computational models of cholera transmission with varying assumptions about the epidemiologic context and vaccine mechanism, we explore the minimum single-dose efficacy needed to avert at least as many cases as a two-dose regimen in a reactive vaccination campaign with limited vaccine. In a simple model not taking into account indirect effects (i.e. herd protection), we find that early vaccination with a single-dose that confers about at least 42% protection against clinical disease would avert more cases than a two-dose regime with the same quantity of vaccine. As vaccination is delayed and indirect effects are taken into account, using a single dose with even lower efficacy will avert more cases than a two-dose campaign. When considering logistical delays and epidemic specific transmission dynamics from recent large outbreaks in Haiti, Zimbabwe, and Guinea, we find that with realistic quantities of vaccine, use of a single-dose regimen would have averted more cases than a two-dose regimen. Through synthesis of clinical and immunological evidence with data from recent cholera outbreaks, we show that a single dose of OCV may be an important tool for emergency response. If future empirical studies are consistent with current single-dose efficacy evidence, our results help create a case for the international licensing of a single-dose OCV regimen.
THE IMPORTANCE OF HISTORICAL DATA IN SIMULATING MALARIA EPIDEMIOLOGY AND CONTROL INTERVENTIONS: APPLICATION TO MULTIPLE SITES IN MADAGASCAR

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Planning malaria interventions requires prediction of likely impacts of different intervention strategies. Simulation models can provide such predictions but weak information about pre-control levels of transmission, intervention coverage and access to healthcare often makes it challenging to correctly parameterize them. We consider a number of low malaria transmission sites in Madagascar, with available historical prevalence and entomological inoculation rate (EIR) estimates (by mosquito sampling), giving disparate estimates of historical exposure. Information about implementation of Long Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS), and access to healthcare, collated from malaria surveys and on-going cross-sectional studies were used to parameterise simulations of malaria transmission, prevalence and burden within the OpenMalaria platform. Multiple parameterisations were considered using various sources of data for pre-intervention transmission level, intervention coverage and access to healthcare. In some sites the simulated impact of existing vector control programs matches reasonably well the malaria prevalence measured in a recent national survey. In others it predicts lower than observed prevalence, very likely because the models do not capture residual local transmission foci. The simulations suggest that the most cost-effective vector control strategy would be to scale-up LLINs or IRS only, depending on the transmission level. Indeed, preliminary results show no additional benefit of IRS where LLINs were used. These preliminary results suggest that historical prevalence data, combined with current coverage information are potentially adequate for planning intervention strategies. The outcome of intervention scale-up is essentially unpredictable if baseline information is poor. Reproducing the observed epidemiology of malaria through simulations both provides confidence in the use of the model but serves as a basis for prospective studies that support decision-making, including cost-effectiveness analyses.
resistance is characterised by slow parasite clearance (SPC). Partner drug resistance is associated with late clinical failure (LCF) or late parasitological failure (LPF). An individual-based malaria transmission model is used to estimate the impact of each resistance scenario on clinical incidence, severe incidence and parasite prevalence across Africa. We find that scenarios with high levels of recrudescence infections (LCF/LPF) resulted in far greater increases in clinical incidence compared to scenarios with high levels of SPC. Across Africa, we estimate that partner drug resistance at levels similar to those observed in parts of Africa for AS+SP could result in 39 million additional cases over a five year period, a 2.7% increase compared to a scenario with no resistance. Artemisinin resistance similar to levels observed in Pailin, Cambodia could result in an additional 29 million cases over the same period. Artemisinin resistance is potentially a more pressing concern than partner drug resistance due to the lack of viable alternatives. However, widespread partner drug resistance, characterised by SP resistance in parts of Africa, would result in greater increases in malaria morbidity than if widespread artemisinin resistance were to develop at levels currently observed in Pailin, Cambodia.

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MODELING THE IMPACT OF COINFECTION ON PERSISTENCE AND INFECTIVITY OF PLASMODIUM FALCIPARUM

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Each year nearly 200 million people are infected with the malaria parasite, Plasmodium falciparum. One of its most notable features is the variable course and duration of infection experienced by different individuals, ranging from high parasite density, acute and often severe infections to persistent, chronic infections that are often undetectable by microscopy. Levels of acute and chronic infections vary across different transmission settings, and what disturbs the delicate balance between parasite growth and immune control, leading to bursts of parasite growth or clearance of an infection, remains an open question. Field studies examining persistence of infection have used a variety of different genotyping methods, but due to limitations, it is difficult to determine the extent of mixed infections, and nearly impossible to determine if the reemergence of parasitemia is due to a new infection or recrudescence of an existing one. Mathematical models, despite limited knowledge of mechanistic details of host-parasite interactions, have qualitatively reproduced single parasite dynamics observed in patient data. Here we adapt a discrete model by Recker et al. (PLoS Pathogens, 2011) of blood-stage parasite dynamics including innate and adaptive immune responses. We analyze simulated output to examine how coinfecting strains, particularly from similar clones that elicit overlapping immune responses, impact infection length and infectiousness. We find that the level of both innate and adaptive immune responses present at the time of coinfection as well as the similarity of the coinfecting strains significantly alters the duration of both the resident and coinfecting strains, particularly during chronic infections. Timing of coinfection also influences the infectivity of the coinfecting strains, likely altering transmission patterns at a population level. Duration of infection and infectivity are critical epidemiological parameters for predicting the efficacy of control strategies, particularly with the looming problem of emerging drug resistance.

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MEASURING THE PATH TOWARDS MALARIA ELIMINATION

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As an area approaches local elimination it becomes increasingly difficult to quantify transmission accurately. This is because countries that manage to control local transmission to relatively low levels but receive large numbers of imported cases from outside its borders are likely to see a considerable number of autochthonous cases. The reproduction number R (the average number of persons infected by a malaria case) is a new method of measuring malaria transmission though, to date, its use has remained largely theoretical and it has had limited practical impact. Here, we present an operational framework to determine whether programs are successful at controlling local transmission of falciparum malaria. It is based on a new and simple method to test the hypothesis R>1 from standard surveillance data consisting of the numbers of local and imported malaria cases. We apply this approach to Swaziland, a country which embarked on an elimination campaign in 2008. Thirty six percent (52/143), 45% (170/377) and 67% (153/229) of investigated cases were imported in 2010, 2011 and 2012 respectively. This indicates that the status of controlled non-endemic malaria was reached in 2011 and 2012 but not in 2010. This provides evidence that since 2010, Swaziland has halted endemic transmission at the national level, and that malaria would be eliminated if the current level of control was continued and importations ceased. The method offers a simple and practical solution to quantify transmission as the disease becomes increasingly rare. Instead of just aiming for zero local cases, programs should use these metrics to set a series of intermediate milestones which are easy to test and show progress towards disease elimination. Evidence like this will be essential to enable the successes of countries like Swaziland to be maintained and replicated elsewhere.

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SPATIAL DYNAMICS OF MALARIA TRANSMISSION IN THE EMOD MODEL FOR CAMPAIGNS TARGETING SUSTAINED REGIONAL ELIMINATION IN SOUTHERN ZAMBIA

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As malaria control programs move towards regional elimination campaigns, it will be increasingly important to understand the dynamics of malaria transmission within and between adjacent regions with different characteristics. Within the framework of the EMOD model, we have constructed a network of 116 interconnected simulated populations corresponding to the spatial distribution of households in Gwembe and Sinazongwe districts in southern Zambia. We use a gravity model that includes local and regional roadways to infer the relative rates of migration between nodes. For each simulated population, we use geographically-specific values for larval habitat, acquired immunity, ITN usage, case management rates, drug-regimen compliance, and mass-screen-and-treat (MSAT) coverage levels to the extent that they may be inferred from surveillance data. Our simulations are able to reproduce the geographically-variable pattern of reduced rapid diagnostic test (RDT) detectable parasite prevalence from 2012 to 2013. Finally, we extend this spatial-simulation framework to predict the impact of different drug-delivery, vector-control, and case-management parameters on the potential to sustain regions of local elimination. When anti-malarial treatment, with both curative and short-term prophylactic effects, is distributed regardless of RDT status, substantial regions of the simulated geography are parasite-free within a few years. However, sustained success almost certainly requires significant improvements in case-management and vector-control activities.
from these models do not always apply to *P. vivax* due to the occurrence of relapse infections months to years after the primary infection. These models are extended to consider relapsing malaria using intuitive, high-school level mathematics to illustrate the qualitative relationships between parameters of epidemiological importance: *P. vivax* parasite prevalence, the basic reproduction number $R_0$, the proportion of infections due to relapses, and the density of *Anopheles* mosquitoes. These relationships are illustrated via examples from malaria control campaigns in The Solomon Islands, Vanuatu and Papua New Guinea where notably different trends were observed in *P. vivax* and *P. falciparum* transmission following the introduction of vector control and mass drug administration. Evidence is presented for three key results, and their implications are discussed with examples from past malaria control programmes. (i) At similar parasite prevalence, *P. vivax* has a greater basic reproduction number than *P. falciparum* ensuring more stable transmission and greater difficulty in reducing $R_0 < 1$. (ii) Although vector control is effective at reducing the transmission of all malarias, it is predicted to be approximately twice as effective at reducing *P. falciparum* prevalence compared to *P. vivax* prevalence, depending on the expected number of relapses. (iii) In the absence of drugs such as primaquine for preventing relapses, *P. vivax* elimination timelines are predicted to be substantially longer than *P. falciparum* timelines due to the reduced effectiveness of vector control and the extended time to relapse infections. As a relapsing malaria, *P. vivax* presents a number of epidemiological challenges that must be considered if it is to be successfully controlled and eliminated.
Markers that accurately differentiated any two of the three groups included haptoglobin, IL-10, IL-6, and TNF-alpha. Among markers that accurately classified bacterial disease in the training set, only haptoglobin (AUC-ROC > 0.85 in all comparisons) performed well in the validation set, where it misclassified only one bacterial patient. The overall sensitivity of haptoglobin (> 0.995 mg/mL) for bacteria was 96% with a +Likelihood-Ratio of 3. In a Classification Tree (CT) signature, TNFR2 (or equivalently IL-10) and TIMP-1 were added to haptoglobin to further classify non-bacterial patients into malarial or viral etiologies. Signatures based on Support Vector Machine (SVM) and regression models showed comparable performance and included haptoglobin, IL-10, MMP9, and CK-MB. The overall sensitivity of the CT/SVM signatures for bacteria were 96%/91%, for malaria 81%/96%, and a total of 20%/17% virus patients were misclassified. Blood-proteins can constitute biomarkers for pediatric respiratory disease amenable to deployment as rapid diagnostic tests. These biomarkers are sensitive for the crucial diagnosis of bacterial infection while achieving moderate to good specificities for viral and malarial infections. These markers should allow diagnosis in malaria endemic and non-endemic areas.

LOW-COST NON-IMAGING ULTRASOUND (LOCONIUS) FOR PNEUMONIA DETECTION IN RESOURCE-CONSTRAINED SETTINGS

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Pneumonia is the leading cause of child mortality. Its diagnosis is currently based on chest X-rays (CXR) and clinical/laboratory criteria, which require trained professionals and a complex infrastructure. In low-resource settings clinical criteria are the only available resources, but these lack sensitivity and specificity. New, simple, low-cost methods for diagnosis of pneumonia are urgently needed. Ultrasound (US) has been increasingly used to diagnose pneumonia in children. Although US has a high sensitivity and specificity when compared to CXR, its major limitation is the lack of specialists to interpret US images in resource-poor settings. The goal of our study is to develop a specialist-independent system that uses real-time US information interpreted through mathematical algorithms for diagnosis of pneumonia in children. Pulmonary US data were obtained from 30 pneumonia and 30 healthy children presenting to local hospitals in Lima, Peru. Images from US videos were processed to obtain vectors of illumination density (10 pixels per vector) along the depth-axes (475 pixels per image). Vectors skipping one column and corresponding to pulmonary infiltrates on US images were classified as pneumonia as our gold standard. 76,964 vectors were used to train and test a single layer neural network classification system. This neural network was used to classify 38,482 vectors from images not used in developing the algorithm, with an observed accuracy of 92% for recognizing pneumonia. The same data were analyzed in a logistic and multinomial multiple statistical regression. On preliminary analysis, we found 94% accuracy in the classification by the neural network. A preliminary multiple regression model achieved a 93.5% sensitivity and 91.4% specificity, with an accuracy of classification expressed by the area under the ROC curve of 0.97 (i.e. 97% of probability to correctly classify a pair of random samples). We expect that further refinement of this model will lead to an automated algorithm for pneumonia that will permit accurate diagnoses using US in low-resource settings.
UNEXPECTEDLY HIGH FREQUENCY OF VIRAL AGENTS IN ASYMPTOMATIC CHILDREN AND LOW INFLUENZA REPRESENTATION IN PNEUMONIA CASES AMONG CHILDREN IN RURAL NORTH PAKISTAN


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Pneumonia is a major cause of child mortality and morbidity in developing countries; the contribution and dynamics of various viral pathogens is not clear, affecting the implementation of optimal prevention and treatment strategies. Oshikhandass, a rural village in NE Pakistan, was previously studied from 1989-1996, where pneumonia was the main cause of mortality in children <5 years (disease incidence 44/100 child years). This study was repeated (after Hib vaccine introduction) with weekly surveillance of children <5 from Apr. 2012-Mar. 2014 to determine changes in epidemiology and describe the frequency of viral agents in nasopharyngeal swabs. PCR from Apr. 2012-Nov. 2013 used the Lumixin® platform to detect 20 viruses (RSV, influenza, enterovirus/rhinovirus, parainfluenza, adenovirus, corona virus, metapneumovirus, bocavirus), while the Taqman® method was used Dec. 2013-Mar. 2014 to detect 4 viruses (RSV, influenza). From Feb. 2013, age, sex and neighborhood matched controls with no respiratory symptoms were added to the study. On average, 809 children were followed monthly with 238 pneumonia episodes detected (14.7/100 child years, average age 22.5 months). Pneumonia incidence was highest during winter months with significant variation between the 2012-13 and 2013-14 winters (54.6 and 10.1 episodes/100 child years, respectively). Among all 232 cases tested, 77% had a detectable virus. Detection rates were 25.0/15.6, influenza 3.4/1.6, enterovirus/rhinovirus 70.3/41.9, parainfluenza 8.8/7.0, adenovirus 4.4/9.3, corona virus 1.1/4.7, metapneumovirus 1.1/0.0, bocavirus 3.3/2.3. Unexpected findings include: 1) a substantial portion (54.7%) of asymptomatic children carried viruses, only slightly below the rate for their matched counterparts with pneumonia (69.0%); 2) the low prevalence of influenza viruses despite high sensitivity of the assay used. Further investigation on severity of disease is underway, but it seems that public health interventions aiming to reduce pneumonia burden should not focus on influenza vaccination as a priority.
18, infected with *S. japonicum*, otherwise healthy, and pregnant at 12-16 weeks gestation. Women (N=380) were enrolled and treated with overencapsulated Praziquantel (60 mg/kg in split dose) or placebo and admitted for 24 hours. The following efficacy outcomes were ascertained: birthweight (primary), maternal hemoglobin and iron status at 32 weeks gestation, maternal gestational weight gain, newborn hemoglobin and birth weight. In addition, safety data were collected including toxicity profiles pre and post dosing, abortion and miscarriage rates, and congenital anomalies. Though all analyses for these efficacy and safety outcomes are complete and data locked, we cannot report these until pharmacokinetic studies are completed (expected summer of 2014). We will present the impact of treatment on the aforementioned outcomes. Results from this trial will provide important data from a well controlled study to inform policies regarding treatment of this high risk group.

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**IMPACT OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS ON VACCINE-INDUCED IMMUNE RESPONSES**

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In light of recent global health initiatives to increase vaccine rates for the world's most vulnerable populations, there is an urgent need to address the issue of vaccine efficacy in populations where helminth infection rates are high. Helminths such as schistosomes are remarkable in their ability to modulate host immune responses, which helps to promote their survival. Immunoregulation begins early in schistosome infection and is characterized by hyperresponsiveness to parasite antigens and other bystander antigens, suggesting that a schistosome infection at the time of vaccination could negatively impact the induction of a protective response to the vaccine. To investigate the impact that concurrent helminth infection might have on how individuals respond to vaccine antigens, we recruited participants from Kisumu Polytechnic College in Kisumu County, western Kenya. At study enrollment participants were screened for schistosomiasis and soil transmitted helminths (STHs) and assigned to a group based on helminth status. The vaccines were then administered: tetanus toxoid (single dose), hepatitis B (doses at 0, 1 and 6 months), and meningococcus A+C (doses at 0 and 2 months). Helminth infections were treated a week after the second hepatitis B boost. A baseline blood draw, a blood draw 2 months after the start of vaccinations and 2 months after the final hepatitis B boost were obtained for evaluation of humoral and cellular immune responses to the vaccine antigens. CD3+/CD4+/CD25high regulatory cells were also determined at each time point to assess their impact on vaccine responsiveness. Preliminary data show changes in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ.

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**EVALUATION OF ORGANOMEGALY AND OTHER MORBIDITIES FOLLOWING TWO ROUNDS OF PRAZIQUANTEL MASS DRUG ADMINISTRATION AMONG KENYAN SCHOOL CHILDREN. THE SCORE PROJECT**

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One of the goals of schistosomiasis control among school age children is to reduce morbidity. However, monitoring and evaluation of the impact of deworming projects usually is limited to parasitological outcomes as there are not well defined markers of schistosomiasis morbidity other than for persons with severe hepatosplenic disease. In a subset of a larger study comparing mass drug administration approaches in school children, we measured organomegaly and anemia at baseline and after two years to determine whether praziquantel treatment approaches made a difference in morbidity outcomes. Children from 12 schools were enrolled; children from 6 of the schools received school-based treatment (SBT) once after baseline assessment while children from the other 6 schools lived in villages that received community-wide treatment (CWT) once a year. Children were examined for Schistosoma mansoni infection using the Kato-Katz method, venous blood was collected to test for malaria parasites and hemoglobin levels, and abdominal ultrasound was performed by a trained ultra-sonographer. The differences between baseline and follow up schistosomiasis prevalence and intensity were not significant for either SBT or CWT schools, or between the treatment approaches. However, ultrasound-detected liver pathology (P< 0.001) and anemia (P< 0.001) were significantly higher at the 2 year follow up for both groups, as was the percent of students with malaria infection (P<0.001). Because anemia and the type of liver pathology we detected are both associated with malaria, we believe that the increased morbidity that we observed at follow-up was likely due to the increased malaria and could have masked any benefits of the schistosomiasis treatments. Our results indicate a need for continued monitoring of morbidity markers in deworming projects, including assessment of co-infecting organisms that can affect morbidity, to determine the best intervention strategies to control *S. mansoni* infection as a public health problem among school age children.

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**GLYCAN MICROARRAY-ASSISTED ANALYSIS OF ANTI-GLYCAN ANTIBODY RESPONSES UPON INFECTION OR VACCINATION WITH SCHISTOSOMA MANSONI**

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Schistosomiasis is a chronic and potentially deadly parasitic disease that affects millions of people in (sub)tropical areas. Immunity to Schistosoma can be acquired, but this takes many years of exposure, multiple infections and treatments, and maturation of the immune system. Most antibodies generated are directed against the numerous schistosome glycans, but the precise structure of the glycan antigens and the relation to immunity are poorly understood. Anti-glycan antibodies can be studied efficiently using glycan microarrays. We have generated a microarray containing hundreds of naturally occurring glycans isolated from different life stages of *S. mansoni*. To study the specificity and nature of anti-glycan antibodies in schistosomiasis, we have applied this microarray to the analysis of anti-glycan IgG and IgM in sera from different age groups within an *S. mansoni*-endemic community in Uganda. The most intense IgG responses are against highly fucosylated glycans associated with cercarial and egg glycoproteins and glycolipids. We observed age-dependent differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. Also within age groups we observed differences between groups with high and low infection intensities. In addition we have used sera from baboons protected against challenge infection after vaccination with irradiated *S. mansoni* cercariae to study longitudinally the development of anti-glycan responses. The strong responses against cercarial lipid-derived glycans as well as cercarial O-glycans are gradually induced by repeated vaccination raising the question if these responses contribute to the high level of protection observed in the vaccinated baboons. Shotgun glycan microarrays allow the definition of groups of schistosome-infected individuals as well as glycan element clusters to which antibody responses are generated in different cohorts and settings.
MULTIPLEX SEROLOGICAL ASSESSMENT OF SCHISTOSOMIASIS IN MBITA DISTRICT, WESTERN KENYA

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Schistosomiasis control programs centered on mass drug administration (MDA) reduce parasite prevalence and morbidity in treated individuals. Currently, program impact in areas endemic for Schistosoma mansoni is measured primarily by assessing changes in infection prevalence and intensity as measured by stool examination. Antibody-based tests potentially have an advantage over currently used antigen-based tests, and a newly developed multiplex bead assay allows for the analysis of multiple antigens at one time. A total of 935 serum samples collected from individuals (1-85 years) living in communities on the shores of Lake Victoria in western Kenya were tested for antibody responses to 36 antigens, including two Schistosoma spp. antigens (soluble egg (SEA); adult worm microsomal (Sm25)). Stool and serum samples were collected at three time points: May-July 2012; December 2012; May-July 2013. One MDA was conducted in August 2012. Parasitological data were available for 916 (98.0%) of the samples. Overall prevalence of S. mansoni at baseline was 35.6% by stool examination, and modestly decreased to 29.6% 9-11 months after MDA with praziquantel. However, intensity of infection significantly decreased after treatment (p=0.005), and importantly, heavy intensity infections decreased from 16.1% to 7.5% (p<0.001). Antibody responses to both SEA and Sm25 were associated with age (p<0.001), and older individuals were more likely to have higher antibody responses than children under five years. Antibody responses to both antigens were significantly associated with intensity of infection, with responses increasing as egg burdens increased (p<0.001). Overall responses to Sm25 significantly decreased after one round of MDA (p<0.001). The observed decrease in Sm25 response after treatment has not been previously reported. Additional samples from this study area will be tested to further evaluate this observation. Detection of antibodies to Sm25 or other defined antigens have the potential to be a useful tool for monitoring the impact of schistosomiasis treatment programs.

THE ADIPOSE TISSUE DERIVED STEM CELLS (ASC) CHANGED THE ACTIVATION PROFILE OF IMMUNE SYSTEM CELLS IN SCHISTOSOMA MANSONI ACUTE INFECTION

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Mesenchymal stem cells (MSC) have the ability of self-renewal and differentiation into various mesodermal cell lineages. Recently, it has been observed that MSC have potent anti-proliferative and anti-inflammatory effects in autoimmune and inflammatory diseases as a new strategy for immunosuppression. The control of inflammation in infectious and parasitic diseases by MSC was not evaluated so far. The present study aimed to evaluate if the adipose tissue derived stem cells (ASC) could be able to regulate the inflammation in an experimental acute Schistosoma mansoni-infection model by immune system activation analysis. The ASC were isolated from C57BL/6 mice, expanded in vitro and characterized phenotypic and functionally. These cells were injected by the tail vein into C57BL/6 mice (n=5) in two, four, six and eight weeks post-infection with S. mansoni. Fifteen and thirty days after the ASC injection, the splenocytes were obtained and lymphocytes activation evaluated by the expression of CD25, CD69, CD28 and CTLA-4 molecules. The IL-2, IFN-γ, TNF-α, IL-17, IL-4, IL-6 and IL-10 cytokine levels were measured in the serum blood by flow cytometry. The results showed a decrease (p<0.05) in TCD4+ regulation as determined by CD25, CD69 and CTLA-4, mainly six weeks post-infection and after fifteen or thirty days post-injection of ASC. Interestingly, the cytokines analysis revealed a different profile in the IL-2 and IL-4 levels. Both cytokines showed significant levels after eight weeks post-infection and fifteen days post-injection of ASC. In conclusion, our results shows that the ASC can modulate the immune response in S. mansoni, mainly after six weeks post-infection, and suggest that ASC can be evaluated for the control of the granulomatous reaction in this disease.

ESTIMATING ACCURACY OF PARTICIPANT RECALL FOLLOWING AN INTEGRATED MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES

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Coverage Surveys (CS) assess quality of implementation of mass drug administration (MDA) and provide feedback for ministries of health and drug donation programs. CS independently determine if individuals in targeted areas received medication during previous MDA, and rely on individuals’ correct recall of medications taken. As neglected tropical disease programs integrate, multiple medications may be offered during MDA, potentially diminishing the strength of CS as a tool. We assessed individuals’ ability to recall multiple medications received in integrated MDA, over time. Niger’s 2012 MDA distributed ivermectin (IVM), albendazole (ALB), praziquantel (PZQ), and azithromycin (AZM) to eligible individuals. During MDA, observers accompanied distributors and created a Gold Standard Register (GSR), independent from the distributor register. All persons living in households in villages were registered, and it was noted whether or not they took medications, regardless of their eligibility. Households were systematically selected to be revisited for CS at 2, 6, or 12-months post-MDA. During CS, respondents were shown pills. During CS, surveyors and respondents were blinded to MDA register responses. Of 4423 individuals registered from 806 households at GSR, 3455 (78.1%) responded at CS. Response rate at 2, 6, and 12-months post-MDA was 84.4%, 80.7%, and 68.3%, respectively. At GSR, 93.9% (3243 of 3455) ingested one or more medications, while 93.5%, 93.5%, 88.7%, and 91.2%, ingested IVM, ALB, AZM, and PZQ, respectively. At CS, 95.1% (3287 of 3455) recalled having ingested one or more medications, while 86.2%, 88.7%, 89.0%, and 82.0%, recalled ingesting IVM, ALB, AZM, and PZQ, respectively. IVM concordance (% agreement between GSR and CS) at 2, 6, and 12-months post-MDA was 82.1% (95% CI: 79.1-85.1%), 86.6% (CI 83.6-89.6%), and 80.8% (CI 77.2-84.4%), respectively. ALB concordance at 2, 6, and 12-months was 85.2% (CI 82.5-87.9%), 87.3% (CI 84.5-90.0%), and 85.0% (CI 81.8-88.3%), respectively. AZM concordance at 2, 6, and 12-months was 79.9% (CI 76.7-83.2%), 82.9% (CI 79.2-86.5%), and 82.5% (CI 79.2-85.8%), respectively. PZQ concordance at 2, 6, and 12-months was 74.5% (CI 70.9-78.2%), 82.6% (CI 79.6-85.7%), and 80.3% (CI 77.0-83.6%), respectively. CS correctly measured overall MDA coverage, though it is less reliable for drug-specific coverage. We confirm the strength of CS as an MDA evaluation tool.
and have higher living standards. Women, who were eligible as girls, have better educational outcomes, are more likely to grow cash crops, and reallocate labor time from agriculture to entrepreneurship. These results suggest that health interventions that are too late in life to affect cognition or height can still have long-run impacts on labor market outcomes by affecting the amount of time people spend in school or work.

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THE IMPACT OF MASS DRUG ADMINISTRATION WITH ALBENDAZOLE ALONE ON LYMPHATIC FILARIASIS IN THE REPUBLIC OF CONGO

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Implementation of mass drug administration (MDA) with ivermectin plus albendazole (Alb) for lymphatic filariasis (LF) has been delayed in Central Africa, because ivermectin sometimes causes severe adverse events in people with very high Loa loa microfilariaemia (MF). Albendazole has activity against Wuchereria bancrofti, and it is safe for use in patients with loiasis, and WHO has recently recommended use of Alb MDA together with vector control for control of LF in areas with co-endemic loiasis. We are presenting preliminary results from a planned 3-year community trial that is assessing the impact of MDA with Alb on LF in a village of the Republic of Congo. Baseline results (September 2012) from 773 subjects revealed a filarial antigenemia rate of 17.3% (ICT) and a MF rate of 5.3% (140 µl night blood smear). The population was offered 000 mg of Alb at baseline and 6 months later. Therapeutic coverage for the population > 2 years of age was – 85%. A second cross-sectional evaluation at 12 months (741 tested) showed that Alb MDA had not yet reduced ICT or MF rates in the community (16.6 and 4.2%); however, MF counts in MF-positive subjects were reduced by 60% (geometric mean reduced from 199.4 to 79.6 Mf/ml, P = 0.01). The effect of Alb was more dramatic in those 38 people who were Mf positive at baseline and retested at 12 months: 37% had total Mf clearance, and Mf counts in those with > 10 Mf/ml at baseline were reduced in average by 82.1% (range: 4.3-100%). In addition, ICT scores (a semi-quantitative measure of filarial antigenemia) were also reduced after Alb MDA. MDA also dramatically reduced the hookworm infection rate from 6.5% to 0.6%, with less impressive effects on Ascaris and Trichuris. These preliminary results suggest that semiannual community MDA with Alb is a useful tool for control of LF and STH that is feasible for use in areas where loiasis is co-endemic. Evaluation at 24 months is planned for October 2014.

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COST-EFFECTIVENESS OF COMMUNITY-WIDE, INTEGRATED PREVENTIVE CHEMOTHERAPY FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS

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World Health Organization (WHO) recommendations for preventive chemotherapy against helminth infections emphasize treatment of school-age children. We aimed to evaluate the cost-effectiveness of expanding integrated preventive chemotherapy to adults in high-burden settings. We developed a dynamic, age-structured transmission and cost-effectiveness model that simulates integrated preventive chemotherapy programs

THE SOCIO-ECONOMIC IMPACT OF CONTROL OR ELIMINATION OF FIVE NEGLECTED TROPICAL DISEASES

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There is renewed commitment to control and eliminate neglected tropical diseases (NTDs) as defined by the WHO and endorsed by the London Declaration. We estimated the economic impact of meeting the targets for the five diseases eligible for mass drug administration: lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiasis, and trachoma. We performed a systematic literature review to identify information on costs related to the diseases or related health outcomes, considering out of pocket payments (OPP), productivity loss, and impoverishment. Cost estimates per person were combined with projections of the number of people suffering from clinical manifestations per NTD, country, and year, for the periods 2011-2020 and 2021-2030, comparing the ideal scenario in which the targets are met with a counterfactual scenario (assuming that the prevalence (%) of clinical manifestations remains constant at its pre-control level). The averted burden is calculated as the difference between the two scenarios. The limited information available on the costs of illness shows that the productivity loss associated with clinical manifestations are about 3% for infestation and mild symptoms, about 15% for more severe manifestations like lymphedema and hydrocele, and as high as 38% and 79% for severe vision loss and blindness. The total productivity cost averted globally by reaching the goals for the five above-mentioned diseases may amount to US$240 billion in the period 2011-2020 and US$390 billion in the period 2021-2030, excluding OPPs as well as productivity loss due to premature mortality. Soil-transmitted helminthiasis accounted for approximately half of this amount. There is considerable uncertainty in these estimates because of scarce literature. Nevertheless, even with conservative assumptions, the averted costs of achieving the London Declaration targets are high. Results will be discussed with respect to the impact on poverty.

SHOULD SCHOOL BASED DEWORMING BE SUBSIDIZED? LONG RUN EVIDENCE FROM A RANDOMIZED CONTROL TRIAL IN KENYA

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Recent research suggests that preventative and non-acute health investment in the developing world is often highly responsive to subsidies, but what level of subsidy should governments provide? Using data from a deworming randomized control trial in Kenya this paper shows that by combining data on take up elasticities with data on labor market impacts and tax rates one can establish the optimal subsidy. To derive this optimal subsidy we first develop a framework where individuals may generate a positive fiscal externality, through consumption or labor income tax, by investing in human capital. Applying this framework to the case of deworming in Kenya, we find that fully subsidizing child deworming raised adult earnings generating sufficient future gains in government revenue to make possible Pareto improving reductions in tax rates. Consistent with previous reports, we estimate differing labor market impacts of child health investments by gender. Ten years after the start of the deworming program, men who were eligible to participate as boys work 3.5 more hours each week, spend more time in entrepreneurship, are more likely to hold manufacturing jobs with higher wage earnings, and have higher living standards. Women, who were eligible as girls, have better educational outcomes, are more likely to grow cash crops, and reallocate labor time from agriculture to entrepreneurship. These results suggest that health interventions that are too late in life to affect cognition or height can still have long-run impacts on labor market outcomes by affecting the amount of time people spend in school or work.
for schistosomiasis and soil-transmitted helminths (STH). We utilized data on prevalence and intensity of infection with Schistosoma mansoni and STH from surveys in Côte d'Ivoire, and simulated a community of 15,000 children and adults. Transmission parameters were calibrated according to prevalence and dispersion of each worm. We simulated drug administration with praziquantel+albendazole among: (i) school-age children only or (ii) school-age children and adults. We assumed 75% coverage for the intervention. Treatment costs for a child and adult were estimated at US$1.09 and US$1.94, respectively. The incremental cost-effectiveness ratio (ICER) was calculated in 2014 US$ per disability-adjusted life year (DALY) averted, comparing expanded treatment of both child and adult populations against current WHO guidelines that target children alone. We defined strategies as highly cost-effective if the ICER was less than the GDP per capita of Côte d'Ivoire (US$1,244 in 2014). An integrated preventive chemotherapy program for schistosomiasis and STH was highly cost-effective in treatment of children alone (ICER: US$620/DALY averted) compared to no treatment. Expanded coverage of both children and adults (ICER: US$653/DALY averted) was highly cost-effective compared to treatment of children alone, and remained highly cost-effective even if treatment costs for adults were 4-fold greater than school-based strategies. Expanded treatment of adults yielded a 50% increase in DALYs averted for children, suggesting that treating adults can lower the prevalence and infection intensity in children. Integrated, community-wide preventive chemotherapy programs for schistosomiasis and STHs may be highly cost-effective. These results support re-evaluating global guidelines for helminth control programs and possibly expanding coverage to adults.

SOECIOECONOMIC INEQUALITIES IN THE BURDEN OF NEGLLECTED TROPICAL DISEASES

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It is generally assumed that neglected tropical diseases (NTDs) are concentrated in poor populations, but evidence remains scattered. We describe between and within country poor-rich inequalities in burdens of London Declaration NTDs, including NTDs controlled through preventive chemotherapy (onchocerciasis, blinding trachoma, lymphatic filariasis, schistosomiasis, soil-transmitted helminths (STH)) and those controlled through intensified disease management (Chagas disease, leprosy, visceral leishmaniasis, human African trypanosomiasis (HAT)). First, using data from surveys in Côte d'Ivoire, and simulated a community of 15,000 children and adults (80 per cluster, aged 1-9 years) were examined using the WHO cluster survey method in Nigeria, TF in year 1 was 29.8%, 14.3%, 21.0%, and 16.3% per site, and cluster survey prevalence was 16.4% [95% CI: 14.8-18.0]; for year 2, TF was 4.75%, 9.25%, 6.00%, and 8.75% and 10.9% [95% CI: 9.5-12.5]; for year 3, TF was 10.0%, 16.0%, 9.0% and 15.0%, and 17.2% [95% CI: 14.8-19.7]. In BF, TF in year 1 was 9.6%, 10.2%, 15.2% and 7.9%, and cluster survey prevalence was 5.9% [95% CI: 3.8-7.6]; for year 2, TF was 1.6%, 2.6%, 5.0%, and 1.4% and 1.6% [95% CI: 0.0-3.71]; for year 3 TF was 0.8%, 3.0%, 2.8%, and 0.8%, and 1.8% [95% CI: 0.0-3.9]. Schistosomiasis prevalence for sites in BF in year 1 was 0.3%, 0.0%, 0.0%, and 0.0%; for year 2, 0.3%, 0.0%, 0.0%, and 0.0%, for year 3, 0.0% in all sites. STH prevalence for sites in BF in year 1 was 0.0%, 0.0%, 0.0%, and 0.3%; for year 2, 0.3%, 0.0%, 0.3%, and 0.0%; for year 3, 0.3%, 0.3%, 2.3%, and 0.3%. LF prevalence for sites in BF in year 1 was 0.0% in all sites and in year 3, 0.3%, 0.0%, 0.5%, and 0.0%. We conclude that integrated sentinel sites are feasible. Prevalence trends between sentinel sites and trachoma cluster surveys in both countries indicated that using sites might be a reliable indicator to determine when the prevalence is low enough to conduct the WHO recommended cluster survey.

CAN WE IGNORE THE INFORMAL PROVIDERS FOR THE TREATMENT OF CHILD DISEASES IN INDIA?

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Diarrhea is one of the two leading child killers in India. When asked about care seeking behavior for a child with diarrhea, 83% of caregivers in Uttar Pradesh, India, reported going to a private “doctor”. Due to the dearth of qualified health providers in rural India, the private doctor (called Rural Health Provider – RHP) is generally an unqualified provider who nevertheless, has gained the trust of the community. Childhood diarrhea is optimally treated through a short course of ORS and zinc. However, the RHP routinely dispenses unnecessary anti-diarrheals and antibiotics. Under the Diarrhea Alleviation through ORS and Zinc (DAZT) project, FHI 360 partnered with local NGOs to identify the RHPs and educate them on the improved treatment of diarrhea through ORS and zinc. The trained NGO

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health worker, emulating the marketing strategy of the pharmaceutical industry, repeatedly visited the RHP to provide him with the benefits of ORS and zinc treatment. The partners initially listed 27 000 RHPs and drug sellers in 12 districts representing a population of 34 million people. After categorizing the RHPs according to their work load and potential, the list was reduced to 22 000 RHPs. A tracking study in August 2013 showed that 54% of RHPs dispensed ORS and zinc treatment from a baseline of 23% ORS use and 1% zinc use in 2010. With the increasing demand for ORS and zinc, the pharmaceutical companies became motivated to supply to the rural areas. The market became large enough to attract several companies who are maintaining the demand and providing uninterrupted supply to the RHPs. As a sign of a sustainable market, 42 brands of zinc were inventoried in the area of intervention, up from seven at the start of the intervention three years ago. Changing RHPs’ treating behaviors towards rational treatment of critical childhood diseases shows potential to improve health outcomes. This simple approach could be replicated for other MNCH interventions in an integrated way aiming at reducing maternal, newborn and child mortality.

PREVALENCE OF ANEMIA AND FACTORS ASSOCIATED WITH SEVERE ANEMIA AMONG UNDER FIVE CHILDREN ADMITTED AT BUGANDO MEDICAL CENTRE, MWANZA, NORTHWESTERN TANZANIA

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Anemia is a major public health problem in developing countries, contributing significantly to morbidity and mortality among under-fives. About 43% of under-fives are anemic worldwide, and two-thirds reside in Sub Saharan Africa. Even where blood transfusion is available for treatment there is still a significant case fatality rate of 6-18%. This study aimed to determine the prevalence of anemia, factors associated with severe anemia and morphological types of anemia among anemic under-fives admitted at Bugando Medical Centre (BMC). This hospital-based, cross-sectional study was conducted between November 2012 and February 2013. Selected laboratory investigations were done using standard operating procedures. A total of 448 under-five children were eligible but detailed history taking and physical examination was available on 309. The overall prevalence of anemia was 77.2% (346/448). Mild, moderate and severe anemia were 16.5%, 33% and 27.7% respectively. Of 239 children with moderate and severe anemia 22.6% (54/239) had iron deficiency. Majority of the anemic children (37.5%) had microcytic hypochromic anemia. The factors associated with severe anemia included malaria parasitaemia [OR = 4.0 95% CI (2.1 - 7.8); p-value <0.001], presence of sickle haemoglobin [2.0 (1.1 - 3.5); 0.018] and unemployment of the parent [2.2 (1.2-4.0); 0.007]. In conclusion, the prevalence of anemia among under-fives admitted at BMC was high. Iron deficiency anemia was the leading type. Factors strongly associated with severe anemia were malaria parasitaemia, presence of haemoglobin S and unemployement among caretakers.

EFFECT OF LOW BIRTH WEIGHT AND INTRA-UTERINE GROWTH RESTRICTION ON STUNTING AND WASTING IN A COHORT OF INFANTS IN THE PHILIPPINES

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The proportion of babies born low birth weight (LBW) and intra-uterine growth restricted (IUGR) has increased over the past few decades in both high and low-income nations. A recent meta-analysis in Europe found that after controlling for socio-economic factors, LBW had a negative effect on health outcomes during childhood. In other studies, LBW infants had lower mean weight, length, and head circumference at one year of age. However, these studies have been conducted in higher income settings, and it is expected that infants in lesser-developed settings may be even less likely to experience catch up growth. The aims of this study were to compare rates of stunting and wasting at six and 12 months of age among LBW, IUGR, and normal weight infants born in Leyte, Philippines. Pregnant women were recruited at 12-16 weeks gestation as part of an NIH-funded trial. Newborns were weighed within 48 hours of delivery, and LBW was defined as weight < 2.5 kg, and IUGR as weight <3rd percentile for gestational age. WHOAnthro was used to derive height-for-age (HAZ) and weight-for-height (WHZ) Z-scores at six and 12 months old. We found that overall 20.6% (72/349) of infants were LBW, 24.6% (86/349) were IUGR, and 2.3% (8/349) were premature. These findings suggest that the primary cause of LBW was IUGR rather than prematurity. Overall, LBW and IUGR infants had significantly lower mean HAZ and WHZ at six and 12 months of age than normal infants. The odds ratio (OR) for stunting (HAZ< -2.0) at 6 months of age among LBW infants compared to normal infants was 3.56 (P = 0.0002), and for wasting (WHZ < -2.0) was 2.13 (P= 0.0002). At 12 months of age, the ORs for stunting and wasting were 2.09 (P = 0.061) and 1.16 (P= 0.6217), respectively. These results suggest that in this low-resource setting where infants often lack sufficient macro and micronutrients required for the rapid growth of infancy, catch up growth may be significantly delayed if it occurs at all. Further follow up of these children will be conducted to determine whether these effects persist throughout childhood.
PAPER-BASED YEAST BIOSENSORS FOR ANTIBIOTIC DETECTION
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Paper-based tests have the potential to serve as inexpensive tools to address analytical questions both inside and outside of the laboratory setting. In this study, Saccharomyces cerevisiae were used to construct the first example of a yeast whole-cell, paper-based biosensor device. This test is sensitive to antibiotics in the tetracycline family and could potentially address questions of pharmaceutical quality as well as antibiotic contamination in liquids. This biologically-based paper analytical device or “BioPAD” can qualitatively discriminate the presence/abscence of doxycycline over a range of 30 - 10,000 µg/mL. Using a BioPAD, a doxycycline dosage form (tablet) commonly used for malaria prophylaxis, was confirmed to contain the antibiotic with 92% and 95% success, evaluated by eye and computer-assisted image analysis respectively, with no false positives by either method. Stored at 4°C these tests were found to remain viable for greater than a year. This research demonstrates the utility of whole yeast cells in paper-based pharmaceutical testing, while highlighting the potential for the development of yeast-based BioPADs to address a range of qualitative analytical questions, especially in low resource settings.

EFFECT OF COOKING METHODS ON THE CONCENTRATION OF OXYTETRACYCLINE RESIDUE IN CHICKEN
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Antibiotics are used in poultry industry to obviate disease, enhance growth and increase production. However, the use of these veterinary drugs often results in the accumulation of violative levels of residues in tissues. Consumption of such poultry meat would potentially adversely affect human health through the development of resistant pathogenic microorganisms and hypersensitivity reactions in sensitized individuals. Although meat is always heat treated before consumption, which should ordinarily render the residues innocuous, some drugs are heat stable and therefore would persist at residue violative levels even after heat treatment. Since tetracyclines are the most frequently used antibiotics in poultry production in Nigeria, this study was therefore embarked up on to find out the effect of cooking methods (boiling, microwaving and roasting) on the concentration of oxytetracyclines (OTC) in poultry meat and organs. Muscle and liver tissues were harvested from birds that were treated with OTC either by intramuscular injection or orally in drinking water and analysed for residues using the three plate test (TPT) and enzyme-linked immunosorbent assay (ELISA). TPT at two different pH levels reduced the inhibition zones of raw liver between 56% for microwaving, boiling and roasting respectively but the difference was not statistically significant. A significant (P<0.05) decrease in OTC concentration was however noticed between raw microwaved and roasted liver samples by ELISA at microwaving (1.85%), boiling (2.83%) and roasting (3.17%) respectively. Roasting has a higher reduction effect on oxytetracycline residue.

EXPLORING THE EFFECTIVENESS OF AN EDUCATION INTERVENTION DELIVERED THROUGH LOCAL GROCERIES TO IMPROVE CHILD FEEDING PRACTICES IN RURAL COMMUNITIES. A CASE CONTROL STUDY
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Malnutrition is one of the most life-threatening conditions in early childhood. It causes about 35% of all deaths in children below 5 years and 50% of child mortality in sub-Sahara Africa. The condition can be effectively addressed by local solutions such as using local food grocers who on daily basis make contacts with child caregivers. Using case-control design in 5 villages in rural south eastern Tanzania, we evaluated an intervention involving training local grocers to select and provide appropriate meals to caregivers with children below two years. The trained grocers also trained their client caregivers on appropriate feeding of children. Ten local food grocers (2 per village) were recruited and each grocer was assigned to 5 randomly selected households with children <2 years. The grocers were retrained monthly, and were asked to help select appropriate foods for and advice their clients on appropriate feeding practices. Monitoring was done monthly. Primary outcome was performance of caregivers on key feeding indicators: breastfeeding, caloric density, nutrients density and food safety and feeding style. Main secondary outcome was growth, measured by weight and length and Z-scores for weight-for-age and length-for-age. We enrolled 27 children from intervention and 27 from control groups and all caregivers in intervention group received recommended training from the assigned grocers. Children in intervention group had higher breastfeeding scores than controls (25 [93%] vs 20 [74%], p = 0.068), higher caloric density scores (22 [81%] vs 21 [78%], p = 0.735), higher scores on nutrient density/versus (22 [67%] vs 14 [52%], p = 0.268) and higher scores on recommended food safety and feeding style targets than control (23 [85%] vs 12 [44%], p = 0.002). Overall, intervention group performed better in all indicators of good feeding practices than controls (19 [70%] vs 8 [29%], p = 0.003). Local food groceries in rural areas, if empowered with knowledge on proper feeding practices and malnutrition can improve child feeding practices and nutritional status of children. Since mothers and caregivers make more contact with food grocers in their communities than they do to health facilities, this approach could significantly improve coverage with appropriate nutritional education.

NUTRACEUTICALS: THE WAY FORWARD TO PREVENT ADVERSE HEALTH EFFECTS OF A CASSAVA-DOMINATED DIET
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More than 600 million people rely on drought-tolerant cassava as staple food, mostly under the tropics. Farming systems are dominated by harsh environmental conditions that mostly allow growing bitter cyanogen-containing cassava (manioc a.k.a tapioca or yuca) as staple crop. In times of war or agro-ecological crisis (and food shortages), populations are forced to adopt shortcuts in cassava processing, exposing themselves to cyanide poisoning and outbreaks of konzo, a permanent and irreversible paralytic disease. Cyanide is a mitochondrial toxin known to induce oxidative damage as evidenced by ongoing research that indicates high serum levels of isoprostanes F2 in proportion to motor and cognitive deficits in children relying on insufficiently processed cassava as staple food.
food. In anticipating interventional trials, we asked whether local food crops may be used as nutraceuticals (food with health benefits) to mitigate the neurotoxicity effects of cassava. We analyzed methanolic extracts of green vegetables (n=12), mushrooms (n=8), yams (n=2) and herbal teas (n=2) varieties that are consumed in a konzo-affected area using the 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) free radical-scavenging assay. Scavenging activities of methanolic extracts (2 - 40μg/ml) were compared using trolox and quercetin as references. The phytochemical screening was done by Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-UV/DAD) using Quercetin, hyperoside, rutin and chlorogenic acid as references. Most extracts displayed efficient concentration-dependent inhibitory effects. The scavenging activity of extracts (mean ± SEM as reported) aligned as follows: herbal teas > green vegetables > yam > mushrooms. TLC and HPLC-UV/DAD analysis has demonstrated the presence of polyphenolic compounds, which may explain the observed free radical-scavenging effects. In conclusion, green vegetables contain rutin (cassava leaves and Hibiscus), hyperoside (in Hibiscus), and chlorogenic acid (in raphia) lookalike compounds, which may explain their antioxidant activities. Select food crops may be promoted as nutraceuticals in campaigns to mitigate the health effects of cassava neurotoxicity when adherence to modern interventional trials is not guaranteed.

THE BURDEN OF RABIES IN TANZANIA AND ITS IMPACT ON LOCAL COMMUNITIES

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Rabies remains a major public health threat in many parts of the world and is responsible for an estimated 55,000 human deaths annually. The burden of rabies is estimated to be around US$20 million in Africa, with the highest financial expenditure being the cost of post-exposure prophylaxis (PEP). However, these calculations may be substantial underestimates because the costs to households of coping with endemic rabies have not been investigated. We therefore aimed to estimate the household costs, health-seeking behaviour, coping strategies, and outcomes of exposure to rabies in rural and urban communities in Tanzania. Extensive investigative interviews were used to estimate the incidence of human deaths and bite exposures. Questionnaires with bite victims and their families were used to investigate health-seeking behaviour and costs (medical and non-medical) associated with exposure to rabies. We found that a large proportion of bite victims do not obtain PEP (28%) and that compliance is relatively poor (~10% drop out rate) amongst those who do obtain PEP. The average costs incurred by bite victims was ~US$40 but varied from US$0 (for ~16% of bite-victims who were provided PEP free-of-charge and the further 28% of victims who did not seek PEP) to over US$300 (for ~16% of bite-victims who were provided PEP free-of-charge). The average costs incurred by bite victims was ~US$40 but varied from US$0 (for ~16% of bite-victims who were provided PEP free-of-charge and the further 28% of victims who did not seek PEP) to over US$300 (for ~16% of bite-victims who were provided PEP free-of-charge). The average costs incurred by bite victims was ~US$40 but varied from US$0 (for ~16% of bite-victims who were provided PEP free-of-charge and the further 28% of victims who did not seek PEP) to over US$300 (for ~16% of bite-victims who were provided PEP free-of-charge).
was experienced daily (53%) and treated successfully with modern rather than traditional medicine (72% [95% CI 63-82] vs 19% [95% CI 11-28], p<0.001). Respondents were more likely to have used (89% [95% CI 83-96] vs 71% [95% CI 61-80], p=0.002) and less likely to have refused (5% [95% CI 2.1-9.3] vs 27% [95% CI 17-36], p<0.001) modern rather than traditional medicine. 21% cited long waits and distance as reasons to avoid local healthcare posts. 95% had previously attended a medical boat. In conclusion, amongst pilot project respondents, there was a lack of understanding of diagnosed illnesses and a desire for further education. There was a high burden of undiagnosed pain, likely linked to agricultural work. While traditional and modern medicine were both used, more patients had taken modern medicine, especially analgesics. Individuals avoided using local healthcare posts due to limited access and almost all had had prior contact with mobile boat-clinics. Mobile boat-clinics may have a unique opportunity to target locally relevant healthcare issues (sun-exposure and headaches, self-physiotherapy for musculoskeletal pain) with simple educational interventions (pamphlets, community-advocate training) whilst healthcare infrastructure improves.

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PAPER ANALYTICAL DEVICES FOR THE SCREENING OF SUBSTANDARD MEDICATIONS IN WESTERN KENYA: A COMPARISON OF TEST RESULTS

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Low quality medications are a global health challenge that has been associated with death and the development of drug resistance. The Paper Analytical Device (PAD) combines liquid chromatography and color reactions on a patterned paper to form an inexpensive method to screen for substandard drugs. The PAD overcomes challenges encountered, mostly in developed countries, in monitoring the quality of drugs, that is, cost of analysis and inadequate technical expertise. We aim to compare the interpretation of test results obtained in the optimization and validation of the PAD. The project's study site is Moi Teaching and Referral Hospital - Academic Model Providing Access To Healthcare - AMPATH. Samples of amoxicillin, ampicillin, amoxicillin-clavulanic acid tablets, azithromycin, ciprofloxacin and acetaminophen tablets were purchased using secret shoppers from registered and unregistered pharmacies in western Kenya. To determine the medication content, the user applies each sample onto the PAD and dips one edge of the PAD into water. After development, the user takes a picture of the utilized PAD and employs a color based key to interpret the results. The interpretation of the PAD test results is carried out by Kenyan pharmacy personnel and University of Notre Dame collaborators and the outcomes reported. The PAD outcomes are reported as “Pass” for samples which displayed similar color patterns to the standard or “Suspicious” for samples which did not. These drugs samples are also being analyzed using high performance liquid chromatography (HPLC) in order to validate the PAD. Of the 139 samples tested in Phase 1 and number of outcomes reported and compared, we found a reader agreement of > 90% in the interpretation of PAD test results. The high reader agreement indicates that the PAD test results can easily be interpreted and therefore may be used to address the challenge of inadequate technical expertise in resource limited settings. We also, anticipate having HPLC results for tested drug samples over the next few months.

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DETERMINANTS OF COVERAGE AND AVAILABILITY OF ZINC AND ORAL REHYDRATION SALTS (ORS) IN THREE NIGERIAN STATES

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Globally, diarrhoea is responsible for 11% of all under-five deaths in 2010, and Africa accounts for over 40% of the global diarrhoea mortality burden. In Nigeria, it is the third largest cause of death in children under age five after malaria and pneumonia. While it is well established that WHO recommended treatments such as Oral Rehydration Salts (ORS) and Zinc can prevent the majority of diarrhoea associated deaths, the coverage of these treatments is not ubiquitous in Nigeria. It is estimated that only 25% of children with diarrhoea receive ORS and less than 1% receive zinc. In light of this, Clinton Health Access Initiative (CHAI) and Nigeria’s Ministry of Health (MOH) established a 4-year program with the goal of scaling up the use of ORS and zinc for treatment of childhood diarrhoea in 8 Nigerian States. By increasing the use of Zinc and ORS to 80% in four years, it is estimated that the scale-up will save over 228,000 lives. The program aims to create a competitive market for ORS and Zinc by the end of 2015 by 1) securing conducive policy environment; 2) ensuring availability and affordability of zinc and ORS 3) increasing provider awareness about Zinc and ORS; and 4) generating awareness and demand among caregivers. The purpose of the study is to evaluate the efficacy of CHAI strategies and identify other potential predictors of Zinc and ORS uptake that occur during the period of the program. The evaluation employs a pre-post design using cross-sectional surveys of both households and health providers in the intervention areas. A multi-stage cluster sampling technique was employed to systematically sample households and healthcare providers (primary healthcare providers, private patent medicine stores, chemists and pharmacies) across 3 Nigerian states. Baseline data were collected between November 2013 and March 2014, with a total of 2,700 caregivers and 900 health providers surveyed. Questionnaires collected data on caregivers’ care seeking attitudes and perspectives, provider knowledge and attitudes, medicine audits, and mystery patient information. Results from this baseline work will provide valuable insight on the determinates of coverage and availability of Zinc and ORS and can inform program implementation and policy at both the National and Global level.

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ESTIMATING THE IMPACTS OF ROTAVIRUS VACCINATION ON GENDER DISPARITIES IN INDIA

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This study aims to assess the gender disparities in rates of vaccination coverage amongst girls and boys in India over time, and estimate their potential impacts on the introduction of rotavirus vaccination, across various geographic and household economic settings. A Microsoft Excel based spreadsheet model is used to model the expected health and economic outcomes disaggregated by gender of the child, for one annual birth cohort of children during the first five years of life. Three data sets have been used in the model: National Family Health Survey-3 2005-6, District Level Health Survey-3 2007-8 and Coverage Evaluation Survey 2009 to estimate the changes over time, in 3 highest mortality states and 6 regions of India. There is an overall increase in vaccination coverage in India. The gap between boys and girls in vaccination coverage over time is estimated to have reduced. Increase in gender parity is estimated to increase in benefits and decrease in cost effectiveness ratio per DALY. It is of immense importance to sustain the efforts of bridging the gender gap in health care utilization and creating enabling conditions to introduce the proposed universal rotavirus vaccination. Preferential treatment to boys increases the risk of mortality amongst girls along with increasing the cost of implementation of vaccination programs.
QUANTIFYING THE IMPACT OF ACCESSIBILITY ON PREVENTATIVE HEALTHCARE IN SUB-SAHARAN AFRICA

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Poor physical access to health facilities has been identified as an important contributor to reduced uptake of preventative health services, and is likely to be most critical in low-income settings. However, the relationship between physical access, travel behavior, and the uptake of healthcare remains difficult to quantify. Here we analyze individual travel patterns of 14,816,521 people across Kenya and show that long travel times to health facilities are strongly correlated with increased mobility in geographically isolated areas. We provide regional and localized estimates of the disparity between estimated travel times to facilities, as a standard measure of access, and observed mobility patterns, and compare these measures to data on the uptake of two preventative healthcare interventions in an area of western Kenya: childhood immunizations and antenatal care. We show that even in areas with equal physical access, mobile phone-derived measures of mobility predict which regions are lacking either type of care, highlighting the potential utility of this approach to map the uptake of preventative healthcare.

PERCEPTIONS ABOUT PROVIDERS AS DETERMINANTS OF APPROPRIATE TREATMENT SEEKING BEHAVIOR FOR SUSPECTED MALARIA IN CAMBODIA

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Seeking treatment for suspected malaria from an appropriate provider facilitates correct case management, contributing to malaria control and elimination and protection of antimalarial drug efficacy. Analysis of data from a nationally representative cross-sectional household survey in Cambodia was conducted to identify individual perceptions about providers associated with appropriate malaria treatment seeking behavior. 1012 individuals (334 age 0-15, 678 age 15+) were identified through screening members of randomly selected households for symptoms of malaria fever occurring in the past 2 weeks. Seeking treatment from an appropriate provider (public health facility or village malaria worker) was the main outcome. Nineteen percent of adults age 15+ and 18% of children <15 sought care at an appropriate provider. Potential covariates included individual preference for appropriate providers on measures of access and quality of care (e.g. cost and convenience of care, respectful and knowledgeable providers, effective medicines). Adjusting for age, gender, education, wealth, and artemisinin-resistance zone in a full logistic regression models, significant determinants of appropriate treatment seeking among adults at 15+ were viewing appropriate providers as: most convenient (AOR=2.9, 95% CI=1.0-4.6); most respectful (AOR=2.9, 95% CI=1.7-2.8), and most knowledgeable (AOR=4.0, 95% CI=1.2-13.6) relative to adults who did not view an appropriate provider as convenient, respectful, and knowledgeable. Relative to adults with fever only, people with additional symptoms were less likely to visit an appropriate provider (AOR=0.24, 95% CI=0.09-0.7). Determinants of appropriate treatment seeking for children <15 included viewing appropriate providers as possessing: lowest cost of transport (AOR=15.3, 95% CI=2.8-82.5); and lowest cost treatment (AOR=20.6, 95% CI=1.9-218.3) relative to caregivers who did not view an appropriate provider as having the lowest cost for treatment and transport. Interventions to improve access to appropriate providers that address cost of transport and treatment may improve appropriate malaria treatment seeking behavior for children. Promoting appropriate providers as knowledgeable and respectful, and encouraging provider practices that reflect these qualities are interventions that may improve appropriate treatment seeking behavior among adults.

PERFORMANCE AND RELIABILITY OF DYXEN M² MULTIPLEXED ASSAY SYSTEM FOR IMMUNITY ASSESSMENT FOR THE DEMOCRATIC REPUBLIC OF THE CONGO - DEMOGRAPHIC HEALTH AND SURVEY

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Childhood vaccine-preventable diseases continue to be a major public health problem in terms of global morbidity and mortality. In endemic regions the only available tools of diagnosis are based on clinical and serological criteria. In support of the 2014 Democratic Republic of Congo -Demographic Health Survey (DRC-DHS) and in collaboration with University of California, Los Angeles, Fielding School of Public Health, UCLA-FSPH an immunity assessment is being undertaken using dried blood spots (DBS). The Dynex Technologies M²™ multiplexed immunoassay platform with a Measles, Mumps, Rubella, Varicella and Tetanus (MMRVT) panel is being used in which 10 polystyrene beads have been coated and immobilized within 54-well M² plates: separate assay beads with MMRVT antigens; positive controls with horseradish peroxidase, total human IgG, goat anti-human IgG; negative controls with MRC-5 and E6 cell lysate. Positive control DBS are 5-donor normal defibrinated serum and negative control DBS are pooled normal IgG-stripped serum. DBS are extracted into 1ml PBS, 0.5% tween20, 5.0% dried milk and processed on a modified Dynex DS2® automated ELISA system. DBS from 32 reference sera and 7-point dilutions of pooled sera were independently processed in DRC and USA. Assay cutoffs were set by reference to 13 simplexplex and 1 multiplex FDA-approved ELISA kits. 423 DBS were collected as a pilot study from children visiting Kinshasa health centers and 9906 DBS during the principal DRC-DHS survey. Including optimization runs data from 60 plates comprising 32,400 data points have been collected including 423 pilot DBS and 1150 DHS samples, with the remaining to be completed by MMDDY. Having been deployed to a substantially resource-limited environment the Dynex M² multiplex immunoassay system has proven to be a very robust assay platform with excellent sensitivity and specificity. This system in conjunction with DBS processing offers a very cost-effective reliable multiplexed immunoassay processing system for use in country-wide assessment of immunity status in challenging environments.

FACTORS ASSOCIATED WITH FREQUENT SICK VISITS FOR FEBRILE ILLNESS AMONGST CHILDREN ENROLLED IN A MALARIA VACCINE TRIAL IN SIAYA DISTRICT, WESTERN KENYA

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Prompt care-seeking is essential to prevent severe disease and death from malaria. We investigated whether social and demographic factors affected number of sick visits for febrile illness including malaria among
children participating in a malaria vaccine trial in Kenya. From January 2010 to January 2011, 800 infants aged 6-12 weeks were recruited into the vaccine trial. Of these 446 with complete social and demographic data were analyzed. The association between number of sick visits, dichotomized as high (>18) and low (<18), and child age, sex, maternal age, maternal education, wealth quintile, sex of household head, distance from health facility, and religion was measured using bivariate and multivariate models. Wealth index was created using a list of household assets and principal component analysis was used to divide households into quintiles, poorest, poor, middle, rich and richest. The number of sick visits per participant during the 12 month period of follow-up ranged from 0-45 (median = 12, mean = 12.9). In multivariate analysis, children from the poor and middle income quintiles were significantly more likely to have a high number of sick visits than those from the wealthiest quintile (OR=3.13, 95% CI 1.12-8.74; OR=2.80, 95% CI 1.02-7.71, respectively). Compared with households practicing Catholicism, Protestant and Indigenous religion households were significantly more likely to have a high number of sick visits (OR=4.36, 95% CI 1.34-14.19; OR=3.14, 95% CI 1.11-8.88, respectively). No significant associations were found between other variables and number of sick visits, including distance to health facility (OR=1.06, 95% CI 0.42-2.67). Even though prior studies have shown wealth to be associated with increased care-seeking for children (Taff 2005, Chuma 2007), we found in this trial setting, where transport reimbursement and free health care were provided, a high number of sick visits was associated with the lower income quintiles. Distance to health facility was not associated with number of sick visits. It might be that in this resource poor setting, removing the barrier of transport through reimbursement and providing free health care could increase health seeking.

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EPIDEMIOLOGY OF LABORATORY CONFIRMED RUBELLA CASES IN ETHIOPIA, 2013

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Rubella is a contagious rash illness. Trans-placental infection leads to serious fetal disorder called Congenital Rubella Syndrome (CRS). Worldwide, more than 110,000 infants are born with CRS each year; most of these occur in developing countries where information is limited on the epidemiology and vaccine not introduced. This study was conducted to see the annual prevalence, seasonal and geographic variation and age distribution of rubella infection. Serum/plasma samples and demographic data were collected from measles/rubella suspected cases from all the 9 regional states and 2 city administration of Ethiopia, January-December 2013. The samples were tested for rubella IgM by ELISA. The data was analysed by Epi Info software version 3.5.4. Among 3,587 patient samples tested, 858 (23.9%) were positive for rubella IgM. The positivity rate was higher among females (26%) than males (22.2%). The highest rubella positivity rate, 27.5% (287/1043), was seen among children 5-9 years. During March and August, the highest 33.6% (179/533) and lowest 10.7% (17/159) prevalence rates were seen respectively. The highest positivity rate was seen in Amhara 36.7% (301/821) followed by SN NPR 36.1% (227/628), 29.4% (35/119) of Tigray, 15.3% (64/417) of Addis Ababa and the lowest 13.4% (190/1416) in Oromiya. In conclusion, Rubella in Ethiopia, becomes an increasing non-reportable public health problem and the infection is seasonal mostly affecting children. The current prevalence of rubella cases calls for conducting CRS surveillance in infants, surveillance among pregnant mothers, reporting and necessitates the introduction of rubella vaccine into the national routine immunization services.

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INTERNATIONAL AID AND NATURAL DISASTERS: A PRE AND POST-EARTHQUAKE LONGITUDINAL STUDY OF THE HEALTHCARE INFRASTRUCTURE IN LEOGANE, HAITI

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Little is currently known about the interaction between international aid donors and local healthcare systems in the context of the healthcare recovery following natural disasters. Using data collected prior to and following the 2010 Haiti earthquake, we detail the response of aid agencies and their interaction with local healthcare providers in Leogane, the town closest to the earthquake’s epicenter. We collected geocoded data in Leogane, Haiti during three time periods: five months prior to the earthquake, one year after the earthquake, and three and a half years after the earthquake. During each visit we collected the same census data, including healthcare facility services, funding source, and receipt of aid. Our findings demonstrate that both one and three years after the earthquake there were marked increases in the total number of healthcare facilities, inpatient beds, and surgical facilities as compared with the pre-earthquake period, and that international aid has been a driving force behind the recovery. Twelve out of thirteen new healthcare facilities that have opened since the earthquake have been aid-financed, and seven out of eight healthcare facilities that were rebuilt after the earthquake were aid-financed. Despite increases in free, aid-financed healthcare following the earthquake, private Haitian healthcare facilities have remained at a constant number. Reconstruction efforts have not been fully sustainable, as the planned phase-out of several aid-financed facilities will leave Leogane with fewer inpatient-beds and emergency medical services as compared with the pre-earthquake period. We hope that our assessment of the recovery effort thus far in Haiti will help frame policy decisions regarding how best to support local healthcare systems in both the acute and long-term phases of disaster recovery efforts.

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SAMUEL LAPSLYE AND WILLIAM SHEPPARD: MISSIONARY MEDICINE, MEDICAL ENTOMOLOGY AND REALPOLITIK IN THE CONGO, 1890-1917

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The dawn of the 20th century marked the height of European colonialism on the African continent. By 1900, the major European powers had carved the continent into sectors of social, cultural, political, and military control. Christian missionaries, Protestant and Catholic alike, soon followed by the thousands. Although many were European, some hailed from the United States. Among the best-known Americans were William Sheppard and Samuel Lapsley, Presbyterian missionaries who worked in the Belgian Congo from the early 1890s to the era of The Great War. As tales of Dr. David Livingstone spread widely in the late 1800s, it is likely that the Reverend Mr. Sheppard and the Reverend Mr. Lapsley would have expected the usual missionary tribulations. They would have been all too familiar with the experiences of the faithful who had preceded them: suspicious—and possibly warlike—natives, linguistic and cultural barriers, fierce animals, and sweltering tropical climates. As such, they arrived in the Belgian-controlled Congo in the early 1890s armed with hundreds of pounds of equipment and supplies, not least, Bibles. The work of Sheppard and Lapsley, however, soon took them not only into the changing world of late 19th-century tropical medicine but also into the grim underside of Congolese life under Belgian control. Christian missionaries, Protestant and Catholic alike, soon followed by the thousands. Although many were European, some hailed from the United States. Among the best-known Americans were William Sheppard and Samuel Lapsley, Presbyterian missionaries who worked in the Belgian Congo from the early 1890s to the era of The Great War. As tales of Dr. David Livingstone spread widely in the late 1800s, it is likely that the Reverend Mr. Sheppard and the Reverend Mr. Lapsley would have expected the usual missionary tribulations. They would have been all too familiar with the experiences of the faithful who had preceded them: suspicious—and possibly warlike—natives, linguistic and cultural barriers, fierce animals, and sweltering tropical climates. As such, they arrived in the Belgian-controlled Congo in the early 1890s armed with hundreds of pounds of equipment and supplies, not least, Bibles. The work of Sheppard and Lapsley, however, soon took them not only into the changing world of late 19th-century tropical medicine but also into the grim underside of Congolese life under Belgian control. This paper will examine only their medical and scientific efforts amidst the changing paradigms in tropical medicine but human-rights efforts in the Congo.
AFRICA

public sector.

clinics and better training in communication skills for public care providers and particularly for disadvantaged women, better management of public facilities constitute the main care providers for the general population were significantly less satisfied than those attending private clinics. The providers. We found that although women tended to be highly satisfied in inadequate space and neatness and inadequate communication with care the following dimensions of antenatal care (ANC): inadequate privacy, with public facilities (after adjustment) included their unhappiness, with the following dimensions of antenatal care (ANC): inadequate privacy, inadequate space and neatness and inadequate communication with care providers. We found that although women tended to be highly satisfied with both private and public ANC facilities, those attending public clinics were significantly less satisfied than those attending private clinics. The main complaints were related to the physical environment, technical process and provision of information or reassurance. Because public facilities constitute the main care providers for the general population and particularly for disadvantaged women, better management of public clinics and better training in communication skills for public care providers may help to retain women patients and improve the quality of ANC in the public sector.

DEVELOPMENT OF ADVANCED SERO-ASSAYS TO BROADEN DIAGNOSTIC AND SURVEILLANCE CAPABILITY IN WEST AFRICA

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West Africa is home to a wide variety of viral infectious diseases, many of which go untreated in the local populations. Lassa virus causes a hemorrhagic fever that occurs throughout the region, with fatality rates approaching 30%. It is endemic in Sierra Leone, where the Kenema Government Hospital (KGH) houses a Lassa fever ward and laboratory. Approximately 600 suspected Lassa fever cases are received here annually from throughout the country, however of these, only 30-40% of samples test positive for Lassa virus; therefore, there is a large number of patients presenting with diseases of unknown origin. Severely limited resources at KGH hinder treatment and diagnostic capacity of Lassa fever, as well as of the additional undiagnosed infections that present there. Here, we have approached these two issues - diagnostic capability for acute Lassa infection, and surveillance of additional circulating viruses in west Africa-by developing an array of diagnostic assays for detection of hemorrhagic fever and arthropod-borne viruses utilizing Magpix technology (Luminex, Austin, TX) and evaluating their clinical performance on site at KGH. Magpix assays detecting Lassa-specific IgM and Lassa antigen in serum were significantly faster and more sensitive compared to traditional ELISAs, which are the current standard at KGH. Identifying past exposures to possible circulating viruses using IgG detection was carried out in a multiplex format, detecting antibodies to Ebola, Marburg, Rift Valley fever, Crimean Congo hemorrhagic fever, Lassa, and an array of flaviviruses and alphaviruses in one well. These assays have shown to be a valuable asset in a clinical setting, significantly improving upon current acute Lassa fever diagnostics at KGH as well as generating a large pool of data that gives insight into the scope of viral diversity and prevalence in West Africa.

USING ITERATIVE PRACTICE FOR DEVELOPING PUBLIC HEALTH ICONOGRAPHY TO PREVENT SOIL-BORNE HELMINTHS

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Shoes are a proven method for disrupting the transmission of soil-borne helminths (STH). However, shoe-wearing practice is often uncommon in areas where STH are prevalent, largely because the health benefits of wearing shoes may not be apparent to local populations. This is especially true in non-literate populations where information dissemination through traditional means (e.g. posters, billboards) is difficult. We launched a public health intervention that combines an animated image (a “lenticular image”) depicting the efficacy of shoes in preventing STH infection with “flip-flop”-style sandals. The image adheres directly onto the shoe, so that health information is linked to the primary means of intervention. Here, we describe the multi-phased, iterative design process used to develop the image and the process of its deployment and social uptake in rural communities in Uganda. We gathered standard iconographies that depicted specific elements of the image to show that shoes can interrupt STH. Elements included abstract constructs such as “healthy” and “don’t”, alongside concrete concepts such as “hoofworm” and “sandals”. Iconographies and color schemes were evaluated, deconstructed, and reconstructed over five focus group sessions. A resulting suite of “infection” and “prevention” messages were tested with 30 individuals across three study communities. The final versions of ‘infection’ and ‘prevention’ were selected based on the frequency of “correct” comprehension. Shoes with the image were then deployed to individuals in communities with high rates of hoofworm infection. We found participants’ overall shoe-wearing practices improved, but varied by activity and individual characteristics. This study demonstrates that holographic iconography can be an effective means of disseminating public health information to non-literate populations. It also demonstrates the importance of incorporating culturally appropriate images into pictorial depictions of public health messages. Interactive design processes are an efficient way to develop such messages.

FEASIBILITY OF A ONE-WEEK COURSE TO TEACH BASIC ULTRASOUND SKILLS TO PHYSICIANS IN RESOURCE-LIMITED SETTINGS

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Portable ultrasound is an inexpensive and highly effective tool. Ultrasound provides real time data that can assist in diagnosis and therapeutic procedures, and may contribute to better outcomes in resource limited settings. However, the training required before a physician gains competency remains controversial. The optimal method of teaching, and which ultrasound views to teach are also questionable. This study aimed to assess whether a one-week intensive course would result in physicians ability to use ultrasound in daily clinical practice competently. The project was conducted at Bugando Medical Centre in Mwanza, Tanzania. The curriculum involved learning basic ultrasonography skills, and the ability to obtain and interpret views of the heart, peritoneal space and liver, perisplenic space, inferior vena cava, pelvis, and thorax. The curriculum involved daily lectures on theory, followed by practical sessions with healthy subjects and in-patients from medical wards. Informed consent was obtained from all participants. Candidates were required to complete a minimum of twenty-five total scans on different patients. Candidates submitted several still-images, which were evaluated for adequacy and...
demonstration of normal or abnormal anatomy. Competency was also assessed by a practical examination at the end of the course involving an ultrasound examination of a standardized patient. All participants completed a minimum of twenty-five ultrasounds of each of the ten views. All candidates’ passed the formative examination, with a median of 96%. Adequate images were submitted by all candidates, with a demonstration of a variety of pathology. Self-reported confidence was shown to be significantly greater after the completion of the course. The steep but rapid clinical learning curve demonstrates that an intensive training course to attain basic bedside ultrasound skills is feasible in a resource limited setting. Integration of a similar curriculum may be possible to enhance medical education and diagnostic accuracy in resource-limited settings.

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ACCURACY OF ULTRASOUND AS A TELE-MEDICINE COMPONENT USING A COMMERCIALLY AVAILABLE TELE-CONFERENCE SYSTEM

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Point-of-care (POC) ultrasound has been shown to have many benefits in patient care. Many international providers are not trained to perform or interpret US results. As a component of the telemedicine (TM) system, POC US has the potential to provide diagnostic capability in locations with limited diagnostic services. Our hypothesis is that US transmission using a commercially available teleconferencing (TC) system will yield similar results to DICOM image transmission as well as comparable picture quality. Similarly, the accuracy of diagnosis will be unchanged. This was a prospective observational trial of transmitted US video using an internet based peer-to-peer communication system (Skype) compared to images transmitted via a DICOM system. US images were obtained from patients presenting to the ED. After consent, limited, POC US exams were performed using a Sonosite US. The images were stored using a DICOMM protocol and transmitted using a TC system. The TC system consisted of a USB to VGA image capture device (Epiphan) connected to the VGA output in the minidock of the US system. The image capture device connected to a Dell laptop and using Skype, images were transmitted to a Mac Pro. Video recordings were made of the transmitted image and stored as mp4 files. Images of the transmitted and non-transmitted images were of the same patient and were of the same US exam. Three ED physicians with hospital credentials for ED US reviewed the US exams using both methods in random order. 148 examinations (74 transmitted and 74 non-transmitted) were reviewed. The kappa statistic was calculated separately for each diagnostic test, and for all tests combined, to examine agreement between each of three reviewers and the gold standard. Kappa for Reviewer #1 was consistently in the “almost perfect agreement” range (0.8 < κ < 1). For the Gallstones and IUP diagnostic tests, all reviewers had κ values in the “almost perfect agreement” range (0.8 < κ < 1). There were statistically significant differences between the scores for the transmitted vs. non-transmitted for all three types (image detail, image resolution, and image quality) for Reviewers 1 and 3 only. In particular, non-transmitted scores were significantly higher than transmitted scores. POC US transmitted via commercially available systems may be a vital and accurate diagnostic option for international providers where other diagnostic radiological resources are unavailable.

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TIME TRENDS FOR CHILD DIARRHEA PREVALENCE AND ORS USE IN A DOMINICAN REPUBLIC PERI-URBAN COMMUNITY

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Reducing child diarrhea prevalence and increasing oral rehydration solutions (ORS) use are important national policy aims for diarrhea control in low- and middle-income countries. Available national level data for some countries has identified gaps in attaining these aims. While informative, national level data may obscure variation across sub-national levels and may miss patterns within high-risk communities. Low-income peri-urban communities are particularly important given that they contain rapidly expanding populations and have conditions that may place children at high-risk for diarrhea (e.g., overcrowding, inadequate water and sanitation infrastructure). The aim of this study was to determine whether the prevalence of child diarrhea is decreasing and ORS use is increasing over time within a low-income peri-urban community of the Dominican Republic. The study community has received several health interventions initiated by non-governmental organizations over the last several years that included water, sanitation and health education projects. Information on child diarrhea and ORS use between 2009 and 2013 was extracted from a dataset derived from an ongoing child growth-monitoring program (GMP) located in the study community. All children in the community under four years of age were eligible to participate. The prevalence of child diarrhea among attendees at the GMP was determined within different time blocks over the 4 year study period. ORS rate was based on reported use within diarrhea cases over time. Data were available for 293 children from one or more appointments. Diarrhea prevalence remained relatively constant at 26 (SD: 2.6)% over the 4-year period with no reduction over time. The mean rate of ORS use over the 4-year period was 38 (SD: 3.4)% with no evidence of increase over time. Despite various health initiatives in this community, the burden of child diarrhea remained high and ORS use remained low. Examination of potential factors impeding progress on diarrhea control is still required, particularly in high-risk communities.

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DEVELOPING SUSTAINABLE CAREER DEVELOPMENT SUPPORT FOR AFRICAN RESEARCHERS

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Developing global health researchers to proficiency is crucial to support the strengthening of health research capacity, yet researchers based in low and middle-income countries in Africa face a disadvantage in their career development to proficiency through limited access to research training, resources and appropriate support and mentorship. Currently much of this support comes from externally funded capacity development programs who invest in developing individuals, strengthening research training and enhancing research environments. However, continued support post program ends remain a challenge. Led by senior African researchers from each of the Malaria Capacity Development Consortium’s (MCDC) African partner institutions, structured and supported Career Development Groups (CDGs) have been set up to advance the career development of researchers within their institutions, in addition to developing sustainable research training and systems of support for researchers within the institution. Oversight of the CDGs sits within the offices of the provost, deans of faculty and schools of postgraduate studies to ensure integration within institutional processes and policies, and sustainability beyond the MCDC program. The CDGs have undertaken a baseline assessment of existing institutional career development support and in wider consultation drawn up plans for development. Plans are specific to institutional need and context, aligned to strategic institutional objectives and incorporate the needs of other capacity development programs based at the institution. Activities and strategies of support will vary but with Personal Development Planning (PDP), formal mentoring and support in postgraduate supervision being key strategies that will be embedded within institutional career development support for researchers and research staff. An evaluation will be undertaken on the impact of the career development support as well as the process of using CDGs as a new way of working within African institutions to support the sustainable development of global health researchers in LMICs in Africa.
THE FIRST REPORTED CASE OF BABESIOSIS IN A RESIDENT OF OREGON

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Babesiosis is a tickborne infection caused by intraerythrocytic parasites of the genus Babesia. Most U.S. zoonotic cases have been caused by B. microti, which is endemic in the Northeast and upper Midwest. Sporadic cases caused by other Babesia species have been documented in various U.S. regions. The first reported case caused by B. duncani (the WA1-type parasite) occurred in Washington State in 1991. Here we describe the first known case of babesiosis diagnosed in Oregon—a parasitologically confirmed case of B. duncani infection, in a 69-year-old otherwise healthy asplenic man, who lived in a rural forested area of Deschutes County in central Oregon. He did not recall tick exposures or have a history of transfusions. On June 30, 2009, he was evaluated in an urgent-care center because of a several-day history of a febrile illness (maximum temperature, 40°C); his hematocrit was 37.3%, and his platelet count was 39 x10^9/L. The marked thrombocytopenia prompted manual review of a blood smear. Intraerythrocytic forms were noted, which were confirmed by a hospital pathologist as Babesia parasites; the parasitemia level was 3%. On July 1, he was hospitalized and began combination therapy with clindamycin plus quinine. When he was discharged on July 6, he had been afebrile for >48 hours and his parasitemia level was <1%. During a follow-up evaluation on July 9, no parasites were found on blood-smear examination and antimicrobial therapy was discontinued. In reference diagnostic testing at CDC, molecular and serologic analyses were negative for B. microti but were positive for B. duncani (the indirect fluorescent antibody titer was 4096); B. duncani also was isolated by inoculation of jirds (Mongolian gerbils). Clinicians and laboratorians should be aware that babesiosis can be caused by agents that are not detected by assays for B. microti. To date, parasitologically confirmed cases of B. duncani infection have been documented in Washington, California, and Oregon. The many unknowns about B. duncani include its geographic distribution, tick vector, and reservoir hosts.

DETECTION OF ANAPLASMA PHAGOCYTOPHILUM, BABESIA MICROTI AND BORRELIA BURGDORFERI IN IXODES SCAPULARIS COLLECTED FROM LOCATIONS SURROUNDING THE RESIDENCE OF A LOCALLY-ACQUIRED CASE OF HUMAN BABESIOSIS IN MARYLAND, USA

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The Delmarva Peninsula has long been recognized as home to populations of Ixodes scapularis infected with Borrelia burgdorferi, although reports of tick surveillance are few. Babesia microti has never been reported from I. scapularis there, although there is only one published investigation of Ba. microti in the area. In 2009, Maryland Department of Health and Mental Hygiene (DHMH) reported the investigation of an autochthonous case of human babesiosis on the Maryland Eastern Shore of the Delmarva Peninsula. The patient was also infected with B. burgdorferi. Subsequently, a team from DHMH and U.S. Army Public Health Command (USAPHC) performed a preliminary investigation of vector ticks in locations surrounding the patient’s residence. Sixteen I. scapularis adults were collected by flagging at the patient’s yard and a nearby site. PCR testing at the USAPHC detected B. burgdorferi in 8/16 of the ticks; 2 of these 8 were coinfected with Ba. microti, and 1 of the 8 was coinfected with A. phagocytophilum. PCR positives were reconfirmed with second PCR at USAPHC, and Ba. microti positive tick samples were sent to Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, Georgia, for reconfirmation with nested primers targeting the rrs-rrnD gene. To our knowledge, these are the first Ba. microti positive I. scapularis collected in Maryland, and the human case is the first locally acquired babesiosis infection reported from Maryland. B. burgdorferi infection can be quite robust in the mid-Atlantic region, so finding 50% of these ticks positive for B. burgdorferi was not completely unexpected. However, the regional prevalence of infection with A. phagocytophilum is low (2-3%), and Ba. microti has never been reported from I. scapularis in Maryland, so detection of these pathogens in a small sample of ticks was remarkable. Further investigation at this location of nymphal I. scapularis, the stage most likely to transmit human diseases, is warranted.

IMPACT OF CLIMATE CHANGE ON TICK-BORNE RELAPSING FEVER BORRELIOSES DISTRIBUTION IN WEST AFRICA

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Tick-borne relapsing fever (TBRF) borreliosis due to Borrelia crocidurae, is a major cause of morbidity in most rural areas of Senegal where its distribution, previously limited to the Sahel, spread to Sudan savannah areas during the 1970s. We report, here, studies conducted in 12 West African countries to investigate the distribution of ticks Ornithodoros sonrai, the occurrence of B. crocidurae infections in vectors and small mammals and their relationship with climatic change. From 2002 to 2012, we investigated the occurrence of O. sonrai in rodent burrows in 210 study sites from West African countries. Ticks collected in each site and their Borrelia infections were genetically characterized by sequencing. We collected small mammals in Mauritania, Senegal, Mali, Niger and Benin. They were tested for Borrelia infections by thick blood film, blood or brain inoculation into white mice and/or by PCR. Out of 8,716 burrows examined, 973 (11.1%) were found colonized by Ornithodoros ticks identified as O. sonrai. Only sites in Senegal, Mali, the Gambia and Mauritania were found positive for this vector. B. crocidurae infections were highlighted in 245/1,121 (21.8%) of ticks tested by nested PCR and 70/842 (8.3%) of rodents and insectivores. All infected mammals were collected in areas where we found the vector. The southern limits of O. sonrai and B. crocidurae corresponded approximately to the 750-mm isohyet. O. sonrai ticks and B. crocidurae are massively distributed north of latitude 13°30’S. Although climatic factors are clearly associated with the distribution and the recent spread of TBRF in the westernmost part of West Africa, the vector appears absent from climatically suitable areas of Burkina Faso and Niger. It is only in central and eastern Mali that the distribution of the vectors was associated with the principal riverbed of Niger River and its mains inflows.

SPILLOVER AND GENETIC DIVERSITY OF RICKETTSIA PARKERI IN NORTHERN VIRGINIA

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Spotted Fever group Rickettsia (SFG) cause a spectrum of disease worldwide, with symptoms ranging from mild to severe febrile illness. In the United States the most notable is R. rickettsii, the causative agent...
of Rocky Mountain Spotted Fever (RMSF). Several other SFGR have been linked to human infection, many of which cause similar clinical symptoms and contribute to misdiagnosis. Previously thought to cause only zoonotic infections, R. parkeri was recognized as a human pathogen in 2002, and has subsequently been linked to twenty cases of spotted fever rickettsiosis in the U.S. and Argentina. The primary vector of R. parkeri is Amblyomma maculatum, which has spread beyond its historic range to occupy foci across the central and southeastern U.S. In 2010, R. parkeri-infected A. maculatum was discovered at a landfill in Fairfax County, Virginia, where sympatric populations of many tick species exist. Initial findings discovered R. parkeri infection of other ticks near the landfill, including Dermacentor variabilis, Rhipicephalus sanguineus, and Haemaphysalis leporispalustris, which are documented to bite humans. This is significant, and highlights the potential for spillover into vectors not typically associated with R. parkeri. Preliminary analysis of a relatively conserved outer membrane protein (gene rompB) indicates 97-99% sequence homology among R. parkeri samples. Low diversity implies that infection of novel vectors may be attributable to spillover from sympatric populations co-feeding on infected hosts. Further sequence analysis of more variable gene targets is ongoing; preliminary findings indicate that genetic variation may exist, which, if confirmed, will provide evidence for the introduction of multiple R. parkeri variants. This work represents a novel approach to understanding the ecology of R. parkeri in North America. Pathogen spillover of multiple genetic variants into novel vectors could lead to increased transmission and propagation of the disease cycle, resulting in an increase in spotted fever rickettsiosis cases.

CONTROL OF TUNGIASIS (SAND FLEA DISEASE) IN EAST AFRICA: SELF-DIAGNOSIS AS A MEANS TO TARGET TREATMENT IN ENDEMIC AREAS

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In East Africa, tungiasis (sand flea disease) has re-emerged in epidemic proportions. This has prompted Ministries of Health and non-governmental organizations to conceive control measures. To target treatment in a cost-effective manner, diagnostic tools are required allowing a rapid assessment of the prevalence and severity of tungiasis at the community level. We investigated the sensitivity, specificity and predictive values of self-diagnosis in rural Uganda and Kenya and determined factors which might have an impact on the validity of this diagnostic approach. The study was carried out in the Makoma Primary School, Bugiri County, Uganda, and in Shivakala village, Kakamega County, Kenya. All the primary school pupils (n = 589) and all the inhabitants of Shivakala village (n = 386) present on two consecutive days were individually asked whether they thought they had tungiasis on at least one foot. Immediately after the self-diagnosis, the individual was carefully examined for the presence of embedded sand fleas, and tungiasis-associated morbidity was assessed semi-quantitatively. In Makoma Primary School, the prevalence of tungiasis was 71.9% (95% CI 66.6 - 76.6). In Shivakala village, the prevalence was 70.3% (95% CI 62.2 - 77.3) in school-age children and 56.5% (95% CI 51.5 - 61.3) in the general population. At both locations, the ratio of infected males to females was 1.2. Whereas in the Ugandan primary school the sensitivity of self-diagnosis was very high (85.1%; 95% CI 80.0 - 89.1), the specificity was rather low (69.6%; 95% CI 59.5 - 78.0). In contrast, in the Kenyan village the sensitivity of self-diagnosis was high (97.1%; 95% CI 92.8 - 98.9), but the specificity was low (66.5%; 95% CI 59.3 - 73.0). In school-aged children the positive predictive value was 98.5 (95% CI 92.0 - 99.7), and the negative predictive value was 56.3 (95% CI 44.8 - 67.3). It was concluded that self-diagnosis can be relied upon to enable targeted community based control measures against tungiasis, but that the validity of this diagnostic approach has to be determined in different settings.

EFFECTIVE TREATMENT OF TUNGIASIS WITH DIMETICONE: A TARGETED TOPICAL APPLICATION IS SUPERIOR TO WETTING THE SKIN OF THE WHOLE FOOT

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Tungiasis (sand flea disease) is a neglected tropical disease associated with debilitating acute and mutilating chronic morbidity. In the endemic areas patients attempt to remove embedded sand fleas with inappropriate sharp instruments, such as safety pins, thorns or sharpened pieces of wood - a hazardous procedure by itself. Recently, we demonstrated that a single topical application of a mixture of two dimeticones with low viscosity onto the skin of the feet effectively killed embedded sand fleas and significantly reduced local inflammation within seven days. We attempted to increase the efficacy of the new therapeutic approach by targeting the dimeticone to the abdominal rear cone of the parasite which protrudes over the corneal layer of the epidermis. 59 children from Bulidha sub-county, Bugiri District, Eastern Uganda, aged from 5 to 12 years, with a total of 311 embedded sand fleas were included in the study. The left and the right foot were randomized to either receive the “simple” or the targeted application with the dimeticone, respectively. The viability of the embedded parasites was assessed by a handheld digital video microscope and the degree of inflammation was determined by means of an inflammation score. The lesions were observed daily during 7 days. After the “simple” treatment 87.3% (95% CI 81-92%) of the embedded fleas lost all viability signs within seven days, and after the targeted treatment 97.5% (95% CI 94-99%; p = 0.008) giving a difference of 10.2% (95% CI 7-14%) in efficacy. We conclude that the targeted application is superior to wetting the skin of the whole foot with dimeticone. The targeted treatment requires less dimeticone, can be performed by the patient himself and is a promising tool for control measures aiming at the reduction of tungiasis-associated morbidity. In view of the high efficacy and safety of the topical application of dimeticone, the hazardous extraction of embedded sand fleas with sharp instruments is no longer warranted.
mL) began losing weight at 7 dpi, reached a nadir on 9 dpi, and regained weight from 13 dpi. The spleen, lung and liver from both groups, and the kidney from lethal dose group had highest bacterial loads on 6 dpi, except the kidney in the sublethal dose group which peaked on 12 dpi. Further studies, especially study of human immune responses after Orientia positive mite bites in endemic areas, will benefit the understanding and control of scrub typhus well as development of vaccine and other prevention measures.

THE EFFECT OF AMBLYOMMA MACULATUM FEEDING ON RICKETTSIA PARKERI INFECTION IN RHESUS MACAQUES

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Rickettsia parkeri is an emerging pathogen in the spotted fever group of Rickettsia that is transmitted by the bite of the Gulf coast tick, Amblyomma maculatun. The immune response to spotted fever group Rickettsia has been studied extensively in mice via intradermal, intraperitoneal, and subcutaneous routes of infection. However, these studies did not evaluate the effect of tick infestation on the immune response and rickettsial transmission. Since hard ticks feed on vertebrate hosts for several days, they must be able to counteract the host immune response. Several studies have shown that tick salvia alters several aspects of both the innate and adaptive arms of the immune system. However, the effect of this immunomodulation on Rickettsia transmission and pathology in the vertebrate host has not been examined. We hypothesize that by modulating the host immune response, tick feeding enhances infection and pathology of pathogenic SFG Rickettsia sp. in non-human primates.

In order to assess this interaction in vivo, we will use rhesus macaques to compare intradermal needle inoculation of R. parkeri alone to inoculation during Rickettsia-free A. maculatum feeding and A. maculatum feeding without Rickettsia administration. For up to one month post inoculation, skin and lymph node biopsies, and blood will be collected to evaluate pathology, quantification of rickettsial load, and assess the acute phase response and cytokine concentrations. Comparison of the disease course, pathology and immune responses to infection in the presence or absence of tick feeding, will help define the role of the tick in spotted fever group Rickettsia transmission and develop a primate model of Rickettsia parkeri rickettsiosis.

CHARACTERIZATION OF THE BACTERIAL COMMUNITY OF SPECIES AND POPULATIONS OF DERMACENTOR Ticks

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Our metagenomic analysis (rrs fragment pyrosequencing) of the bacterial communities present in 23 adult Dermacentor variabilis (Dv) ticks from Atlanta, Georgia and southeastern Ohio identified 27 taxonomic groups; however, only 6 were present in at least 3 ticks - 2 types of Rickettsia, 3 types of Francisella endosymbiont (FE) and a Rickettsiella. Dermacentor ticks are classic vectors for Rickettsia (7 ticks) while the FE was found in higher abundance and in all ticks, 15 (both sites) had two types of FE. In order to select other tick samples for further metagenomic analyses and to determine whether there was any relationship between the presence of Rickettsia and FE agents, additional Dv adults from 8 sites on a coastal barrier island in Georgia and 6 ecologically variant sites in Ohio, and D. occidentalis (Do) from 6 sites in southern California were collected. 

Their DNA was analyzed by semiquantitative EVAGreen assays (qPCR) for fragments of ompA (Rickettsia) or rrs (FE). The Rickettsia species were identified by sequencing ompA, ompB, and/or 17 Kd antigen gene amplicons. Nearly all samples again contained FE while Rickettsia was found in lower prevalence and abundance irrespective of species. Primers for 11 genes commonly used in multi-locus sequence typing (MLST) for pathogenic Francisella (PF) were tested by PCR with the Dv FE but products were obtained for only 4 of them. Three MLST targets exhibited no genetic variation between Dv populations but they had <90% homology to PF; however, a small number of 16S genotypes were detected at each site including some new variants. While the quantity of FE detected by qPCR varied significantly between ticks and tick locations for both Dv and Do, it did not correlate with either tick sex or the presence of a Rickettsia. The physiological importance of the widespread dominant prevalence of different Francisella endosymbionts to Dermacentor ticks is unknown; if (and how) their presence may alter these ticks' responses to acquisition, growth and transmission of Rickettsia also needs experimental investigation.
CLINICAL VALIDATION OF NEW AND EXISTING ANAPLASMA PHAGOCYTOPHILUM REAL-TIME PCR ASSAYS

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Anaplasma phagocytophilum, an obligate intracellular bacterium, is the causative agent of human granulocytic anaplasmosis (HGA). Characterized by fever, headache, myalgia, leukopenia, and thrombocytopenia, HGA is a vector-borne disease carried by Ixodes tick species. While HGA has a low fatality rate, the risk increases with delayed treatment or when the elderly or immunocompromised are infected. Currently, our laboratory uses a SYBR Green assay that targets the 16S gene of Anaplasma and Ehrlichia, which requires sequencing confirmation to distinguish the species. The msp2 gene encodes an outer membrane protein unique to Anaplasma. Depending on the isolate, A. phagocytophilum may contain >80 copies of msp2 variants. An existing real-time PCR multiplex assay targets the msp2 gene of A. phagocytophilum and the 23S gene of Borrelia burgdorferi (Courtney JW et al., 2004). In this study, we validate the A. phagocytophilum msp2 primer/probe set as a singleplex real-time PCR assay for testing clinical specimens in the CDC Rickettsial Diagnostic Laboratory. A second set of primers were designed to flank the original amplicon (77-bp) extending the product to provide a template (213-bp) suitable for DNA sequencing. Primer/probe concentrations were optimized and analytical specificity was tested with exclusivity panels of environmental DNAs (33) and bacterial near neighbor DNAs (41). Sensitivity of the assays was determined by spiking known concentrations of A. phagocytophilum into PBS and blood. The assays have a limit of detection of 10 copies of the msp2 gene. Assay verification was performed by testing a blind panel of DNA extracted from blood with high, medium, and low concentrations of the organism. Results from the blinded panel correlated with expected values. Assay reproducibility (inter-assay and intra-assay) was also evaluated, generating consistent results. In conclusion, two sensitive and specific real-time PCR assays have been validated and can be used as effective diagnostic tools for the detection of A. phagocytophilum in clinical blood specimens.

SEROLOGICAL SURVEILLANCE OF RICKETTSIA DISEASE IN NORTHEASTERN AND CENTRAL CAMBODIA

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Rickettsioses are arthropod-borne diseases caused by intracellular bacteria of the genera Rickettsia and Orientia and are classified into 3 groups: spotted fever group (SFG), typhus group (TG) and scrub typhus group (STG). Rickettsial diseases generally present as a febrile illness that can range from moderate to severe symptoms. Limited data has been generated on the prevalence of rickettsial diseases in Cambodia. To investigate further, we have conducted febrile syndromic surveillance in the Northeastern provinces of Cambodia near the Lao PDR border and a second region within central Cambodia from July 2010-April 2011. Subjects who reported to local clinics with an acute fever greater than 37.5°C were enrolled. Data thus far includes a total of 390 enrolled patients who tested negative for influenza, dengue and malaria were checked for the presence of specific antibodies against SFG (R. rickettsii), TG (R. typhus), and STG (O. tsutsugamushi) by ELISA. Convalescent sera positive for antibodies specific to one of the three rickettsial groups were detected in 116 of 390 (29.7%) samples and positive in 77 (19.7%), 23 (5.9%) and 48 (12.3%) of cases for SFG, TG and STG, respectively. Titrations were performed on positive convalescent specimens and corresponding acute specimens. Recent infections, defined by a fourfold rise in antibody titers, or seroconversion were determined in 13 (11.2%) of SFG, 3 (3.9%) of TG and 4 (8.3%) of STG rickettsiosis. These results suggest that rickettsial infections may be a common etiology of fever for this area. Continued monitoring of this population and molecular characterization of these specimens will provide further insight to the epidemiology of rickettsiosis in this area.

RICKETTSIA SPECIES DETECTED IN DERMACENTOR VARIABILIS FROM NORTH CAROLINA

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We screened Dermacentor variabilis (American dog tick) for the genus Rickettsia under the hypothesis that less <10% of the ticks would harbor rickettsiae. To test our hypothesis, we extracted genomic DNA from 532 Dermacentor variabilis that were collected from several NC counties over years 2009 and 2010. Rickettsia species were identified by PCR amplification of 23S-5S intergenic spacer fragments combined with reverse line blot (RLB) hybridization with species-specific probes. Thus far, amplification of 100 genomic DNA samples has produced 61 (61%) samples, which showed a band of the correct size on agarose gels, and are considered to contain rickettsiae. PCR-RLB of 33 ticks showed that 96% (32/33) of the samples hybridized with a Rickettsia genus-specific probe. With species-specific probes, R. amblyomnii was found in the majority of the ticks (21/33); other Rickettsia species detected included R. massiliae (3/33), R. canadensis (2/33), R. montanensis (2/33), R. bellii (1/33) and R. conorii (4/33). Of the 33 samples, 10 samples showed hybridization with more than 1 species of Rickettsia and 3 samples contained unknown Rickettsia species. DNA sequencing to confirm the detection of Rickettsia and to verify species identifications obtained through RLB hybridization assays is in progress. Based on the ticks examined thus far, the presence of Rickettsia in D. variabilis is more common than we hypothesized. Our analysis of D. variabilis is ongoing and should provide informative results about the prevalence and species of Rickettsia harbored by this tick species.

STUDY OF SEROPREVALENCE AND VECTORS OF RICKETTSIA IN THE COLOMBIAN CARIBBEAN

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The first clinical signs and symptoms of spotted fever caused by Rickettsia are nonspecific, so can be easily confused with other febrile infections. The medical diagnosis is even more difficult because there are no diagnostic tests during the acute phase of the disease. Thus, clinical suspicion based on epidemiology, ecology and distribution of vectors, followed by subsequent clinical manifestations of the disease becomes the best strategy for a timely diagnosis. Conduct studies to establish risk areas is necessary to prevent deaths by rickettsiosis, therefore the objective of this work was to determine the seroprevalence in human populations and natural infection with Rickettsia in ticks in the department of Sucre, located in the Colombian Caribbean. A blood sample was taken from people, in the rural area of five municipalities of this area, who signed an informed consent and underwent an interview to establish risk factors. An indirect immunofluorescence test was performed to detect anti-Rickettsia IgG antibodies. The independence between the seroprevalence and risk factors was tested by a Chi-square test at a 5% level of significance. Additionally, ticks were captured from animals and drag sampling on vegetation, which were taxonomically identified and processed in order
to detect Rickettsia DNA by PCR. 320 human samples were taken, where 20 of them were seropositive (6.2%). Seropositive individuals were mainly elderly men (p<0.05), who were engaged in agriculture. 10,217 ticks were captured, D. nitens (7922), larvae of Amblyomma sp. (909), R. sanguineus (882), R. (B.) microplus (372), A. cajennense (96), A. dissimile (23), H. leporispalustris (6), A. ovale (5) and A. auricularium (2). Rickettsia felis DNA was detected in R. sanguineus and D. nitens, with minimum infection rates of 7.4 and 1.9 respectively. This study provides evidence of the presence of bacteria from the genus Rickettsia in ticks, and its circulation in human population from rural areas of the department of Sucre.

Q FEVER AND RICKETTSIOSIS IN U.S. MARINES DEPLOYED TO AFGHANISTAN

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Many dangers face military personnel when deployed in combat zones, but one of the most formidable opponents is infectious diseases. Operation Iraqi Freedom and Operation Enduring Freedom have brought more than 100,000 US troops to Central Asia exposing them to a multitude of pathogens that they may not have been exposed to otherwise. Several of these include the causative agents of the operationally relevant arthropod borne diseases Q fever (Coxiella burnetii) scrub typhus (Orientia tsutsugamushi), murine typhus (Rickettsia typhi), and spotted fever group rickettsioses (Rickettsia sp.). Since 2003, more than 150 cases of Q fever have been confirmed among troops deployed to Iraq and Afghanistan, though the symptoms of infection often mimic a multitude of other infections and can often be misdiagnosed. To assess the risk of these pathogens among military personnel in Afghanistan we initiated a survey of close to 1000 Marines deployed to Afghanistan for at least one continuous year. The DOD Serum Repository provided serum from pre-deployment and post-deployment blood draws, paired for each Marine. Analysis of these sera revealed sero-conversion of approximately 6% for Q fever, and 0.5% for spotted fever, but no sero-conversions for typhus group or scrub typhus. This study leads to a greater understanding of the infectious disease risk in Central Asia and provides valuable information for diagnosis and treatment of acute febrile illness in the area.

SCRUB TYPHUS IN AFRICA

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Since a report in 1951 evidence has been slowly increasing that scrub typhus exists in sub-Saharan Africa. The previous evidence included two cases (Cameroon and Congo) and evidence of O. tsutsugamushi-specific antibodies in humans (Ruanda-Urundi). With the recent reports of scrub typhus in Dubai and Chile, outside the previously accepted scrub typhus group or scrub typhus. This study leads to a greater understanding of the molecular regulation of the immunological response to rickettsial infection in ticks, the mechanisms of which may define vector competence.

A STUDY OF RICKETTSIAL AND ORIENTIA INFECTION AMONG ABATTOIR WORKERS AND THE DETECTION OF RICKETTSIA IN TICKS, DJIBOUTI

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Little is known about rickettsial infection in Djibouti located in the Horn of Africa. A study was conducted to examine the exposure to vector-borne and zoonotic pathogens in a high-risk environment, an abattoir in Djibouti. Human serum samples were collected from the abattoir workers in September 2010 and followed up every 4 weeks during a 20 week long period. Ticks were collected from the freshly-slaughtered cattle. 49 baseline and 11 follow-up human serum samples were tested for IgG antibodies against whole-cell antigens of SFGR, TGR and orientiae by enzyme-linked immunosorbent assays (ELISAs). Eight (16%), two (4%), and three (6%) were found seropositive for IgG antibodies against SFGR,
TGR and orientiae, respectively. Analysis of paired sera for 11 workers showed one was seroconversion for antibodies against orientiae during the study period, and the positive reactions were confirmed by Western blot assays using recombinant protein Kpr56 and Kpr47b. No seroconversions were observed for antibodies against SFGR or TGR. DNA extracted from 189 tick pools (contain 1-3 ticks) including 6 tick species from 72 cattle imported from Ethiopia were tested for the infection of Rickettsia using a Rickettsia genus-specific quantitative real-time PCR (qPCR) assay (Rick17b) and the positives were further tested by a qPCR assay specific for Rickettsia africae (RafriG). Overall, 32 (17%) pools of ticks from 26 (36%) cattle were infected with Rickettsia, of which 25 (47%) were R. africae, the causative agent of African tick-bite fever. Three types of R. africae variants were identified from Amblyomma variegatum ticks (but none from A. lepidum) by PCR and sequencing of a fragment of ompB. In addition, R. sp. S strain was detected in 2 (6%) pools of Hyalomma marginatum ticks. Although future studies are needed to determine the extent and impact of rickettsial infections in Djibouti, healthcare providers should be aware of possibility of these infections among their patients.

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RICKETTSIA AFRICAЕ VARIANTS

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Rickettsia africae causes African tick-bite fever (ATBF) and is one of the main causes of fever and illness in travelers returning from sub-Saharan Africa. R. africae belongs to the spotted fever group rickettsiae (SFGR) and is transmitted to humans by ticks of the genus Amblyomma found throughout sub-Saharan Africa. An infection rate in these ticks in endemic areas is high, and may reach 100%. Recent studies have shown existence of R. africae variants in ticks from several countries in Africa and New Caledonia. The genetic variants have mainly been detected in Amblyomma variegatum ticks and are closely related to wild type R. africae, but have not been associated with ATBF. The variability observed is mainly due to nucleotide substitution but some may have deletions and/or insertions leading to premature stop codons in the amino acid sequence. The impact of these mutations to the infectivity of the R. africae variants is currently unknown but we hypothesize that it may result in nonfunctional proteins and preclude human infection. The variability observed among R. africae genotype underscores the need to undertake comparative assessment of variants associated with clinical ATBF and those circulating within the tick population to understand the role that these variants play in the epidemiology of ATBF.

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AN ONLINE TOOL FOR MAPPING INSECTICIDE RESISTANCE IN MAJOR ANOPHELES VECTORS OF HUMAN MALARIA PARASITES AND A REVIEW OF INSECTICIDE RESISTANCE STATUS FOR THE AFROTROPICAL REGION

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Rapid evolution of insecticide resistance in malaria vectors threatens to erode gains made in malaria control unless action is urgently taken. Upto-date insecticide resistance data are urgently needed for inform vector control policy and programmes. A new online geospatial mapping tool, IR Mapper, is introduced herein. A systematic search of peer-reviewed literature was performed and Anopheles phenotypic and resistance mechanisms data extracted to Microsoft Excel 2010 database. IR Mapper (www.irmapper.com) was built on ArcGIS for JavaScript. Additional integrated functionalities allowing triangulation of entomological monitoring data with epidemiological data include Plasmodium falciparum and P. vivax endemicity layers, entomological inoculation rate layers, and an “Add Own Data” functionality enabling users to add and temporarily view their own data on the application, and print user-generated maps. IR Mapper contains 8, 596 data points from 1, 587 georeferenced localities in 55 countries for data collected between 1954-2013. Susceptibility reports were available for 53 countries; 43 reported resistance confirmed to at least one insecticide. One or more types of resistance mechanisms were detected in 37 countries. In the Afrotropical region pyrethroids and DDT were commonly tested in susceptibility assays than carbamates or organophosphates. Between 2001 and 2012, there was an increase in cases of confirmed resistance of An. gambiae s.l. to pyrethroids (from 41 to 87%) and DDT (from 64 to 91%). Resistance mechanism assays in An. gambiae mainly focused on target site mutations with very few reports on elevated metabolic enzyme assays. Mapper is a dynamic tool that collates resistance information from various sources and in various formats into a single platform. Mapper is useful for investigating temporal and spatial trends in Anopheles insecticide resistance, informing rational deployment of vector control interventions based on the evidence, as well as for pointing out gaps in resistance monitoring for appropriate action.

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INSECTICIDAL PROPERTIES OF LOCAL PLANTS USED AGAINST ANOPHELES GAMBIAE, MALARIA VECTOR IN BURKINA FASO, WEST AFRICA

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Malaria remains a serious public health problem killing mostly in Africa. It is estimated to 207 millions of cases and 627000 deaths the burden of malaria in 2012. Malaria control is still heavily dependent of insecticides for vector control but the emergence of resistance to insecticides in Malaria vector population can jeopardize this control effort. The main objective of this study was to evaluate, larvicidal and adulticidal properties of essential oil of local plants, namely, Ocimum canum, Hyptis suaveolens, Hyptis spicigera and Lantana camara extracts on Anopheles gambiae and their inhibiting activity of acetylcholinesterase (ACHE). Young branches with leaves were collected from local plants and the extraction of essential oil was processed. Adults of 3-5 old days and third-fourth stage larvae were used for bioassay tests based on WHO protocols. Different concentrations have been used for each plant essential oil and results have been assessed as mortality and Knock down rate of adults after 1 hour exposure. Larvae mortality after 24 hours and the lethal concentration were calculated for each essential oil. The inhibition effect of essential oils on acetylcholinesterase activity was assessed using Ellman’s spectrophotometric method. All tested essential oils exhibited adulticidal and larvicidal activities. The LD50 and LD90 lethal doses value observed were 0.82% and 1.55% respectively on adults for L. camara. On larvae, the LC50 and LC90 values of this oil were 61 and 125 ppm respectively. The high inhibitory activity on acetylcholinesterase was observed with O. americanum and H. suaveolens respectively. The high inhibitory activity on acetylcholinesterase was observed with O. americanum and H. suaveolens essential oil with 50% inhibitory concentration (IC50) of 0.21 and 0.55 µg/ml respectively. Our results highlighted that essential oils of these plants have a potential as insecticides for malaria vector control and can be considered as an interesting source of natural and ecofriendly substances for vector control.
FIELD EFFICACY OF VECTOBAC GR AS A MOSQUITO LARVICIDE FOR THE CONTROL OF ANOPHELINE AND CULICINE MOSQUITOES IN NATURAL HABITATS IN BENIN, WEST AFRICA

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The efficacy of Vectobac GR (potency 200 IU/mg), a new formulation of bacterial larvicide Bacillus thuringiensis var. israelensis Strain AM65-52, was evaluated against Anopheles gambiae and Culex quinquefasciatus in simulated field and natural habitats in Benin. In simulated field conditions, Vectobac GR formulation was tested at 3 dosages (0.6, 0.9, 1.2 g granules/m²) against An. gambiae and 1, 1.5, 2 g granules/m² against Cx. quinquefasciatus) according to manufacturer’s product label recommendations. The dosage giving optimum efficacy under simulated field conditions were evaluated in the field. The efficacy of Vectobac GR in terms of emergence inhibition in simulated field conditions and of reduction of larval and pupal densities in rice fields and urban cesspits was measured following WHO guidelines for testing and evaluation of mosquito larvicides. Vectobac GR caused emergence inhibition of ≥80% until 21 [20–22] days for An. gambiae at 1.2 g/m² dose and 28 [27–29] days for Cx. quinquefasciatus at 2 g/m² in simulated field habitats. The efficacy of Vectobac GR in natural habitats was for 2 to 3 days against larva and up to 10 days against pupae. In conclusion, treatment with Vectobac GR caused complete control of immature mosquito within 2-3 days but did not show prolonged residual action. Larviciding can be an option for malaria and filariasis vector control particularly in managing pyrethroid-resistance in African malaria vectors. Since use of larvicides among several African countries is being emphasized through Economic Community of West Africa States, their epidemiological impact should be carefully investigated.

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EFFECTS OF COMBINATIONS OF A SUNLIGHT-ACTIVATABLE PORPHYRIN AND NEEM TREE PARTS AGAINST LARVAE OF ANOPHELES GAMBIAE S.L., POTENTIAL COMPLEMENTING TOOLS FOR INTEGRATED STRATEGIES OF INSECTICIDE RESISTANCE MANAGEMENT

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Meso-tri(N-methyl-pyridyl), mono(N-dodecyl-pyridyl)porphine is a porphyrin known as C12. Loaded on ground cat food pellets (CF), the C12 has rapid killing effect on larvae of Anopheles gambiae s.l. The present study aimed at screening combinations of C12 and neem portions with the ability of both rapid killing and delayed-acting effects (additive effect) and subsequent residual efficacy (synergistic effect) on larvae of the malaria vectors using outdoor tray experiments. C12-neem combinations were obtained by incubating finely ground neem leaves (NL), bark (NB), fruit (NF) and 10% NeemAZA-loaded cat food (CFNA) in 500-μM C12 solutions. Batches of 60 larvae of wild or laboratory colonies (2nd - 4th instars) were exposed outdoors with 20 - 30 mg of C12-neem products in water samples from typical water bodies. Larvae treated with CF and C12-CF free of neem served for negative and positive controls respectively. Three consecutive experiments were run allowing to determine respectively larval mortality efficacy after i) a short time of 48 h post-treatment, ii) a prolonged-exposure up to 9 days post-treatment and iii) residual efficacy as larval mortality and subsequent inhibition of pupation up to 9 days post-treatment. Alike the C12-CF, C12-neem combinations particularly C12-NF and C12-NL yielded rapid killing efficacy of ~100 % larval mortality after 48 h post-treatment in spring water. This effect decreased in turbid water types. However, a prolonged-exposure allowed to achieve a delayed-effect of 70 - 100% mortalities in turbid water type after 4 - 5 days post-treatment. Upon a prolonged-exposure time of 9 days in spring water, C12-NF and C12-CFNA yielded residual effects of 70 - 100% larval mortalities. However, C12-CF yielded such residual activity for only 21% mortality. C12-NF possesses at least an additive efficacy on larvae of An. gambiae s.l. in this preliminary study. Further investigations should help to reinforce efforts towards the development and validation of such larvicidal tools in the context of integrated management of insecticide resistance.

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EVALUATION OF THE FACTORS AFFECTING THE FUNCTIONALITY OF INDOOR RESIDUAL SPRAYING AND LONG LASTING INSECTICIDE NETS PROGRAMS USED FOR MALARIA CONTROL IN WESTERN KENYA

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Indoor Residual Spraying and long-lasting insecticidal nets have been extensively used for malaria prevention and control in Kenya. However there are concerns that certain factors greatly affect the efficacy of these programs. We evaluated the persistence of ICON and deltamethrin on the sprayed mud walls and the bio efficacy of Long Lasting Insecticide nets. Wall bioassays were performed on artificial mud walls and filter papers sprayed with ICON and Deltamethrin using mosquitoes collected from seven sites in western Kenya and Kisumu strain as a control. Net cone bioassays was performed on nets collected from the fields using wild mosquitoes from Bungoma and Bungoma and Kisumu susceptible strain was used as a control. Chemical analysis of the netting material was done using gas chromatography. Kisumu strain was susceptible to the insecticides with 100% mortality. ICON persisted on the mud wall for six months where as Deltamethrin persisted on the Mud walls for four months. Sprayed artificial walls showed lower mortality rates compared to sprayed filter papers. ICON had high mortality rates on the mosquitoes compared to Deltamethrin. Mosquitoes from Chulaimbo, Ahero, Emakakha and Kisian showed susceptibility to both deltamethrin and ICON with the mortality rates ranging between 80%-85% but mosquitoes from Bungoma and Emutete had lower mortality rates to both ICON and Deltamethrin with mortality rates ranging between 69%-74%. Wild mosquitoes showed low mortality rates to LLINs nets collected from the field (60%-75%), compared to the control strain (100%). Net chemical analysis results indicated that there was no difference in the net chemical content ranging between 0.06 wt% - 0.13 wt%. In conclusion, insecticide resistance, persistent of the insecticide on the sprayed surfaces, bioavailability of the insecticide and the physical condition of long-lasting insecticide nets affect the efficacy of indoor residual spraying and Long Lasting Insecticide nets, therefore these factors should be considered in malaria control programs.
NEAR-INFRARED CAN DETECT PRESENCE OF WOLBACHIA IN FEMALE AND MALE Aedes aegypti

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Vector control strategy utilizing endosymbiotic bacterium, Wolbachia has been reported successful in Australia. The ability of Wolbachia pi marginLeftients to shorten the lifespan of mosquitoes and block dengue transmission simultaneously makes it a very effective vector control intervention. The success of such an intervention is determined by its ability to rapidly spread in a given mosquito population. Currently, Wolbachia infected mosquitoes can only be differentiated from non-infected population by use of Polymerase Chain Reaction (PCR) assay. However, PCR is costly and time consuming. In this study, we applied the Near-infrared spectroscopy to detect the presence of two pathogenic strains of Wolbachia pi marginLeftientis; wMel and wMelPop in male and female Aedes aegypti reared in the laboratory. Using cross validation technique, the accuracy of differentiating infected from non-infected females of the wMel and wMelPop Wolbachia was 86.5% and 85%, respectively. wMel and wMelPop infected males were predicted with an accuracy of 86% and 96% respectively. Furthermore, independent female sets of wMel and wMelPop were predicted with an accuracy of 84% and 97%, respectively whereas independent male sets were predicted with an overall accuracy of 90%. Where processing of large field samples are required to evaluate the efficacy of strategies involving Wolbachia, NIRS could be a complementary diagnostic tool as thousands of samples can be scanned daily. However, we recommend a further assessment of the accuracy of this tool to detect Wolbachia infected wild mosquitoes relative to PCR.

OVERDOMINANCE OF KDR MUTATION IN ANOPELLES GAMBIAE ON A BEHAVIORAL TRAIT UNDER INSECTICIDE SELECTIVE PRESSURE

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The anthropophilic Anopheles gambiae malaria mosquito females need a blood meal to mature eggs. Faced to the new physical and chemical barrier (i.e. the pyrethroid insecticide treated bed net), Anopheles mosquitoes exhibit evolutionary responses such as the Kdr mutation that provides physiological resistance to the pyrethroid insecticides family. Nevertheless, the impact of physiological resistance specifically Kdr mutation on the short-range of host seeking behaviour, when the host is under treated net remains poorly studied. In the present study, we investigated the ability of An. gambiae s.s. of the three genotypes of the Kdr mutation to find a hole in a piece of net (either untreated or insecticide treated) to reach a bait in a wind tunnel. Homozygous resistant mosquitoes were the less efficient to pass through an untreated holed net indicating for the first time a cost for the Kdr mutation in An. gambiae. This reduced performance was likely to be due to a reduced host-seeking activity as revealed by video-tracks analysis of the mosquitoes. When faced to insecticide treated net, heterozygous mosquitoes were the most efficient evidencing overdominance of the Kdr mutation under pyrethroid selective pressure. Our findings brought valuable new insights in the study of heterosis with an evidence of overdominance of a single gene through behavioral traits. These striking results raise a lot of questions the evolution of such insecticide resistance mutations in natural conditions.

ENTOMOLOGICAL MONITORING OF INDOOR RESIDUAL SPRAYING INTERVENTION IN LAKE VICTORIA BASIN REGIONS, TANZANIA

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In response to the success of indoor residual spraying (IRS) intervention in Kagera region, which resulted to the decline of entomological indices. The Government of United Republic of Tanzania through financial support of the US President’s Malaria Initiatives decided to scale-up the IRS programme in all regions of the Lake Victoria basin, Tanzania. Entomological monitoring activities were carried out at seven representative sentinel districts in Mwanza, Mara, Geita and Kagera regions where IRS intervention have been implemented from July 2011 - August 2012. Mosquitoes were collected using CDC light traps, Pyrethrum spray catch (PSC) and pit trap (PT) method to determine malaria vector species, abundance and sporozoites rates. Vector species were identified by PCR and sporozoite rates were determined by ELISA technique. To determine residual effect of insecticide on various sprayed surfaces, eight houses were selected in each sentinel site. Two to five days old 20 laboratory susceptible female Anopheles gambiae s.s. (Kisumu strain) were used. A total of 1713 anopheline mosquitoes were collected. Out of these, CDC light traps collected 879(51%), PT 755(44.5%) and PSC 79(4.7%). A total of 637 anopheline mosquitoes were identified to species. Predominant malaria species were An. arabiensis accounted 52.4%, An. funestus 24.8%, An. gambiae s.s 17.9% and An. parnensis 4.9%. A total of 972 mosquitoes were tested for sporozoites. The prevalence of sporozoite rates was 1.01% for P. falciparum. Bioassays results indicate that wood and cement surfaces have long retention rate. In conclusion, mosquito abundance indicates that high numbers of mosquitoes were collected indoors using CDC light traps. Generally, anopheline mosquitoes were collected in high number in the period of May and June in all sentinel sites. The IRS intervention may have contributed to the decline of malaria in the area.

PYRETHROID KNOCKDOWN RESISTANCE (KDR) HAPLOTYPES IN ANOPELLES GAMBIAE POPULATIONS FROM MALARIA SURVEILLANCE SITES IN THE GAMBIA

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Knockdown resistance (kdr) is a target-site resistance mechanism implicated for DDT and pyrethroid insecticides resulting from point mutations at the domain II of the voltage-gated sodium channel gene of Anopheles gambiae s.s. Indications of DDT and pyrethroid resistance among An. gambiae s.l. in The Gambia were reported in a recent bioassay study. The aim of the present study was to investigate the underlying target-site resistance mechanism and associated kdr haplotypes in the same mosquito population. A combination of allele-specific polymerase chain reaction (AS-PCR), sequencing and a high resolution melting (HRM) assay modified by use of primers further away from kdr point mutation on the sodium channel gene was employed to describe the kdr genotypes in over 1000 Gambian An. gambiae specimens from four sites analysed in previous bioassays. Five haplotypes of kdr mutations to pyrethroids describing, L1014S, kdr east and L1014F, kdr west mechanisms

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were identified with a total frequency of 15% mainly in Anopheles arabiensis. With the modified HRM assay, four unique melt profiles could be distinguished for kdr mutant genotypes representing six mutant haplotypes from polymorphisms at positions 1013 (T/C) and 1014 (T/A). Four kdr wild-type haplotypes resulting from 1013 (T/C), 1014 (T/A), 1033 (G/T), 1042 (C/G) and 1059 (A/T) substitutions could also be identified by different clusters of melting profiles and sequenced. The contribution of these different haplotypes to vector survival and impact on vector control remains undetermined. These results provide evidence of different pyrethroid kdr haplotypes in The Gambia and present HRM as a useful assay for rapid scanning of kdr-based insecticide resistance in a mosquito population.

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ELECTROPHYSIOLOGICAL RESPONSES OF DIFFERENT ANOPHELES GAMBAE RESISTANT STRAINS TO INSECTICIDE AND HOST ODORS

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Major mean of malaria vector control are based on use of insecticides. Their efficiency is threatened by widespread resistance mechanisms. In addition to the physiological resistance mechanisms already well studied, the issue of the behavioral modulation as cause or consequence of the resistance is largely overlooked. Nevertheless there are evidences that insecticide-based control tools alter mosquito behavior before any contact, suggesting that the mosquitoes can detect the presence of the insecticide. In the present study, we tested this hypothesis by investigating the olfactory responses of different resistant strains of Anopheles gambiae to host odors and volatile insecticide chemicals. We used An. gambiae females aged of 5-8 days old from different strains sharing the same genetic background but insecticide resistance mutations kdr and Ace1R. We run electroantennograph (EAG) experiments with different genotypes for these loci (SS, RS, RR) and compared the electrical responses of the antennae. We investigated the responses to the following odors: Octenol, human sweat odour, permethrin, deltamethrin, bendiocarb, propoxur. We described the dose-response relationship to all of these chemicals (except human sweat odour) and run a comparative analysis of the responses of each genotype for the two insecticide resistance loci. Genotypes responded differentially to the odor suggesting that resistance mutations impact olfactory sensitivity of mosquitoes to their environment. Moreover, sensitive strain, Kisumu, responded to permethrin, evidencing that mosquitoes are able to smell insecticide molecule.

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DETECTION OF G119S ACE-1R MUTATION IN FIELD-COLLECTED MOSQUITOES USING ALLELE SPECIFIC LOOP MEDIATED ISOTHERMAL AMPLIFICATION METHOD (AS-LAMP)

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Malaria vectors have developed resistance to the four families of insecticides available for public health purpose. The resistance to organochlorines, pyrethroids, organophosphates and carbamates has increased along with the increasing of the 1014F kdr and the G119S ace-1R mutations frequency in Burkina Faso. The spread of kdr and ace-1R mutations in malaria vectors is the main constraint for effective insecticide based control of malaria. There is an urgent need to improve resistance management using existing insecticides and new tools to assess resistance level on time for quick decision-making. With the aim of developing a fast and reliable tool to detect ace-1R mutation, we report an allele specific LAMP (AS-LAMP) method to detect the ace-1R mutation. We designed specific primers with the mutation on the 5’ end of the inner primers (BIP and FIP) and additional mismatch nucleotides to increase the specificity and distinguishing between the resistant and wild type of ace-1R alleles. Plasmids inserted with targeted DNA sequence as well as genomic DNA were used as DNA template to set up the detection method. The reaction was processed in a real time turbidimeter at 63°C for 75 min and detected by the turbidity values as well as by naked eye. The results have been confirmed by direct sequencing and the specificity and sensitivity were compared to RT-PCR using more than 100 mosquitoes. The primers designed for LAMP can distinguish between the wild type allele (ace-1S) and the mutated type allele (ace-1R). The detection time for the wild type homozygous is 50 min and 64 min for the heterozygous using the wild type specific primers. Using these primers, there is no amplification for the resistant type (ace-1R) until 75 min. For the ace-1R resistant type, the detection time is 51 min for the resistant homozygous and 55 min for the heterozygous, there is no amplification for the wild type (ace-1S) until 75 min when using the resistant type primers. The gel electrophoresis of the LAMP products confirmed that the amplification is primer-DNA specific, the primers can only amplify their target specific DNA. Comparing the AS-LAMP to RT-PCR for ace-1R detection, the sensitivity and specificity of the two methods were globally similar This fast and reliable detection method which can be performed with just a water bath at 63°C and yields a result detectable by the naked eye, can be used for resistance monitoring at the site of transmission for quick decision-making.

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LABORATORY SELECTION FOR PYRETHRIOD RESISTANCE IN FIELD ANOPHELES SINENSIS, THE MAJOR ASIAN MALARIA VECTOR

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Anopheles sinensis (Diptera: Culicidae) is one of the major malaria vectors in China and other Asian countries. Increased attention has been shifted to this species due to its wide distribution, high abundance, and modest susceptibility to malaria parasites. The rapid spread of insecticide resistance becomes the major obstacle for insecticide-based strategy of vector control, which has placed current national efforts in malaria elimination at risk. Since we already have colonized the insecticide-susceptible in An. sinensis, the establishing of insecticide-resistant population is essential for the genetic approaches to further understand the molecular mechanism of insecticide resistance and to enhance resistance surveillance in this species. In 2013, the natural An. sinensis mosquitoes (F0) were collected from regions, where pyrethriod resistance had been demonstrated in central China, and identified by the morphological characters on both adults and eggs to avoid the species contamination. The adults (F1) 3 days post emergence were treated by standard WHO tube bioassay with 0.05% deltamethrin, and the survivors 24 h post exposure were left for blood-feeding, followed by the forced mating method to overcome the low-mating rate. Eggs from each generation were reared through to adults and eggs to avoid the species contamination. The adults (F1) 3 days post emergence were treated by standard WHO tube bioassay with 0.05% deltamethrin, and the survivors 24 h post exposure were left for blood-feeding, followed by the forced mating method to overcome the low-mating rate. Eggs from each generation were reared through to adults and selected for resistance to deltamethrin in the same way. After the continuous pyrethriod selection, the survival rate in F3 generation significantly increased to 99.16% and 99.73% in male and female populations, respectively. Two types of kdr mutations (L1014F: TTG T T TT; L1014C: TTG T GT) at codon position 1014 of the para-type sodium channel gene were detected during the selection process, and the kdr mutation frequency reached to 100% in F2 and F3 generations, with a predominant kdr mutation L1014F. As a conclusion, we have selected and colonized the deltamethrin-resistant population from field An. sinensis, which would provide the fundamental material for genetic studies in this important malaria vector.
UNDERSTANDING THE VARIATION OF INSECTICIDE RESISTANCE IN ANOPHELES GAMBIAE S.S FROM CÔTE D’IVOIRE: TOWARD ALTERNATIVE OPERATIONAL STRATEGIES FOR MALARIA ELIMINATION

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Malaria control is heavily dependent on the use of insecticides that target adult mosquito vectors via insecticide treated nets (ITNs) or indoor residual spraying (IRS). In Côte d’Ivoire, where IRS is not implemented, ITNs and more recently LLINs remain the main control measure. We used different approaches based on literature review, field collections, Geographical Information System (GIS) and IR mapper tool to investigate the report the spatial and temporal variation of resistance to WHO-approved insecticide for LLINs and IRS. Overall, mosquitoes collection covered the period from 1993 to 2013, with 60 % of records from 1993-2003 and 40 % between 2004 and 2013. Data were stratified according to the four ecological zones of the country with pyrethroids (OP) and organochlorines (OC) largely tested compared to carbamates and organophosphates (X2 = 10.8, p<0.05). Organophosphate (52.2%) and carbamates (47.8%) were tested in 23 sites mainly, with no fenitrothion and pirimiphos methyl resistance detected in ecological zone 2, but multiple insecticide resistance detected in only four sites mainly located in zone 1. Within OP and OC, permethrin, deltamethrin and DDT were largely tested between both decades. Temporal trend based on three clusters gathering three yearly mean mortality data per insecticide (exception for DDT in cluster 1, no available data for 1993) showed non-significant increase of susceptibility to deltamethrin in cluster 2 (X2= 3.879, p= 0.144). We then checked the trend between clusters for each insecticide across zones. Overall, no significant difference was observed in ecological zone 1 between cluster 1 and 2 these insecticides (P> 0.05). Mortality to DDT has significant increase in in zone 3 (X2 = 49.94, p< 0.001), while non-significant variation was detected in 4 (X2 = 1.07 p= 0.300). Data of this study provide valuable information to National malaria control programme for vector control intervention. Deltamethrin could be used for vector control in several areas of ecological zones 3 and 4. In ecological zone 2 where no resistance to fenitrothion and pirimiphos methyl have been detected for most investigated sites, OP could be a potential alternative for IRS. Finally, in ecological zone 1, where multiple insecticide resistance was detected in four sites, new strategies and tools for vector control are needed for vectors control.

TARGET-SITE RESISTANCE MECHANISMS KDR AND ACE-1 PRESENT UNIQUE CHALLENGES TO REGIONAL MALARIA ELIMINATION IN THE AMERICAS

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In the region of the Americas, malaria transmission intensity tends to be low and/or focal, and as such, the Americas are seen as a region where malaria elimination may potentially be achieved. However, a variety of anopheline vector species displaying a wide range of breeding, bloodfeeding, and resting behaviors present a challenge for vector control strategies in the Americas. Strategies that successfully control malaria vectors elsewhere are not as well-characterized in the Americas, and little research has been conducted on how insecticide resistance might further compromise efforts for malaria control, and ultimately, elimination. For example, Anopheles albimanus are highly resistant to multiple classes of insecticides in coastal Peru, including an area where Plasmodium falciparum was recently re-introduced. Our data suggest that voltage-gated sodium channel target site resistance (kdr) has been present in albimanus throughout Mesoamerica for at least 20 years. To date, the kdr polymorphisms we have detected in An. albimanus throughout the region are L1014C (associated with permethrin, deltamethrin and beta-cypermethrin resistance in other anophelines) and L1014F (associated with resistance to DDT and a broad range of pyrethroids in other anophelines). We have also identified a novel mutation on the ace-1 gene and our data suggest that ace-1 gene duplication and balancing selection are allowing resistant phenotypes to persist at high levels with minimal fitness costs. Even in the absence of routine insecticide application for vector control, resistance selection pressure throughout coastal Mesoamerica and South America is likely maintained through the use of agricultural pesticides, as An. albimanus habitats are often associated with lowland crop cultivation. The high frequency of alleles associated with resistance to pyrethroids, DDT, carbamates, and organophosphates in these populations is worrisome and could pose significant vector control challenges as interventions are scaled up as part of regional malaria elimination strategies.

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WIDESPREAD EMERGENCE OF PYRETHROID RESISTANCE AND PRESENCE OF KNOCK DOWN RESISTANCE (KDR) MUTATIONS IN INDIAN Aedes aegypti POPULATIONS

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Aedes aegypti, primary vector of yellow fever in Americas, is the primary vector for dengue and chikungunya in India. Resistance to DDT, temephos, permethrin and deltamethrin has been reported in this species in America, Brazil, China, Thailand, Indonesia, Vietnam and many other tropical and subtropical countries; however there is scanty information about insecticide resistance of this species in Indian populations. We collected immature of Ae. aegypti from different geographical regions of India; Haryana, Bangalore, Chennai, West Bengal, Bhopal and Khandwa. Larvae/ pupae were allowed to emerge into adults. WHO standard insecticide susceptibility tests for DDT (4%), permethrin (0.75%) and deltamethrin (0.05%) were carried out on 2-3 days old adult females. We found varying susceptibility for different insecticides in different populations. Very high level of resistance against DDT was observed in Kolkata population (12.07% mortality) moderate resistance in Bangalore, Chennai and Haryana (55.13% - 66.16% mortality) and incipient resistance in Bhopal and Khandwa populations (91.11% mortality). Deltamethrin resistance was found to be moderate level in Bangalore, Chennai, (48.48% - 80.83% mortality) and low level in Bhopal, Kolkata and Haryana (82.48% - 83.58% mortality). Permethrin was still effective in most of the population showing moderate level of resistance Kolkata population (74.29 % mortality) and low level in Bangalore, Chennai, Haryana and Bhopal populations (92.31% - 98.37% mortality). Two populations (Haryana and Kolkata) tested for Malathion were found susceptible (100% mortality). Here we also studied a knock down resistance (kdr) mutation reported worldwide F1534C and found it few populations. This mutation was absent in Bangalore, Chennai, Khandwa and Bhopal populations. In Haryana population the frequency for mutant allele is very low 0.095 but the high degree of resistance in Kolkata population can be correlated to the high frequency of mutant allele 0.48. Resistance against pyrethroids is alarming and has negative effect on the success of pyrethroid based personal protection methods. Understanding of resistance mechanism is helpful for effective vector control strategies.

INSECTICIDE SUSCEPTIBILITY OF ANOPHELES GAMBAE S.S MOSQUITOES IN IBADAN, SOUTHWEST NIGERIA

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The emergence of insecticide resistance in Anopheles mosquitoes has great implications for malaria control in Nigeria. This study aimed to determine the dynamics of insecticide susceptibility levels and frequency of knockdown resistance (kdr) mutations in wild An. gambiae s.s. (Diptera: Culicidae) from Ojoo and Bodija areas of Ibadan, South-west, Nigeria. Insecticide susceptibility to pyrethroids, organophosphates carbamates and organochlorines was assessed using WHO bioassays. A subset of the mosquitoes exposed to pyrethroids and DDT was used for species and molecular form identification and their kdr genotyping was determined using the Taqman assay. The mosquitoes were resistant to pyrethroids and DDT but completely susceptible to organophosphates and carbamates. All samples were identified as An. gambiae s.s. Bodja sample comprised of the S form (91.4%) and the M form (8.1%) while one MS hybrid was recorded. All the mosquitoes screened in Ojoo belonged to the S form. The 1014F kdr mutation was detected at a frequency of 24.52% and 5.8% in Bodja and Ojoo respectively. No correlation was observed between kdr genotypes and resistance phenotypes. The results indicate that metabolic resistance probably plays an important role in the resistance observed and calls for a need to implement insecticide resistance management strategies.

EFFICACY OF OLYSET DUO® (A PYRIPROXYFEN AND PERMETHRIN BI-TREATED NET) AGAINST PYRETHROID RESISTANT ANOPHELES GAMBAE IN BENIN, WEST AFRICA

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Pyrethroid resistance threatens to undermine the efficacy of longlasting insecticidal nets (LNs) in Africa and without prompt action, the fragile gains in malaria control could be reversed. Alternative compounds which can replace or complement pyrethroids on LNs are urgently needed. Pyrpyroxfen (PPF), an insect growth regulator, reduces the fecundity and fertility of adult female mosquitoes. Mixing pyrethroids and PPF on LNs could provide personal protection through the pyrethroid and reduce vector abundance through the sterilising effects of PPF. Experimental hut studies were performed in Benin, West Africa to assess the efficacy of Olyset Duo, a new LN incorporating permethrin (pyrethroid) and pyrpyroxifen against pyrethroid resistant Anopheles gambiae which are difficult to control with current LNs. Comparison was made with LNs treated with permethrin alone (Olyset Net) and pyrpyroxifen alone (PPF LN). Laboratory bioassays were performed with resistant and susceptible strains of An gambiae to substantiate the hut studies. Mortality of wild pyrethroid resistant An gambiae in the experimental huts was significantly higher with Olyset Duo (40-50%) than with Olyset Net (20-30%, P<0.05). Olyset Duo also provided significantly higher levels of personal protection than Olyset Net. The reproductive rate of bloodfed pyrethroid resistant mosquitoes surviving in huts with the pyrpyroxifen treated LNs was significantly reduced relative to the control (71-100% reduction with Olyset Duo and 91-100% with PPF LN). Cone bioassay and tunnel test results were consistent with the experimental hut studies and showed complete sterilisation and life shortening effects in mosquitoes surviving exposure to Olyset Duo and PPF LN. The mixture net (Olyset Duo) demonstrated superior performance to the pyrethroid net (Olyset Net) in terms of mortality and personal protection; and also sterilised and reduced the lifespan of surviving mosquitoes. Combining PPF with pyrethroids on bednets shows potential for improved malaria vector control and management of pyrethroid resistance.

HOW LONG DO BED NETS LAST? PHYSICAL DURABILITY AND INSECTICIDE ACTIVITY OF LONG-LASTING INSECTICIDAL NETS AFTER THREE YEARS IN USE, CRUZEIRO DO SUL, BRAZIL

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As regional malaria control efforts in the Americas begin to consider the possibility of elimination, the role of vector control tools such as long-lasting insecticidal nets (LLINs) must be well-defined. With the number of malaria cases reported in the Americas yearly, LLINs have become...
an integral part of malaria control in the Brazilian Amazon region. The monitoring of LLIN durability is crucial to guide vector control programs and refine distribution and replacement strategies. In 2007, public health authorities in Brazil distributed 7,000 PermaNet 2.0 LLINs in Cruzeiro do Sul, Acre State covering all sleeping spaces in selected areas of the municipality. We evaluated the physical integrity and insecticide content of LLINs distributed in three high-transmission areas of Cruzeiro do Sul municipality after nets have been in use for 3 years. Physical integrity was evaluated by counting and measuring the holes on each of the five panels of the net (4 lateral sides and roof). Cone bioassays were used to assess the bioefficacy of the LLINs and a standard colorimetric assay was used to estimate the amount of deltamethrin available on the surface. Of the 27 evaluated LLINs, the lateral panels showed the greatest amount of physical damage, although most holes (61.3%) were <1.5 cm in diameter. Bioassay results indicated that only two LLINs achieved mosquito mortality >80%. The colorimetric test showed that 59.3% (16/27) of the nets had insecticide on the surface ≥0.36 µg/net, which is considered the minimal efficacy threshold. In summary, most LLINs contained holes. However, most were small, so the LLINs likely still provided some degree of physical protection against host-seeking mosquitoes. More importantly, bioefficacy was severely impaired. The colorimetric data also showed depletion of insecticide levels on the surface of the nets. These data suggested that the lifespan of LLINs in this part of Brazil should be further evaluated at time periods <3 years to help refine the planning of LLIN distribution and replacement strategies.

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ACCURACY OF GOOGLE EARTH FOR HOUSEHOLD ENUMERATION AND PLANNING OF AN INDOOR RESIDUAL SPRAYING CAMPAIGN FOR MALARIA IN CHIBOMBO DISTRICT, ZAMBIA

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Satellite imagery is increasingly used for public health research and operations. Indoor residual spraying (IRS) for malaria requires extensive planning including enumeration of structures and procurement of insecticides. Using free satellite imagery from Google Earth may be more efficient and cost effective than ground based processes for planning and monitoring IRS campaigns. In 2014, a 5 x 15 km area was selected and all structures were enumerated using Google Earth imagery; location, number of rooms in the house, and wall material based on the roof (tin, thatch or asbestos) was recorded. These were compared to IRS data from 2011 captured by the mSPRAY tool which collects GPS coordinates of households sprayed, the number of rooms in the house and the wall surface type. One hundred structures from the mSPRAY data and 25 visited by field staff were analyzed to compare Google Earth enumeration to ground processes. Google Earth was able to identify household locations within 10-meter accuracy. The number of rooms determined based on Google Earth showed strong agreement to data captured at the household with a mean difference of 0.26 (95% confidence intervals =-0.01, 0.52, p=0.06, 100% agreement to ground visits). Among thatch roof structures, agreement was 100%; tin or asbestos roof structures, however, frequently have unfinished walls meaning they are porous instead of non-porous. This may vary regionally; ground visits will help determine the proportion with finished or unfinished walls. Previously, ground enumerations required 25-30 trained technicians to visit each household in a designated area and conduct an extensive questionnaire for 30 days resulting in an estimated 7,200 structures enumerated (~900 person days). In comparison, a single trained GIS technician was able to enumerate the same structures in only 6 days (6 person days) using Google Earth. Google Earth and satellite-based enumerations may be useful and more cost effective in both planning for and conducting an IRS campaign to ensure high IRS coverage.

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CROSS RESISTANCE TO ALTERNATIVE MOLECULES AFTER LONG-TERM USE OF PERMETHRIN AND TEMEPHOS IN Aedes aegypti FROM YUCATAN, MEXICO

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Aedes aegypti chemical control campaigns have applied exclusively two insecticides, permethrin and temephos, during the last 12 years in Mexico. Recent reports of insecticide resistance threaten the effectiveness of such mosquito control strategy. An updated list of insecticides for vector control re-introduced the use of organophosphates, carbamates and other pyrethroids type I and II. Therefore, it is very important to track the susceptibility status to these molecules, as well as cross resistance patterns. We performed bottle bioassays using seven adulticides, including permethrin, bifenthrin, transfluthrin and deltamethrin; and the organophosphates chlorpyrifos and malathion, and one carbamate, bendiocarb. We also ran WHO larval assays using temefos. We calculated the lethal concentrations that killed 50% (LC50) of two Yucatan mosquito collections - Hunucma and Vergel – that differ in permethrin resistance profiles. LC50 for each insecticide were used to calculate the resistant ratios (RR) relative to the New Orleans susceptible strain. We found that the Vergel collection is highly resistant to permethrin (RR = 98 fold) and this phenotype also confers resistance to other pyrethroids type I and II (RR= 92-117 fold); however, it remained susceptible to organophosphates and carbamate adulticides (RR = 2 - 7 fold). Hunucma had moderate levels of resistance to pyrethroids (RR= 3-17 fold) and was also susceptible to organophosphates and carbamates (RR = 3 - 5 fold). Vergel and Hunucma were resistant to temefos by 21 and 17 fold relative to New Orleans, respectively. Temefos resistance phenotype in larvae seems to be unrelated with adult resistance against molecules that share the same mode of action, organophosphates and carbamates. These results indicate that a rotation scheme for insecticides could be suitable in these locations.

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USE OF A SEMI-FIELD SYSTEM TO EVALUATE THE EFFICACY OF TOPICAL REPELLENTS UNDER USER CONDITIONS PROVIDES A DISEASE EXPOSURE FREE TECHNIQUE COMPARABLE WITH FIELD DATA

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Before topical repellents can be employed as interventions against arthropod bites, their efficacy must be established. Currently, laboratory or field tests, using human volunteers, are the main methods used for assessing the efficacy of topical repellents. However, laboratory tests are not representative of real life conditions under which repellents are used and field testing potentially exposes human volunteers to potential disease. There is, therefore, a need to develop methods to test efficacy of repellents under real life conditions while minimizing volunteer exposure to disease. A lotion-based, 15% N, N-Diethyl-3-methylbenzamide (DEET) repellent and 15% DEET in ethanol were compared to a placebo lotion in a 200 sq m (10 m x 20 m) semi-field system (SFS) against laboratory-reared Anopheles arabiensis mosquitoes, and in full field settings against wild malaria vectors and nuisance-biting mosquitoes. The average percentage protection against biting mosquitoes over four hours under SFS and field setting was determined. A Poisson regression model was...
higher for \textit{An. darlingi} than for \textit{An. albimanus}. In addition, \textit{An. darlingi} showed a highest probability of occurrence in the endemic area Urabá-Bajo Cauca-Alto Sinú. This information contributes to future studies on the eco-epidemiology of these species.

\section*{Role of \textit{Anopheles balabacensis} in Transmission of Simian Malaria in Kudat Division, Sabah, Malaysia}

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Simian malaria, \textit{Plasmodium knowlesi} is currently a cause of human malaria leading to fatal infections. High proportion of knowlesi malaria cases were reported in Kudat Division. A study was conducted in Kudat division, Sabah, Malaysia from June 2013 to February 2014 to determine the vectors. A total of 1192 anopheline mosquitoes belonging to 13 species were collected from two localities in Pulau Banggi and one in Kudat using human landing catch (HLC). The mosquitoes were dissected, and the mid gut and salivary glands were examined for oocysts and sporozoites. From the study, \textit{Anopheles balabacensis} was the predominant anopheline, consisting 90.74\% of the total collection. They bite early in the night which accounts in 61.17\% came to bite before 2200 hours. There are 27 \textit{An. balabacensis} positive for malaria parasites, of which 6 was positive for sporozoites, 10 for oocysts and 11 had both. From the ongoing molecular work, mono-infection of \textit{Plasmodium inui} was detected in both midgut and salivary glands while mixed infection of \textit{P. inui}+\textit{P. knowlesi} and \textit{P.inui}+\textit{P. cynomolgi} were detected in salivary glands only.

\section*{Ecological niche model of \textit{Anopheles darlingi} and \textit{An. albimanus} (Diptera: Culicidae) in Colombia}


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The potential distribution of two primary malaria vectors in Colombia, \textit{Anopheles albimanus} and \textit{Anopheles darlingi} and the environmental factors that influence habitat occupancy were evaluated. Presence records were obtained from published literature from 1997-2011 and field collections performed between 2012 and 2013. Two ecological niche models (ENM) were developed per species using environmental and topographic layers: elevation, land cover and 15 of 19 bioclimatic layers from WorldClim. The models were evaluated using the area under the curve (AUC). The first model included 15 bioclimatic layers and altitude, while, a land cover layer was incorporated in the second model. AUC for the two \textit{An. albimanus} models was 0.94, with altitude being the most influential environmental variable. These results were consistent with the predicted potential distribution for \textit{An. albimanus}, mainly spanning low land areas of the Atlantic and Pacific Coasts. AUC for \textit{An. darlingi} was 0.97, and altitude was also the variable contributing the most to both models. Land cover was the second variable in contribution to the second \textit{An. darlingi} model (24.4\%), which is consistent with previous reports of a strong association of this species with forested covers. The potential distribution range was

\section*{Phenotypic stability in extrinsic incubation of West Nile virus strains in \textit{Culex tarsalis}}

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Temperature is a critical factor that regulates the efficiency with which West Nile virus (WNV) is transmitted by its mosquito vectors. The interaction between rates of WNV extrinsic incubation, vector biting and vector survival are key determinants of the virus’s range as well as the potential for its amplification to outbreak levels. Previous studies have suggested that extrinsic incubation rates of WNV are higher among recent WNV strains, while others have questioned this assertion. Here, we compare the founding North American NY99 strain with two other representative isolates from WNV’s initial incursion into California’s southeastern deserts and a recent strain from a hyperendemic area of California’s Central Valley. We expand on a previous degree-day model to compare the probabilistic range of extrinsic incubation periods among these strains, and we had expected that our more recent strains would exhibit accelerated extrinsic incubation compared to NY99, in agreement with the previously described emergence of a selectively advantaged strain in North America. However, at the two temperatures studied (22 and 30°C), we found no difference in the extrinsic incubation period in \textit{Culex tarsalis} between NY99 and the recent California isolates. We present our comparative findings among strains and to earlier literature, along with a model-based assessment of the epidemiological implications for the efficiency of WNV transmission.

\section*{Biting cycles of yellow fever and malaria vectors on UDS Navrongo campus in the upper east region of Ghana}

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Yellow fever and malaria are both infectious vector borne diseases with high mortality rates among humans and some animals. While yellow fever has a history of a 10 to 12 years cycle in Ghana with minor ones occurring at three-yearly intervals malaria is found at all times. Behavior such as host seeking, biting activities, resting and disease transmission potentials have considerable epidemiological importance for planning and implementing vector control programmes. However very little information is available on these parameters for both \textit{Aedes} species and \textit{Anopheles} species in the Navrongo area. We studied the comparative biting behavior of \textit{Aedes} and \textit{Anopheles} mosquitoes within the Navrongo campus of UDS. Female adult mosquitoes were collected both indoor and outdoor between the hours of 05.00 and 09.00 GMT, 15.00-17.00 GMT and 18.00-06.00 GMT using human landing collections. All mosquitoes were grouped and identified morphologically using appropriate taxonomic keys. 27 \textit{An. gambiae} complex were identified to species and molecular forms of \textit{An. gambiae} s.s. using PCR-RFLP. Also, the presence of the kdr gene mutation was estimated in the \textit{Anopheles} population. A total of 1072 mosquitoes were collected. Out of this, 61.8 \% were \textit{Anopheles}, 22.7 \% were \textit{Aedes} and 15.5 \% were \textit{Culex}. \textit{An. gambiae} complex was predominant of the \textit{Anopheles} genera constituting 59.6 \% whereas \textit{Ae. aegypti} was predominant (85.6 \%) of the \textit{Aedes} genera. \textit{Aedes} mosquitoes had a bimodal biting pattern peaking at 05.00 - 07.00 hours GMT in the morning and 17.00 - 19.00 hours GMT in the evening as compared to the \textit{Anopheles} species which had a unimodal biting behaviour peaking at 24.00 - 02.00 hours GMT. All the 27 \textit{An. gambiae} were \textit{An. gambiae}
susceptibility of wild and laboratory-reared An. darlingi to infections with *Plasmodium vivax* using membrane feeding assays (MFA). The study was carried out in Iquitos (Peru) in 2009-2010 and seven patients with positive thick smears for *P. vivax* malaria were enrolled in the study. Sexual and asexual parasites stages were recorded and subsequent mosquito feeding was performed using the MFA technique. To detect number of oocysts, mosquito midgut dissections were performed 8 days postinfection (pi) and counted by microscopy. To determine the proportion of infectious mosquitoes, salivary glands were dissected 14 days pi and performed an ELISA assay to detect VK210 and VK247 *P. vivax* strains. A total of 1333 An. darlingi females, 701 wild and 632 laboratory-reared, were used for this study. Our results showed that there was no statistically significant difference between oocysts means of wild and laboratory-reared An. darlingi (*p* =0.105). Likewise, no statistically difference was identified between sporozoites detection in the two groups (*p* =0.137, *p* =0.170, respectively). ELISA assays showed that VK210 was the most common strain, representing 59% and 63% in wild and laboratory-reared mosquitoes, respectively. In conclusion, the MFA might be a realistic ex-vivo surrogate technique for measuring first insights into malaria transmission, and provides the basis for human-mosquito interaction studies such as transmission-blocking vaccines studies.

**SUSCEPTIBILITY TO *PLASMODIUM VIVAX* AND INSECTICIDE RESISTANCE OF *ANOPEGES CAMPESTRIS* AND AN. SUBPICTUS ALONG THAI-CAMBODIAN BORDER**

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Changing malaria prevalence from *Plasmodium falciparum* to *P. vivax* along the Thai-Cambodian border has been observed. We have investigated the potential vectors that may contribute to this change in two selected endemic areas, Sa Kaeo and Chantaburi provinces. Two potential vectors, An. campestris and An. subpictus, were collected by cow bait or human landing catch during the wet and dry seasons of 2012-2013. In Sa Kaeo, 17.8% of 2,355 mosquitoes from 22 species collected were An. campestris. In Chantaburi 88% of 433 mosquitoes from 7 species collected were An. subpictus. Susceptibility to *P. vivax* for these mosquitoes was studied in laboratory using F1 to F15 progeny and artificial membrane feeding. Both species were found susceptible to *P. vivax*. The parasite developed normally to oocysts in the midguts and sporozoites in the salivary gland. In contrast, development of *P. falciparum* was not observed in either species when compared with An. dirus, the major malaria vector in Asia. Study of insecticide susceptibility indicated that An. campestris (F1 to F3) from Sa Kaeo had potential to resist 0.1% Bendiocarb, 1% Fenitrothion and 4% DDT. An. subpictus (F1-F3) had shown to resist 0.15% Cyfluthrin, 0.05% Lambda-cyhalothrin, 5% Malathion, and 0.05% Deltamethrin. The change in species composition and malaria vector biology on the Thai Cambodian border may contribute to the shift in the *P. falciparum* to *P. vivax* prevalence; therefore, the malaria control program including vector control will have to be planned accordingly.

**SEASONALITY OF MULTIPLE BLOOD FEEDING BEHAVIOR IN ANOPHELINE MOSQUITOES AND IMPLICATIONS FOR MALARIA TRANSMISSION IN NCHENELENG DISTRICT, ZAMBIA**

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As part of the Southern Africa International Centers for Excellence in Malaria Research (ICEMR) project, mosquito collections were performed during August-September 2012 and March-May 2013 in Nchelenge District, Luapula Province, Zambia. Located along the environs of Lake Mweru and Kenani Stream, Nchelenge experiences hyperendemic...
transmission and has the highest malaria infection rate in children under the age of 5 years despite implementation of indoor residual spraying (IRS) and long-lasting insecticide-treated net (LLIN) distribution. Center for Disease Control light traps (CDC LTs) and pyrethroid spray catches (PSCs) were performed at three villages along Lake Mweru and two villages along Kenani Stream. The collections revealed that during the dry season, *Anopheles funestus* sensu stricto is the dominant vector near both the lake and stream. In contrast, during the wet season, *An. gambiae* sensu stricto is the dominant vector in the lakeside villages, whereas *An. funestus* s.s. is the primary vector with secondary contribution from *An. gambiae* s.s. in the streamside villages. Both vector species are highly anthropophilic and *An. funestus* has a higher *Plasmodium falciparum* sporozoite rate than *An. gambiae*. In the wet and dry seasons, it was found that the *P. falciparum* infection rate of the vector populations was higher near the streamside villages than those of the lakeside villages. The multiple blood feeding rate for *An. funestus* is higher during the dry season, whereas the multiple blood feeding rate for *An. gambiae* is higher in the following wet season. As a result, the entomological inoculation rates (EIRs) for each vector during the dry and wet seasons are underestimated. The results also suggest that there are spatial differences in vector composition and their multiple blood feeding rates, which contribute to differences in human malaria risk during the dry and wet seasons. Overall, the vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and implications for malaria control in high-risk areas.

**INCONSISTENCY IN THE RELATIVE PERFORMANCE OF HUMAN LANDING CATCHES AND LIGHT TRAPS IN SAMPLING ANOPHELES POPULATIONS ACROSS DIFFERENT AREAS OF AFRICA**

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The need for surveillance of adult host-seeking mosquitoes is of foremost importance in determining levels of disease transmission and for appropriate allocation of interventions. The gold standard for estimating mosquito - human contact rate has thus far been based on Human Landing Catch (HLC), where human volunteers catch mosquitoes that land on their exposed body parts. This approach is necessitates exposure to potentially infectious mosquitoes, such a risk it is unethical calling the need for safer and accurate tools. Centers for disease control light traps (LT) have been used widely in malaria endemic setting as an alternative tool to HLC in estimating human biting rate (HBR). Here, multi-sites paired mosquito collections of LT against HLC are evaluated for their consistency in the sampling indoor host seeking mosquitoes. Regression models were applied to determine the site specific as well as the overall LT sampling efficiency and their trend across increasing mosquito density for two major malaria vectors across Africa, *Anopheles gambiae* sensu lato and *Anopheles funestus* sensu lato. Generally, LT were able to collect more mosquitoes than HLC, though the ratio of LT:HLC varied between sites and mosquito density. Across sites LT had an overall sampling efficiency of =1.07 [0.76-1.51] in sampling *An. gambiae* s.l. and =1.78 [0.90-3.44] in sampling *An. funestus* s.l.. There was variation in sampling efficiency of LT across mosquito densities and only in a few locations did LT sample proportionally to HLC. More often LT either underestimated human exposure by under-sampling or over-sampling at high mosquito densities, in particular for *An. funestus*. Such inconsistency necessitates calibration of LT against HLC for each location and across seasons. Also advises against the use of a single calibration factor across all geographical locations since no evidence of a geographical pattern in the sampling efficiency of LT against HLC was demonstrated.

**IDENTIFICATION OF THE FIRST OVIPOSITION ATTRACTIONT FOR GRAVID MALARIA MOSQUITOES USING NEWLY DEVELOPED METHODS FOR MEASURING OLFATORY ATTRACTION IN ANOPHELES GAMBIAE S.S..**

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Little is known about how *Anopheles gambiae* s.l. find aquatic sites to lay eggs. Some evidence suggests that they use both vision and smell to locate and select breeding sites. Like many nocturnal insects with efficient mechanisms for processing chemical cues, olfaction is suspected to play the major role in this process and has been the subject of many investigations often with contradictory conclusions. Over 2000 gravid mosquitoes were evaluated individually. Every mosquito was given two cups with oviposition media in a cage. We measured the number of eggs laid by individual mosquitoes and determined whether they laid eggs in more than one cup. Thereafter we developed cage experiments that takes into account newly described behaviour. In addition, a semi-field test was developed using modified BG sentinel traps. This test measured olfactory attraction of gravid females to substrates over time and space. Together these two tests enabled the screening and description of an assortiment of oviposition substrates. The number of eggs laid by individual mosquitoes varied widely. In addition individual mosquitoes skip-oviposited: laying their eggs in more than one cup on the same night. This is the first report of within-cage skip oviposition in the species and demonstration of how it could lead to an illusion of substrate preference with common egg count bioassays. A new two-tier two-choice cage test that adjusts for large variation and eliminates the risk of artefact preferences as a result of skip oviposition was developed and used to identify the first confirmed attractant (patent pending) for gravid *An. gambiae* s.s. Our study confirms that gravid mosquitoes use olfactory cues to select breeding sites. These findings pave the way for the development of new strategies for controlling malaria vectors; one that will also help target insecticide resistant and outdoor biting populations previously left out by indoor residual spraying and long lasting insecticidal nets.

**ANOPHELES DARLINGI RESTING BEHAVIOR USING INTERCEPTION SCREENS IN THE PERUVIAN AMAZON**

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*Anopheles darlingi*, the main malaria vector in the Neotropics, is highly anthropophilic. However, extrinsic drivers of host seeking and oviposition are poorly understood. Analysis of anopheline flight patterns within villages could enhance malaria risk assessment and control. Data from preliminary studies using a single barrier screen were problematic because several factors may have affected mosquito behavior. The aim was to
INVESTIGATING THE ROLE OF DIVERSE ANOPHELES IN TRANSMITTING ARTEMISININ-RESISTANT PLASMODIUM FALCIPARUM IN CAMBODIA - A CHALLENGE FOR MALARIA CONTROL AND ELIMINATION

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Camodia is a hotspot for the rapid evolution of artemisinin (ART)-resistant Plasmodium falciparum parasites, which endanger the effectiveness of all ART-based combination therapies (ACTs), and harbors an exceptional level of P. falciparum and Anopheles diversity. Subpopulations of ART-resistant and -sensitive parasites have recently been identified, but the role of diverse anophelines in transmitting them has not yet been investigated. We approached this question by screening field-collected anophelines from three Cambodian provinces for P. falciparum infection and infecting multiple Southeast Asian and other regional anophelines with ART-resistant and -sensitive parasite strains from Cambodia. More than 10,000 Anopheles mosquitoes from three provinces (Pursat, Western Cambodia, where ART resistance is established; Preah Vihear, Northern Cambodia, where ART resistance is emerging; and Ratanakiri, Eastern Cambodia, where ART resistance is rare) were screened for Plasmodium infection. Contrary to the current dogma that only one or two vectors efficiently transmit malaria in this region, we found 14 distinct anophelines infected with Plasmodium (at least 4 infected with P. falciparum and at least 10 infected with P. vivax) using nested PCR. Molecular analysis of rDNA ITS2 loci identified 27 distinct Anopheles species and revealed only a 50% concordance between morphological and molecular identification methods. P. falciparum infections of An. dirus A isolates from Western Cambodia and An. minimus, An. gambiae s.s., and An. stephensi lines were achieved in the laboratory. Rates of infection of these and diverse Cambodian anophelines by ART-resistant and -susceptible parasite isolates will be presented. The ability of multiple vectors to carry ART-resistant parasite subpopulations presents a startling challenge for the control and elimination of falciparum malaria in this region.
EFFORT OF HOLDING CONDITIONS ON THE DETECTION OF CHIKUNGUNYA AND VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN MOSQUITO POOLS

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Emerging and re-emerging arboviruses continue to be a threat to global public health. With the recent introduction of chikungunya virus (CHIKV) into the Caribbean and its potential spread across the Americas, there will be a need to increase surveillance of mosquito populations for viruses. Due to the tropical climate of many of the affected areas, it will be difficult to maintain a cold chain as the samples travel from collection sites to laboratories for testing. We determined how suboptimal holding temperatures affected the ability to detect viruses in pools of mosquitoes. Adult female Aedes albopictus and Ae. taeniorhynchus were inoculated with CHIKV or Venezuelan equine encephalitis virus (VEEV) suspensions, respectively, and placed at 26°C for 7 days. One infected mosquito was then added to a vial of 24 negative mosquitoes and then held at -70°C, -20°C, 4°C, 22°C, or 35°C for selected time intervals. Mosquito pools were triturated in cell culture media and processed for detection of CHIKV and VEEV. Samples were analyzed for both infectious virus by plaque assay and for viral RNA with real-time RT-PCR. At high temperatures the amount of infectious virus decreased rapidly, but virus in samples held at 4°C or lower remained relatively stable. In contrast, viral RNA was detectable from pools held at all temperatures and holding times by real-time RT-PCR, although Ct values increased as temperatures and holding times increased. These findings suggest that if viral RNA detection is the goal of surveillance efforts, then mosquito pools do not need to kept at 4°C. This enhances the feasibility of field-based arbovirus surveillance programs where maintaining a cold chain may not be a possibility.

SOCIOMETRIC AND ECOLOGICAL FACTORS OF Aedes aegypti PRODUCTIVITY IN DHAKA, BANGLADESH: IMPLICATIONS FOR DENGUE VECTOR CONTROL

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In the absence of commercially available dengue vaccine, the primary option for formulating dengue prevention strategies is vector control programming. Although numerous dengue vector control efforts have been implemented, none of them has proved to be sustainable in our research, we have argued that the general failure of vector control practices demands consideration of the interrelationships within the socio-economic system and ecological system. The first hypothesis we tested was that most Aedes aegypti pupae are being produced in a few types of household containers, and socio-economic characteristics of households are responsible for possession of container types. The second hypothesis tested in our study was that the risk of exposure to dengue virus (DENV) is directly associated with the magnitude of Aedes productivity. Two household (n=1200) level entomological surveys were conducted in the City of Dhaka (2011, 2012). During our second survey in 2012, blood samples from human population were also collected from members of the same households. During the 2011 entomological survey, a total of 3,651 immature Aedes was counted in 1,501 containers with water in 826 premises. The first hypothesis was supported by the data, revealing that 82.1% of total Aedes pupae were produced from only 9 types of containers. We applied multiple criteria, e., container use, two-step cluster analysis based on surrounding ecological variables of containers, and multivariate nominal regression model of container types, to classify the containers and to evaluate the role of household’s socio-economic factors.
for possessing the specific containers types. Most pupae were 3 of 10
types of container usage categories. The cluster technique revealed that
most pupae were produced from in-house, tap water-filled containers
with no vegetation nearby, and under shade. Household income ($2 = 11.9,
df = 4, p = 0.01) and purpose of storing water ($2 = 16.9, df = 4, p = 0.003)
were most important explanatory variables to affect possession of productive
containers types. ELISA and PRNT results of collected blood samples
showed high seroprevalence (80%) and 95 new DENV infections among
survey population. Interestingly, the correlation of number of pupae from
a household and seropositivity led us to infer that household income, the
purpose of water storage, and indoor potted plants are the most significant
factors of Aedes productivity and seroconversion.

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MOSQUITOCIDAL NECTAR DELIVERY: EVALUATION OF
CANDIDATE PLANT SPECIES
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A transgenic toxic protein delivered via nectar could be used to kill or
weaken mosquito males, which feed on nectar, and females, which require
nectar for energy to seek a blood meal. In addition, transgenic nectar
proteins could be used to deliver any peptide, such as a hormone, a lectin,
or antipathogen peptide, directly to the gut of wild mosquito populations.
However, most mosquito-attractive plants listed in the literature are
not amenable to genetic transformation. We selected plants with known
insect/plant symbioses and published plant transformation protocols for
evaluation of mosquito feeding attractiveness and the nectar protein
profile. In all tests, cages with 20 (10m/10f) *Culex pipiens or Aedes aegypti*
were tested and delivered similar results. Mosquitoes survived best in
cages with Impatiens plants (*Impatiens walleriana*), followed by passion
flower vines (*Passiflora edulis*), tropical milkweed (*Asclepias curassavica*),
red trumpet flower vines (*Campsis radicans*) and 10% sucrose control,
followed by castor bean (*Ricinus communis*). With red dye added to the
nectaries, mosquitoes were seen to ingest dye within a few hours, with
the speed and percentage of acquisition following the same order of plant
species. When Impatiens with dyed nectaries was placed in a large cage
with three other species with undyed nectaries plus an undyed sucrose
tube, 80% of the mosquitoes were dyed within one day, implying that
Impatiens nectar would be sampled by mosquitoes even in a complex
garden setting. Finally, the nectar protein profile was examined and
Impatiens nectar was found to contain a 20 kDa protein at 3 mg/ml. Since
*Impatiens walleriana* has a facile transformation protocol, currently in use
in the authors’ laboratory, produces seeds easily, and belongs to a mainly
tropical genus containing 800-1000 species, it represents an excellent
candidate plant system for the creation of a mosquitocidal nectar plant.

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THE UBIQUITIN PROTEASOME SYSTEM IS REQUIRED FOR
VENEZUELAN EQUINE ENCEPHALITIS VIRUS REPLICATION
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Proteins destined for degradation are tagged with multiple copies of
ubiquitin. Traditionally, the proteasome was restricted to disposing
of misfolded proteins or general recycling in the cell. Recently, the
ubiquitin proteasome system (UPS) has been depicted in roles such as
signal transduction and intracellular trafficking. Many viruses have
been implicated in utilizing or modulating the UPS to enhance viral
replication and/or to sustain a persistent infection. The mosquito-
borne virus Venezuelan Equine Encephalitis Virus (VEEV) belongs to the
Togaviridae family and is considered an important biodefense pathogen
and select agent. There are currently no approved vaccines or therapies
to treat the disease, therefore it imperative to identify novel targets for
therapeutic development. An initial screen of a limited FDA-approved
drug library indicated that Bortezomib (Velcade) inhibited replication of
the attenuated TC-83 strain of VEEV in infected cells without apparent
toxicity to the cell. We hypothesized that a functional UPS is required for
efficient VEEV replication. Proteasomal inhibitors deplete free ubiquitin
that may be needed to modify viral proteins for efficient viral budding.
Bortezomib, a depeptidyl boronic acid, specifically and reversibly inhibits
the 26S proteasome. We have shown that at non toxic concentrations
Bortezomib proved to be a potent inhibitor of VEEV replication in the
human astrocytoma cell line U87MGs. Bortezomib inhibited both the
virulent Trindad Donkey strain and TC-83 strain of VEEV. Additional
studies with virulent strains of Eastern Equine Encephalitis Virus and
Western Equine Encephalitis Virus depicted that Bortezomib is a broad
spectrum inhibitor of the New World alphaviruses. Time of addition assays
showed that Bortezomib may affect early time points in the viral life cycle
thus influencing viral replication. Mass spectrometry analyses indicated
that VEEV capsid was ubiquitinated, which was validated by western blot.
Subsequent studies revealed that capsid is stabilized in the presence of
Bortezomib in treated cells while the nuclear and cytoplasmic distribution
of capsid did not undergo any marked changes. Ongoing studies are
focused on elucidating the mechanism by which Bortezomib inhibits
alphavirus replication. This study will aid future investigations in identifying
host proteins as potential broad spectrum therapeutic targets for treating
VEEV infections.

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SINDBIS AND BUNYAMWERA VIRUSES CIRCULATE AMONG
PATIENTS WITH FEBRILE ILLNESS IN MFANGANO ISLAND OF
LAKE VICTORIA WESTERN KENYA
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Bunyamwera and Sindbis viruses are medically important arboviruses in the
tropical and subtropical regions of the world, with an estimated 2.5 billion
people being at risk. Early detection of any arbovirus infection has great
importance for the clinical management of patients, surveillance, and to
prevent possible disease outbreak. The aim of this study was to investigate
if patients with acute febrile illness without *Plasmodium* detectable by
microscopy or RDTs were arbovirus positive. Patients whose ages ranged
from 13 to 70 years with a mean age of 29 were used for the study. There
was preponderance of females (65%) over males (35%) among the
patients studied. Whole blood was sampled (n=105) from consenting
febrile patients at Sena Health Centre, Mfangano Island of Lake Victoria,
Kenya. Information on body temperature, type of occupation (indoor/
outdoor), clinical manifestation, nature of exposure (direct or indirect) to
animals and type of treatment received were collected using a briefed
close ending questionnaire. Arbovirus specific RNA sequences extracted
directly from blood were amplified by reverse transcriptase, multiplex PCR
and amplicons resolved by High Resolution Melt (HRM) for virus detection
and differentiation. Four patients (3.8%) tested positive for Sindbis virus
while two representing (1.9%) had Sindbis virus infection. Among all
the patients studied, 102 (97.1%) received anti-malarial treatment while
3/29 (9.9%) were treated with antibiotics. These findings demonstrate the
presence of arboviruses among febrile illness cases in Western Kenya and
the possibility of these viruses being the aetiological agents of in the cases
exist. In addition, inadvertent use of antimalarial and antibiotics treatments
for malaria negative cases should be discouraged. This study underscores
the need to differentially diagnose febrile illnesses for arboviruses to guide
and direct appropriate treatment and management.

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DEVELOPMENT OF EIÑAT VIRUS, A HOST-RESTRICTED
ALPHAVIRUS, AS A VACCINE VECTOR

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Eiñat virus (EIv) is an alphavirus isolated from a pool of mosquitoes collected in Israel. It replicates efficiently in insect cells but is unable to replicate in vertebrate cells. EIv is host-restricted at two points in its replication cycle: 1) attachment/entry, and 2) viral RNA replication. The host-restricted nature of EIv provides an opportunity to develop it as a potential “pseudoinactivated” vaccine vector. Our goal was to develop EIv chimeras as a new platform for alphavirus vaccines. For proof-of-concept, we chimerized EIv with eastern equine encephalitis virus (EeeeV), a highly virulent alphavirus that can cause fatal disease outbreaks in both humans and equids with high case-fatality rates. Our central hypothesis was that a chimeric alphavirus containing the non-structural protein genes of EIv and the structural protein genes of EeeeV would retain the vertebrate host restriction of EIv and provide safe, effective protection against lethal challenge with EeeeV. To test this hypothesis, we generated a chimeric EIv/EeeeV infectious cDNA using standard cloning techniques, and rescued the virus in insect cells. We then performed immunogenicity and safety experiments in mice. After a single vaccination, EIv/EeeeV protected mice against lethal challenge with EeeeV and produced a higher titer of neutralizing antibodies when compared to a commercial EEE vaccine for horses. Additionally, EIv/EeeeV showed no neurovirulence in suckling mice after intracranial inoculation. These results suggest that the chimeric EIv-based alphavirus vaccine platform represents a safe and efficient approach to protect against EeeeV and other highly pathogenic alphaviruses in mice.

DEVELOPMENT OF A CHIKUNGUNYA VIRUS RNA REFERENCE REAGENT FOR STANDARDIZATION OF NUCLEIC ACID TESTS

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Chikungunya virus (CHIKV) is an Alphavirus transmitted by the mosquitoes Aedes aegypti and Aedes albopictus, the same vectors that transmit Dengue viruses (DENV), a group of four Flaviviruses that often co-circulate with CHIKV in the same geographical area. CHIKV has caused explosive epidemics in Africa, Asia and Indian and Pacific Ocean islands. It recently appeared in the Caribbean islands, posing risk to the rest of the Americas. Some CHIKV human infections are asymptomatic. Most cause a febrile illness similar to that of DENV, characterized by high fever, polyarthralgia, headache, back pain, myalgia, nausea, vomiting, and rash. There are no vaccines or specific treatments for DENV or CHIKV. However, DENV infections require prompt differential diagnosis and not infrequently hospitalization to prevent fatalities. Laboratory diagnosis for these viruses is made by serology, viral isolation or by nucleic acid test (NAT), the most sensitive method. There are no FDA-approved CHIKV diagnostic or blood screening assays. The lack of a reference reagent for CHIKV is a barrier for proper evaluation of NAT assays. This work aims to produce a well-characterized CHIKV RNA Reference Reagent (CHIKV-RR) for use as a standard for evaluation of performance of existing assays, and to facilitate the development of NAT assays that fulfill the requirements for blood screening. The CHIKV-RR candidate was produced by (a) expansion of a CHIKV clinical strain in Vero cells; (b) heat-inactivation (HI) of the viral stock; (c) preliminary in-house titration of the HI CHIKV stock; (d) validation of results in external collaborative studies; (e) formulation of the Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration CHIKV-RR. Preliminary results showed that the CHIKV stock has a concentration of ~10^6 PCR-detectable units/ml. The final formulation of the CHIKV-RR was shipped to collaborators for a second round of testing. External results are expected within 3 months, when it will be subjected to statistical analysis for assignment of a final number of units.

EFFECT OF THE LYSOSOMOTROPIC COMPOUNDS ON CHIKUNGUNYA VIRUS IN VITRO

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Alphaviruses are mosquito-borne viruses that primarily alternate between the arthropod vectors and vertebrate hosts. These viruses utilize the class-II fusion protein-mediated viral membrane fusion in the acidic intracellular compartments during the process of viral entry. The treatment of lysosomotropic compounds has been extensively used to characterize the viral entry of alphaviruses in mammalian cell lines and have been found effective for the inhibition of viral entry. Such characterization has not been performed for the analysis of chikungunya virus (CHIKV) entry in mosquito cell lines. In this study, three lysosomotropic compounds, ammonium chloride, chloroquine and monensin were tested to evaluate the inhibitory effect of viral entry in vitro. The entry of CHIKV is assessed by the fluorescent signals from the expression of the green fluorescent protein (GFP) genetically engineered into the viral genome. The inhibition of viral entry was assessed at concentrations which maintained the viability of the cells. The effective dose50 of each compound was calculated based on the percentage of reduction in the fluorescent signals and the concentrations of the compounds. The results and the possible mechanisms responsible for the entry of CHIKV and alphaviruses into mosquito cells will be discussed.

INSIGHTS INTO THE SPREAD OF CHIKUNGUNYA IN BANGLADESH FROM A SEROREPREVALENCE STUDY

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Chikungunya virus is an emerging infectious disease in Southeast Asia. The first chikungunya outbreak in Bangladesh was reported in 2008 in Chapai Nawabganj, a district in northwest Bangladesh bordering India. Two more outbreaks occurred in 2011 in the same district. It is unclear whether these outbreaks are markers of large-scale transmission throughout the district or isolated events. We conducted a serosurvey in Chapai Nawabganj to better understand chikungunya dispersal and to determine factors associated with exposure. Between March — May 2013, we randomly selected 40 communities and selected all household members to participate in the study until we enrolled 30-50 individuals per community. We collected a blood sample and socio-demographic characteristics and typical travel behavior from all participants. We used IgG ELISAs (NovallsATM, Dietzenbach, Germany) to identify past chikungunya infection. We used a multivariate logistic regression model with a random intercept for each community to identify risk factors. Overall 6.7%
arthralgia affecting more than six million people worldwide. The causative virus, Chikungunya fever, is an important emerging arboviral disease.

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RESTRICTS CHIKUNGUNYA VIRUS REPLICATION

INTERFERON-INDUCED EXORIBONUCLEASE, ISG20, ELUCIDATING THE MECHANISM BY WHICH THE VACCINE RESTRICTS CHIKUNGUNYA VIRUS REPLICATION

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Alphaviruses represent a diverse set of arthropod borne viruses, many of which are important veterinary or human pathogens. One of the most medically relevant alphaviruses, Chikungunya virus (CHIKV), is the cause of an ongoing outbreak of arthritic disease in the Caribbean islands and South America and its spread to North America appears likely. Other alphaviruses such as Venezuelan equine encephalitis virus (VEEV) and Semliki Forest virus (SFV) cause encephalitis disease. The role of viral genetics determining these different disease phenotypes is unclear. E2, the alphavirus receptor binding protein, has been implicated as a critical determinant of tissue tropism and host range. For example, previous reports have demonstrated that domains A and B of E2 contain residues important for host range expansions; however, little research has been performed to implicate which critical domain (e.g., A, B, or C) of this protein confer variations in cell tropism. Therefore, we created chimeric viruses between a normally arthritic virus (CHIKV) and a normally encephalitic virus (VEEV or SFV) to probe the effect of each domain on cell tropism both in vitro and in vivo. Chimeric CHIKV/VEEV and CHIKV/SFV were not viable, likely due to high sequence dissimilarity between the viruses. In contrast, CHIKV/SFV chimeras were rescued successfully and replicated well in several cell lines, albeit to lower levels than parental viruses. All chimeric viruses were attenuated as compared to parental viruses. When delivered intracranially to type-I interferon deficient mice, the virus containing domain A and to a lesser extent domain B from SFV caused neuron degeneration and demyelination, similar to parental SFV. The chimera containing domain A from SFV appeared to cause more perivascular cuffing, similar to SFV. Transfer of these two domains from SFV to CHIKV altered tropism to reflect that of SFV. This indicated that the different domains of E2 may be playing an important role in determining cell tropism for alphaviruses. Further study of these domains may provide useful insight into alphavirus pathogenesis.

OUTBREAK IN THE MIAMI METRO AREA

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Currently, there are no approved vaccines or antivirals available for the treatment of CHIKV or related arthritogenic alphaviruses. We have previously demonstrated a crucial role for type I interferon (IFN) in the protection against musculoskeletal CHIKV disease in mice. Using a systems biology approach for identifying novel interferon stimulated genes (ISGs) with potent anti-alphaviral activity, our lab has identified a subset of genes with antiviral activity against CHIKV and its relatives. Among the genes identified with the greatest anti-alphaviral activity was the 20 kDa, nuclear exoribonuclease, ISG20. Using an inducible over-expression system and dominant-negative inhibitor, we have characterized this antiviral activity against CHIKV infection and identified early genomic translation as an early target of ISG20 function. Interestingly, over-expression of ISG20 also controlled the upregulation of a subset of other ISGs, independently of type I IFN production. Ongoing experiments are examining the mechanism, direct or indirect, by which ISG20 abrogates CHIKV genomic translation and the extent of host regulatory networks influenced by ISG20 upregulation. Our results indicate a crucial role for ISG20 in IFN-mediated protection against CHIKV infection and may reveal targets for future therapeutic intervention.

DISSECTING E2 PROTEIN DOMAINS INVOLVED IN ALPHAVIRUS CELL TROPISM

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Alphaviruses represent a diverse set of arthropod borne viruses, many of which are important veterinary or human pathogens. One of the most medically relevant alphaviruses, Chikungunya virus (CHIKV), is the cause of an ongoing outbreak of arthritic disease in the Caribbean islands and South America and its spread to North America appears likely. Other alphaviruses such as Venezuelan equine encephalitis virus (VEEV) and Semliki Forest virus (SFV) cause encephalitis disease. The role of viral genetics determining these different disease phenotypes is unclear. E2, the alphavirus receptor binding protein, has been implicated as a critical determinant of tissue tropism and host range. For example, previous reports have demonstrated that domains A and B of E2 contain residues important for host range expansions; however, little research has been performed to implicate which critical domain (e.g., A, B, or C) of this protein confer variations in cell tropism. Therefore, we created chimeric viruses between a normally arthritic virus (CHIKV) and a normally encephalitic virus (VEEV or SFV) to probe the effect of each domain on cell tropism both in vitro and in vivo. Chimeric CHIKV/VEEV and CHIKV/SFV were not viable, likely due to high sequence dissimilarity between the viruses. In contrast, CHIKV/SFV chimeras were rescued successfully and replicated well in several cell lines, albeit to lower levels than parental CHIKV. All chimeric viruses were attenuated as compared to parental viruses. When delivered intracranially to type-I interferon deficient mice, the virus containing domain A and to a lesser extent domain A from SFV caused neuron degeneration and demyelination, similar to parental SFV. The chimera containing domain A from SFV appeared to cause more perivascular cuffing, similar to SFV. Transfer of these two domains from SFV to CHIKV altered tropism to reflect that of SFV. This indicated that the different domains of E2 may be playing an important role in determining cell tropism for alphaviruses. Further study of these domains may provide useful insight into alphavirus pathogenesis.

ELUCIDATING THE MECHANISM BY WHICH THE VACCINE RESTRICTS CHIKUNGUNYA VIRUS REPLICATION

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Chikungunya fever is an important emerging arboviral disease characterized by painful and often persistent, flaring polyarthritis and arthralgia affecting more than six million people worldwide. The causative agent, chikungunya virus (CHIKV), is a positive sense, RNA virus from the genus Alphavirus, family Togaviridae, whose geographic distribution has recently increased to include Europe, the Caribbean and South America. Currently, there are no approved vaccines or antivirals available for the treatment of CHIKV or related arthritogenic alphaviruses. We have previously demonstrated a crucial role for type I interferon (IFN) in the protection against musculoskeletal CHIKV disease in mice. Using a systems biology approach for identifying novel interferon stimulated genes (ISGs) with potent anti-alphaviral activity, our lab has identified a subset of genes with antiviral activity against CHIKV and its relatives. Among the genes identified with the greatest anti-alphaviral activity was the 20 kDa, nuclear exoribonuclease, ISG20. Using an inducible over-expression system and dominant-negative inhibitor, we have characterized this antiviral activity against CHIKV infection and identified early genomic translation as an early target of ISG20 function. Interestingly, over-expression of ISG20 also controlled the upregulation of a subset of other ISGs, independently of type I IFN production. Ongoing experiments are examining the mechanism, direct or indirect, by which ISG20 abrogates CHIKV genomic translation and the extent of host regulatory networks influenced by ISG20 upregulation. Our results indicate a crucial role for ISG20 in IFN-mediated protection against CHIKV infection and may reveal targets for future therapeutic intervention.

MODELING THE POTENTIAL FOR A CHIKUNGUNYA OUTBREAK IN THE MIAMI METRO AREA

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Once found only in parts of Africa, southeastern Asia, and the Indian subcontinent, chikungunya has spread globally in the past decade with major outbreaks occurring in parts of Europe, La Réunion Island, and most recently in the Caribbean. The expansion of the disease has been facilitated in part by global travel, increased vector competence due to mutations in the virus, and increased ranges of habitats of the primary vector species, Aedes aegypti and Ae. albopictus. Expansion of the disease into the Western Hemisphere coupled with recent outbreaks and autochthonous transmission of dengue fever (which is vectored by the same species) in the U.S. raises concern that a chikungunya outbreak in the U.S. is imminent. Southern Florida, including the densely populated Miami metropolitan area, is particularly susceptible to an outbreak due to the tropical climate, cohabitation of Ae. aegypti and Ae. albopictus, and heavy travel between the region and tropical locations worldwide, particularly Central and South America and the Caribbean. To assess the potential for an outbreak in the Miami metro area, we developed a mathematical model parameterized to study chikungunya dynamics in this region. We utilize the model to study the impact that timing, location, and size of an introduction have on the ability of an introduction to lead to an outbreak. Further, we study the influence of variation in viral strains and differential competence between vectors on disease dynamics. We calculate the probability that introductions will lead to outbreaks in the Miami metro area and discuss the potential for the disease to spread to other parts of the U.S. We also highlight the flexibility of the model by proposing its utility in studying the potential for outbreaks of other diseases, such as dengue fever, in the same region.
HOST-PATHOGEN INTERACTION DYNAMICS OF HUMAN ASTROCYTES INFECTED WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS

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Venezuelan Equine Encephalitis Virus (VEEV) is an emerging arthropod-borne virus responsible for causing acute encephalitis, and often death, in animal and human hosts. VEEV is a priority pathogen that has previously been weaponized. Due to its continued environmental persistence in the Americas, it represents a significant threat to U.S. public health and economic security. The increased circulation and spread in the Americas of VEEV, and other encephalitic arboviruses such as Eastern Equine Encephalitis Virus (EEEV) and West Nile virus (WNV), underscores the need for research aimed at characterizing the pathogenesis of viral encephalomyelitis as a foundation for the development of novel medical countermeasures. In this study, we have sought to characterize the host-pathogen dynamics of VEEV in the human neuronal cell line U87MG by carrying out RNA sequencing of poly(A)+ mRNAs. We aim to identify critical alterations in the host transcriptome that take place within the first 24 hours following VEEV infection. Triplicate samples were collected at 4, 8, and 16 hours post-infection and RNA-Seq data acquired using an Ion Torrent PGM. Significant differentially expressed genes were part of the following super pathways: immune response IFN alpha/beta signaling, immune response IL-2 activation and signaling, regulation of nuclear SMAD2/3 signaling, and development glucocorticoid receptor signaling. Specifically we observed an increase in interferon regulated genes IFIT1, IFIT2, IFIT3, and OASL following VEEV infection. We also observed an increase in EGR-1 and differential expression of a number of genes that are involved in the EGR-1 pathway including ADAMT21, IFIT1, IFIT2, IFIT3, and OASL following VEEV infection. We also observed an increase in EGR-1 and differential expression of a number of genes that are involved in the EGR-1 pathway including ADAMT21, IFIT1, IFIT2, IFIT3, and OASL following VEEV infection. Data from these studies will be used to pinpoint differentially regulated genes and corroborate previously identified putative predictors of disease progression. As a whole, these data sets elucidate differentially regulated genes in the context of the host’s genetic background. Furthermore, this approach has the potential to enable discovery of genes not previously associated with particular disease states, and in so doing, lead to the development of improved vaccines, diagnostics and therapeutics.

POTENTIAL PROTECTIVE ACTIVITY OF DENGUE NANOVACCINE DELIVERY BY BCG/CHITOSAN NANOPARTICLE

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In this study, we report the development and testing of a novel dengue nanovaccine (DNV) composed of UV-inactivated DENV-2 (UVI-DENV-2), strain 16681 and BCG/chitosan nanoparticles (BCG/CS-NPs). We prepared BCG/CS-NPs by emulsion-polymerization method, yielding 299.1 ± 1.67 nm particles in size. The adjuvant properties of BCG/CS-NPs were tested using primary human immature DCs. A significant increase in the activation markers of DCs (CD80, CD86 and HLA-DR) and stimulation of IL-12 production were observed. By these properties, we used BCG/CS-NPs as an adjuvant and vaccine delivery system for the UVI-DENV-2, a vaccine immunogen. The inactivation and antigenicity of UVI-DENV-2 were confirmed by plaque assay and ELISA using specific monoclonal antibodies (4G2, 3H5 and 2H2). To prepare DNV, we loaded UVI-DENV-2 into BCG/CS-NPs which yielded DNV of 372.0 ± 11.21 nm particle in size. We also showed that up to 98.6% of UVI-DENV-2 found on DNV. We tested the immunogenicity of DNV in Swiss albino mice. Mice were vaccinated with three DNV doses, 15 days apart. The results showed that DNV stimulated anti-dengue IgM/IgG antibodies along with dengue-neutralizing antibodies in a dose-dependent manner, peaking 15 days post-dose three when using 10 µg of DNV per dose. We also demonstrated the cell-mediated immunity properties of DNV by flow cytometry. We showed an increase in the frequency of interferon (IFN)-producing CD4+T cells and CD8+ T cells after stimulation with 10 µg of DNV. We developed a mouse dengue challenge model to test the protective qualities of the DNV. Mice were vaccinated with three doses (10 µg each) of DNV and challenged with 10^6 PFU of a mouse-adapted DENV-2 (NGC strain) 14-day post dose three. Serum and spleen were collected at various dates post-challenge to measure viremia by RT-PCR. The decreasing of viremia in vaccinated mice indicated a potential use of the DNV in protecting against dengue virus infection.

COMMUNITY KNOWLEDGE AND ATTITUDES AND HEALTH WORKERS’ PRACTICES REGARDING NON-MALARIA FEBRILE ILLNESSES IN EASTERN TANZANIA

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Although malaria has been the leading cause of fever for many years, with improved control regimes malaria transmission, morbidity and mortality have decreased. Recent studies have increasingly demonstrated the importance of non-malaria fevers, what has significantly improved our understanding of etiologies of febrile illnesses. A number of non-malaria febrile illnesses including Rift Valley Fever, dengue fever, Chikungunya changes in cellular dynamics upon DENV infection. Specifically, we are able to pinpoint differentially regulated genes and corroborate previously identified putative predictors of disease progression. As a whole, these data sets elucidate differentially regulated genes in the context of the host's genetic background. Furthermore, this approach has the potential to enable discovery of genes not previously associated with particular disease states, and in so doing, lead to the development of improved vaccines, diagnostics and therapeutics.
virus infection, leptospirosis, tick-borne relapsing fever, Brucellosis and Q-fever have been reported in Tanzania. This study aimed at assessing the awareness of communities and practices of health workers on non-malaria febrile illnesses. Twelve focus group discussions with members of communities and 14 in-depth interviews with health workers were conducted in Kilosa district, Tanzania. Transcripts were coded into different groups using MaxQDA software and analyzed through thematic content analysis. The study revealed that the awareness of the study participants on non-malaria febrile illnesses was low and many community members believed that most instances of fever are due to malaria. In addition, the majority had unrealistic beliefs about the possible causes of fever. In most cases, non-malaria febrile illnesses were considered following a negative Malaria Rapid Diagnostic Test (mRDT) result or persistent fevers after completion of anti-malaria dosage. Therefore, in the absence of mRDTS, there is over diagnosis of malaria and under diagnosis of non-malaria illnesses. Shortages of diagnostic facilities for febrile illnesses including mRDTS were repeatedly reported as a major barrier to proper diagnosis and treatment of febrile patients. Our results emphasize the need for creating community awareness on other causes of fever apart from malaria. Based on our study, appropriate treatment of febrile patients will require inputs geared towards strengthening of diagnostic facilities, drugs availability and staffing of qualified health workers.

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NEW INSIGHTS INTO THE MOLECULAR EVOLUTION OF DENGUE VIRUS TYPE 4 IN PUERTO RICO OVER TWO DECADES OF EMERGENCE

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Dengue has emerged globally as a human health problem since the 1950s and is now the major arboviral disease infecting hundreds of millions of people annually. While some cases are asymptomatic, others develop a febrile illness (dengue fever) or even progress to severe dengue which can be fatal. Dengue is caused by any of four related viruses (DENV-1 to -4) that are maintained in endemic transmission in large urban centers of the tropics by Aedes mosquitoes. Since the late 1960s, Puerto Rico (PR), a major population center in the Caribbean, has experienced increasingly severe epidemics following the introduction of multiple dengue serotypes. A particularly severe outbreak in 1998 was dominated by a novel DENV-4 strain that evolved in PR, replacing earlier forms and spreading throughout the region. Here we show that this DENV-4 strain is genetically distinct based on unique changes in the NS2A and NS5 genes. Its replacement of earlier forms in Puerto Rico progressed rapidly from 10% of samples in 1994 to 95% in 1997, suggesting that strong natural selection played a role in its fixation. This study confirms that dengue viruses evolve through rapid lineage turnover driven in part by natural selection, important contributors to its ongoing emergence in human populations. In vitro experimental infection did not support higher replication rate as the cause of severity in the 1998 outbreak, however similar experiments are now being analyzed from Aedes aegypti mosquitoes.

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POOR CORRELATION BETWEEN BLEEDING AND THROMBOCYTOPENIA IN DENGUE

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Thrombocytopenia is a common finding in Dengue. Advice for prophylactic platelet transfusions in some guidelines has led to many un-necessary platelet transfusions. Threshold of platelet transfusions is often set at 20x10^9/L, an arbitrary figure set without clinical evidence. Hence, it is important to study the relationship of bleeding in Dengue to low platelet count. We studied all the patients with dengue infection who were admitted under and referred to the principal investigator at Asiri Hospitals PLC, Colombo, during a period of 24 months from 1st of June 2011. Dengue infection was confirmed by positive antigen and/or antibody test. DHF was diagnosed in patients who had ultrasonically confirmed fluid leakage. Study included 254 patients with 142(55.9%) males and 112(44.1%) females. Mean age was 28.28 yrs. While 118 of them had DHF, 136 had DF. Bleeding manifestations were seen in 47(18.5%) patients; GI bleeding (n=6, 2.4%), gum bleeding (n=4, 1.6%), PV bleeding (n=31, 12.2%) and occult bleeding (n=6, 2.36%). Bleeding in DHF patients was significantly higher than that of DF patients. (DHF: 36, DF - 11, p=0.000). Bleeding was significantly associated with low platelet count when all the Dengue patients were considered with a mean platelet count of 34.02x10^9/L (range 80-5x10^9/L) in bleeders and 53.66x10^9/L in those who had no bleeding (p=0.000). When only the patient with platelet counts <80x10^9/L were analysed, there was a significant difference in the two groups of patients with platelet counts >20 and <20x10^9/L (p=0.022). However, when the patients with minor bleeds (gum bleeding) were excluded, there was no significant difference between these two groups (p=0.136). Mortality rate in this study was zero. Of the total, 33(12.99%) had platelet counts <20x10^9/L, but only 10 of them had any bleeding. None had prophyactic platelet transfusions. Therapeutic blood transfusions were required in 18(7.1%) and 01 had both platelet and blood transfusions as they were hemodynamically unstable. Mean lowest platelet count of this group was 30.44x10^9/L and 56% had lowest platelet counts above 20x10^9/L. This study confirms that the transfusion threshold of 20x10^9/L in Dengue is arbitrary and relationship with clinically significant bleeding in Dengue and low platelet counts is not straight forward. Therefore, recommendations on prophylactic platelet transfusions need to be reconsidered.

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DENGUE HEMORRHAGIC FEVER VIRUS DETECTION IN Aedes MOSQUITOES, IBADAN NIGERIA

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Dengue Hemorrhagic Fever is cause by arthropod-borne virus that is transmitted to humans through mosquitoes bite. The subtropical climate of southern Nigeria is often hot and humid which promotes mosquitoes breeding. The consequences are reports of feverish illnesses most often attributed to malaria or label as fever of unknown origin when treatment is intractable. This study was carried out in Ibadan, southwest Nigeria to Detect Dengue Hemorrhagic Fever Virus in mosquito vectors and as a possible cause of fever of unknown origin in order to shed more light on the ecology of infectious disease in the locality. Five hundred and ten anthropophilic mosquitoes were captured, identified and processed for detection of arboviruses by molecular techniques. Nucleic acid was extracted from mosquitoes pool following homogenization in cell culture medium. Extracted RNA was thereafter transcribed to cDNA with random Hexamers. Thereafter, primers that primarily target alpha and flaviruses were used for gene amplification. Subsequently, gene specific primers for yellow fever, West Nile, Chikungunya, rift valley and dengue viruses were used to amplify conserved regions of the different gene segments. Amplified products were thereafter separated in agar gel electrophoresis and viewed in UV light with a transilluminator. Out of 17 mosquito pools, 7 Aedes species pool tested positive for Dengue Haemorrhagic Fever Virus. DENV-1 was found in 6 pools, DENV-2 in 0 pools, DENV-3 in 0 pools and DENV-4 was tested positive in 5 pools. We detected Dengue Haemorrhagic Fever virus in Aedes mosquitoes in the study area, this provide recent data on Dengue Haemorrhagic Fever Virus ecology in Nigeria. It is also an indication that arboviruses may contribute to the burden of fever of unknown origin in the sub-region and should be considered for public health interventions.

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in febrile and symptomatic children, from semi-urban areas of Burkina Faso. Due to the contextual/local limitations, it was important to explore different ways to obtain the information and to analyze the samples. Therefore, filter paper use was a feasible tool to perform molecular analyses needed to confirm the presence of the virus. These findings reveal the need to address research and actions towards non-malaria diseases in the region. In this case thinking about dengue as one of the causes for febrile diseases. (More data about the viral sequencing and epidemiological description of the cases will be available for presentation at the conference)

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PRESENCE OF DENGUE FEVER IN SEMI-URBAN AREAS OF TWO HEALTH DISTRICTS IN BURKINA FASO

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The global incidence of dengue has grown dramatically in recent decades. In Africa, particularly in Burkina Faso, it has been neglected or ignored, the last description of acute cases were back in 1982. All fever cases are systematically considered presumptive malaria cases. During a regular populations survey, unexplained persistent fevers facing anti-malaria treatment were observed. We therefore conducted an epidemiological investigation for non-malaria cases. The study was conducted in a panel of febrile children 0-10 years old, with temperature of 37.8°C or higher, living in semi-urban areas of Kaya and Zorgho. Rapid tests from SD Bioline Dengue Duo (AgNS1-IgG/IgM) were added to a panel of malaria rapid test. Samples on filter paper were taken from every patient with a rapid dengue test positive and for every tenth negative patient to perform qRT-PCR analyses. A total of 264 children were included, 153 from Kaya and 111 from Zorgho. Among them, 6.7% (18/264) had positive results in the two districts. In Kaya, 9.8% (15/153) positive cases were reported and 2.7% (3/111) in Zorgho. From all positive results, 11.1% were NS1 antigen (2/18) and 88.9% IgG/IgM (16/18) antibodies. The qRT-PCR results show the presence of DENV2, further analyses are ongoing to characterize the virus and to define the possible presence of other serotypes. Thirty years after from its last report, dengue presence has been document

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MOLECULAR DETECTION OF DENGUE VIRUS SEROTYPES CO-CIRCULATING WITHIN MOSQUITOES IN PERI-DOMESTIC ECOSYSTEMS IN NIGERIA

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Dengue Virus (DENV) causes devastating health impacts with an estimated 500,000 infections annually. The first DENV-1 isolate in Africa was reported from Nigeria. Reports of importation of DENV from West Africa into Europe have been increasing, yet the burden of the disease in Africa has not been estimated. Surveillance activities are weak or non-existent in many African countries. There is currently no vaccine against DENV, although several research activities are underway for an effective tetravalent vaccine. In this study we provide recent epidemiological data of co-circulating DENV serotypes in mosquitoes involved in peridomestic transmission. This is by far the most elaborate study in Nigeria with comparison between habitats where non-human primates are kept and aboreal habitats without nonhuman habitats where humans visit. The modified CDC light trap was used to catch both Aedes albopictus and Ae. aegypti from Agodi garden Ibadan; University of Ibadan zoo, botanical garden, botany forest Oyo State. They were sorted and identified morphologically into 26pools of 50mosquitoes each. It was screened after RNA extraction using primers designed to target and amplify the nonstructural proteins region of the genome using a thermalcycler and the amplicon was observed using a trasilluminator. Temperature of diurnal activity was between 26-36°C. The number of positive pools are as follows; DENV-1 (11), DENV-2 (14), DENV-3 (16), DENV-4 (10). DENV was detected in all 26pools and the minimal rate of infection was DENV-1 (22.2%), DENV-2 (44.4%), DENV-3 (77.8%) and DENV-4 (44.4%). Aedes albopictus was found to be more abundant all year round compared to Aedes aegypti, both competent in transmitting DENV. Co-circulation of four DENV serotypes within peridomestic cycles has been established in Nigeria. There is the need for immunological investigation of host factor interaction within the area, this may enhance vaccine production. Also, sustained surveillance activities in hospitals are encouraged to confirm clinical cases and improve patient care.

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THE ROLE OF DENGUE VIRUS MATURATION IN VECTOR INFECTION

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Dengue, a mosquito-borne flavivirus, affects up to 100 million people a year with over 40% of the global population currently at risk. Infections range from mild febrile illness to severe dengue hemorrhagic fever and dengue shock syndrome, both of which can be lethal. Present primarily in tropical and subtropical regions, dengue’s expansion into the U.S. and other areas has become a major public health concern. Due to antibody-dependent enhancement of a secondary infection, a dengue vaccine should contain all serotypes or risk putting vaccinated individuals at higher risk for severe disease. While progress has been made to develop a vaccine with the four established serotypes, the discovery of a fifth
possible serotype presents a significant setback. With no vaccine or specific treatment for dengue, understanding the viral interaction with the mosquito vector is particularly important. Knowledge regarding dengue pathogenesis in the mosquito could lead to novel treatments and preventative measures. Dengue is a positive-stranded RNA virus which contains three functional proteins: capsid protein C, envelope protein E, and membrane protein M. Immature virus within the cell is coated with immature M protein (prM). This protein is cleaved by furin during eocytosis, which results in mature particles coated with E protein. This process is imperfect and can lead to the release of viral particles in various stages of maturation. The infectivity of these particles has been well-documented in mammalian cells, with the addition of prM-antibody rendering immature particles infectious. However, since mosquitoes lack antibody receptors, the mosquito-specific infectivity of immature virus particles remains unclear. We and others have found that completely immature dengue particles are not infectious in mosquito cells in vitro yet the in vivo infectivity remains to be seen. It is possible that enzymes or other factors may render the immature stages of the virus infective to the mosquito. Recent and ongoing experiments have given us valuable insight into the function of these immature virus particles when it comes to vector infectivity. Results point to exciting new roles for viorn in various stages of maturity when taken up by the mosquito vector.

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HIGH PREVALENCE OF FLAVIVIRUS EXPOSURE AMONG PREGNANT WOMEN IN MYSORE, INDIA

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Dengue is a flavivirus infection whose prevalence has been steadily growing in recent years, especially in the developing world. It has also been shown to affect pregnant women by increasing the rate of cesarean section deliveries and risk of pre-eclampsia. This pilot study describes the baseline incidence of dengue in a sample of pregnant women in Mysore, India. While 349 samples (89%) were seropositive for dengue, there was no significant association with demographic factors that were studied. The high numbers of flavivirus infection however, indicate that further research is crucial to better understanding and implementation of preventive measures.

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MOLECULAR CHARACTERIZATION OF DENGUE VIRUSES IN THE PHILIPPINES 2008-2013

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Dengue is the most important arboviral disease in the Philippines with over 166,000 cases from January to November 2013. All four serotypes of dengue are found in the Philippines. However, to our knowledge, there have been no studies to characterize dengue strains at the molecular level in the Philippines. Molecular characterization is important to monitor changes in circulating strains. The aim of this study was to characterize the dengue strains circulating in the Philippines from 2008-2013. To date, 34 serotype 1, 18 serotype 2, and 24 serotype 3 samples have been sequenced and analyzed. Substitution rates were analyzed using BEAST software giving mean rates of 1.99x10-3 substitutions/site/year for serotype 1, 1.3136x10-3 substitutions/site/year for serotype 2, and 8.3243x10-4 for serotype 3. Single Breakpoint Recombination (SBP) and Genetic Algorithm for Recombination Detection (GARD) analysis showed potential breakpoints at 157 in capsid-premembrane gene of serotype 1 in seven samples and 194 in CprM gene and 587 in the Env gene of Serotype 3 in 4 samples. Initial analysis of the recombinant sequences of serotype 3 indicates recombination between genotypes I and IV. The recombination in both dengue serotypes 1 and 3 was first detected in samples from 2010 and serotype 1 recombinants were also detected in samples from 2011. The occurrence of these recombinants may be linked to virulence and improved transmission. These are probable factors in the evolution and dynamics of the dengue virus in the Philippines. No recombination was found in serotype 2 samples. More samples will be sequenced and analyzed with a target completion date of June 30, 2014.

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VIROLOGICAL AND SEROLOGICAL INVESTIGATION OF THE FIRST DENGUE FEVER OUTBREAK IN ETHIOPIA

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Dengue virus (DENV) continues to put billions at risk of life-threatening disease annually. Infection is enhanced when DENV is opsonized with sub- or non-neutralizing antibodies that augment entry into monocytes and dendritic cells through Fc-gamma receptors (FcγRs), a process termed antibody-dependent enhancement of DENV infection (ADE). It has also been suggested that besides augmenting entry, ADE occurs through other intrinsic factors activated by FcγRs, a process termed ADE. We recently reported a role for leukocyte immunoglobulin-like receptor B1 (LILRB1) in downregulating activating Fcγ-mediated signaling. We recently reported a role for leukocyte immunoglobulin-like receptor B1 (LILRB1) in downregulating activating FcγR-mediated induction of the interferon stimulated genes (ISG). Co-ligation of LILRB1 by antibody-opsinized DENV recruits the phosphatase SHP-1 to dephosphorylate Syk and hence reduce ISG induction. In this work, we test the hypothesis that reduced Syk...
A DENGUE HUMAN INFECTION MODEL TO EVALUATE THE PROTECTIVE EFFICACY OF THE LIVE ATTENUATED TETRAVALENT DENGUE CANDIDATE VACCINE TV003

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Dengue virus (DENV) has become the most important arbovirus worldwide with estimates of as many as 400 dengue infections occurring annually. These infections result in approximately 100 million symptomatic cases of which more than 2 million are classified as severe disease and approximately 21,000 result in death. Neutralizing antibody, the accepted immunologic endpoint chosen to move candidate DENV vaccines forward, was not predictive of protection against DENV illness in the field in a recently completed Phase 2b efficacy trial of the lead DENV candidate vaccine CYD. These results have made it more difficult for vaccine manufacturers to determine which candidates should be further evaluated in large efficacy trials in endemic areas where vaccine failure could predispose subjects to more severe disease should they subsequently become infected with DENV. A dengue human infection model (DHIM) would be useful in down-selecting candidate vaccines prior to testing in endemic areas as well as identifying putative correlates of protection. We have developed a DHIM to evaluate the protective efficacy of the live attenuated tetravalent dengue vaccine TV003 developed by the U.S. National Institutes of Health. In Phase I clinical trials, TV003 induced neutralizing antibody to DENV-1, DENV-2, DENV-3, and DENV-4 in 92%, 76%, 97%, and 100% of vaccinees, respectively. It induced seroconversion to all four DENV serotypes in 74% of vaccinated subjects and to three or more serotypes in 98%. Because the seroconversion rate to DENV-2 was lowest, we sought to evaluate protection elicited by the vaccine against a heterotypic strain of DENV-2. We vaccinated 24 flavivirus-naive subjects with a single dose of TV003; an additional 24 subjects received a placebo. Six months later, all 48 subjects received a challenge dose of 1,000 PFU of rDEN2Δ30, an under-attenuated DENV-2 vaccine candidate. DEN2Δ30 was previously demonstrated to induce viremia in 100% of inoculated subjects with a mean peak virus titer of 2.5 log10 PFU/mL. In addition, 80% of subjects developed a maculopapular rash and 40% developed neutropenia. The study is powered to detect 60% efficacy of TV003 to prevent viremia caused by rDEN2Δ30 (the primary outcome). Secondary outcomes include protection against rash and neutropenia. Preliminary clinical, virological, and efficacy outcomes of the trial will be presented.
ASC expressed CCR2 (48.3%) whereas only a minor population expressed CCR7 (29.4%) or CCR9 (22.8%). Low frequencies of ASC expressed beta1 (3.9%) or CD122 (5.7%) whereas ICOS, CD103 and CD137 could not be observed (less than 1%). The results suggested that large proportions of ASC may distributed to various organs and tissues including lymph nodes, lung, skin, gut and bone marrow whereas minor proportions mobilized to the central nervous system. The results obtained in this study provide novel insights on the target distribution of ASC to specific organ and tissue where it plays protective or disease enhanced roles during acute dengue infection.

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HOUSEHOLD LEVEL ECONOMIC BURDEN OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

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Dengue virus (DENV) is an arbovirus with global distribution. DENV was reintroduced to Peru in the 1990s and has been reported in Puerto Maldonado (population ~80,000) in the Peruvian southern Amazon Basin since 2000. This region also has the highest human migration rate in the country, much of it from areas where DENV is not endemic. Several studies have reported on the economic burden of dengue disease from societal, health care system or governmental perspectives but few have focused on the financial burden at the household level. We therefore sought to assess the household income diverted to costs incurred when a household member contracts DENV and to compare costs between recent migrants (RM) and longer-term residents (LTR), defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. We administered a standardized questionnaire to persons diagnosed with dengue disease at Hospital Santa Rosa in Puerto Maldonado from December 2012 to March 2013. We compared direct and indirect medical costs and compared between RM and LTR. Demographic data, socioeconomic characteristics and assets were also compared between RM and LTR. Of the 80 persons who completed the survey, 28 (35%) were RM. Each dengue disease episode cost the household an average of US$ 105 (SD=107), representing 24% of their monthly income. Indirect costs were the greatest expense (US$ 56, SD=87), especially lost wages. LTR households had a higher average monthly income than RM ones (p=0.041) and were significantly more affluent based on wealth index (p=0.002). Costs did not differ significantly between RM and LTR households. The study highlights the very significant financial burden incurred by households when a family member suffers dengue disease, especially for RM since their overall monthly income is lower.

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COMPARING FLUORESCENT FOCUS ASSAYS (FFA) WITH PLAQUE ASSAYS (PAS) IN DENGUE CONFIRMED CASES

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Plaque formation assays and mosquito inoculations are two widely used techniques to quantify dengue viruses. Each of these techniques has its limitations; for example, mosquito inoculations are sensitive but require regular maintenance of a mosquito colony, and the sensitivity of plaque assays (PA) depends on the type of cell line used and the ability of a particular virus to induce a desired cytopathic effect (CPE). The sensitivity of C6/36 Aedes albopictus cells to dengue virus (DENV) infection has been well documented; however, DENV causes little apparent CPE in this cell line. Fluorescent focus assays (FFA) are a variation of PA where focused areas of infection are visualized by detecting viral antigen with monoclonal antibody. To quantitate DENV infections in cell culture, we performed serial dilutions of all four DENV serotype seeds, infected Vero 76 and C6/36 cells using the FFA in 8-well chamber slides, and performed PA using BHK-21 clone 15 cells in 24-well plates. We also titrated 8 and 15 samples positive for DENV-2 and DENV-4 by viral isolation, respectively. Seed viruses showed higher titers of DENV-1, DENV-3 and DENV-4 using FFA in 2-3 days in both cells lines compared with 5-7 day in PA. Compared with PA, FFA in C6/36 cells resulted in higher titers of DENV in 14 samples (61%), similar titers in 2 samples (9%), and lower titers in 2 samples (9%). Only 3 samples (13%) were positive by FFA in Vero cells. Although more expensive than PA, FFA does not require the development of CPE in the infected cell monolayer. In conclusion, C6/36 cells are ideal for DENV titration in humans, increasing sensitivity and producing more accurate quantifiable DENV results.

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DESIGNING GOAL-ORIENTED DISEASE SURVEILLANCE NETWORKS: A DENGUE VIRUS CASE STUDY

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With public health facing increasing budget constraints, disease surveillance is at a critical juncture. To design effective surveillance under resource constraints, we propose a four-step process that produces a surveillance system through systematic evaluation and integration of candidate data streams. This methodology quantifies the performance of individual data streams in terms of the specified surveillance objectives, and prioritizes them for incorporation into surveillance systems. We demonstrate the utility of this method by designing a multi-objective Dengue surveillance network for Puerto Rico. Networks designed using this approach were able to effectively monitor regional and local Dengue outbreaks, track serotype dynamics, and provide early warning for large outbreak years. This system was predicted to perform at least as well as the existing system and only utilized 20% of the existing providers.

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LONGITUDINAL ANALYSIS OF SERUM AVIDITY FOLLOWING SECONDARY DENGUE VIRUS TYPE 2 INFECTION IN PATIENTS WITH DIFFERENT DISEASE SEVERITY

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The four dengue virus serotypes (DENV1-4) are responsible for the most prevalent mosquito-borne viral illness in humans. DENV causes a spectrum of disease ranging from self-limiting dengue fever (DF) to severe, life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Secondary (2°) DENV infection is associated with more severe disease, which is attributed to cross-reactive antibodies and T cells. However, antibodies and T cells can also mediate protection. The effectiveness of the antibody response is dependent on both the affinity and avidity of the antibody/antigen interaction, where avidity depends on the antibody isotype and the number and affinity of individual binding sites. We sought to determine how antibody avidity developed over time following 2° infection with DENV2 and whether avidity differed according to disease severity. We analyzed sera from 41 2° DENV2-infected subjects (DF, n=15; DHF, n=15; DSS, n=11) from an ongoing hospital-based study of pediatric dengue in Managua. IgG avidity against DENV2 whole virion antigen (DENV2 strain N172) was measured in samples collected during the acute and convalescent phases as well as 3, 6, 12, and 18 months post-illness using a urea avidity enzyme-linked immunosorbent assay.

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(ELSUSA). With a stringent cutoff of background absorbance <0.2 OD, a positive control absorbance of >5X background OD, and positive control within one standard deviation from a quality control plate, the avidity was calculated as a ratio between percent IgG bound in 9M urea-treated wells compared with PBS-treated wells. Longitudinal analysis of serum IgG avidity against DENV2, regardless of disease severity, paralleled our previously published kinetics of avidity in 2° DENV3 infections. Our data show a substantial increase in avidity from acute to convalescent phase followed by a decrease in avidity from the convalescent phase to 3-months post-infection, then a plateau. During the acute phase, individuals who would develop more severe disease demonstrated higher serum avidity against DENV2. In contrast, at 18 months post-infection, sera from individuals with more severe disease displayed lower avidity when compared to sera from cases with less severe disease. Taken together, these data show that the kinetics of serum avidity following 2° DENV2 and DENV3 infection are similar. In addition, the data suggest that serum avidity levels may correlate with disease severity.

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QUAD-COLOR B CELL FLUOROSPOT: A NOVEL APPROACH FOR ANALYZING THE SEROTYPE SPECIFICITY AND CROSS-REACTIVITY OF ANTIBODY-SECRETING CELLS IN DENGE VIRUS-INFECTED INDIVIDUALS

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The 4 dengue virus serotypes (DENV1-4), members of the flavivirus genus, cause the most prevalent mosquito-borne viral illness in humans, with >3 billion people at risk for infection and up to 96 million cases annually worldwide. DENV infection can result in self-limiting dengue fever or more severe life-threatening disease. Severe disease is thought to be associated with the presence of serotype cross-reactive antibodies (Abs) that enhance viral uptake into target cells during a heterologous secondary infection. In contrast, type-specific Abs after a primary infection are important for homotypic protection. However, little is known about the protective role of cross-reactive Abs. To date, studies have analyzed DENV-specific B cells using ELISPOT assays and flow cytometry or by generating monoclonal Abs (MAbs) from memory B cells (MBCs) or plasma cells. We have developed a novel approach to determine the frequency of serotype-specific and serotype cross-reactive MBC responses on a per-cell basis. Using a modified version of an ELISPOT and applying recent advances in fluorescent detection (FluoroSpot), we can evaluate MBC specificity to all 4 DENV serotypes simultaneously in individual cells. The MBCs from cryopreserved peripheral blood mononuclear cells (PBMCs) of DENV-exposed individuals were stimulated in vitro for 3-5 days to become Ab-secreting cells and then incubated in ELISPOT plates coated with a human IgG capture Ab for 1-2 days. Subsequently, OptiPrep-purified virions from the four serotypes (Nicaraguan DENV1-4) were added to bind to DENV serotype-specific and cross-reactive Abs secreted by the Ab-secreting B cells. Visualization was achieved using four mouse anti-E Domain III serotype-specific MAbs that were labeled with different fluorophores. Serotype-specific MBCs and MBCs cross-reactive to 2, 3, or 4 DENV serotypes were detected in different individuals, with type-specific and cross-reactive MAbs hybridomas used as controls. We are currently applying this method to analyze longitudinal samples (acute, convalescent, and 3, 6, 12, and 18 months post-illness) from primary and secondary DENV infections in a hospital-based dengue study in Nicaragua. This new technique will further the understanding of how MBC specificity evolves over time, how the serotype-specific and cross-reactive responses associate with disease outcome, and how serial infections with heterotypic serotypes drive MBC specificity.

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A NATURALLY OCCURRING SINGLE POINT MUTATION IN THE ENVELOPE PROTEIN CHANGES THE NEUTRALIZATION PROFILE OF DENV2 ASIAN 1 VIRUSES

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The four serotypes of dengue virus (DENV1-4) cause the most medically important arthropod-borne viral disease worldwide. Each serotype is comprised of several genotypes. A DENV2 genotype replacement event in Vietnam, where the previously dominant Asian-American (AA) genotype was entirely replaced by the Asian 1 (A1) genotype, was observed during 2003-6. We investigated the possible role of pre-existing immunity in driving genotype replacement by generating reporter virus particles (RVPs) representing the two genotypes and analyzing their neutralization profiles by patient sera collected in Vietnam prior to (1997-8) and after (2006-7) the replacement event. The Vietnamese 2007 samples neutralized the A1 better than the AA genotype, as did sera from post-secondary DENV2 infections in a Nicaraguan cohort. However, among A1 isolates...
that shared the 5 amino acid changes in charge or side chain between the E genes of A1 and AA – N83K, D203N, T226K, G228E, and H346Y – one A1 isolate had a different neutralization profile when analyzed using antigenic cartography with non-human primate antisera. Six A1 isolates were poorly neutralized by both homologous and heterologous antisera, but the outlier A1 was strongly cross-neutralized by DENV1 and DENV3 antisera and had a distinct substitution, K160Q. This mutation was observed in strains isolated at the end of the Vietnam DENV2/A1 epidemic, in a clade that persisted for multiple years. To test if this amino acid substitution determined differences in neutralizing antibody titers with human antisera, the A1 RVP with 160Q was mutagenized to K. Interestingly, the dominant neutralizing phenotype was lost in the K160K A1 RVP when tested with Nicaraguan cohort antisera from post-secondary DENV2 infections (1.7-fold reduction, p<0.001), post-primary DENV3 infections (2.4-fold reduction, p<0.001), and post-primary DENV1 infections (1.5-fold reduction, p<0.05). We are analyzing changes in neutralization titer to 160K and 160Q variants by a panel of monoclonal antibodies sensitive to virion structure to determine if mutations at position 160, located in a valley on the surface of E Domain I, alter the global structure of the virus. In sum, our findings from independent analyses indicate that a clade of viruses circulating for multiple years in Vietnam had a single point mutation in the E protein (K160Q) that significantly altered polyclonal neutralization, in particular by heterologous antisera.

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DIAGNOSTIC UTILITY OF ATYPICAL LYMPHOCYTES TO IDENTIFY DENGUE AND SEVERE DENGUE

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Dengue is the most prevalent mosquito-borne viral disease of humans worldwide. Dengue virus (DENV) infection can cause a range of outcomes, from asymptomatic infections and undifferentiated fever to dengue fever and severe illness. Prompt and accurate diagnosis of dengue, as well as the identification of severe cases, remains a challenge, in particular in resource-limited settings. Atypical lymphocytes, aka Downey cells or reactive lymphocytes, have been described in several infectious, autoimmune, and idiopathic disorders, including severe dengue. Atypical lymphocytes can be identified morphologically and quantified in blood smears simply using a light microscope. Here, we analyzed the diagnostic value of atypical lymphocytes, quantified as percentage of total lymphocytes, for the identification of 1) confirmed dengue cases and 2) severe dengue cases among suspected dengue cases. A total of 1,522 suspected dengue cases, aged 6 months to 14 years and enrolled in a prospective, hospital-based study in Managua, Nicaragua, from August 2005 to November 2013 were included (964 laboratory-confirmed dengue-positive and 558 dengue-negative cases). Daily measurements of atypical lymphocytes were performed (total: 4,416; range: 1-6 per case). On days 1 and 2 of illness, no difference was observed in the proportion of atypical lymphocytes between dengue-positive and dengue-negative cases. However, from day 3 on, the proportion of atypical lymphocytes was significantly higher in dengue-positive cases (p<0.01). On day 5, the best cut-off value for atypical lymphocytes was estimated to be 22%, with a sensitivity of 64.6% (95%CI: 61.0-68.8) and a specificity of 62.3% (57.1-67.3) for dengue positivity. We then compared severe dengue cases, defined using the WHO 1997 classification, 6% (95% CI: 4-8%) using the WHO 2009 classification, and 3% (95% CI: 0.2-9.3%) using standardized intervention categories. Models using the cut-point of total serum cholesterol <85mg/dL had cross-validated areas under the curve (AUC) ranging from 0.70-0.8. These results indicate the relatively specific ability of cholesterol to discriminate patients with severe dengue outcome from patients with mild dengue outcome when they first present to the hospital.

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LOWER CHOLESTEROL LEVELS ARE ASSOCIATED WITH SEVERE DENGUE OUTCOME

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Dengue virus is a flavivirus of worldwide importance, with approximately 9.97 billion people across 128 countries at risk of infection and up to 96 million dengue cases annually. Previous studies have shown that lipids and lipoproteins may play a role in modifying virus infectivity and the host’s immune response to infection. However, the relationship between total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and severe dengue outcome is unclear. We analyzed data from 789 laboratory-confirmed pediatric dengue cases and 447 other febrile illnesses (OFI) in a prospective hospital-based study in Managua, Nicaragua, between August 2005 and January 2013, and used three different classifications of dengue severity: World Health Organization (WHO) 1997, WHO 2009 and standardized intervention categories. To examine the association between total serum cholesterol at first presentation and risk of severe dengue outcome, relative risks and 95% confidence intervals (CIs) were calculated using modified Poisson models with robust standard errors, adjusted for day of illness and other confounders. We found that total cholesterol, HDL-C and LDL-C decreased over the course of illness and differed across disease outcome groups. Greater decreases were observed in LDL-C compared to HDL-C. Multivariate models showed that lower levels of total serum cholesterol were associated with severe dengue outcome compared to OFI and mild dengue outcome. At first presentation, for each 10 mg/dL decrease in total serum cholesterol, the risk of severe dengue outcome increased by 14% (95% CI: 6-22%) using the WHO 1997 classification, 6% (95% CI: 2-11%) using the WHO 2009 classification, and 3% (95% CI: -0.2-9.3%) using standardized intervention categories. Models using the cut-point of total serum cholesterol <85mg/dL had cross-validated areas under the curve (AUC) ranging from 0.70-0.8. These results indicate the relatively specific ability of cholesterol to discriminate patients with severe dengue outcome from patients with OFI and mild dengue outcome when they first present to the hospital.

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MOLECULAR SURVEILLANCE OF DENGUE VIRUS (SEROTYPES 1 TO 4) AND ITS SEVERAL GENOTYPES IN A MEDIUM SIZE CITY OF SOUTHEASTERN BRAZIL, DURING EIGHT EPIDEMICS YEARS

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Dengue is a viral infection transmitted by the bite of an infected Aedes mosquitoes. This illness is endemic throughout the tropics and subtropic (100 countries worldwide) with a geographic distribution similar to that of malaria, but more risk in urban and residential areas. The four serotypes
of dengue virus (DENV 1-4) (family Flaviviridae, genus Flavivirus) are antigenically and genetically distinct and their genotypes vary in virulence. Wherefore, the detection and analysis of spatial and temporal transition are essential. In this work we looked the Dengue viruses circulation in Sao Jose do Rio Preto (a 434K inhabitants) city from 2006 to 2014. Sao Jose do Rio Preto show subquente tropical and humid weather, ideal for the proliferation of vector and has annual epidemics and hyperendemic situations. We used serum samples of suspected DENV patients provided by the Public Health to profile DENV circulation by PCR. We analyzed 2,162 cases from January 2006 to January 2014. We amplified 1372 (63,45%) samples of DENV: 389 (28,35%) were positive for DENV-1, 177 (12,90%) for DENV-2, 494 (36,00%) for DENV-3, 312 (22,74%) DENV-4 and 4 (0,29%) DENV-1/4 coinfection. Studying the movement of DENV from 2006 to 2014 we get to see the introduction, extinction and re introduction of serotypes in the city. Up to now, we got 20 sequences of DENV-1 (inferred E protein) and 5 sequences of DENV-1 (full genome); 26 sequences of DENV-2 (E protein) and 4 sequences of full genome; and 31 sequences of DENV-4 (E protein) and 4 sequences of full genome. The phylogenetic reconstruction of serotypes 1, 2 and 4 show that the samples identified in this study grouped with genotypes that circulating in Brazil (genotypes V, Asian American and American, for types 1, 2 and 4 respectively). We have identified two lineages of DENV 1 circulating in the region ad the others serotypes showed no lineages divergences. Some E protein aa substitutions were detected between the two strains of DENV-1. This data shows that the phylodinamics of dengue circulation can be much more complex than expected even in a medium size city and reinforces the importance of DENV constant surveillance.

COMPARISON OF DENGUE TRANSMISSION INTENSITY ESTIMATES OBTAINED FROM DIFFERENT DATA TYPES

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With more than 2.5 billion individuals at risk and an estimated 390 million infections per year globally, the vector-borne viral infection dengue continues to be a major public health problem. Prior knowledge of local dengue transmission is essential to design effective vector control and vaccination strategies. Yet estimates of global distribution and transmission intensity remain highly ambiguous. Most of the models estimating dengue transmission risk have utilised notification data which depend heavily on the quality of the surveillance system. Furthermore since the majority of dengue infections are asymptomatic, assessing the true burden of dengue is difficult. We estimated the force of infection (λ) by fitting a catalytic model to age-stratified seroprevalence IgG data from 7 countries using a Metropolis-Hastings Markov Chain Monte Carlo algorithm. We also estimated λ and the probability of detection of sequential infections by fitting a catalytic model to case-notification data from Puerto Rico. We find that the estimates of the force of infection vary substantially depending on the type of data fitted, with seroprevalence data resulting in higher estimates of λ on average, and that reporting rates and sequential infections play a major role in how dengue burden is currently assessed. Although IgG data are unable to differentiate between the dengue serotypes, they can identify past asymptomatic infections, are easy and cheap to obtain and can provide an insight into local dengue dynamics. On the other hand, case notification time series data, although subject to reporting bias, is more readily available. Combining the two data sources allows a more robust estimation of dengue transmission intensity and helps to identify areas where clinical dengue surveillance may require improvement.

ESTIMATING DENGUE INTER-SEROTYPE INTERACTIONS FROM AGE-STRATIFIED SEROTYPE SPECIFIC DATA

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With an estimate of 390 million infections per year globally, dengue continues to be a major public health problem. While the majority of infections are asymptomatic, the presence of multiple serotypes circulating simultaneously in a population increases the risks of severe infection due to antibody-dependent dependent enhancement (ADE). Although costly compared to IgG ELISAs, plaque reduction neutralisation tests (PRNTs) can determine the infecting serotype of past infections, are cheaper than PCR and are considered the gold standard for routine serotyping. The serotype with the highest titre is regarded as the most recent infecting serotype. However due to the cross-reactivity of anti-dengue antibodies it is difficult to definitively determine primary and secondary infecting serotypes from PRNTs alone. Thus it is essential not only to identify populations previously exposed to dengue and thus at risk of severe dengue upon heterologous infection, but to estimate the degree of immune-mediated inter-serotype interactions. We developed a catalytic model to estimate serotype-specific forces of infection (λ) and the level of interaction between serotypes (susceptibility enhancement-inhibition following a primary infection) from cross-sectional PRNT data. The model was fitted to multiple PRNT datasets from 1985 - 2010 using a Metropolis-Hastings MCMC algorithm. We find that if one serotype has a significantly higher λ than the others, the basic reproduction numbers are driven by the dominant serotype. We can infer the probable order of sequential heterologous infections by comparison of the interaction parameters assuming interaction was dependent solely on the primary or secondary infection strain. All estimates were comparable to previously published values of R0 estimated from incidence data. Our models provide an insight into dengue transmission intensity in different countries at different time points and highlight the complex transmission dynamics of dengue.

METHOD DEVELOPMENT AND CHARACTERIZATION OF IN VITRO RELATIVE POTENCY (IVRP) ASSAY FOR A TETRAVALENT RECOMBINANT SUBUNIT ENVELOPE-BASED DENGUE VACCINE CANDIDATE

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Dengue is a widespread and potentially fatal disease, with 50 - 100 million infections per year, and over 2.5 billion persons at risk globally. Dengue has four serotypes and a potential vaccine should elicit strong and balanced neutralizing responses to each type to reduce the risk of dengue hemorrhagic fever. Dengue envelope glycoprotein has been the focus of subunit-vaccine development as it is the primary target of neutralizing antibodies. We have developed a tetravalent (DEN1, 2, 3, 4) recombinant subunit envelope (E) protein based Dengue vaccine candidate. An in vitro relative potency (IVRP) assay has been developed as an alternative to the in vivo immunogenicity assay to support product release and characterization. In this method, Dengue antigens are detected by neutralizing monoclonal antibodies (mAbs) specific for E protein of each serotype. The mAbs for IVRP were selected by neutralization activity, binding affinity and kinetics, and stability-indicating attributes. These mAbs were further characterized by other methods including Biacore and Western blot. The IVRP assay uses a sandwich ELISA format, and measures relative potency of the Dengue
antigen sample as compared to a reference standard. The IVRP method is highly specific for each serotype, has a precision of 10-20% RSD, and can be completed in 2-3 days. The relationship between antigenicity measured by the IVRP and immunogenicity measured by the mouse potency assay was evaluated by testing several heat stressed Dengue antigen samples in both methods. The results showed that stressed samples had decreased antigenicity by IVRP and decreased immunogenicity by mouse potency assay relative to untreated control samples. This study also suggests that the IVRP assay is stability-indicating. With good specificity, precision, stability-indicating attributes, and quick turnaround, the IVRP assay is suitable for use as potency release and stability assay for subunit based Dengue vaccine candidates. Furthermore, this method may serve as a good alternative to the variable and time-consuming mouse potency assay for vaccine product release for licensure in the future.

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MOSQUITO SALIVA FROM THE DENGUE VECTOR Aedes aegypti MODULATES ENDOTHELIAL CELL PERMEABILITY IN VITRO: A NEW MECHANISM FOR VIRAL DISSEMINATION?

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Dengue is the most important mosquito-borne viral disease, affecting >40% of the world’s population, especially in tropical and sub-tropical areas where the main mosquito vector, Aedes aegypti, circulates. Infection with any of the four dengue virus serotypes (DENV1-4) causes a wide spectrum of illness from dengue fever to severe, potentially fatal disease mainly associated with plasma leakage, hemorrhage and organ failure. The infection initiates when a DENV-infected female Aedes mosquito probing for blood injects saliva, together with virus, into the skin of its human host. Mosquito saliva is known to enhance replication and pathogenesis of many arthropod-borne pathogens. In DENV infection, A. aegypti saliva has been shown to both hinder infection of dendritic cells in vitro and increase DENV titers in infected mice. However, the early events after DENV inoculation into the skin and the role of mosquito saliva in regulating virus dissemination is not well understood. Here we examine the ability of salivary gland extract (SGE) from female A. aegypti to modulate endothelial cell permeability in vitro. Briefly, cultures of human pulmonary microvascular endothelial cells (HPMEC) grown on a transwell permeable membrane system as a model of endothelial barrier function in vitro were exposed to SGE (1 SGE unit=5 µl) through either the apical (AP) or basolateral (BL) membrane, and endothelial permeability was examined by continuously measuring the trans-endothelial electrical resistance (TEER). A sharp reduction in TEER after treatment with 2 SGE units was detected with both AP and BL treatment (60%, p<0.001 and 30%, p<0.05 reduction, respectively). The AP effect was greater after the second dose of SGE compared to BL (TEER ratio: 0.35-0.66), while the BL effect persisted for a longer time (up to 48 hours). These results suggest distinct mechanisms for modulating endothelial permeability by SGE. A complex mixture of anti-hemostatic, anti-inflammatory, and immunomodulatory compounds has been identified in saliva of blood-sucking arthropods. Experiments to determine the effect of SGE on the intercellular junctions by confocal microscopy and to identify the mechanism(s) conditioning the increased endothelial permeability in vitro are ongoing. These findings may suggest a new biological mechanism associated with modulation of endothelial permeability that may facilitate systemic viral dissemination.

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THE ROLE OF UBQUITIN-PROTEASOME PATHWAY IN DENGUE VIRUS EGRESS: LEARNING FROM THE Aedes aegypti MIDGUT

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The mosquito-borne dengue virus (DENV) is a cause of significant global health burden. In the mosquito vector, the virus first establishes infection in the midgut before systemic spread to the organs to cause persistent infection. In the midgut, however, infectious DENV titers reaches a peak approximately 8 days after an infectious blood meal but decreases thereafter despite continual viral RNA replication. The mechanism that decouples viral RNA replication and infectious particle production in the midgut may constitute a novel innate immune response employed by Aedes mosquitoes against DENV. Using RNA interference studies, we show in vivo that down-regulation of ubiquitin proteasome pathway (UPP)-related genes, including proteasomal subunits, B2 and B5 decouples RNA replication from infectious titer production. Mechanistically, inhibition of proteasomal function prevented virus egress but not DENV assembly by exacerbating endoplasmic reticulum (ER) stress within the unfolded protein response. The translational attenuation due to increased ER stress led to reduced protein levels of Exoc7, an exocyst complex component required for exocytosis. Our study thus identified a mechanism that contributes to the mosquito midgut barrier. This mechanism also appears to be amenable for clinical translation as inhibition of UPP in primary monocytes with the licensed proteasome inhibitor, bortezomib, inhibited DENV titer even at low nanomolar drug concentration. In conclusion, we suggest here that functional UPP is necessary for virus egress through exocytosis and the inhibition of which could be an effective therapeutic strategy.

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ALARM SIGNALS FOR DENGUE OUTBREAKS: A MULTI-CENTER STUDY IN ASIA AND LATIN AMERICA

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Dengue outbreaks threaten both endemic countries and those with low or no stable transmission. Outbreaks are detected late, and response is often inadequate; early detection of outbreaks is challenging, since no established set of indicators exist for defining an outbreak. Candidate alert signals have been defined, but a systematic literature review found no satisfactory analyses or validations of these indicators and our expert meetings in 2012 and 2013 found that no country uses dengue outbreak alert signals routinely for an early response. Within the context of a WHO/TDR-led Research Work Package, part of the European Union funded IDAMS research consortium (International Research Consortium on Dengue Risk Assessment, Management and Surveillance; www.idams.eu), a model dengue outbreak contingency plan based on the available evidence collected through country case studies and literature reviews, is being developed. The aim is to test the validity of alarm signals for dengue outbreaks through a retrospective study in 5 countries (Brazil, Mexico, Dominican Republic, Vietnam and Malaysia), including a large number of districts and 5-10 years of observation. The following candidate alerts are being explored for their ability to predict dengue outbreaks with a defined probability: increased number of dengue cases compared to previous years; entomological indices; levels of sero-positivity in blood samples;
changes in predominant serotype, age shift; climate data (temperature, rainfall and relative humidity). For each covariate, the Shewhart Method is used to quantify correlations between outbreak ‘alarms’ with known dengue outbreaks. Information about the ongoing analytical process and the results to date will be presented and discussed.

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**USE OF LOCAL OCEANOGRAPHIC INFORMATION TO PREDICT DENGUE IN ECUADOR**

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Dengue fever, a mosquito-borne viral disease, has increased in distribution, prevalence and severity throughout the tropics and subtropics. In this study, we explore the use of local oceanographic variables to predict dengue transmission in the southern coastal of Ecuador. We hypothesize that local oceanographic conditions, such as invasion of warmer water masses from the Panamá Bay and strengthening of the cold Humboldt Current, interact with the El Niño-Southern Oscillation (ENSO) to influence local climate and dengue transmission. We developed a statistical mixed effects model to evaluate the influence of local oceanographic variables (weekly sea surface temperature (SST) and sea sub-surface temperature in front of Puerto Bolivar and other coastal localities), ENSO, and local climate on the dengue standardized morbidity ratio from the city of Machala, El Oro Province (weekly SMR, 2003-2013). We found that SST at Puerto Bolivar, El Oro Province, was positively associated with dengue (3-month lag). We also found that the Coastal El Niño index, which was developed in this study using the SST of El Niño 1+2 region, was positively associated with dengue (6-month lag). These findings indicate that local oceanographic variables provide some predictive lead for forecasting dengue outbreaks, and consequently, the potential to be integrated into operative tools of epidemic management, such as climate-driven early warning systems.

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**CLINICAL AND HEMATOLOGICAL PARAMETERS THAT DIFFERENTIATE DENGUE VERSUS OTHER FEBRILE ILLNESSES IN VENEZUELA**

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Dengue is the most important vector-borne viral disease worldwide. Dengue can be asymptomatic or present a wide range of manifestations from mild dengue fever to more severe disease. To date, there are no vaccines or antiviral treatments for dengue. At the early stage of the disease, general signs and symptoms of dengue can be confused with other febrile illness (OFI) and a late dengue diagnosis can be fatal. Dengue in Maracay, Venezuela, is hyperendemic with co-circulation of the 4 serotypes. In this setting, a longitudinal observational study was set up in 2010 to identify clinical and laboratory parameters that could differentiate patients with dengue versus OFI. Patients presenting with fever and dengue clinical criteria were recruited from 3 designated health centers. Dengue infection was confirmed by IgM ELISA and/or RT-PCR. Patients were followed daily with clinical examination and sequential blood sampling at determined intervals up to 30 days. Severe cases were treated in a tertiary hospital and followed daily until discharge. Hematological parameters and serum levels of selected biochemical markers were determined in acute phase blood samples. Between August 2010 and August 2013, 254 individuals met the inclusion criteria of which 44% were positive for dengue, 31% of positive patients developed alarm signs, while only 6% developed severe dengue. All four serotypes were detected in patients, with DENV-3 predominating. Using logistic regression and mixed effect models clinical, hematological and biochemical parameters where analyzed comparing the first 3 days with days 4-7 after fever onset. Rash, hemorrhagic manifestations including a positive tourniquet test, a concomitant decrease in platelets (~10,000 platelets/day) and white blood cell count and the absence of sore throat were associated with dengue infection in the first 3 days of the disease. A higher proportion of patients with hemorrhagic manifestations were present in days 4-7 after fever onset. A final model of determinants will be presented.

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**GLOBAL ECONOMIC COST OF DENGUE CASES TREATED IN THE MEDICAL SYSTEM**

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As the most important vector-borne viral disease, dengue is a serious and growing global public health problem, with 2.5 billion (b) people at risk. To estimate the global cost of symptomatic dengue cases treated in the professional health-care system, we reviewed the literature for numbers and cost of dengue cases by country. Using Bhatt et al. (2013) and Brady et al. (2012), we identified 134 countries with active dengue transmission. Shepard et al (2010 and 2013), supplemented by recent field data from India, Mexico, and Philippines, provided the number of dengue cases in the Americas and Southeast Asia. We adjusted the WHO reported dengue cases for South Asia using an expansion factor estimated for India (282). For the rest of the world, we multiplied the number of apparent dengue infections from Bhatt et al. (2013) by 16.25%—the ratio of cases in medical settings to total cases projected in Southeast Asia by Bhatt et al. (2.92 million (m)/17.96 m). To estimate the economic cost of dengue illness (both direct and indirect), we reviewed the literature for 5 studies reporting overall economic cost of dengue complemented by recent field studies from 3 countries. Collectively, these provided the per capita cost of dengue illness for 58 countries. We estimated the log of this per capita cost as a function of log of GDP per capita and log of dengue incidence per 100,000 population (p.<0.001, R²=0.66) and calculated estimated values for the remaining 76 countries. Bootstrapping the selection of 58 countries used with replacement generated a range of estimates. Multiplying the cost per capita times the population gave the aggregate cost of dengue by country, region, and globally. Preliminary results show a total of 30 m locally transmitted symptomatic dengue cases annually treated in the medical system in 134 countries with transmission. The estimated annual global aggregate cost of dengue illness is $8.65 b (range: $3.98-$8.80 b) or $1.58 (range: $0.73-$1.61) per capita. Of this, $3.77 b (44%) was incurred in 8 countries in South Asia; $1.95 b (23%) in 28 countries in East Asia and the Pacific, $2.47 b (29%) in 44 countries in Latin America and the Caribbean; $0.31 b (4%) in 42 countries in Sub-Saharan Africa, $0.14 b (2%) in 6 countries in the Middle East and North Africa, $5.6 m (0.06%) in 4 countries in Europe and Central Asia; and $3.4 m (0.04%) in North America. If control strategies could reduce dengue appreciably, billions of dollars would be saved globally.
VIRUS E LINKER REGION IN MOSQUITOES

CHARACTERIZATION OF A MUTATION IN THE YELLOW FEVER VIRUS E LINKER REGION IN Aedes aegypti Mosquitoes

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The linker region of the flavivirus envelope (E) protein connects the C-terminus of the domain I and the N-terminus of the domain III and was first hypothesized to accommodate the mechanical forces resulting from structural rearrangement that occur during viral membrane fusion. Available evidence further demonstrated the importance of the linker region in viral particle assembly and secretion of progeny virions. These conclusions were derived from in vitro models since all the mutations in the linker region of DENV-2 E protein are lethal and prevent production of infectious virions, thereby precluding in vivo studies. We evaluated the phenotypic effect of a methionine to isoleucine mutation generated at position 299 in the yellow fever virus (YFV) E gene that occurred during Yellow Fever virus propagation. Using the YFV reverse genetics system and serial passage of the wild-type Asibi strain to derive the attenuated 17D vaccine virus, we characterized the infection, replication and dissemination phenotypes of the mutant and parental viruses in mosquitoes. Further research is needed to evaluate more fully the risk of vertical transmission of such viruses via this route. Although vertical transmission of Dengue Fever and West Nile has been documented previously, little information exists regarding transmission of Yellow Fever via breast milk.

MERCADEO VIRUS: A NOVEL INSECT-SPECIFIC-FLAVIVIRUS

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The genus Flavivirus (Flaviridae, Flavivirus) is a heterogeneous group of viruses; some of them are important arthropod-borne-virus for both human and veterinary medicine. Recently an explosion of insect-specific flaviviruses has been documented. Although, many flaviviruses have been isolated from humans, field-collected-mosquitoes and birds in Panama, insect-specific flavivirus has never been detected in this Central American country. During 2011, mosquitoes were collected in Darien province close to the border of Colombia, where an outbreak of alphaviral encephalitis in both human and equines was reported in 2010. A number of virus isolates were obtained in C6/36 mosquito cell culture from specific pools of Culex Melanoconion spp. and Cx. Culex spp. Structural and antigenic characterization placed these viruses in the flavivirus group and their complete genome sequence was obtained using illumine next generation sequencing. Phylogenetic analysis based on the ORF of two isolates shows that our isolates grouped within the insect-specific clade of flaviviruses, closely related to cell fusing agent and Nakiwogo virus that were isolate in Uganda. Based on the location of the mosquito collection site, we named this virus strains as “Mercadeo virus”. Here, we report the discovery and characterization of an insect-specific flavivirus in Panama. Our results highlight the importance of combined viral isolation, structural, antigenic and genetic characterization for virus discovery and taxonomic designation.

HEAT SHOCK COGNATE PROTEIN 70 ISOFORM D IS REQUIRED FOR CLATHRIN-DEPENDENT ENDOCYTOSIS OF JAPANESE ENCEPHALITIS VIRUS IN C6/36 CELLS

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Japanese encephalitis virus (JEV) generally enters host cells via receptor-mediated clathrin-dependent endocytosis that requires the involvement of the 70 kDa heat shock protein (Hsp70). Hsc70 is one member of the Hsp70 family and is mainly constitutive; thus, it may be expressed in the absence of stress. In C6/36 cells, Hsc70 is up-regulated in response to JEV infection. Since Hsc70 shows no relationship with viruses attaching to the cell surface, it probably does not serve as the receptor although it has been reported to exist on the cell face. In contrast, Hsc70 is evidently involved in virus penetration and the resultant acidification of intracellular vesicles, suggesting that it is highly involved in clathrin-mediated endocytosis. The effects of Hsc70 occur particularly at a late stage of viral entry into host cells. Furthermore, we found that Hsc70 is composed of at least three isoforms, with isoform D being the one that helps JEV penetrate C6/36 cells via clathrin-mediated endocytosis. This study provides relevant evidence that sheds light on the regulatory mechanisms of JEV infection in host cells.
THE OBSERVED AGE DISTRIBUTION OF CONFIRMED CASES

INFERRING THE YELLOW FEVER FORCE OF INFECTION FROM THE OBSERVED AGE DISTRIBUTION OF CONFIRMED CASES

ANALYSIS OF SEROLOGICAL SURVEYS FOR YELLOW FEVER IN AFRICA

ROLE OF ENDOGENOUS FLAVIVIRAL GENETIC ELEMENTS IN A VECTOR MOSQUITO

IDENTIFICATION OF THE FIRST CASE OF IMPORTED ZIKA FEVER TO THE UK: A NOVEL SAMPLE TYPE FOR DIAGNOSTIC PURPOSES AND SUPPORT FOR A POTENTIAL NON-VECTOR-BORNE ROUTE OF TRANSMISSION
and Imported Pathogens Laboratory at Porton Down and a panel of serological tests based on stated travel was performed. Dengue testing revealed positive IgM, but negative IgG and PCR. This pattern has been reported previously due to cross-reactivity between the flaviviruses. The sample was then tested by PCR for ZIKV and found to be positive. Further samples were taken on day 28 after rash onset. Blood and urine from both patients were tested in parallel, along with a semen sample from Patient 1. The semen was the only sample found to be positive for ZIKV by PCR. The reasons for persistence in the semen are not yet clear, but this case is significant not only as the first case of Zika Fever imported to the UK, but also as support for the possibility of sexual transmission, though not in this case, and highlights an additional sample type for the confirmation of infection in the future.

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**IXODES SCAPULARIS SALIVA ENHANCES POWASSAN VIRUS TRANSMISSION TO THE HOST, INFLUENCING ITS DISSEMINATION AND THE COURSE OF DISEASE**

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Powassan virus (POWV) is transmitted to humans by infected ticks, and successful transmission of POWV can occur within one hour of *Ixodes scapularis* attachment. Successful tick feeding is facilitated by a collection of pharmacologically active factors/proteins in tick saliva which are secreted into the feeding pool that the tick creates on the vertebrate host. The skin serves as the interface of host-virus-tick interactions; therefore, we sought to understand how tick saliva immunomodulates the tick-host interface and whether it facilitates POWV transmission and dissemination. To achieve this objective, groups of naïve BALB/c mice were infected intradermally in the hind footpad with either POWV alone or with POWV plus *Ixodes scapularis* salivary gland extract (SGE). A third group consisted of mice injected with media only. Mice from each group were sacrificed at 1, 3, 5, 7, and 8 days post infection (dpi). Organs were harvested from each sacrificed mouse and viral titers were determined by detecting POWV using quantitative real-time PCR. Starting at 3 dpi, mice infected with POWV+SGE had viremias that were at least 0.5Log greater than the viremias detected in the mice receiving POWV only. At 3 and 5 dpi the viremias of the POWV+SGE group were statistically significantly higher than the POWV only group. A similar pattern of higher viral loads in the POWV+SGE group was detected in the popliteal lymph nodes; however, there was no significant difference between groups on any of the days. POWV was not detected in the brains of mice until 5 dpi. From 5 dpi until experiment completion, the POWV+SGE infected mice had higher titers than the POWV only group, with 5 dpi being statistically significant. The onset of clinical symptoms occurred one day earlier (4 dpi) in the POWV+SGE group. Overall, this data indicates that *I. scapularis* SGE enhances POWV transmission to the host and influences the course of disease. To further examine the effect that *I. scapularis* saliva in the presence of POWV has on the host’s immune response, we fed POWV-infected and uninfected *I. scapularis* nymphs on naïve mice for 3 or 6 hours. 5-um sections were taken near the mouthparts of each feeding tick and infiltrating immune cells and POWV-infected cells at the tick bite site were identified. The location, timing, identity, and quantity of immune cells determined via histology enabled us to define the quality and kinetics of the host response to tick-borne POWV infection.

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**OUTBREAK OF YELLOW FEVER VIRUS DISEASE IN UGANDA IN 2010-2011: WERE OTHER INFECTIONS INVOLVED IN THE OUTBREAK?**

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An outbreak of yellow fever in northern Uganda was confirmed on 23 December 2010 after samples tested at the US Centers for Disease Control and Prevention (CDC) were positive for Yellow Fever Virus (YFV). Uganda is endemic for YFV however nearly 40 years had passed since the previous recorded transmission of YF in Uganda. The first documented outbreak in the country was in 1939-41. Single cases of yellow fever disease were documented in 1952, 1959, 1964 and 1971. Cases of illness of unknown cause had been reported since October 2010 from twelve districts in northern Uganda. These cases mainly concerned males aged 20 to 34 years mostly presenting with severe frontal headache, fever, lethargy, abdominal pain, diarrhea and vomiting, as well as with hemorrhagic signs. Blood and tissues samples from patients were sent to the U.S. CDC Special Pathogens laboratories in Atlanta, Georgia. Yellow fever virus was identified as the culprit. Then the CDC laboratory in Ft. Collins, Colorado, confirmed the finding. Overall, 181 cases met the YF suspected case definition; there were 45 deaths (case fatality rate 24.9%). Molecular sequencing revealed 92% homology to the YF virus strain Couma (Ethiopia), East African genotype. Suspected YF cases had fever (100%) and unexplained bleeding (97.8%), but jaundice was rare (11.6%). Over 200 blood samples were tested by RT-PCR and by IgM ELISA, including many samples from people who met the suspected case definition. However, only a total of 13 cases were laboratory confirmed from five districts. It was during a dry spell and the limited number of mosquitoes collected were not positive for YFV. Although this is acknowledged as the largest YF outbreak ever reported in Uganda. It remains unclear if all of the cases of disease were caused by YFV given that only a small number of suspected cases were laboratory confirmed to be YFV infections, and they were randomly scattered in five districts. There could have been other flaviviruses which probably were not tested for.

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**MOSQUITO SALIVA-MEDIATED ENHANCEMENT OF YELLOW FEVER VIRUS INFECTION**

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Mosquito-borne viruses like those in the genus Flavivirus must interact with both the mosquito and mammalian host in order to complete its life cycle and, to this end, have evolved complex strategies to replicate and disseminate within these different environments. Dengue and West Nile viruses take advantage of the complex mix of proteins in mosquito saliva in order to enhance infectivity in the mammalian host following the bite of an infected mosquito. Our recent work indicates that a non-catalytic serine protease is responsible for mosquito saliva-mediated enhancement of infectivity of these viruses and can be abrogated by the addition of a serine protease inhibitor or specific knockdown of the protein. We now report that in a murine model of the closely related yellow fever virus (YFV), early infection is enhanced in the presence of mosquito salivary gland extract (SGE) by as much as 5,000-fold both at the subcutaneous site of infection and in visceral tissues. However, despite this early enhancement, mice infected in the presence or absence of SGE, develop similar peak virus load and manifestation of clinical disease. Surprisingly, animals infected in the presence of SGE were more likely to survive infection, suggesting the development of a more effective or less damaging adaptive
immune response. We are currently examining the specific role that SGE-enhancement of infection plays in the development of cell-mediated and humoral immunity toward YFV.

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YELLOW FEVER RE-EMERGENCE AFTER FIFTY YEARS, ETHIOPIA, 2013

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In Ethiopia from 1960-1962 there was an outbreak of 100,000 cases of yellow fever (YF), which caused 30,000 deaths. Since 1962, however, no cases of yellow fever had been reported in Ethiopia. In May 2013 we received reports of a suspected outbreak of yellow fever from South Omo Zone situated within the Rift Valley in southern Ethiopia. We investigated to confirm the outbreak, identify risk factors and institute control measures. We used a clinical case definition to identify suspected YF cases and conducted a medical record review at all hospitals and health facilities in the Zone. Laboratory confirmed cases were confirmed by IgM serology at the Institut Pasteur in Dakar. We also conducted an age-matched case control study that included 30 suspected cases and 60 controls, who were neighbours of suspected cases without symptoms of disease. We identified 6 laboratory confirmed and 124 suspected YF cases from 28 November 2012 to 6 June 2013 from four rural districts of South Omo Zone. There were 53 deaths (case fatality rate of 41%). The overall attack rate was 30/100,000, and the highest attack rate (45/100,000) was from South Ari District. The median age of the 130 cases was 27 years, and 73 (56%) were males. Significant risk factors associated with illness included attending a mass gathering (OR=4.0, 95% CI=1.3-6.2) and presence of a person with YF in the village (OR=3.7, 95% CI=1.5-9.4). Working in a forest was a non-significant risk factor (OR=0.92, 95%CI=0.4-2.4). We confirmed the first outbreak of yellow fever in Ethiopia in 50 years. The outbreak was contained with a mass vaccination campaign of more than 500,000 people. We recommended heightened surveillance for hemorrhagic illness throughout Ethiopia.

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TAU ABNORMALITIES, INFLAMMATION AND AXONAL DAMAGE IN MURINE CEREBRAL MALARIA

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Cerebral malaria (CM) is a potentially fatal complication of disease associated with Plasmodium falciparum infection. Despite complete clearance of the parasite with anti-malarial treatment, approximately 20% of CM survivors develop long-term neurological deficits; however, the mechanisms that mediate this are not well understood. Neuronal injury has been linked to neuroinflammatory impairment in several neurodegenerative diseases and may contribute to the deficits seen in CM. In this regard, damage to neuronal axons has been observed in both human and murine experimental CM (ECM). Furthermore, improper regulation of tau, an axonal protein important for microtubule stability and cytoskeletal organization, has been demonstrated in mouse and human disease. We hypothesized that the neuronal injury observed in ECM results, in part, from abnormalities in tau. Improper regulation of tau results in an increase in its phosphorylated levels. We quantified protein levels of two forms of phosphorylated tau known to be pathological in Alzheimer’s disease (Ser396/404; Ser202) in several brain regions of mice with ECM and compared our findings with uninfected mice and mice infected with a less neurotropic malarial strain. In the same regions, we also quantified the level of SMI 32, a marker of axonal damage. Phosphorylated tau and SMI 32 were elevated throughout the brains of mice with neurological defects to the atypical tau regulation in ECM. Our goal is to further establish abnormal tau as a hallmark of CM. This protein may prove to be a viable target to ameliorate both the neuronal damage and subsequent neurocognitive impairment which occur during disease.

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CRYOPRESERVATION OF PLASMODIUM VIVAX SPOROZITES

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Plasmodium vivax represents a great challenge to malaria control because of the ability to relapse of dormant form known as hypnozoite in the liver. Research efforts to understand hypnozoite biology are limited due to the availability of P. vivax sporozoites. Mahidol Vivax Research Unit (MVRU) is one of few centers in the world that has facilities to produce the sporozoites by feeding mosquitoes on blood from P. vivax-infected patients. The ability to cryopreserve sporozoites is therefore essential to support liver-stage malaria research community. Hydroxyethyl starch 6% (HESTAR), a currently used cryoprotectant in cryopreservation of P. vivax sporozoites at MVRU, retains sporozoite viability up to 30% after thawing. In this study, the protective effect of different combinations of cryoprotectants on sporozoite viability and infectivity was investigated in order to improve the cryopreservation protocol. Sporozoites were harvested from infected mosquitoes and cryopreserved at slow freezing rate of -1°C/min to final temperature of -80°C before plunging in liquid nitrogen. Frozen sporozoites were thawed in a 37°C water bath for a few seconds and viable sporozoites were counted under hemocytometer. Addition of 10% DMSO and 250 mM sucrose into HESTAR solution increased number of viable sporozoites after thawing. The combination of PBS, 0.5% BSA, 250 mM sucrose, and 10% DMSO demonstrated highest sporozoite recovery. However, reduction of sporozoite infectivity was observed in all cryoprotectants tested.

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FOLIC ACID METABOLISM IS DISRUPTED IN A NON HUMAN PRIMATE MALARIA MODEL

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Plasmodium falciparum is a leading cause of severe malaria. Due to the challenge of studying P. falciparum pathophysiology in human populations, non-human primate (NHP) models have been developed. As part of an effort by the Malaria Host-Pathogen Interaction Center (MaHPIC), we hypothesized that high-resolution metabolomics (HRM) would further our understanding of the biochemical changes ensuing in the early phase of P. coatneyi infection in Macaca mulatta. As part of a larger intervention study, we collected serum samples from 4 healthy animals at 2 time points: 15 days before experimental infection (baseline) and 5 days after challenge (early infection). All animals developed malaria after intravenous injection of 4x10⁴ P. coatneyi infected erythrocytes/kg obtained from an infected donor monkey. At the early infection blood collection, parasitemias were low and animals did not require antimalarial treatment. Samples underwent randomization followed by HRM. We used xMSanalyzer for data extraction and obtained >20,000 metabolites (unique m/z with retention time). Paired analyses for differences due to infection were performed using the Linear Models for Microarray package (LIMMA, R).
the Southeast Asian Ovalocytosis (SAO), Gerbich negativity, and variant vaccine platform. The presence of red cell polymorphisms including strengthens the support to include these invasion proteins into the malaria P. falciparum ligands expressed in demonstrated that EBA-140, Rh-1 and EBA-175 were the major invasion EBA-175 and EBL-181 and PfRh genes such as Rh-1, Rh-2a, Rh-2b. We quantitative real-time reverse-transcription polymerase chain reaction were quantified from the schizonts stage of each isolate. We employed Duffy binding ligand (PfDBL) and P. falciparum (NsTsCr; 21,74%). The expression of two invasion ligands; isolates in Timika invaded red blood cells through type A receptor that showing receptor sensitivity or resistance to those enzymes. Most field mg/ml chymotrypsin. Eight invasion profiles were found in this study or chymotrypsin (C). Sixty nine isolates were subjected to various enzyme their invasion based on their sensitivity to neuraminidase (N), trypsin (T) surface. We reported various receptors used by the parasite ligands during Plasmodium falciparum invasion is a complex process involving several parasite ligands and their receptors expressed on the red blood cell surface. We reported various receptors used by the parasite ligands during their invasion based on their sensitivity to neuraminidase (N), trypsin (T) or chymotrypsin (C). Sixty nine isolates were subjected to various enzyme treatments including 50 μM neuraminidase, 1 mg/ml trypsin, or 1 mg/ml chymotrypsin. Eight invasion profiles were found in this study showing receptor sensitivity or resistance to those enzymes. Most field isolates in Timika invaded red blood cells through type A receptor that was resistant to all enzyme treatments (Nl/TrCr, 28.99%) and type B that was sensitive to neuraminidase and trypsin, but resistant to chymotrypsin (NsTsCr, 21.74%). The expression of two invasion ligands; P. falciparum Duffy binding ligand (PfDBL) and P. falciparum reticulocyte homolog (PfRh) were quantified from the schizonts stage of each isolate. We employed quantitative real-time reverse-transcription polymerase chain reaction (QRT-RT-PCR) to detect the expression of PfDBL family including EBA-140, EBA-175 and EBL-181 and PfRh genes such as Rh-1, Rh-2a, Rh-2b. We demonstrated that EBA-140, Rh-1 and EBA-175 were the major invasion ligands expressed in P. falciparum of Timikan isolates. The current study strengthens the support to include these invasion proteins into the malaria vaccine platform. The presence of red cell polymorphisms including the Southeast Asian Ovalocytosis (SAO), Gerbich negativity, and variant hemoglobin (HbE) were not found to affect parasite invasion.

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THE EXPRESSION OF PLASMODIUM FALCIPARUM INVASION LIGANDS AND THEIR RECEPTORS IN CLINICAL ISOLATES FROM TIMIKA, PAPUA, INDONESIA

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Plasmodium falciparum invasion is a complex process involving several parasite ligands and their receptors expressed on the red blood cell surface. We reported various receptors used by the parasite ligands during their invasion based on their sensitivity to neuraminidase (N), trypsin (T) or chymotrypsin (C). Sixty nine isolates were subjected to various enzyme treatments including 50 μM neuraminidase, 1 mg/ml trypsin, or 1 mg/ml chymotrypsin. Eight invasion profiles were found in this study showing receptor sensitivity or resistance to those enzymes. Most field isolates in Timika invaded red blood cells through type A receptor that was resistant to all enzyme treatments (Nl/TrCr, 28.99%) and type B that was sensitive to neuraminidase and trypsin, but resistant to chymotrypsin (NsTsCr, 21.74%). The expression of two invasion ligands; P. falciparum Duffy binding ligand (PfDBL) and P. falciparum reticulocyte homolog (PfRh) were quantified from the schizonts stage of each isolate. We employed quantitative real-time reverse-transcription polymerase chain reaction (QRT-RT-PCR) to detect the expression of PfDBL family including EBA-140, EBA-175 and EBL-181 and PfRh genes such as Rh-1, Rh-2a, Rh-2b. We demonstrated that EBA-140, Rh-1 and EBA-175 were the major invasion ligands expressed in P. falciparum of Timikan isolates. The current study strengthens the support to include these invasion proteins into the malaria vaccine platform. The presence of red cell polymorphisms including the Southeast Asian Ovalocytosis (SAO), Gerbich negativity, and variant hemoglobin (HbE) were not found to affect parasite invasion.

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EFFECTS OF LOW MOLECULAR WEIGHT HEPARIN AND ANTIMALARIAL DRUGS ON ROSETTE FORMATION AND CYTOADHERENCE OF PLASMODIUM FALCIPARUM

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The main pathophysiology of severe malaria is due to the mature stage of Plasmodium falciparum–infected red cells sequestered in the capillary vessels of the brain and other vital organs known as sequestration. This sequestration is due to cytoadherence, the ligands-receptors interactions and rosette formation which are defined as the adhesion of two or more uninfected red cells to the infected cells. Many studies have shown that the heparin sulfate, which is negatively charged through sulfate groups, was used in the adjunctive treatment of malaria. However, there is no report on the effects of low molecular weight heparin (Sevuparin) on the cytoadherence of P. falciparum isolated from patients. We therefore investigated the effects of low molecular weight heparin on rosette formation and cytoadherence of Plasmodium falciparum isolates in Thailand. P. falciparum–infected blood samples at trophozoite stage was incubated with Sevuparin at 37°C for 30 minutes and an hour before performing the rosetting and cytoadherence assay, respectively. The results shown that Sevuparin (62.5–1000 μg/mL) inhibit rosette formation of Pfalciparum (N=43) in vitro, (P<0.01) in dose dependent manner. The concentration of Sevuparin at 400 μg/mL significantly inhibited cytoadherence (N=24) (P=0.02). This study provides the novel knowledge for understanding the pathophysiology of malaria and improving treatment of malaria patients. The low molecular weight heparin with less anticoagulation activities might be used as adjunctive treatment in severe malaria patients.

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THE IMPACT OF DYSREGULATED ANGIOGENESIS ON BIRTH OUTCOMES IN EXPERIMENTAL PLACENTAL MALARIA

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Each year ~125 million pregnant women are at risk for malaria infection. Placental malaria (PM) due to Plasmodium falciparum infection has a profound impact on maternal and child health, including preterm birth and low birth weight (LBW), and results in an estimated 100 000 infant deaths per year. Previous work by our lab showed that PM-mediated LBW outcomes in both human and murine studies are associated with dysregulated angiogenesis including modifying the angiopoietin (Ang)-Tie2, endoglin, and VEGF pathways. Coordinated expression of these angiogenic factors is required for normal placental development during pregnancy. Furthermore, recent studies have implicated complement activation (e.g. C5a) in mediating these changes. Although we uncovered a role for angiogenesis and complement in the pathobiology of PM, the temporal regulation of these factors has not been precisely mapped. Thus, the objective of this study is to compare the kinetics and levels of factors that regulate vasculogenesis and angiogenesis including VEGF, soluble VEGF Receptor-1 (sFlt-1), endoglin, Placental Growth Factor, Ang-1, and Ang-2, required for the normal placental vascular development and gestation. Using the established BALB/c mouse model of PM, we will measure maternal, fetal, and placental levels of the various angiogenic factors throughout gestation by qRT-PCR and ELISA. Further, to establish a causal role for Ang-1 in mediating PM outcomes, we will use heterozygous and conditional Ang-1 knockout mice to determine the impact of Ang-1 deficiency on angiogenesis and fetal growth in an experimental PM model. Finally we will investigate the therapeutic utility of angiogenic factors such as recombinant Ang-1 and determine if adverse birth outcomes in PM can be reversed by interventions to restore normal angiogenesis. Overall, these studies will improve our understanding of the specific roles of the angiogenic factors in normal and malaria-complicated pregnancies and may help identify novel interventions to prevent poor birth outcomes associated with PM.
FUNCTIONAL MICROENGINEERED MODEL OF THE HUMAN SPLENON-ON-A-CHIP

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We have developed a new functional microfluidic device that mimics the hydrodynamic behavior and filtering functions of the spleen, the minimal functional unit of the red pulp of the spleen able to maintain its filtering functions. Unlike any other previous microfluidic devices, the human splenon-on-a-chip incorporates for the first time two compartments with different flow velocities (according to the physiological flow division) and two physical barriers representing the reticular mesh and the interendothelial slits (IES) where cells first slow down increasing the hematocrit and then traverse the IES in a unidirectional matter. To validate the use of this platform, several experiments were carried out with different types of red blood cells (RBCs). As a proof of concept, we described that old RBCs showed less deformability than freshly drawn RBCs when traversing the microconstrictions. Posterior analysis allowed studying the passage and deformability of infected RBCs through the IES using peripheral blood of BALB/c mice experimentally infected with the Plasmodium yoelii 17X-GFP strain. Results showed that infected reticulocytes are significantly more deformable than non-infected reticulocytes, in agreement with the higher deformability of reticulocytes parasitized by P. vivax, a human malaria parasite with reticulocyte tropism. These results suggest that the device is able to reproduce physiological conditions and to distinguish different types of RBCs by means of deformation/mechanical properties. Presently, we are determining if there is hemolysis during the pass of blood through the device, if there is pitting in the absence of macrophages and the rheological properties of malarial infected RBCs.

ORGAN-SPECIFIC TISSUE FACTOR ACTIVITY IS SIGNIFICANTLY ALTERED IN EXPERIMENTAL MALARIA

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Sequestration of Plasmodium falciparum-infected erythrocytes in the brain or the maternal blood space of the placenta results in two severe clinical manifestations of this disease, cerebral malaria (CM) and placental malaria (PM), respectively. Hyperinflammation consisting of increased expression of Tumor Necrosis Factor-α (TNF) and activation of macrophages in affected organs are associated with CM and PM. It is well established in other models of disease that inflammatory damage leads to systemic activation of coagulation and severe thrombosis. Recent evidence showing the existence of a procoagulant state in patients suffering from CM or PM indicates the inflammation-coagulation cycle may play a significant role in malaria pathogenesis and may provide useful diagnostic and therapeutic targets. However, the extent to which coagulation is responsible for the pathogenesis of these diseases is incompletely understood. Thus, nonpregnant C57BL/6J mice were infected with 10⁷ P. berghei ANKA, a virulent murine malaria species capable of inducing CM, or 10⁷ P. chabaudi AS, a non-CM strain, then serially sacrificed between days 3 and 6 post-infection and assayed for TF procoagulant activity in brain, liver, and lung tissues. TF activity was significantly higher in brain (>1400-fold; P<0.01) of mice that succumbed to CM 5 and 6 days post-infection relative to uncomplicated malaria cases. In mice sacrificed on days 3 and 4 post-infection, all three organs exhibited a trend toward reduced TF activity, most notably in the brain (50-fold decrease). Preliminary data show reductions in TF activity at day 4 of P. chabaudi AS infection in the brain (>360-fold, P = 0.057), lung (>1200-fold, P = 0.016), and liver (>4400-fold, P = 0.057) compared to uninfected controls. Increased TF activity in the brains of mice that succumb to CM, but not those with uncomplicated P. berghei ANKA infection, suggests a significant role for coagulation in the pathogenesis of experimental CM. Ongoing experiments seek to further elucidate critical cellular sources of TF with the use of TF-floxed/cre mice. In addition, the importance of TFN in malaria-associated TF activity is being assessed with TNF and TNF receptor (TNFR) null mutant mice. Finally, to determine the effect of TF-driven coagulation on inducing placental pathology during PM, placentae from TF-, TNF-, and TNFR-modified P. chabaudi AS-infected mice are also being assessed.

MALARIA-RELATED ANEMIA IN COLOMBIA

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Anemia and other hematological changes associated with malaria infections vary with the level of malaria endemicity, demographic variables and malaria immunity, among other factors. Information on cofactors associated with malaria-related anemia in Latin America is scarce. This
study aimed at characterizing erythrocytes indices in patients living in endemic areas of Colombia with unstable malaria transmission. A total of 929 malaria patients were enrolled between 2011 and 2013 in three areas of Colombia: Tierralta, Tumaco and Quibdó. Demographic and epidemiological information related to malaria was obtained at the time of diagnosis, and automated complete blood cell counts were performed on samples collected from all participants. Multiple linear regression analyses were then carried out to determine the relationships between erythrocytes indices and independent variables. *Plasmodium falciparum* was found to be the most prevalent species in Tumaco (84%) and Quibdó (70%), whereas *P. vivax* was predominant in Tierralta (92%). Variable degrees of non-severe anemia (Hb 7.1-10.9 g/dL) were present in 21% of the patients, with similar distribution in the three study sites. Severe anemia (Hb<7 g/dL) was almost absent (0.3%). Afro-descendants from Quibdó presented lower hemoglobin levels than other ethnic groups regardless of the parasite species. Only in Tumaco, patients with *P. vivax* displayed lower hemoglobin levels than those found in *P. falciparum*-infected patients. In Tierralta, hemoglobin levels, hematocrit and RBC counts were negatively associated with days of illness. Moreover, in Tierralta and Quibdó, a direct relationship was found between the number of previous malaria episodes and hemoglobin levels. Both *Plasmodium* species appear to have similar potential to induce malarial anemia, but distinct cofactors at each endemic setting in Colombia seem to modify this clinical manifestation. Early diagnosis and prompt treatment are likely preventing more frequent and serious anemia in Colombia, and the target age in these low transmission settings has shifted towards adolescents-young adults. Moreover, previous malaria history appears to induce protection against anemia development.

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**HEME INDUCED ACTIVATION OF TOLL-LIKE RECEPTOR 4 IS ASSOCIATED WITH REDUCED HEMATOPOIETIC STEM CELL VIABILITY AND APOPTOSIS IN SEVERE MALARIA**

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*Plasmodium falciparum* infections are responsible for malaria associated mortalities ranging between 700,000 and 900,000 cases annually. Approximately 30% of the deaths occur after treatments indicating an urgent need for adjunctive therapies. Endothelial progenitor cells (CD34+/VEGFR2+) play an important role in the repair and regeneration of vascular endothelium as well as in neovascularization during cerebrovascular diseases, yet they are depleted by an unknown mechanism in severe malaria. Hematopoietic stem cells (HSC) are CD34+ precursors to EPC and diseases, yet they are depleted by an unknown mechanism in severe endothelium as well as in neovascularization during cerebrovascular diseases. Changes in the function of HSC could function to induce malarial anemia as it correlates with seventy of malaria. Among TLRs, TLR4 played a significant role in the heme-induced pathogenesis of malaria. In conclusion, *Plasmodium* infection activates TLR4 and subsequently induces apoptosis in HSC resulting in decreased numbers of circulating EPC and limited vascular repair in severe malaria pathogenesis.

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**PREVALENCE AND DIVERSITY OF HAEMOPARASITES IN BIRDS AT THE RESERVA NACIONAL ALLPAHUAYO MISHANA (RNAM), IQUITOS, PERU, 2013**

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Birds, like most wild animals, are frequently found to be affected by an enormous range of parasites, both endoparasites and ectoparasites, which represent a potential health risk for both humans and wild animals as well as a threat to the conservation of biodiversity. There is no information from Peruvian Amazon about the existence of these blood parasites, despite having the second richest ornithological diversity worldwide. The lack of research on wild birds in the Peruvian Amazon motivated us to investigate the presence of 3 hemoparasites that are genetically closely related (*Plasmodium*, *Hemoproteus*, and *Leucocytozoon*) in the Allpahuayo-Mishana National Reserve (AMNR). This is the first study in the Peruvian Amazon focus on identifying the presence of blood parasites in wild birds, and will provide invaluable information needed to assess the risk of emerging diseases that can affect human populations. For this purpose, a series of 10 parallel mist nets were installed in the study area from March to April 2013. A total of 203 birds belonging to 22 families and 62 species were captured. Our preliminary microscopy results revealed that 64 birds belonging to 28 species (31% of the total), were positive for *Plasmodium/Hemoproteus*. Casicus cela was the species with the highest prevalence of parasites and mixed infections with Microfilaria and *Plasmodium/Hemoproteus*.

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**GENOME-WIDE ANALYSIS OF CHANGES IN BEWO CELLS INDUCED BY CS2 PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES, SCHIZOGONIC PRODUCTS AND CYTOKINES**

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Placental malaria (PM) changes the microenvironment at the intervillous space of the placenta which is essential for fetus growth by exposing the syncytiotrophoblast (ST) to *Plasmodium falciparum* infected erythrocytes (IE), *P. falciparum* schizogonic products (SP) and inflammatory cytokines leading to low birth weight babies. Changes of ST functions occur during PM, however it is not known what contribute to these changes. We analyzed gene expression changes in the BeWo cell line produced by the binding of IE, IE and parasite SP, and cytokines produced by THP-1 monocytes co-cultured with IE. BeWo cells were sycntialized for 72 hours using 10 µM forskolin in triplicate cultures and exposed to intact CS2 IE, intact CS2 IE plus SP, and conditioned medium (CM) produced by THP-1 cells upon exposure to IE and co-cultured for 48 hrs. Gene expression changes were then analyzed by the microarray. Functional annotation of differentially expressed genes (fold change ≥1.5 and adjusted P value ≤0.01) was determined using GO-term context with DAVID version 6.7 and altered pathways were identified using Pathway Miner (BioRag). Conditioned medium containing inflammatory cytokines and chemokines, but not IE alone or IE with SP, significantly altered genes associated with...
biological processes and pathways in BeWo cells that are important for fetus and placental growth. The categories of genes enriched (P ≤0.05) among down-regulated genes in BeWo cells treated with CM included, genes linked with vasculogenesis, blood vessel formation and negative regulation of vasocostriction. Growth pathways significantly altered (P ≤0.05) included insulin, insulin like growth 1, mammalian target of rapamycin, transforming growth factor beta 1, platelet derived growth factor, epidermal growth factor signaling and prostaglandin synthesis and regulation. Cytokines and chemokine dyoregulate pathways and biological processes in BeWo cells that are involved in placental vascular development and remodeling. These results may explain why placental insufficiency occurs as a result of malaria infection.

HEMATOPOIETIC STEM/PROGENITOR CELL SOURCES TO GENERATE RETICULOCYTES FOR PLASMODIUM VIVAX

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The adaptation of Plasmodium vivax to in vitro culture remains an important challenge. The preference of P. vivax for immature erythrocytes (reticulocytes) constitutes one of the main hurdles. Three different sources of hematopoietic stem/progenitor cells (HSPC) i.e; umbilical cord blood (UCB), bone marrow (BM) and adult peripheral blood (PB) were expanded for 5 days and differentiate for 14 days to obtain reticulocytes. The HSPC population could be efficiently expanded and produce reticulocytes that are equally permissive to P. vivax. Interestingly the permissiveness of HSPC-derived reticulocytes was higher than the reticulocyte-enriched blood. Our findings might suggest that P. vivax has a preference for immature reticulocytes opening new perspective in the research on P. vivax invasion mechanism.

TACI IS REQUIRED TO CONTROL PARASITEMIA LEVELS POST PY NL INFECTIONS

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The TACI (transmembrane activator and calcium-modulator and cyclophilin ligand) receptor binds BAFF (B-cell activator factor) and APRIL (a proliferation-inducing ligand), two cytokines critical for B-cell survival and plasma cell generation. To assess the role of TACI in malaria, TACI/KO and the wild type (WT) C57BL/6 mice were infected (i.p.) with 1 million Plasmodium yoelii (Py) NL parasites and parasitemia was assessed. Results showed that the level of parasitemia was significantly elevated in TACI/KO mice (55.2% at day 28) compared to wild type WT mice (12.4% at day 16), and the parasitemia clearance was substantially delayed in the TACI/KO (28 days) relative to WT control (21 days). Subsequently, anti-Py NL antibodies and BAFF and APRIL concentrations were measured. In addition, to determine the role of B cells in protection, adoptive B-cell transfers were also done. Measurement of serum BAFF and APRIL levels revealed that in TACI/KO mice, the highest levels of BAFF (33.2 ng/ml) and the lowest levels of APRIL (119 ng/ml) were detected at peak parasitemia. Additionally, less anti-Py NL IgG-antibodies were produced by the TACI/KO mice up to Day 22 post infection when compared to WT mice, but the differences in antibody levels vanished between TACI/KO and WT mice at day 28 post infection. Both, WT and TACI/KO mice were immune to a second Py NL challenge with peak parasitemia less than 1% and parasitemia clearance by day 10 following the secondary infection. Importantly, adoptive transfer of “immune” B-cells conferred protection in both WT and TACI/KO mice against Py NL challenge. Conclusion: TACI plays a key role in the control of Py NL infections.

PHENOTYPIC CHARACTERIZATION OF THE PLASMODIUM FALCIPARUM NOT1 (PF3D7_1103800) GENE KNOCKOUT AND HEAT SHOCK ANALYSIS OF THE PIGGYBAC MUTANT LIBRARY

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Ongoing research in our lab uses genetic approaches to identify new potential targets for therapeutic treatments. The objective of this work is to characterize PF3D7_1103800 and identify other genetic factors in the piggyBac mutant library affected by heat shock. The Not1 gene, PF3D7_1103800, is a part of a larger gene regulatory system in eukaryotes known as the CCR4-NOT complex, which is involved in mRNA transcription and degradation. Our research on the asexual stages of the major malaria parasite Plasmodium falciparum uses the phenotypic analysis of genetic mutants. The primary phenotypic screens are growth attenuation and changes in virulence factors. My dissertation focuses on the screening for genes involved with stress responses to increased temperature. In the process of a whole genome random transposon mutagenesis of P. falciparum, a knockout of Not1 was isolated. Characterization of protein extracts from mutant and wild type parasites were analyzed by immunoblots with antibodies to EBA-175 and GAP45. The IFA localization of these invasion-related proteins in the Not1 mutant was compared with wild type patterns. Not1 and other piggyBac mutants were analyzed for altered sensitivity to febrile temperatures during culture through growth analysis with a flow cytometer. The immunoblots display altered expression at mid to late trophozoite stages in the Not1 mutant when compared to the wild type. The IFAs for the Not1 mutant reveal patterns similar to that of the Caf1 mutant, which is also a part of the CCR4-NOT complex and found to be important in parasite egress. Of the more than 40 parasites tested, 2 were significantly increased in growth and 18 were significantly decreased compared to the wild type, including the Not1 knockout. Validation of the Not1 mutant phenotype should result in delayed growth and invasion of the parasite. Furthermore, exposure of the piggyBac mutant library to increases in heat may reveal genetic factors involved with the intraerythrocytic cycle that lead to new drug targets.

IRON SUPPLEMENTATION AFTER SEVERE MALARIA IS ASSOCIATED WITH DECREASED HEME OXYGENASE-1 LEVELS ONE MONTH AFTER ILLNESS

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Iron treatment in malaria endemic areas can increase the risk of malaria and death. One mechanism by which iron supplementation increases the risk and severity of malaria may be by increasing oxidative stress. To assess changes in oxidative stress after treatment with iron in children with severe malaria, we assessed baseline and 4-week levels of heme oxygenase-1 (HO-1), an enzyme that is upregulated under oxidative stress, in Ugandan children with cerebral malaria (CM) (n= 51), severe malaria anemia (SMA) (n=39) and community children (CC) (n=52). We hypothesized that iron treatment would increase oxidative stress, as measured by HO-1. Upon enrollment, children who were iron deficient (zinc protoporphyrin (ZPP) ≥80) either received daily iron therapy immediately (n=62) or 4 weeks later (n=54). All children with SMA and CM and 26 CC had a ZPP>80, and were randomized to immediate vs. delayed treatment. 26 CC had a ZPP>80 and did not receive iron. Children with CM and SMA had similar baseline HO-1 concentrations, and did not differ in the immediate vs. delayed subgroups. Baseline HO-1 concentrations were lower in CC than in children with CM or SMA (P<0.0001). At 1 month, HO-1 concentrations in children in the CM and SMA delayed iron groups were increased compared to the immediate iron groups (both P<0.05) and the
iron sufficient group (both P<0.001), but HO-1 levels in the iron deficient community control group did not differ between immediate and delayed groups. There was no correlation of HO-1 levels at 1 month in children with CM or SMA who received either immediate or delayed iron treatment with readmission (P>0.05). Contrary to our hypothesis, iron treatment after malaria was associated with significantly lower HO-1 concentrations at 4 weeks. This suggests that iron treatment reduces oxidative stress in iron deficient children after malaria. Future research will explore potential mechanisms for this process.

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SEQUESTRATION MECHANISMS OF PLASMODIUM VIVAX-INFECTED ERYTHROCYTES

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We have previously demonstrated that Plasmodium vivax-infected erythrocytes (Pv-iEs) cytoadhere to endothelial cell receptors and form rosettes, a typical feature observed for P. falciparum. Rosette is a cytoadhesion phenotype where infected red blood cells can adhere to non-infected red blood cells and in falciparum malaria rosettes are normally associate to poor clinical outcomes and severity and might improve parasite infectivity. Nevertheless, it has recently shown that Pv-iEs harvested from vivax patients form rosettes, however correlation with severity and infectivity was not observed. These observations prompt us to investigate the biological role of rosetting formation of Pv-iEs. Thus, using a panel of 59 P. vivax Brazilian isolates, we showed that rosettes are mostly observed at late stages, they are formed mostly by normocytes and parasitemia levels correlate with rosetting rates. Nonetheless, we noticed that autologous plasma improved significantly rosette formation, suggesting a key role of plasma components. Indeed, we found positive correlation between IgM levels and an inverse association of IL6 and IL10 with rosetting rates. Moreover, antibodies towards to VIR proteins disrupt rosettes, which are less likely to be phagocytosed in vitro. Thus, we hypothesize that rosetting probably acts as a mechanism of immune evasion.

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AN ADJUSTABLE GAS-MIXING DEVICE TO INCREASE FEASIBILITY OF IN VITRO CULTURE OF PLASMODIUM FALCIPARUM PARASITES IN THE FIELD

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A challenge to conducting high-impact and reproducible studies of the mechanisms of Plasmodium falciparum drug resistance, invasion, virulence, and immunity is the lack of robust and sustainable in vitro culture in the world. While the technology exists and is routinely utilized in developed countries, various factors—from cost, to supply, to quality—make it hard to implement in malaria endemic countries. Here, we design and rigorously evaluate an adjustable gas-mixing device for the in vitro culture of P. falciparum parasites in the field to circumvent this challenge. The device accurately replicates the gas concentrations needed to culture laboratory isolates, short-term adapted field isolates, cryopreserved previously nonadapted isolates, as well as to adapt ex vivo isolates to in vitro culture in the field. We also show an advantage over existing alternatives both in cost and in supply. Furthermore, the adjustable nature of the device makes it an ideal tool for many applications in which varied gas concentrations could be critical to culture success. This adjustable gas-mixing device will dramatically improve the feasibility of in vitro culture of Plasmodium falciparum parasites in malaria endemic countries given its numerous advantages.

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MOLECULAR MARKERS OF RADIATION INDUCED ATTENUATION IN INTRAHEPATIC PLASMODIUM FALCIPARUM PARASITES

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Radiation attenuated sporozoite (RAS) vaccine has been shown to be promising for malaria vaccine development. While the host requirements for sterile immunity are still not fully understood, we performed genome-wide transcriptional profiling of untreated versus radiation-treated intrahepatic parasites on days 3 and 6 post-infection of human HepG2 hepatocytes by Plasmodium falciparum sporozoites of the 3D7 strain to identify parasite gene targets of growth attenuation and enhanced immune protection induced by radiation. Using this approach, we found that 180 parasite genes had significantly altered transcriptional expression in response to radiation on day 3 and 6 post-infection in hepatocytes. Among these novel biomarkers, we identified a signature of eight candidate parasite genes that associated with functionally diverse pathways that may regulate radiation induced cell cycle arrest of the parasite within the hepatocyte. In addition, a repertoire of 14 genes associated with protein translation is transcriptionally overexpressed within the parasite by radiation. We hypothesize that this radiation induced enhanced translational activity may be a contributing factor for the sterilizing immunity observed by RAS vaccination by increasing the pool of parasite antigens available for immune presentation either as processed epitopes on the hepatocyte surface or as released antigens from apoptotic infected hepatocytes. These results have significantly increased the repertoire of novel targets for 1) generating genetically attenuated parasite vaccine candidates 2) subunit vaccines against the hepatic stage cycle and 3) biomarkers of safety to define proper attenuation.

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COMPARATIVE GROWTH ANALYSIS OF MALARIA PARASITE, PLASMODIUM FALCIPARUM, USING 3H-HYPOXANTHINE INCORPORATION AND SYBR GREEN FLUORESCENCE

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Differential growth of Plasmodium falciparum in red blood cells (RBC) is an essential feature of malaria parasite biology. High growth rate results in increased parasite biomass which is correlated with disease severity in patients. ‘Growth’ is generally ascertained microscopically or through a surrogate reporting system such as tritium labeled hypoxanthine (3H-Hx). The use of tritium has been the ‘gold standard’ for measuring parasite growth as it effectively mirrors visually determined parasitemia. This method of labeling requires the parasites to be starved of the essential nucleic acid precursor, hypoxanthine (Hx) for 48 hours in order to facilitate uptake of the isotope. SYBR green, a relatively inexpensive fluorescent dye, has been shown to give similar results to 3H-Hx-incorporation in drug assays, providing an alternative method for ascertaining parasite growth. However, a side-by-side comparison of SYBR green and 3H-Hx-incorporation has yet to be completed in the absence of drug. Here
we provide a head to head comparison of the SYBR green and ³H-Hx-incorporation assays in a set of genetically unique progeny to ascertain if these two methods can be used interchangeably in determining parasite growth. Our data shows a correlation between the data sets indicating that the relative growth rate as measured by both assays is comparable. In addition we use the SYBR green assay to measure differences in parasite growth in Hx starved and unstarved conditions. Preliminary data indicates decreased parasite growth in Hx starved conditions. More interesting is the observation that parasites show variable responses to Hx starvation suggesting a possible genetic mechanism may be involved.

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**ASSESSMENT OF KNOWLEDGE AND ADHERENCE TO THE NATIONAL GUIDELINES FOR MALARIA CASE MANAGEMENT IN PREGNANCY AMONG HEALTHCARE PROVIDERS AND DRUG DISPENSERS IN RURAL, WESTERN KENYA**

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Although prompt and effective treatment is a cornerstone of malaria control, information on healthcare provider adherence to malaria treatment guidelines in pregnancy is lacking. Incorrect or sub-optimal treatment can cause adverse consequences to the mother and fetus. We conducted a cross-sectional study from September to November 2013, in all health facilities and randomly selected drug outlets in Siaya County, western Kenya, to assess healthcare provider adherence to and knowledge of national guidelines for treatment of uncomplicated malaria in pregnancy to inform the Kenyan Ministry of Health and relevant stake-holders on knowledge gaps to be targeted. In health facilities, standardized questionnaires were used to interview all healthcare providers and all women of childbearing age who had been assessed for fever. Mystery clients posing as 1st trimester pregnant women or as relatives of women in 3rd trimester collected information from drug outlets. Information on drugs was recorded from prescriptions or after reviewing drugs in patient’s possession. None of the drug outlet dispensers, versus 56% of health facility providers, knew the appropriate treatment for 1st trimester patients, while 39% and 87%, respectively, knew the appropriate treatment for 2nd/3rd trimester. Prescription of the correct drug for pregnancy treatment at the correct dosage, was observed in 66% of cases in health facilities and 50% in drug outlets. Prescribing was more often correct in 2nd/3rd trimester than in 1st (65% vs. 32%, p<0.004, and 38% vs. 0%, p<0.001, at health facilities and drug outlets, respectively). Sulfadoxine-pyrimethamine, which is no longer recommended for treatment of acute malaria, was prescribed and available in 4% of cases in health facilities and 23% of simulations in drug outlets (p<0.001). Exposure to artemether-lumefantrine in 1st trimester, which is contraindicated due to its unknown safety, occurred in 13% and 51% of cases in health facilities and drug outlets respectively (p=0.04); none were a result of quinine stock-out. This study highlights knowledge inadequacies and incorrect prescribing practices in the treatment of malaria in pregnancy. These should be addressed through comprehensive trainings and adequate supervision by the Kenya Ministry of Health to improve the quality of patient care and maximize therapeutic outcomes.

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**RANDOMIZED TRIAL TO COMPARE THE SAFETY, TOLERABILITY AND EFFICACY OF BI-MONTHLY INTERMITTENT PREVENTIVE TREATMENT WITH EITHER MEFLOQUINE-ARTESUNATE OR SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE, WITH THE STANDARD REGIMEN OF DAILY PROGUANIL, FOR THE PREVENTION OF MALARIA AND RELATED COMPLICATIONS IN PATIENTS WITH SICKLE-CELL DISEASE IN NIGERIA**

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Effective prophylaxis should be provided for children with sickle-cell disease(SCD) in malaria endemic areas. The aim of this study was to find out if intermittent treatment with mefloquine-artesunate (MQ+AS) could be used in this way. Patients with SCD were randomly assigned one of three intervention groups. Patients in group 1 received a fixed-dose combination of Mefloquine-Artesunate at each bimonthly clinic visit, those in group 2 sulfadoxine-pyrimethamine plus amodiaquine (SP+3AQ) bimonthly whilst patients in group 3 received daily proguanil. Patients were given diaries and are asked to return to the clinic every two months and whenever they were unwell. Three days after each clinic visit, patients or their carer were interviewed to ask about compliance and adverse events. The primary outcome of the trial was tolerability; secondary outcomes were adherence to the regimen, incidence of malaria and the number of outpatients presentations and hospitalizations over 12 months. 270 SCD patients were followed-up from September 2011 to May 2013, 90 in each study group. Adherence to the bimonthly regimens was excellent, (87% received 6 doses of MQ+AS and 84% completed all courses of the drug; 88% received 6 doses of SP+3AQ and 86% completed all courses). In contrast, adherence to the daily proguanil regimen was poor (10% took tablets less than three days in four). Incidence of mild adverse events during 3 days after each clinic visit, was highest in the MQ+AS group with about 20% of children reporting side effects, the most common of which were nausea, vomiting and abdominal pain, but these symptoms did not affect adherence. Incidence of malaria was low in all groups. The number of malaria cases was 17 (proguanil), 14(SP+3AQ) and 9(MQ+AS), rate ratios compared to proguanil were 0.84 (95%CI 0.4,1.9) for SP+3AQ and 0.55 (95%CI 0.2,1.2) for MQ+AS. The incidence of other illnesses was similar in the groups. Supervised treatments with MQ+AS at the clinic once every 2 months is well tolerated and safe and may be more effective in preventing uncomplicated malaria than daily prophylaxis with proguanil. These results justify a larger trial powered to determine efficacy in preventing malaria and malaria-related complications. This trial is registered with ClinicalTrials.gov: NCT01319448 ISRCTN46158146.

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**SAFETY AND EFFICACY OF DIHYDROARTESINEMIN-PIPERAQUINE FOR TREATING UNCOMPPLICATED FALCIPARUM PREGNANCY-ASSOCIATED MALARIA IN GHANA**

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Dihydroartesinin-piperaquine (DHA-PPQ) was approved for treatment of uncomplicated malaria in Ghana in 2008. However, DHA-PPQ is not used in pregnancy due to the paucity of safety data in this population. In light of anticipated DHA-PPQ use by self-medicating pregnant women, we have studied the safety and efficacy of DHA-PPQ compared to artesunate-amodiaquine (ASAQ) in a non-inferiority trial for treatment of uncomplicated malaria in second/ third trimester pregnancies.
Pregnant women who attended antenatal clinics in two districts of the Ashanti Region were screened for falciparum malaria using both a rapid diagnostic test and microscopy. Those testing positive in both were recruited, individually randomized to DHA-PPQ or ASAQ, and followed up actively on days 1, 2, 3, 7, 14, 28 and 42 after the start of treatment, at delivery and 6 weeks post-partum. During this period, assessment of adverse events, assessment of study drug adherence, sampling of blood for haematological and parasitological assessments and data collection on neonatal morbidity and mortality were performed. The primary outcome was parasitological efficacy by day 42. Analysis was primarily per protocol. Uncorrected cumulative parasitological efficacy by day 42 for DHA-PPQ was 91.2% (95% CI: 86.8%, 95.4%) and for ASAQ it was 87.0% (95% CI: 80.6%, 91.7%). By day 28, it was 93.8% (95% CI: 89.3%, 96.9%) for DHA-PPQ and almost 92.0% (95% CI: 86.5%, 95.4%) for ASAQ. There were no reports of hepatic/renal toxicity or evidence of white blood cell dyscrasia in either study arm. The DHA-PPQ arm had fewer adverse events than the ASAQ arm; vomiting (17% vs 29%; p=0.021), general weakness (40% vs 60%; p<0.001). DHA-PPQ was non-inferior to ASAQ within a margin of 5% and also safer in the second and third trimesters. However, we are cautious in generalizing this conclusion because of the small sample size and our inability to report PCR-corrected efficacy rates. Larger studies are needed to confirm the results of this preliminary study.

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IN VITRO DRUG TRANSPORT OF ANTIMALARIALS; AMODIAQUINE, MELOQUINE AND METHYLENE BLUE

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Identification of P-gp mediated drug-drug interactions that interfere with antimalarial drug absorption would inform optimal drug design combinations in malaria. The study aimed to determine the P-gp substrate and inhibitory activities of amodiaquine (AQ), meloquine (MQ) and methylene blue (MB). Caco-2 cell lines were grown on 0.6 cm² filter inserts at the cell density of 65,000 cells/cm². Bidirectional drug transport studies were done on 21-24 day cells with transepithelial electrical resistance > 300 Ω.cm². AQ, MQ and MB were assayed via HPLC techniques. Rhodamine 123 (Rh123) quantification was done using fluorescence. MQ at 100 micromolar, pH 7.4 showed an apical-basolateral (A-B) permeability of 11 × 10⁻⁶ cm/sec and basolateral-apical (B-A) permeability of 12 × 10⁻⁶ cm/sec. Efflux ratios (ER) for 100 and 10 micromolar MQ suggested diffusion, as they were 1.1 and 1.3 respectively. Other antimalarial drug combinations that included MQ were not altered significantly, nor combinations with the P-gp inhibitor PSC-833. AQ at 100 µM, pH 7.4 showed an A-B permeability of 20 × 10⁻⁶ cm/sec and B-A permeability of 23 × 10⁻⁶ cm/sec, thus more permeable than MQ, but still not evidence of active efflux as their efflux ratios were suggestive of diffusion. B-A permeability of AQ did increase when combined with PSC833, however the statistical power of the test was low. P-gp substrate activity of MB was studied at 10 μM, pH 7.4 and showed an ER of 3.4 dropping to 1.6 in combination with 4 micromolar PSC833. Changes in transport rates in both directions were statistically significant. P-gp inhibitory activities of the three drugs were studied by combining drugs with a known P-gp substrate, Rh123. Rh123 efflux was not altered significantly when combined with any of the antimalarials (MQ, AQ or MB). We conclude that MQ and AQ are transported mainly by passive diffusion. MQ diffusion is not altered by artesunate. MB is subjected to P-gp mediated drug efflux. These drugs did not exhibit P-gp inhibitory activity.

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THE AVAILABILITY AND COST OF ANTIMALARIAL MEDICATIONS IN DRUG OUTLETS IN RURAL SIAYA COUNTY, WESTERN KENYA

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Although antimalarials are free in public health facilities in Kenya, challenges including stock-outs lead to patients seeking treatment in the private sector or directly from drug outlets. Medicine retailers play a major role in the distribution of antimalarials. The Affordable Medicines Facility - malaria program (AMFm) was introduced in June 2010 to make artemisinin combination therapy (ACT) as affordable as less effective alternatives. This study was conducted to determine the availability and cost of rapid diagnostic tests (RDTs) and antimalarials in drug outlets in a high malaria transmission area in rural western Kenya. We conducted a cross-sectional study from September to October 2013 and mapped all drug outlets within the Kenya Medical Research Institute/Centers for Disease Control and Prevention Health and Demographic Surveillance System area in Siaya County. We administered a standardized structured questionnaire to drug outlet personnel to collect data on outlet characteristics and availability, types and prices of RDTs and antimalarials. We identified 181 drug outlets, and 179 agreed to participate. Of these, 13% were registered pharmacies, 25% informal drug outlets, 46% general shops; 13% home-based outlets and 2% other. Only 17 (10%) had RDTs in stock, and 149 (84%) never stocked RDTs. 165 (92%) had ACTs in stock; of these, 98% had Artemether-Lumefantrine (AL); 13% had dihydroartemisinin-piperaquine (DHA-PPQ); 47% had sulfadoxine-pyrimethamine (SP); 34% had quinine (QN) and 8% had amodiaquine (AQ). Among the QN-stocking outlets, 48% had tablets and 62% had a parenteral formulation. The mean (SD) price in USD of an adult treatment course of AL, DHA-PPQ, QN tablets, SP, AQ was 1.01 (0.38), 4.40 (1.15), 2.24 (1.18), 0.62 (0.31), and 0.42 (0.20) respectively; the mean (SD) price in USD of RDTs was USD 0.92 (0.64). There was no significant difference in mean price of AL with or without the AMFm logo (USD 1.01 and 1.07 respectively, p= 0.45). The majority of drug outlets did not stock RDTs suggesting that testing prior to treatment per the national guidelines is unlikely for patients first seeking treatment in the outlets. The first-line treatment, AL, is widely available across outlet types. SP and AQ are not recommended for treatment but are less expensive than AL; the lower cost might encourage preferential use by patients. Restrictive policies should be enforced to prevent access and sale of ineffective antimalarials.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH DIHYDROARTESININ-PIPERAQUINE ON COGNITIVE FUNCTION, SCHOOL ATTENDANCE AND SCHOOL PERFORMANCE IN UGANDAN SCHOOLCHILDREN: A RANDOMIZED PLACEBO-CONTROLLED TRIAL

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Intermittent Preventive Treatment (IPT) is a promising option for malaria control in schoolchildren. However, there is still limited evidence of its benefits on cognitive function and educational outcome in this age group in different transmission setting. Therefore, we have investigated the impact of two dosing schedules of IPT with dihydroartemisinin-piperaquine (DP) on cognitive function, school attendance and school performance in Ugandan schoolchildren living in a high malaria transmission setting. We conducted a randomized, double-blind, placebo-controlled trial in 740 schoolchildren aged 6-14 years. Enrolled children were randomized to receive DP once every month (IPTm), once a school term (four treatments over 12 months) (IPTs) or placebo and followed for 18 months. The outcomes included cognitive function which was assessed using the
Ravan’s matrices (for abstract reasoning) and the code transmission test (for sustained attention), school performance in the end of year mathematics and English exams and school attendance. Analyses were conducted on an intention to treat basis. Compared to placebo, IPTm marginally reduced the risk of missing school due to being sick (12.5% versus 9.5% p=0.06), especially if the illness was malaria (5.1% versus 0% p=0.05). No difference was observed between IPTst and placebo on school attendance. No impact of IPT compared with placebo was observed on sustained attention (mean difference [MD] IPTm -0.01 p=0.920, and IPTst -0.11 p=0.693), abstract reasoning (MD IPTm 0.09 p=0.726, and IPTst 0.52 p=0.228), results in mathematics exams (MD IPTm -0.8 p=0.418, and IPTst -1.5 p=0.676) or results in English exams (MD IPTm 0.5 p=0.551, and IPTst -1.2 p=0.797). IPTm reduced the risk of missing school due to malaria. However, IPT as implemented in this study (IPTm and IPTst) was not effective in improving the cognitive function or school performance of schoolchildren.

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ADHERENCE TO CO-FORMULATED AMODIAQUINE ARTEUNATE VS. ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN FREETOWN, SIERRA LEONE

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Increasing access to and targeting of artemisinin-based combination therapy (ACT) is a key strategy of malaria control programmes. Approaches, such as co-packaging ACTs into blister packs, have been shown to improve adherence. While co-packaging facilitates correct treatment dosing, it does not reduce the quantity or frequency of tablet intake. To overcome these limitations, several ACTs are now produced as co-formulated tablets. Although there have been a number of studies looking at adherence to ACTs, the majority have focused on adherence to artemether-lumefantrine (AL). The aim of this study was to measure the level of caregiver adherence to co-formulated amodiaquine-artesunate (AQAS) compared to AL in Sierra Leone. This open-labelled randomised controlled study was conducted from September 2013 to January 2014 in two public health centres in urban Freetown. Patients 6-59 months presenting for fever at the health facility were randomized to receive either AQAS or AL at the time of prescription. Adherence was assessed using self-report of medication intake and package inspection. Exit interviews of caretakers were conducted at the health facility and follow-up interviews at their home on day four post-prescription. Of 1,950 patients screened, 795 were excluded due to: inappropriate age (29.6%), residence outside of the catchment area (12.3%), prior participation in the study (6.6%), no fever or history of fever (9.1%), and evidence of severe disease (2.0%). Of the 1,155 patients enrolled, 90% completed study follow-up, and 859 (78.5%) received an ACT. Preliminary results from one study site show that there was no difference in adherence to AQAS (96.4%) compared to AL (97.1%) (p=0.56). Reasons for non-adherence were similar for both treatment arms (forgot the last dose or took dose incorrectly). Vomiting was the most cited reason for missing a treatment dose for both groups. Data analysis for the second site is currently on-going. Full results, including comparison of caregiver adherence at both study sites and factors influencing adherence, will be presented.

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POPULATION PHARMACOKINETICS OF ORALLY ADMINISTERED MEfloQUINE IN HEALTHY VOLUNTEERS AND PATIENTS WITH UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA

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The determination of dosing regimens for the treatment of malaria is largely empirical and thus a better understanding of the pharmacokinetic/pharmacodynamic properties of anti-malarial agents, such as mefloquine, is required to assess the adequacy of current treatment regimens and identify sources of sub-optimal dosing which could select for drug-resistant parasites. Mefloquine pharmacokinetics were assessed in 24 healthy adults and 43 patients with P. falciparum malaria. Population pharmacokinetic modelling was conducted using NONMEM. A two-compartmental model with a single transit compartment and first-order elimination from the central compartment most adequately described mefloquine concentration-time data. The model incorporated population parameter variability for clearance (CL/F), central volume of distribution (VCF/F) and absorption rate constant (KA), and identified, in addition to body weight, malaria infection as a covariate of VCF/F (but not CL/F). Simulations predict that falciparum malaria infection is associated with a shorter elimination half-life (407 c.f. 566hr) and time above MIC (766 c.f. 893hr). This is first population pharmacokinetic study to show falciparum malaria to influence mefloquine disposition. Protein binding, anaemia and other factors may contribute to differences between healthy individuals and patients. As VCF/F is related to the earlier portion of the concentration-time profiles, which occurs during acute malaria, and CL/F is more related to the terminal phase during convalescence after treatment, this may explain why malaria was found to be a covariate for VCF/F but not CL/F. These findings highlight the importance of patient characteristics when determining optimal dosing regimens for use in clinical practice; more studies will be needed in different populations.

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ANEMIA IN ACUTE AND CONVALESCENT UNCOMPLICATED FALCIPARUM MALARIA TREATED WITH ARTEMISINE-AMODIAQUINE AND COMPARATORS IN SUB-SAHARAN AFRICA

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Anemia is both a complication of malaria and a potential drug adverse reaction. Recovering from malaria should coincide with increased hemoglobin (Hb) levels, but the process is confounded by poorly-understood parasite, host and drug-related factors. Delayed hemolytic anemia was recently reported after parenteral artemisinin for severe malaria. We analysed 5189 patients with acute uncomplicated falciparum malaria from 8 studies conducted at 14 sites in 9 Sub-Saharan Africa countries during 2002-2009. Median age was 4 years (range 6m - 86y). 64% of the patients were 10 yo. 2380 patients were treated with artemesinate-amodiaquine (ASAQ, 45.8%), 2809 with comparators (54.2%). In ASAQ groups, high parasitaemia was associated with low Hb both at baseline (r=-0.287) and post-treatment at D3, at D7, D14, and D28 (r=-0.165, -0.483, -0.161, -0.382, respectively, p=0.001 for all, Spearman test). Patients before ASAQ treatment had a mean Hb=10.9 g/dL (sd 2.20). Mean changes between D0-D3 were -0.48 g/dL (-4.0%); D0-D7 -0.45 g/dL (-3.2%); D0-D14 +0.54 g/dL (+7.5%); D0-D28 +0.77 g/dL (+9.8%). By multivariate analysis with random effects on the site (9594 Hb records) Hb increased over time (r=0.04, p=0.001) and in older patients (r=0.06, p=0.001) and decreased with higher parasitaemia (r=-0.39, p=0.001).
multivariate survival analysis, patients with higher parasitaemia (adjusted hazard ratio, AHR=1.32, 95% CI 1.19-1.46, p<0.001) and children <5 yo (AHR=1.91, 95% CI 1.55-2.34, p=0.001) and anemia (<10 g/dL) before treatment (AHR=1.26, 95% CI 1.09-1.46, p=0.002) were at higher risk for parasite recurrence. On D0 32.6% (502/1541) patients were anemic, 39.6% (610/1541) on D7, 16.2% (249/1541) on D28. Between D7 and 28, 2.0% (19/931) became anemic, 37.7% (230/610) remained anemic and 62.3% (380/610) became normal. Similar trends were observed with other combination treatments. Anemia and high parasitaemia concur to increase malaria treatment failure. Hb increases by D14 after initial fall. Delayed anemia did not occur in uncomplicated malaria.

SCALE UP OF INTEGRATED HOME-BASED MANAGEMENT IN SENEGAL

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Since 2008, the Senegal National Malaria Control Program (SNMCP) has implemented home-based management of malaria (PECADOM) using rapid diagnostic tests (RDTs) and artesiminin-based combination therapy (ACTs). In 2012, the SNMCP and partners piloted integrated home-based management, including case management of diarrhea with zinc and oral rehydration salts (ORS) and pneumonia with antibiotics (in children under 5 years) as well as malaria in all age groups. Encouraging results prompted the scale-up of integrated home-based management in the southeastern regions of Tambacounda and Kedougou, which suffer from high all-cause mortality. In 2013, 428 villages in Tambacounda and Kedougou selected a home-based care provider (DSDOM). They received four days of classroom instruction on diagnosis and treatment of diarrhea, pneumonia, and malaria, followed by 15 days of practical experience at the health post nearest the village, supervised by the head nurse. Each provider received a drug box, timer, vest and hat, case register, RDTs, ACTs, co-trimoxazole, ORS, and zinc. Health districts received a budget to provide monthly supervision by the nurse and quarterly supervision by the district health management team. From June to December 2013, DSDOMs saw 12,979 patients: 5,705 patients under 5 years and 7,274 patients 5 years and older. Of these, 10,704 (82%) had fever and 98% were tested with an RDT; 7,310 (70%) had positive tests and 7,652 were treated with an ACT. Of children under 5, 2704 (36%) were diagnosed with malaria, 1,041 (18%) were diagnosed with diarrhea, 58 (5%) were treated with ORS alone, 35 (3%) were treated with zinc alone, and 95 (92%) received both. There were 656 (11%) children under 5 diagnosed with pneumonia and treated with co-trimoxazole. Of the remaining 578 patients, 575 (10%) were diagnosed with upper respiratory infection, and symptomatic relief was recommended. Of the 3,217 patients (25%) referred to the health post (all ages), 2,791 (22%) had a negative RDT, 30 (0.2%) were less than two months, 25 were pregnant women (0.2%), and 359 (3%) had severe disease. In 2014, integrated PECADOM will be scaled up to an additional nine regions, and DSDOMs will be trained to identify and refer acute malnutrition among children under 5 years. These efforts should contribute substantially to the achievement of Millennium Development Goals 4 and 6, the reduction of under 5 mortality and the fight against malaria and other diseases.

CHANGE IN MALARIA FALCIPARUM TREATMENT IN A MUNICIPALITY OF ACRE STATE, BRAZIL

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Brazil has around 300,000 malaria cases/year which represents about 60% of cases in Americas. In 2012, Acre state showed a 37% increase in malaria cases compared to 2011. Since 2006 Acre state has been using artesunate+mefloquine (ASMQ) for P. falciparum treatment, started as an efficacy study and adopted as treatment as soon as the drug was registered in Brazil. According World Health Organization recommendations to not use mefloquine in high transmission areas due to its longer half-life and possible development of resistance, the malaria advisory committee has suggested in 2011 to change the treatment and in 2012, treatment has been changed to artemether+lumefantrine (AL). All others Amazonia regions were already using AL since 2006. The aim of the present study was to evaluate if the change in treatment regimens has contributed to increase cases in Rodrigues Alves municipality in 2013. An observational cross sectional study was performed based on primary data and were used as a measure of Odds ratio association to estimate relative risk. Study population was patients treated in health units or by field health workers in routine visits in Rodrigues Alves, with positive thick blood smear for Plasmodium falciparum. Data were analysed with Epinfo 3.5.3. A total of 210 questionnaires were analysed. Respondents were male in 54% and the median age was 15 years (1-80). The slide cure verification on day 7 (d7) was performed in 191 (60%) patients. Treatment with ASMQ has been used in 99 (47%) patients. Three (3%) have not been cured. 111 (53%) have been treated with AL and one (0.9%) has not been cured. The odds ratio was 0.29 and IC95% 0.02-2.84. The results show that individuals treated with AL had 0.29 times more probability to have been cured when compared to subjects treated with ASMQ. However, this difference was not significant statistically, and the protection factor was possible related to a spurious association. In this way it suggests a similar efficacy between the two drugs. On the other hand, possibly there was a reduction of the mefloquine half-life effect to prevent new infections. Monitoring resistance surveillance to antimalarial drugs has prominent role to contain or eliminate the resistant parasites before they spread to higher transmission areas what will put in serious risk the recent progress in malaria control. It is important to create new strategies to detect early decrease of effectiveness of these drugs.
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ARTESUNATE-AMODIAQUINE IN HEALTHY VOLUNTEERS
ACTIVITY OF ARTEMISININ-PIPERAQUINE VERSUS
ANTIMALARIAL PHARMACOKINETICS AND
sterile heterologous protection against sporozoites. Splenic and hepatic lymphocytes harvested from unchallenged
74
CD8 T cells identified using H-2Kd restricted epitope of CS Protein had protection against homologous challenge that we previously reported.
and
both the discovery of new antimalarial agents and the development of their implications for the treatment of malaria.
relationship of the two ACTs will be presented and discussed, including
PK-PD
ACT possessed greater blood schizontocidal activity,
volunteers plasma samples after drug administration to determine which
ex vivo
MS for estimating the PK properties of the partner drugs for each ACT.
were collected up to 42 days and 28 days after the last dose of Artequick
artesunate and 270 mg amodiaquine) for 3 days. Serial blood samples
were administered daily a dose of two tablets of Artequick (each tablet contains 62.5 mg artemisinin
healthy Vietnamese subjects. Group 1 volunteers were administered daily a
artemisinin-piperaquine and artesunate-amodiaquine in two groups of 22
falciparum malaria in south-central Vietnam. Because there is limited
therapies (ACTs) such as dihydroartemisin-piperaquine has been
Western Cambodia and south Vietnam. This is of immense concern as ACTs are currently recommended worldwide for first-line
treatment of uncomplicated Plasmodium falciparum malaria. In Vietnam, dihydroartemisin-piperaquine is the ACT of choice for the treatment of falciparum malaria, with little knowledge of the efficacy and tolerability of alternative ACTs. Recently, we reported Artequick (artemisinin-piperaquine) and Coarsucam (artesunate-amodiaquine) to be highly efficacious (PCR-corrected cure rate >98%) and well tolerated in Vietnamese patients with falciparum malaria in south-central Vietnam. Because there is limited pharmacokinetics (PK) data on these two ACTs, we evaluated the PK of artesunate-piperaquine and artesunate-amodiaquine in two groups of 22 healthy Vietnamese subjects. Group 1 volunteers were administered daily a dose of two tablets of Artequick (each tablet contains 62.5 mg artesinin and 375 mg piperaquine) for 3 days. Group 2 volunteers received a daily dose of two tablets of Coarsucam (each tablet contains 100 mg artemisin and 270 mg amodiaquine) for 3 days. Serial blood samples were collected up to 42 days and 28 days after the last dose of Artequick and Coarsucam, respectively. Plasma samples were assayed by LC/MS/ MS for estimating the PK properties of the partner drugs for each ACT. Furthermore, we carried out a head-to-head comparison of the ex vivo antimalarial activity (pharmacodynamics-PD) of the two ACTs using the volunteers plasma samples after drug administration to determine which ACT possessed greater blood schizontocidal activity, in vitro. The PK-PD relationship of the two ACTs will be presented and discussed, including their implications for the treatment of malaria.

249 PHARMACOKINETICS AND EX VIVO ANTIMALARIAL ACTIVITY OF ARTEMISININ-PIPERAQUINE VERSUS ARTESUNATE-AMODIAQUINE IN HEALTHY VOLUNTEERS

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Reduced susceptibility to artesunate and artemisinin-based combination therapies (ACTs) such as dihydroartemisin-piperaquine has been reported in western Cambodia and south Vietnam. This is of immense concern as ACTs are currently recommended worldwide for first-line treatment of uncomplicated Plasmodium falciparum malaria. In Vietnam, dihydroartemisin-piperaquine is the ACT of choice for the treatment of falciparum malaria, with little knowledge of the efficacy and tolerability of alternative ACTs. Recently, we reported Artequick (artemisinin-piperaquine) and Coarsucam (artesunate-amodiaquine) to be highly efficacious (PCR-corrected cure rate >98%) and well tolerated in Vietnamese patients with falciparum malaria in south-central Vietnam. Because there is limited pharmacokinetics (PK) data on these two ACTs, we evaluated the PK of artesunate-piperaquine and artesunate-amodiaquine in two groups of 22 healthy Vietnamese subjects. Group 1 volunteers were administered daily a dose of two tablets of Artequick (each tablet contains 62.5 mg artesinin and 375 mg piperaquine) for 3 days. Group 2 volunteers received a daily dose of two tablets of Coarsucam (each tablet contains 100 mg artemisin and 270 mg amodiaquine) for 3 days. Serial blood samples were collected up to 42 days and 28 days after the last dose of Artequick and Coarsucam, respectively. Plasma samples were assayed by LC/MS/ MS for estimating the PK properties of the partner drugs for each ACT. Furthermore, we carried out a head-to-head comparison of the ex vivo antimalarial activity (pharmacodynamics-PD) of the two ACTs using the volunteers plasma samples after drug administration to determine which ACT possessed greater blood schizontocidal activity, in vitro. The PK-PD relationship of the two ACTs will be presented and discussed, including their implications for the treatment of malaria.

250 EXPLOITING EVOLUTION TO SUPPRESS THE EMERGENCE AND SPREAD OF DRUG RESISTANCE IN MALARIA

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Rising drug resistance threatens to make malaria untreatable, necessitating both the discovery of new antimalarial agents and the development of strategies to identify and suppress the emergence and spread of drug resistance. Drug resistance can rapidly compromise the effective useful lifetime of antimalarial agents. However, drug resistance emerges in the context of a population and is limited by evolutionary fitness costs. We focused on in-development dihydroorotate dehydrogenase (DHODH) inhibitors. We identified eleven point mutations and gene amplification in the PfDHODH target, as well as several unknown resistance mechanisms. These mutant parasites were often hypersensitive to other PFDHODH inhibitors, which immediately suggested a novel combination therapy approach to preventing resistance. Indeed, a combination of wild-type and mutant-type selective inhibitors led to resistance far less often than treatment with either drug alone. The effects of point mutations in PFDHODH were corroborated with purified recombinant proteins. Comparative growth assays demonstrated that two mutant parasites were less fit than their wild-type parent, and the purified protein of these mutants showed a decrease in catalytic efficiency, thereby suggesting a reason for the diminished fitness. Co-crystallography of PFDHODH with three inhibitors suggested that hydrophobic interactions are important for drug binding and selectivity, and that mutations in the active site tunnel have more serious catalytic and fitness consequences than mutations in the active site lid. Fitness limitations result in a small number of mutational escape pathways being heavily favored in Plasmodium. These escape pathways can be anticipated and blocked, and doing so increases the efficacy of an individual patient’s treatment and protects the useful lifetime of novel therapeutics.

251 MEFLOQUINE VERSUS SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTp): A MULTIPLE-OUTCOME ANALYSIS ON EFFICACY AND TOLERABILITY

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Evaluation of alternative drugs to replace sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment (IPTp) is urgently needed because of the spread of resistance of parasites to the drug, which raises the question of the short-term efficacy of IPTp-SP to prevent malaria in pregnancy. Very few options are currently available, of which none fulfil all the ideal properties for IPTp. Therefore, alternatives need to be evaluated on basis of a benefit-risk ratio. Mefloquine (MQ) is one of the options that have already been evaluated for IPTp. Here, we reanalysed data from the first Beninese trial on IPTp-MQ using a multiple-outcome approach, which allowed the joint assessment of efficacy and tolerability. The trial was conducted in Ouidah, South Benin, and involved 1600 pregnant women, who were randomised in two groups (IPTp with MQ, 15mg/kg, versus IPTp with SP (1500-75mg) administered twice during pregnancy). For the present analysis, the overall superiority of one drug to the other was defined as: superiority on low-birth weight (birth weight < 2500 grams), placental malaria or maternal anaemia (Hb < 10g/dL), non-inferiority on all efficacy outcomes and non-inferiority on tolerability defined as severe adverse events (AE) - either cutaneous or neuro-psychiatric - or low compliance with the treatment (i.e., reluctance to receive the second IPTp dose because of AE at the first dose or refusal to receive the second dose whatever the reason). MQ was found to be overall superior to SP (global P value=0,004). By applying different strategies to deal with missing data and/or including stillbirths, spontaneous abortions and multiple pregnancies in the analyses provided similar results. Whatever
the final decision on the use of MQ for IPTp, such a statistical approach outweighing the benefits/disadvantages of a large prevention strategy should systematically be taken into account by institutional policy makers.

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CYP 2D6 METABOLISM IS ESSENTIAL FOR THE ANTIMALARIAL ACTIVITY OF TAFENOQUINE AND NPC-1161B

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Tafenoquine (TQ) is an 8-aminoquinoline (8AQ) that has been tested in several Phase II and Phase III clinical studies and is currently in late stage development as an anti-malarial prophylactic agent. NPC-1161B is a promising 8AQ in late preclinical development. It has been reported that the 8AQ drug primaquine requires metabolic activation by CYP 2D6 for efficacy in humans and in mice, highlighting the importance of pharmacogenomics in the target population when administering primaquine. A logical follow-up study was to determine whether CYP 2D activation is required for other compounds in the 8AQ structural class. In the present study, the anti-malarial activities of NPC-1161B and TQ were assessed against luciferase expressing Plasmodium berghei in CYP 2D knock-out mice in comparison with normal C57BL/6 mice (WT) and with humanized/CYP 2D6 knock-in mice by monitoring luminescence with an in vivo imaging system. These experiments were designed to determine the direct effects of CYP 2D metabolite activity on the anti-malarial efficacy of NPC-1161B and TQ. NPC-1161B and TQ exhibited no anti-malarial activity in CYP 2D knock-out mice when dosed at their ED100 values (1 mg/kg and 3 mg/kg, respectively) established in WT mice. TQ anti-malarial activity was partially restored in humanized/CYP 2D6 knock-in mice when tested at two times its ED100. The results reported here strongly suggest that metabolism of NPC-1161B and TQ by the CYP 2D enzyme class is essential for their anti-malarial activity. Furthermore, these results may provide a possible explanation for therapeutic failures for patients who do not respond to 8AQ treatment for relapsing malaria. Because CYP 2D6 is highly polymorphic, variable expression of this enzyme in humans represents a significant pharmacogenomics liability for 8AQs which require CYP 2D metabolite activation for efficacy, particularly for large-scale prophylaxis and eradication campaigns.

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A CONSTANT RATE INTRAVASCULAR INFUSION MODEL FOR THE EVALUATION OF INCREASES IN HEMATOCRIT AFTER ARTEMISININ-BASED COMBINATION TREATMENTS OF ACUTE FALCIPARUM MALARIA IN CHILDREN

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A constant rate intravenous infusion model, which employed the principles of constant rate intravenous infusion of drugs, was used to evaluate the kinetics of the increases in hematocrit after artesunate-amodiaquine (AA) or arteether-lumefantrine (AL) treatments of acute, uncomplicated falciparum malaria in 112 children. The model assumed baseline hematocrit was zero and a constant rate increase in hematocrit from baseline after treatment, and involved semi-logarithm plots of the difference between hematocrit at plateau and that at earlier times, against the corresponding times. Hematocrit reached a plateau in a median time of 28 days after treatment started. Mean plateau hematocrit was 6.75% (95% CI [6.0-7.5]) and was similar for AA- and AL- treated children [6.92% (95% CI 6.0-7.83), n = 81 v 6.32% (95% CI 4.9-7.7), n=31, P = 0.45]. Times to plateau were significantly shorter and plateau hematocrit significantly lower in non-anemic compared to anemic children. Declines from plateau were monoexponential with mean half time of 2.49 days (95% CI 2.1-2.79) and were similar in AA and AL-treated children [2.42 days (95% CI 2.07-2.77) v 2.68 days (95% CI 2.03-3.3), P = 0.46]. Hematocrit half times were significantly shorter in non-anemic compared to non-anemic children [2.09 days (95% CI 1.77-2.41, n 59) v 2.94 days (95% CI 2.41-3.46, n 53), P = 0.006] indicating hemopoietic responses may have been accelerated following successful treatment in the former. The constant rate intravenous infusion model permits evaluation of increases in hematocrit following antimalarial treatments and may be used in observational and clinical studies in uncomplicated falciparum malaria.

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COMBINING COMMUNITY CASE MANAGEMENT OF MALARIAL AND SEASONAL MALARIA CHEMOPREVENTION FOR CHILDREN LESS THAN 10 YEARS IN SENEGAL: FEASIBILITY, IMPACT ON MALARIA AND ANEMIA

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Malaria and anemia are commonly associated in tropical regions. Scaling up antimalarial interventions as advocated by the WHO, will contribute to further reduce malaria burden and may induce a significant reduction in the global anemia burden. Malaria control in many epidemiological situations such as in Senegal will require a multi-intervention strategy with the use of combination of antimalarial interventions. Studies are needed, to document the feasibility as well as the impact on malaria and anemia of combining several antimalarial interventions. Relevant studies are also needed to assess potential anemia determinants and risk factors while scaling up antimalarial interventions, for an effective and integrated anemia control. This project aimed to assess the potential benefit of adding seasonal malaria chemoprevention (SMC) to community case management of malaria (CCMm) and to investigate factors associated with anemia among <10 years. The study objectives were covered by undertaking: i) a cluster randomised trial during which eight communities were randomised to receive either CCMm combined with SMC (intervention arm) or CCMm alone (control arm), (ii) a case control study with anaemic and non.anaemic children. The overall adjusted rate ratio for incidence of malaria attacks in intervention and control communities was 0.15, indicating a protective effect of CCMm+SMC of 85% (95% CI: 39.9%-96.3%, p=0.01). The case control study, demonstrated that the main factors significantly associated with anemia in these communities were: malaria parasitaemia (aOR=5.23, 95% CI[1.1-28.48]), sickle cell disorders (aOR=2.89, 95% CI[1.32-6.34]), alpha-thalassaemia (aOR=1.82, 95% CI[1.23-3.5]), stunting (aOR=3.37, 95% CI[1.93-5.88], age ranged from 2 to 4 years (aOR=0.13, 95% CI[0.05-0.31]) and age > 5 years (aOR=0.03, 95% CI[0.01-0.08]). The combination of CCMm and SMC will result in a significant reduction of malaria and anemia burden. However, to achieve an effective control of anemia, integrated approaches taking into account other aetiologies of anemia need to be developed.
OUTLET SURVEY CONTAINING ANTIMALARIALS IN TANZANIA’S PRIVATE SECTOR - RESULTS FROM A NATIONALLY REPRESENTATIVE OUTLET SURVEY

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Artemisinin-containing antimalarials (ACAs) are acknowledged to be the most effective treatment for uncomplicated malaria. There have been reports that in some areas up to a third of antimalarials are “fake”, especially in the private sector, but few studies have been conducted in a representative fashion. Poor quality (falsefilled, substandard or degraded) antimalarials can lead to increased mortality and morbidity and contribute to resistance. In Tanzania the private sector is commonly used as a source of treatment for fever. We purchased ACAs from drug retailers throughout Tanzania mainland and measured the quality of each sample. A nationally representative survey was conducted in 2010 as part of the Independent Evaluation of the Affordable Medicines Facility-malaria. Every drug store, pharmacy and general retailer in 49 wards was visited and a sample of every ACA in stock was purchased. The active pharmaceutical ingredient (API) of the artemisinin and partner component was measured using high performance liquid chromatography and mass spectrometry, followed by multivariate analysis to determine risk factors for poor quality. Of the 1,737 ACAs purchased and analysed, 87.1% were in tablet form and a quarter were WHO prequalified products. All samples contained the artemisinin derivative (ART), with 4.1% being outside the acceptable 85-115% API range. Based on multivariate analysis of the ART component, ACAs manufactured in Europe were 4.02 times more likely to be of poor quality (p=0.01), while WHO prequalified ACAs had 0.05 times the odds of being poor quality (p=0.002). Weak medicine regulatory systems have been observed in a range of low and middle income countries, reflecting lack of financial resources, manpower and capacity. However, these results indicate that important improvements in quality can be achieved by ensuring that only products meeting WHO prequalification are registered and permitted onto the market.

PLASMODIUM FALCIPARUM MALARIA-ASSOCIATED ANEMIA IN UNDER FIVE-YEAR-OLD NIGERIAN CHILDREN: BEFORE AND AFTER ARTEMISININ-BASED COMBINATION TREATMENTS

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The risk factors for, and recovery from anemia in acute falciparum malaria were characterized in 672 <5-year-olds from five geographical areas of Nigeria before and after artemether-lumefantrine or arsunate-amodiaquine treatments. Of these, 294 (43.8%, 95%CI 40.0-47.6) presented with anemia (hematocrit <30%). Before treatment, an age <3 years and duration of illness >3 days were independent risk factors for anemia, and anemia at enrolment and parasitemia ≥100,000/µL after treatment. Malaria-attributable fall in hematocrit was 6.4% (95%CI 6.06-6.84). Mean time to recovery from anemia was 12.7d (95%CI 11.9-13.4), was similar with both treatments, and correlated positively with enrolment parasitemia (P=0.012). Fall in hematocrit per 1000 asexual parasites cleared from peripheral blood after treatment was significantly lower at higher compared with lower parasitemias (P<0.0001). Kinetics of the deficit in hematocrit (from 30%) after treatment began were estimated by a non-compartment model. Declines in hematocrit deficit were monoexponential, with a mean elimination half-life of 0.37d (95%CI 0.35-0.38). Deficit in hematocrit half-lives and efficacy in anemic children were similar for both treatments. Artemether-lumefantrine and arsunate-amodiaquine, influence the risk of anemia after treatment, conserve hematocrit at high parasitemias, and may influence recovery from Plasmodium falciparum malaria-associated non-severe anemia in Nigerian children.

ARTEMETHER-LUMEFANTRINE PHARMACOKINETICS AND PHARMACODYNAMICS IN VERY YOUNG CHILDREN TREATED FOR UNCOMPLICATED MALARIA IN UGANDA

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Artemether-lumefantrine (AL) is the most widely utilized treatment for malaria. Pharmacokinetic (PK) and pharmacodynamic (PD) data are limited in the youngest children, but concern exists over inadequate exposure in young children. Our objective was to describe the population PK/PD of lumefantrine (LR) in children 6 to 24 months of age. Capillary blood samples were collected on filter paper (n=1027) over 21 days during 249 episodes of malaria occurring in 105 children. Samples were assayed using solid-phase extraction followed by HPLC. Population PK analysis of LR with
the software Monolix® (v.4.3) employed a 2-compartment open model with first-order absorption and allometric scaling for CL, Q, V1 and V2. Cox proportional hazards regression, stratified by use of trimethoprimsulfamethoxazole (TS) prophylaxis, was conducted to explore relationships of LR levels on days 7 and 14 with recurrent parasitemia to days 28 and 42. Point estimates for the typical patient in this population were 173 L for V1, 0.94 L/hr for Q, 5,740 L for V2, and 0.021 L/hr for CL. The influence of hematocrit on V1 was the only covariate identified as significantly (p<0.005) affecting PK, with a positive correlation. Multivariate regression revealed that, in those children not taking TS, higher day 7 LR capillary concentrations were significantly associated with lower cumulative risk of recurrent parasitemia by days 28 and 42 (HR 0.7, p<0.001 for both). Children with detectable LR on day 14 (n=15/129) had no recurrent parasitemia by day 28. Additionally, while exposure was not significantly affected by TS usage in the population PK model, day 7 LR levels were significantly higher in children on TS compared to those not on TS (median level 252 vs 209 ng/mL). Our results describe a 2-compartment PK model for LR disposition in children with a significant covariate effect of hematocrit on V1. LR exposure was significantly associated with recurrent parasitemia, and TS significantly impacted that relationship. Additional analyses are underway to inform treatment strategies in the youngest age group.

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RITONAVIR-BOOSTED LOPINAVIR (LPV/R) ANTIRETROVIRAL THERAPY ASSOCIATED WITH LOWER CONCENTRATIONS OF DESETHYLANDOMAQINE IN MALARIA-UNINFECTED HIV-POSITIVE PEOPLE TREATED WITH ARTESUNATE-AMODIAQUINE

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In sub-Saharan Africa, most HIV-infected (HIV+) individuals on antiretroviral therapy (ART) are exposed to malaria. When infected with malaria they require treatment with antartesunate-amodiaquine (ASAQ). Few studies have examined the pharmacokinetics (PK) and safety of ASAQ in HIV+ individuals taking ARTs containing Nevirapine (NVP) or ritonavir-boosted Lopinavir (LPVr). We conducted an open label clinical trial to compare the maximum concentration (Cmax) and area under concentration-time curve (AUC) of amodiaquine (AQ) and desethylamodiaquine (DESAQ) in antiretroviral naive HIV+ individuals and those taking NVP and LPVr-based ART. In step 1 of the trial, malaria uninfected adults (n=6/ART group) received half the standard dose of ASAQ (1 tablet of 100/270mg each) at times 0, 24 and 48hrs. In step 2, another group of malaria uninfected adults (n=25/ART group) received a standard dose of ASAQ (2 tablets of 100/270mg each). We performed data-rich PK blood sampling and assessed treatment emergent hematological and biochemistry abnormalities. In steps 1 and 2, AQ concentrations were well below the HPLC assay lower limit of quantitation (25ng/mL). From step 1 to Step 2, AQ concentrations significantly decreased (560 ng/mL, 95%CI: 412, 95%CI: 324-383) to 40,400 ng.hr/mL (95%CI: 36,285-43,175), p<0.000001. In step 1, there were no significant differences in DESAQ Cmax and AUC among participants in the LPVr-, EFV and ART-naive groups. In step 2, compared with the ART-naive group, participants in the LPVr-ART group had lower DESAQ Cmax (267, 95%CI: 178-375) vs 412, 95%CI: 324-678) ng/mL, p<0.0001 and AUC (27,893, 95%CI: 17,276-41,383) vs 48,172, 95%CI: 35,402-59,336) ng.hr/mL, p<0.0001. However, no significant differences in Cmax and AUC were observed between the NVP-based and ARV-naive groups. In step 2, cases of transaminitis (n=2) and neutropenia were commonly observed in the NVP-ASAQ and ART-naive groups but not in the LPVr group. Thus, LPVr-ART reduces DESAQ plasma concentration and may be associated with reduced efficacy of ASAQ. However, higher DESAQ levels may be associated with liver and hematological toxicities.

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HIGH RESOLUTION MELTING (HRM) ANALYSIS HIGHLIGHTS MALARIA MISDIAGNOSIS AMONG MICROSCOPICALLY NEGATIVE FEBRILE PATIENTS IN WESTERN KENYA, AND HAS IMPLICATIONS FOR BETTER DRUG USE

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Microscopy and rapid diagnostic tests (RDT) are common tools for diagnosis of malaria, but are deficient in detecting low Plasmodium parasitemia, often leading to inappropriate medical prescriptions and patient care based on misdiagnoses of clinical presentations. We developed and applied a molecular diagnostic tool (nPCR-HRM) that combines the sensitivity and specificity of nested PCR (nPCR) and direct PCR-high resolution melting analysis (dPCR-HRM) to screen blood samples from febrile patients for low-parasitemia malaria in a rural malaria endemic setting. Blood samples (n=197) were collected in two islands of Lake Victoria, Kenya, from febrile patients without Plasmodium detectable by microscopy or RDTs. 18S rRNA gene sequences were amplified from extracted DNA by nPCR-HRM, nPCR, and dPCR-HRM to detect and differentiate Plasmodium parasites. Data on administration of antimalarials were also collected. The coupled nPCR-HRM assays detected Plasmodium parasitemia in 62 (31.5%, 95% CI 25-38%), whereas 39 (19.8%, 95% CI 14-25.4%) of the samples respectively. Among low-parasitemia infections detected by nPCR-HRM, only 42 (67.7%) patients were treated with antimalarials, whereas 110 (81.5%) patients not infected with Plasmodium parasites were treated with antimalarials. Our findings demonstrate the limitations of differential diagnostics of febrile illnesses in rural malaria endemic settings that confound epidemiological estimates of malaria, and lead to inadvertent overprescription of antimalarial drugs to non-malaria febrile patients and under-prescription of antimalarial drugs to low-parasitemia malaria patients. nPCR-HRM enhances low-parasitemia malaria diagnosis and can potentially surmount the deficiencies of microscopy and RDT based results in determining malaria parasitemia, and evaluating malaria epidemiology.

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PRESCRIPTIVE DIAGNOSIS MALARIA TREATMENT IN FEBRILE CHILDREN IN PARTS OF SOUTHEASTERN NIGERIA

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Malaria diagnosis in Nigeria has largely been done clinically, based to a larger extent on recognition of symptoms. Parasitological confirmation is a key component of case management of malaria. Clinical diagnosis has poor accuracy and leads to over diagnosis of malaria and poor management of non-malaria febrile illnesses and wastage of antimalarial drugs. Parasitological diagnosis using microscopy was performed among 560 febrile children, 0-12years attending pediatric clinics in Federal Medical Center Owerri and some primary health care centers in Imo State, South Eastern Nigeria after obtaining Ethical clearance. Their blood
samples were collected prior to treatment and were treated for malaria according to the IMCI guidelines and the standard routine practice of the clinic. These children were separated into two age groups: group I (0≤5) and II (>5-12) for data analysis purpose. Out of 560 children (0-12 years) enrolled, 156(27.9%) were actually positive for malaria parasites, while 404(72.1%) were slide negative. Only *Plasmodium falciparum* was found in the positive samples. Children’s age was significantly related to the prevalence of uncomplicated malaria (p=0.000) and a high determinant in explaining 6.4% of the variance in the prevalence of uncomplicated malaria (F=37.915 and p=0.000). Group II (>5-12) had higher parasitaemia prevalence of 44% compared to 20% prevalence found in group I (0≤5) although group I had higher parasitaemia (density count). Only children between 0≤5 years received antimalarials in compliance with the IMCI guidelines. This gave an overtreatment of 83.1% in children 0≤5 years based on blood smear microscopy confirmation. The findings highlight the need for the scaling-up of parasitological confirmation of all patients suspected to stand overtreatment with the ACTs. Improving the diagnostic system for effective health care delivery in Nigeria and other poor endemic areas will not only provide a good platform for malaria treatment/monitoring but also reduce rapid onset of drug resistance, wastage of antimalarials and adequate management of other febrile illnesses in children.

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**LOOP MEDIATED ISOThERMAL AMPLIFICATION (LAMP) FOR MALARIA DIAGNOSIS TARGETING THE APICOPLAST GENOME**

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Malaria still remains a global health burden with an estimated 627,000 malaria deaths worldwide in 2012. The shortcomings of current diagnostic methods are a concern, and the malERA Consultative Group on Diagnoses and Diagnostics has called for improved malaria diagnostic methods. Current malaria diagnostic methods do not rapidly and accurately detect asymptomatic infections which contribute significantly to transmission. As malaria declines and large numbers of samples need to be screened to target intervention measures appropriately, high throughput amplification of nucleic acids via polymerase chain reaction (PCR) become more relied on, however, they are not easily field deployable. Isothermal amplification methods have been developed to address the major shortcomings of PCR methods. Different targets are being amplified, the most common being the conserved small subunit ribosomal RNA 18S locus. In this study, we report the development and optimization of the apicoplast genome of *Plasmodium falciparum* as a target for molecular diagnosis of malaria using a loop-mediated isothermal amplification (LAMP) assay. *P. falciparum* sequences from 15 Gambian isolates and 8 laboratory clones were aligned against the PlasmoDB reference sequence (emb|X95275.2). Primers were designed from a highly conserved region of the consensus sequence, approx. 1.5kb segment of the gene coding for a ribosomal RNA protein (AP|0010):rRNA) and validated in silico. The assay was optimized for temperature, primer, dNTPs and MgCl₂ concentration. 184 archived DNA samples from West Africa and S.E Asia were analyzed against the reference PCR method. End point determination was by naked eye and DNA samples from West Africa and S.E Asia were analyzed against a reference PCR method. The assay was optimized in explaining 6.4% of the variance in the prevalence of uncomplicated malaria (F=37.915 and p=0.000). Group II (>5-12) had higher parasitaemia prevalence of 44% compared to 20% prevalence found in group I (0≤5) although group I had higher parasitaemia (density count). Only children between 0≤5 years received antimalarials in compliance with the IMCI guidelines. This gave an overtreatment of 83.1% in children 0≤5 years based on blood smear microscopy confirmation. The findings highlight the need for the scaling-up of parasitological confirmation of all patients suspected to stand overtreatment with the ACTs. Improving the diagnostic system for effective health care delivery in Nigeria and other poor endemic areas will not only provide a good platform for malaria treatment/monitoring but also reduce rapid onset of drug resistance, wastage of antimalarials and adequate management of other febrile illnesses in children.

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**CHALLENGES OF MALARIA DIAGNOSIS IN PEDIATRIC PATIENTS AT A NIGERIAN HOSPITAL**

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This study compares the performance of routine malaria diagnostic tests, and explores the challenges of malaria diagnosis in paediatric patients in an endemic setting in South West Nigeria. This was a Cross sectional study conducted at the children’s outpatient and emergency units of the University College Hospital, Ibadan, Nigeria. Patients seen between May and August, 2013 were enrolled in the study. The records of all 532 children aged six months to 12 years who received treatment for an acute febrile illness at the hospital during the study period were reviewed. The proportion of children classified as having malaria by clinical diagnosis, Rapid Diagnostic Test (RDT) and blood smear microscopy were compared. Factors associated with test positivity were explored using multivariate analysis. By clinical diagnosis 45.2% of children were diagnosed as having malaria, 37.6% tested positive to malaria parasite on RDT and 19.3% had positive blood smears on microscopy. Logistic regression showed that with RDTs, younger children were less often found to be positive than older children [OR: 0.594 (0.401-0.879)]. A similar lower probability of positivity was found for younger children on microscopy [OR0.624 (0.391-0.996)]. Positive smears were however recorded 3.9 times more often for those who gave a history of fever compared to those who did not [OR: 3.882 (1.154-13.057)]. The true malaria morbidity among these paediatric patients remains questionable due to the differences in the results produced by the different diagnostic methods. The clinical implication of RDT-positive but microscopy-negative samples may be grave if microscopy results are erroneous. Quality control systems and surveillance of routine malaria diagnostics are imperative to limit misdiagnosis of malaria in this endemic setting.

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**EVALUATING THE PERFORMANCE OF ID-FLUORESCENT IN SITU HYBRIDIZATION IN DETECTING MALARIA PARASITES IN WESTERN KENYA**

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Diagnosis of malaria parasites requires highly sensitive and specific diagnostic tools that may help rationalize antimalarial drug use in Kenya. In many cases, the identification of malaria parasites concerns their salient features that can be utilized to distinguish between species and subspecies. Conventional techniques including serology and microscopy (Giema) have limitations making it difficult to meet these requirements. Currently, detecting very low density infection has been done with alternative methods including nucleic acid amplification tests (NAAT) such as Polymerase Chain Reaction (PCR); Loop mediated isothermal amplification (LAMP) and Fluorescent In Situ Hybridization (FISH). These nucleic acid techniques generally involve the use of DNA and RNA probes and have been shown to be very sensitive and specific. However, these techniques are not easily accessible and relatively expensive. Data to-date shows that Plasmodium FISH assays for detection of *Plasmodium* parasites and speciation of *P. falciparum* and *P. vivax* have high sensitivity and specificity and are easy to perform. The FISH assays detect both asexual and sexual stages of the malaria parasites. This current study proposes to evaluate the performance of Plasmodium FISH assays for the diagnosis of *P. falciparum* malaria infections, more specifically *P. falciparum* gametocytes and low parasitemia in patients. The technique will be evaluated against...
malaria. Seventy percent of the facilities did not have basic supplies for parasite-based diagnosis of malaria. However, providers in most of the facilities managed severe malaria over the three months preceding the assessment (May, June and July 2013) was 18, 695 and diagnosis of severe malaria was made in 8.6% of the total malaria cases. Out of the severe malaria cases, about 76% of cases were discharged and mortality was recorded in about 2% of the cases. However, providers in most of the facilities managed severe malaria with injectable arteether (46%), quinine (37%) and artemesunate (29%). Majority of the health facilities (96%) practiced parasite-based diagnosis of malaria but 29% monitored the parasite clearance of patients with severe malaria. Seventy percent of the facilities did not have basic supplies for supportive management of severe malaria. In addition, 67% of the health facilities experienced stock out of parenteral arteether in the previous three months. Case management of severe malaria is still sub-optimal in study sites and to reduce the mortality attributable to malaria, effort must be intensified toward health system strengthening with emphasis on capacity building of health care providers, medical commodity security and improvement in supportive management of severe malaria.

**SEVERE MALARIA CASE MANAGEMENT PRACTICES IN SELECTED STATES IN NIGERIA: NEED FOR URGENT INTERVENTION**

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Severe malaria is a life-threatening medical condition that requires emergency interventions including prompt and effective treatment to prevent death (WHO 2000). The AQUAMAT study showed a relative reduction in mortality of 22.5% with use of parenteral Arteesunate compared to quinine in the management of severe *P. falciparum* malaria and the national policy on malaria diagnosis and treatment has been revised based on this evidence. The goal of the study was to determine baseline capacity and management practices for severe malaria in selected facilities in Nigeria with the aim of designing interventions to address specific gaps identified. A cross-sectional study design was used to assess twenty-four health facilities in three states (Benue, Kogi and Oyo States). Data on capacity of health care providers; three months malaria services provided at different service delivery points; and medical supplies were collected. Double data entry method was used for data entry into SPSS software programme and analysis done with STATA version 10.0. Total number of malaria cases reported in the three states over the three months preceding the assessment (May, June and July 2013) was 18, 695 and diagnosis of severe malaria was made in 8.6% of the total malaria cases. Out of the severe malaria cases, about 76% of cases were discharged and mortality was recorded in about 2% of the cases. However, providers in most of the facilities managed severe malaria with injectable arteether (46%), quinine (37%) and artemesunate (29%). Majority of the health facilities (96%) practiced parasite-based diagnosis of malaria but 29% monitored the parasite clearance of patients with severe malaria. Seventy percent of the facilities did not have basic supplies for supportive management of severe malaria. In addition, 67% of the health facilities experienced stock out of parenteral arteether in the previous three months. Case management of severe malaria is still sub-optimal in study sites and to reduce the mortality attributable to malaria, effort must be intensified toward health system strengthening with emphasis on capacity building of health care providers, medical commodity security and improvement in supportive management of severe malaria.

**MALARIA DIAGNOSIS SERVICE AVAILABILITY - MAPPING OF PRIVATE SECTOR SERVICE DELIVERY OUTLETS IN 7 PMI/MAPS SUPPORTED STATES, NIGERIA**

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The private sector’s role in health services in Nigeria has increased in recent times, and provides approximately 60% of health services. The MAPS project supports the implementation of parasitological confirmation of malaria cases in public health facilities in selected states. A mapping exercise was carried out to identify the private facilities providing malaria diagnostic services and determine the gaps in existing malaria diagnostic capacity with the aim of designing a strategy for improvement. Data were obtained from questionnaires administered to facility owners and location data collected by handheld GPS device. Overall, 394 private health facilities in urban areas of 7 states were mapped; Hospital/Clincs (79.7%) and Stand-alone Laboratories (20.3%). Majority (28.9%) of personnel were Nurses/Midwives, followed by Auxiliary Nurses (21.9%); Medical Specialist (14.3%) and Medical Officers (10.1%); others were CHEWs (8.1%); Medical Lab Scientist (6.3%); Lab Technicians (5.7%); Lab Assistants (3.1%); and CHOs (1.6%). Only 97 (24.6%) health workers had received training on malaria diagnosis; 43.5% of facilities offered both malaria microscopy and rapid diagnostic test (mRDT) while 17.7% and 38.8% offered only mRDT and microscopy respectively. Quality of microscopy was sub-optimal because of poorly trained personnel, re-cycling of slides and sub-optimal reagents. Overall prevalence of clinically diagnosed malaria in the outpatients was 70.3%, and malaria slide positivity rate was 78.8% using available 3-months outpatients/laboratory statistics. A good pool of human resources exists in the private sector and their capacity needs to be built on malaria diagnosis especially mRDT for non-laboratory staff. Quality of malaria microscopy could be improved with re-training of laboratory scientists and increased access to slides and good reagents. Policy framework that provides access to quality malaria diagnostic materials and creates an enabling environment is needed to support the massive scale-up of public campaigns against malaria control.

**SUCCESS OR FAILURE OF CRITICAL STEPS IN COMMUNITY CASE MANAGEMENT OF MALARIA WITH RAPID DIAGNOSTIC TESTS**

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Community case management of malaria (CCMm) by community health workers (CHWs) aims to increase access to correct and timely treatment. Currently the World Health Organization recommends to treat only confirmed malaria cases, rather than to treat presumptively. In general, CCMm with rapid diagnostic tests (RDTs) is considered a good strategy, but no in-depth review of the steps determining its success or failures has been performed. We describe an in-depth review of existing literature to provide a comprehensive overview of the success or failure of critical steps in CCMm with RDTs to identify why certain programs of studies have better results with CCM than others and to identify areas for further research. Our study showed that CHWs performed RDTs correctly, although specificity levels were variable (between 83.2 and 97.9%). Also safety issues such as reusing lancets were seen in certain studies and warrant attention. CHWs showed high adherence to test results but treating patients with a negative RDT for malaria remained an issue in several areas (ranged between 0.2 and 58%). Uptake and acceptance by the community was high, but negative-tested patients did not always follow referral advice. Information on CHW motivation was limited and needs to be further studied. In general CHWs were not satisfied with the financial compensation. However, when asked about the most motivating aspect of their work, community respect and spiritual outcome were mentioned the most. Stock outs occurred in 4 out of 5 studies that reported on these. It is generally believed that CCM with RDT also reduced morbidity and mortality compared to CCM without RDT this could not be concluded form this study. Interestingly the preception of the patients on morbidity showed that CCM with RDT is worse than without RDT. In addition the actual cost benefit of the intervention could not be assessed. Although several studies have looked at it the data is too heterogenic to make a firm conclusion. In conclusion, we showed that trained CHWs can deliver quality care for malaria using RDTs. However, lower RDT specificity could lead to misdignoses of non-malarial diseases. Other threats for CCMm are non-adherence to negative test results and low referral
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TESTING, TREATING AND TRACKING MALARIA IN A LOW RESOURCE SETTING: IMPROVED CASE MANAGEMENT THROUGH EMPOWERMENT OF A MEDICINES AND THERAPEUTICS COMMITTEE AT ENYIRESI GOVERNMENT HOSPITAL IN ATIWA DISTRICT, GHANA

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The World Health Organization initiative, T3: Test. Treat. Track, urges malaria-endemic countries and donors to move towards universal access to diagnostic testing and antimalarial treatment, and to build stronger malaria surveillance systems. Health providers in Ghana have practiced presumptive diagnosis and treatment of malaria for many years. Provider adherence to malaria test results is only around 50%. To operationalize the T3 initiative and achieve universal diagnosis and treatment, there is a need to improve provider adherence. Enyiresi Government Hospital, a district health facility, switched from presumptive diagnosis to treating patients based on malaria test results through strong leadership, training, and inter-departmental collaboration. Hospital leaders empowered the medicines and therapeutics committee (MTC) to implement a policy to treat suspected malaria cases only when a positive diagnosis is received from the laboratory. The MTC developed a peer review mechanism and motivated staff across departments including the outpatient department (OPD), consulting room, laboratory, and pharmacy. As a result, the hospital improved testing of suspected malaria cases from 24% in 2010 to 100% in 2012. During this time, the annual number of diagnosed outpatient department (OPD) cases of malaria declined from 10,243 (25% of total OPD visits) in 2010 to 1,707 (5% of total OPD visits) in 2012. Because of ongoing stewardship by the MTC, the hospital began monitoring the number of malaria cases treated and their outcomes and investigations for other causes of fever were redoubled in malaria test negative cases. Consequently, they noted that malaria morbidity and mortality dropped significantly across all age groups. Resources once used for treating clinically diagnosed malaria were refocused on other diagnoses and the community increased trust in medical care at the facility. Successful implementation of the T3 initiative required a strong leadership commitment, collaboration among departments, effective training, and a steady supply of malaria commodities.

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PERFORMANCE OF THREE CARESTART™ MALARIA RAPID DIAGNOSTIC TESTS AFTER REDUCTION IN MALARIA PREVALENCE IN BAGAMOYO, TANZANIA

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This study aimed at testing the diagnostic performance of three malaria rapid diagnostic tests (RDTs) (CareStart™ Malaria HRP-2, pLDH [Pf-pLDH/pan-pLDH] and HRP-2/pLDH (Pf/pan) Combo Test) with reference to light microscopy for the diagnosis of falciparum malaria in Tanzania. Blood samples were collected from 627 patients suspected to have malaria at th Miswe, and Yombo (Bagamoyo District) primary health facilities from October 2013 to December 2013. The samples were examined immediately by light microscopy and the CareStart™RDTs at the site, second reading of blood slides was done at reference laboratory. Statistical analysis was performed using Stata version 11. Overall 31 of 381 (8.1%) malaria suspected cases were detected by microscopy compared to 5 of 78(6.4%) by CareStart™ Combo kit, 8 of 80 (10.0%) by both pLDH kit and 18 of 223(8.1) by HRP-2 RDT kit. CareStart™Combo RDT kit’s sensitivity and specificity for the diagnosis of malaria were 100.0% (47.8%-100%, 95% CI) and 98.6% (92.6%-100%) respectively, compared to standard microscopy. The sensitivity and specificity of CareStart™ PLDH and HRP-2 were found to be 87.5% (47.3%-99.7%) and 100 % (81.5%-100%), respectively. The CareStart™Combo RDT had positive predictive value of 83.3% (35.9%-99.6%) compared to 50.0% (23.0%-77.0%) for CareStart™ PLDH and 62.1% (42.3%-79.3%) for HRP-2 RDTs. All the three CareStart™ RDTs had the high negative predictive values of >=98.5% (91.8%-100%, 95% CI). In conclusion, the performances of CareStart™ RDTs had variation (CareStart™ Malaria HRP-2 and HRP-2/pLDH (Pf/pan) Combo Test) maintained high accuracy compared to pLDH [Pf-pLDH/pan-pLDH] RDT.

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PERSISTENCE OF PLASMODIUM FALCIPARUM DIAGNOSTIC ANTIGENS AFTER TREATMENT WITH ARTEMISININS: ASSOCIATION WITH PARASITE STAGE AND MECHANISM OF CLEARANCE

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The most sensitive rapid diagnostic tests of Plasmodium falciparum are based on detection of histidine-rich protein 2 (HRP2), but HRP2 persists in the blood after treatment and cannot be used to assess treatment response. We measured antigen levels over a 4-week period in ACT-treated hyperparasitaemic patients with P. falciparum in Uganda and Thailand (prior to the emergence of artemisinin resistance). Antigen clearance was quantified by calculation of area-under-curve (AUC) after normalization to baseline antigen level. Whole blood HRP2 levels showed a wide range of AUCs: Uganda median 415 d % (range 48-2588), Thailand 590 d % (33-1534). The primary determinant of AUC was admission stage; in patients presenting with >75% tiny/small rings (32/40 in Uganda, 24/38 in Thailand), median HRP2 AUCs were respectively 6.4 and 10.7-fold higher than cases with later stages (p=0.0005, <0.0001). Later stages at baseline strongly predicted rapid HRP2 clearance (AUROC = 0.89 for the combined dataset). LDH clearance was relatively rapid and narrower in terms of AUC range: Uganda median 53 d % (range 19-205); Thailand 76 d % (25-141). The stage effect was also reduced with median AUCs in early ring infections only 2.0 and 1.9-fold higher than with late stages (p=0.1665, 0.0002). All seven infections in Thailand showing long parasite clearance half-lives (>4.5h) had late rings at presentation, but paradoxically rapid HRP2 clearance. These data offer a clear explanation for the persistence of HRP2 in whole blood following artemisinin therapy. We propose that in patients who present with mostly young rings at baseline (the majority), parasites are removed by pitting in the spleen and once-infected erythrocytes return to the circulation where they provide the source for persistently high levels of HRP2 and ongoing positivity of HRP2-based RDTs. In contrast, when patients present with large rings/trophozoites, the spleen is unable to pit these larger parasite forms, the entire infected cell is removed by immune clearance or sequestration, and HRP2 clearance is rapid. Consistent with this model, in these less common infections, parasite clearance can be paradoxically slow.
MOLMALQA: A MOLECULAR MALARIA EXTERNAL QUALITY ASSURANCE NETWORK TO SUPPORT CLINICAL TRIALS

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Nucleic acid (NA) tests for malaria parasites are important diagnostic endpoints in many clinical trials of malaria vaccine and drug candidates. While many NA tests are described, there is no formalized external quality assurance (EQA) program that provides site qualification and ongoing EQA for the diverse assays in use. We recruited a network of centers performing controlled human malaria infection (CHMI) clinical trials to participate in an EQA effort. Here, we report on initial and ongoing EQA work. Each clinical site received blinded Plasmodium falciparum-positive and -negative samples of known parasite density that were prepared by the coordinating laboratory according to needs of each recipient site. Sites tested the samples and reported the data to the coordinating center. All sites in the first round of this effort demonstrated 100% specificity, achieved limits of detection consistent with each laboratory’s expectations and demonstrated as expected levels of within-laboratory precision (<10% coefficient of variation). Reported parasite densities generally agreed with expected values (<0.5 log_{10} parasites/mL). Where deviations from expected values were observed, root cause analyses were performed. In one laboratory, such analyses revealed reasons for observed quantitative differences and led to assay recalibration at that site. The MolMalQA effort is recruiting additional participating sites and is developing other reagents and standards to share with the malaria community. These efforts may eventually establish a dedicated long-term EQA program in support of high-quality molecular malaria diagnostics.

TEST AND TREAT PEDiATRIC FEVERS: META-ANALYSIS OF 15 NAiTIVE SURVEYS ASSESSING THE EFFECT OF MALARIA DIAGNOSTIC TESTING ON DRUGS USED BY FEBRILE CHILDREN UNDER FIVE IN DIFFERENT MALARIA RISK CONTEXTS

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In 2010, the World Health Organization revised treatment guidelines to recommend testing all suspected malaria cases prior to treatment. Studies from health facilities demonstrate reduced anti-malarial prescriptions after test introduction, and in some studies, increased antibiotic prescriptions. Many of these studies also indicate widespread non-adherence to test results. Yet, there is no evidence on how testing affects drugs used to treat pediatric fevers at the population level, nor if this effect differs by malaria risk, source of care or child’s age. 15 DHS/MIS in 2009-2013 were included that collected fever prevalence, care-seeking, diagnostic test and drug use, and geocoded survey clusters to link malaria risk estimates to datasets (n=16,831 febrile under-fives taken for care). Mixed-effects logistic regression models quantified the influence of testing on three outcomes (any anti-malarial, ACT and any antibiotic use) after adjusting for data clustering and confounding socioeconomic covariates. Preliminary results suggest large country variability in malaria testing’s effect on treatment patterns. In Uganda, tested pediatric fevers had 1.36 higher odds of antibiotic use (AOR: 1.36, 95% CI: 1.08-1.70) compared to those not tested, and no significant difference in any anti-malarial (AOR: 1.25, 95% CI: 0.97-1.62) or ACT use (AOR: 0.84, 95% CI: 0.67-1.07). Conversely, in Tanzania, tested pediatric fevers had significantly reduced odds of antibiotic use (AOR: 0.48, 95% CI: 0.31-0.74) and significantly higher odds of using any anti-malarial (AOR: 3.68, 95% CI: 2.38-5.68) and ACT (AOR: 1.95, 95% CI: 1.32-2.88). Further analyses will examine this association in all studied countries individually and in the pooled dataset. Results will be stratified separately by malaria risk, source of care and child’s age. Findings could inform future research to better understand reasons for variability in effect across settings or population groups (Final results will be available by June 2014).

AN ENHANCED CHEMILUMINESCENCE SLOT BLOT ASSAY FOR DETECTION OF MOSQUITO STAGE PLASMODIUM FALCIPARUM PARASITE

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Robust, quantitative detection of malaria antigens in preclinical and clinical phases of development as well as surveillance and epidemiological studies, particularly in the mosquito host, necessitates the development of immunoassays with superior sensitivity and reproducibility. In this work, we have developed and characterized a research-grade, quantitative enhanced chemiluminescence-based Slot Blot assay (ECL-SB) for detection of Plasmodium falciparum circumsporozoite protein produced in recombinant form (rPICP) in E. coli and as native protein from Oocysts (POocyst) developing in the midguts of P. falciparum-infected Anopheles stephensi mosquitoes. The ECL-SB detects as little as 1.25 pg of rPICP (linear range of quantitation 2.5-20 pg, R² = 0.9505). We also find that the earliest detectable expression of native PICP in POocyst by ECL-SB occurs on day 7 post infected blood meal. The ECL-SB was able to detect approximately as few as 0.5 day 8 POocysts (linear quantitation range 1-4, R² = 0.9795) and determined that one POocyst expressed approximately 2.0 pg (0.5-3 pg) of native PICP, suggesting that this detection limit agrees with the assay’s sensitivity limit for purified rPICP. The ECL-SB is highly reproducible; the Coefficient of Variation (CV) for inter-assay variability for rPICP and native PICP were 1.74% and 1.32%, respectively. The CVs for intra-assay variability performed on three days for rPICP were 2.41%, 0.82% and 2% and for native PICP 1.52%, 0.57%, and 1.86%, respectively. The ECL-SB also matched microscopic analysis of P. falciparum prevalence based upon POocyst expression on day 8 POocysts in two independent feeding assays, yielding estimates of 83.3% and 75% compared to microscopy’s 85.7% and 80%, respectively. Thus, based on its performance characteristics, ECL-SB could be valuable not only in vaccine development and manufacturing, but also to measure the parasite prevalence in mosquito populations and in studies of transmission-blocking activities in endemic areas.

QUALITY INSPIRED PROJECT - A KEY TO ACHIEVING RESULTS WITH MALARIA INTERVENTIONS

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With an aim to accelerate malaria case management, Tanzania Ministry of Health and Social Welfare (MoHSW) is strengthening its pre-service education program to ensure graduates have the right knowledge and
skills to diagnose and treat malaria. Investment in pre-service education lessens the burden on in-service training since those entering the workforce will have the knowledge and skills they need to provide.

Jhpiego, through MAISHA (Mothers And Infants Safe, Healthy and Alive) program, provided technical assistance to the MoHSW to help develop a pre-service malaria case management-updates Learning Resource Package (LRP), which includes: Facilitator’s Manual, Participant’s Manual, Activity Worksheets and Training Modules addenda. The LRP was developed based on national malaria policy, guidelines and in-service training materials; it is taught using job aids, power point presentations, video demonstration and numerous case scenarios which reflect what actually happens in real life situations at service delivery points. The LRP aims at reinforcing appropriate practices for care of malaria patients and management of commodities with emphasis on parasite-based diagnosis and compliance to results, proper recording and reporting; and management of malaria in special situations and groups. The training package is well organized with laboratory and medical supplies which gives each participant an opportunity for hands-on activity to acquire and strengthen their skills. Checklists to guide Quality Assurance/Quality Improvement (QA/QI) processes have been included in these training materials. The project successfully provided competence-based orientation on malaria case management updates to 210 medical doctors. Annually, it reaches more than 4,000 students from eight Zonal Health Resource Centers and 480 students from Medical Universities. There is a need to incorporate the addenda developed into these training modules for easy use. In the near future, clinical skills-mentorship will be conducted in selected schools using the nationally approved QA/QI checklists.

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CLINICAL SIGNS AND SYMPTOMS CANNOT RELIABLY PREDICT PLASMODIUM FALCIPARUM MALARIA INFECTION IN PREGNANT WOMEN LIVING IN AN AREA OF HIGH SEASONAL TRANSMISSION

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Malaria in pregnancy is a major public health problem in endemic countries. Though the signs and symptoms of malaria among pregnant women have already been described, clinical presentation may vary according to intensity of transmission and local perceptions. Therefore, determining common signs and symptoms among pregnant women with a malaria infection may be extremely useful to identify those in need of further investigation by rapid diagnostic test or microscopy. Six hundred pregnant women attending the maternity clinic of Nanoro District Hospital, Burkina Faso were recruited, 200 with suspected clinical malaria and 400 as controls. Cases were matched with controls by gestational age and parity. Signs and symptoms were collected and a blood sample taken for rapid diagnostic test, microscopy and haemoglobin measurement. A multivariate model was used to assess the predictive value of signs and symptoms for malaria infection. The overall prevalence of malaria was 42.6% (256/600) while anaemia was found in 60.8% (365/600) of the women. Nearly half (49%) of the cases and 39.5% of the controls had a malaria infection (p = 0.03). The most common signs and symptoms among the cases were fever (36%, 72/200), history of fever (29%, 58/200) and headache (52%, 104/200). The positive predictive value for fever was 53% (95%CI:41-64), history of fever 58% (95%CI:37-63) and headache 51% (95%CI:41-61). Signs and symptoms suggestive of malaria are frequent among pregnant women living in areas of intense transmission. Common malaria symptoms are not strong predictors of infection. For a better management of malaria in pregnancy, active screening to detect and treat malaria infection early should be performed on all pregnant women attending a health facility.

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PREVALENCE AND PREDICTORS OF MALARIA MISDIAGNOSIS IN HEALTHCARE SEEKING CHILDREN UNDER SIX MONTHS OF AGE IN UGANDA

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There is limited information on malaria prevalence and diagnosis among infants under six months of age. Little is known regarding how clinicians in limited resource settings diagnose malaria in this age group, given different and overlapping clinical manifestations and known protection against infection such as maternal antibodies. We identified the malaria prevalence and diagnosis practices, and predictors of malaria misdiagnosis in outpatients. We utilised data on individual outpatients under six months from 36 primary health care facilities in Uganda from January to December 2010. Multivariate logistic regression models were used to identify clinical and operational factors associated with misdiagnosis of malaria. Of the 23,587 young infant outpatients, 10,426 (44.2%) were diagnosed with malaria of which only 3,189 (30.6%) were laboratory confirmed. Of the 3,884 patients with a negative diagnostic test result for malaria, 1,213 (31.2%) were misdiagnosed with malaria. The odds of misdiagnosis were higher if the patient was one (aOR 3.64, 95%CI 1.26, 10.5), three (aOR 4.47, 95%CI 1.36, 14.7), four (aOR 6.34, 95%CI 2.03, 19.7) or five (aOR 6.65, 95%CI 1.15, 24.1) months old; had presence of clinical fever (aOR 2.64, 95%CI 1.33, 5.26); visited the health facility during a week when multivitamins were out of stock (aOR 1.62, 95%CI 1.12, 2.33) or visited a health facility with medium to high malaria endemicity (aOR 1.47, 95%CI 1.01, 2.16). The odds of misdiagnosis were lower when the patient was also diagnosed with cough/cold (aOR 0.32, 95%CI 0.19, 0.53) or urinary tract infection (aOR 0.16, 95%CI 0.03, 0.76). Triage status, clinical reattendance, health provider training, availability of antimarial, antibiotic and oral rehydration drugs, and diagnosis with anaemia, pneumonia and diarrhoea did not have an effect on odds of misdiagnosis. In conclusion, we found high rates of malaria prevalence and misdiagnosis in young infants. Clinicians were less likely to trust malaria test results when the infant was older or had fever or when health facility had medium to high malaria endemicity or lacked multivitamins. Clinicians were more likely to trust malaria test results when other diagnoses were made. These findings can inform future interventions to improve the quality of malaria care in infants.

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QUALITY IMPROVEMENT OF MALARIA DIAGNOSTICS WITH RAPID DIAGNOSTIC TESTS WITH THE DEKI READER IN TANZANIAN MILITARY HEALTH FACILITIES

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Malaria is the major cause of mortality and morbidity in Tanzania, where over 90% of the population is at risk for infection. Malaria diagnosis remains problematic, especially in resource-challenged settings. The use
of malaria Rapid Diagnostic Tests (mRDTs) has become a useful alternative to microscopy for diagnostic purposes, however its quality control (QC) is needed to ensure accurate diagnosis. In Tanzania we have successfully used the Deki Reader (DR) as a means of addressing diagnostics quality improvement issues through remote monitoring. The DR is a rugged, mobile in vitro diagnostic device which interprets commercially available RDTs. The DR automatically captures and securely transfers patient diagnostic data, along with the RDT image, to a centralized database over mobile phone networks. All results from the field are accessible from anywhere in the world in real-time by logging into a password protected web portal. Since 2012 DRs have been deployed at five camps overseen by the Tanzania People's Defence Forces (TPDF). Malaria RDT data and images were monitored remotely for quality assurance on a regular basis. When quality issues were detected monitors communicated with users at the testing facilities to investigate and troubleshoot problems. In this ongoing study, the DR was instrumental in remotely identifying false positive and negative mRDT interpretations made by users. Errors were due to either misinterpretation of mRDT weak lines or improper RDT preparation. Initially, approximately 5.5% of all mRDTs processed were incorrectly interpreted by users. After 6 months of remote QC monitoring and interaction with users, the problems decreased to 2.5%. Similarly, RDT preparation problems decreased from 2.3% to 0.1%. In summary, the use of DR reduced quality issues with RDTs, decreased cost of physical site visits, improved the malaria surveillance mechanism and improved patient care. The DR has proven to be a valuable tool for quality assurance of mRDT use and may be useful in other similar settings for malaria control/elimination efforts.

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MALARIAN DIAGNOSTIC AND MONITORING IN REMOTE RIVERINE VILLAGES IN THE AMAZON BASIN DURING ITINERANT MEDICAL MISSIONS

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Malaria affects primarily people living in remote tropical settings. In the Amazon Basin, malaria is concentrated in the riverine villages with limited access to healthcare and diagnostic capacities. Therefore, innovative strategies are needed to deliver diagnostics and treatment to these communities. We describe the contributions of two itinerant riverine medical missions to malaria control. Malaria diagnostics and treatment was provided at two riverine medical missions: 1) the Amazon Hope ship lead by the VineTrust since 2002, and 2) the Rio Napo ship of the Peruvian Navy, which started trips in the Napo River during 2013, as part of multiple medical and social services. NAMRU-6 supports the diagnoses and molecular characterization of cases providing equipment, materials and training. Blood smears and filter paper samples were collected from all suspect cases for malaria diagnosis by microscopy and PCR, respectively. The sensitivity and specificity of microscopy were calculated using PCR as the gold standard. Diagnosis was provided in 20 trips of the Amazon Hope ship in the Marañón, Amazonas, Ucayali, Putinahua and Tigre river basins. A total of 793 febrile cases were tested by microscopy, and 80 (10%) were malaria positive. Three quarters were due to Plasmodium vivax. The mean age was 23 years old and 60% were men. The Rio Napo Ship conducted three trips between July and December 2013, and tested 156 febrile cases for malaria. Microscopy diagnoses identified 46 (29%) positives, 26% due to P. vivax and 3% due to P. falciparum, respectively. The prevalence of P. vivax and P. falciparum by PCR was 28% and 5%, respectively. Compared to PCR, the sensitivity of microscopy for P. vivax was 89% and for P. falciparum was 57%, while the specificity for both species was 98%. Itinerant medical ships can extend malaria diagnostic capacities and surveillance to remote, hard to reach areas. Participation and cost-effectiveness can be improved providing training, basic diagnostic resources and other medical and social services.

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PECADOM PLUS: AN ACTIVE, COMMUNITY-BASED APPROACH TO MALARIA DETECTION AND TREATMENT

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The region of Kedougou has the highest prevalence of malaria in Senegal, and geographic, educational and financial barriers often impede rural populations from accessing care. A home-based care model (PECADOM) has been implemented in Senegal since 2008, addressing many of these barriers to care and care seeking. Volunteer, home-based care providers (DSDOMs) perform rapid diagnostic tests (RDTs) for malaria and administer treatment for cases of uncomplicated malaria, referring complicated cases. However, this passive model relies on patients seeking care from DSDOMs. To reduce barriers to treatment seeking, Peace Corps and the Saraya Health District have piloted an active case finding model in which DSDOMs were paid to conduct weekly sweeps of every household in their village to actively seek patients with symptoms of malaria. RDTs were undertaken on those with symptoms, with free treatment provided for positive cases. The model was implemented in fifteen villages from July through November 2013, the period of highest malaria transmission. Fifteen comparison villages were chosen among villages with the original, passive PECADOM model, and three sweeps were conducted in these villages to estimate and compare prevalence of malaria at the beginning, midpoint and end of the active model pilot. At baseline, point prevalence of symptomatic malaria confirmed by RDT for the total population was 1.1% in both sets of villages (p= .79). During the midline comparison sweeps, the point prevalence was 2.5 times higher (p<.007) in the comparison villages (2.9%) than in the intervention villages (1.1%). At endline, the point prevalence in comparison villages (2.5%) was nearly 16 times higher (p=.003) than in the intervention villages (1.16%), where only 6 cases of symptomatic malaria were found. This pilot study shows that this model of active weekly sweeps by community health workers is both feasible and effective in increasing access to care and reducing malaria prevalence. Due to these promising results, PECADOM Plus will be scaled up to the entire region of Kedougou in 2014.

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QUALITY IMPROVEMENT AND NOVEL USE OF MALARIA DIAGNOSTICS IN VIETNAM


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The National Malaria Control and Elimination Program in Vietnam is currently planning to eliminate malaria by 2030. They are currently conducting baseline assessments and developing methods for malaria diagnostic quality improvement. Quality malaria diagnostics are essential for malaria elimination efforts. False negative diagnostic results in both symptomatic and asymptomatic infected individuals will increase the malaria transmission reservoir. False positive results (malaria is not really
TRANSFORMING ARMY DIAGNOSTICS LABORATORY INTO A RESEARCH READY FACILITY, IMPROVEMENT IN QUALITY ASSURANCE; RUUVU JKT CASE STUDY

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Malaria continues to be one of the diseases affecting many U.S. and Coalition personnel deployed in Sub Sahara Africa. It is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Diagnosis of malaria is based on clinical symptoms which are non-specific, leading to false diagnosis and over use of anti-malarial drugs, increasing the potential of drug resistance, as well as the number of malaria cases. Within Tanzania microscopy continues to be a challenge with the Tanzania People's Defense Force (TPDF) and Tanzania National Service Program (JKT) medical services. Amethyst Technologies LLC (ATL) with its partners developed a comprehensive malaria microscopy and Rapid Diagnostic Testing assessment program to capture information for strengthening of the quality of Malaria Diagnostics at Ruuvu JKT as a potential research site. The need to develop quality malaria diagnosis at Ruuvu JKT was critical in getting the site laboratory ready for malaria research. Information on infrastructure, safety, human resources, training, and diagnosis was obtained in order to establish the minimum guidelines and requirements for quality malaria microscopy. The assessment came up with a quality management plan to provide on-going quality improvement of malaria microscopy and enabled the research team to estimate the amount of malaria transmission. We will present data obtained through continues training, onsite corrective actions supporting activities and discuss how these data were used to develop our Quality Management Program at Ruuvu JKT for research readiness.

MODULAR DESIGN OF ANTIPARASITIC CHEMOTHERAPEUTICS USING PRENYLATION AS NEW TOOL IN DRUG DISCOVERY

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One of seven people in the world is affected by Neglected Tropical Diseases. Treatments of these diseases are insufficient and inefficient and require constant development of new drugs and strategies. Within the universe of small organic molecules, the isoprenoids are the most important and numerous families of metabolites. In this regard, it is useful to focus on the design, synthesis and studies of molecular probes that interfere with different biosynthetic pathways, particularly the isoprene and sterol pathways. The implemented strategies used modern medicinal chemistry approaches like diversity-oriented synthesis, parallel solution library preparation, conventional multistep synthesis, midthroughput screening, bioinformatics and computational chemistry, allowed to generate more than 100 new compounds. The generated libraries were tested against the parasites responsible of the malaria (Plasmodium falciparum chloroquine sensitive and resistant strains), visceral leishmaniasis (Leishmania donovani), HAT (Trypanosoma brucei) and Chagas' disease (T. cruzi). As result of our effort we have found very promising new hits to develop drugs against malaria and other diseases. Our strategy also included action mechanism studies of the lead compounds. To do that, fluorescent tagged isoprenes were synthesized to be incorporated on the lead structure to perform cellular localization by fluorescent microscopy.

EVALUATION OF THE PROPHYLACTIC ACTIVITY OF ORAL 8-AMINOQUINOLINE DERIVATIVES IN C57BL/6 MICE USING AN IN VIVO IMAGING SYSTEM

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As anti-malarial drug resistance escalates, new safe and effective medications are necessary to prevent and treat malaria infections. The US Army is developing 8-aminquinaoline (8-AQ) analogues with improvements in hemolytic toxicity, which is expected to be safer in preventing malaria in deployed military personnel. To compare the prophylactic efficacy of these compounds, a transgenic P. berghei parasite expressing the bioluminescent reporter protein luciferase was utilized to visualize and quantify parasite development by using a real-time in vivo imaging system (IVIS) at 24, 48, and 72 hours post infection. Two standard compounds of 8-AQ, tafenoquine (TQ) and primaquine (PQ) were treated with single or multiple regimens. As an additional endpoint, blood stage parasitemia was assessed periodically over the next 30 days by flow cytometry. C57BL/6 Albino mice were infected intravenously with 50,000 sporozoites by tail vein and treated orally with three multiple doses of TQ and PQ at their minimal curative doses of 5 and 25 mg/kg, respectively. The outcome of this treatment showed no bioluminescence liver signal and no blood stage parasitemia was observed, suggesting both drugs showed 100% causal activity at the doses tested. Single dose oral treatment with 5 mg TQ or 25 mg of PQ, however, yielded different results as only TQ treatment resulted in causal prophylaxis in P. berghei sporozoite-infected mice. TQ is highly effective for causal prophylaxis in mice at a minimal curative single oral dose of 5 mg/kg, which is a five-fold improvement in potency versus PQ. We also tested these drugs in a more sensitive C57BL/6 wild-type strain (black) where mice were infected with only 10,000 sporozoites and treated orally with three multiple doses of TQ at 3 mg/kg or with PQ doses orally at 15 mg/kg. None of the mice demonstrated a bioluminescence liver signal and no blood stage parasitaemia was observed suggesting both drugs showed 100% causal
activity at the doses tested. Accordingly, we conclude TQ is highly effective for causal prophylaxis in C57BL/6 wild-type mice at a minimal curative single oral dose of 3 mg/kg, which is also a five-fold improvement in potency versus PQ where the minimal curative dose required is 15 mg/kg. Both stains are working well to screen the prophylactic activity of oral 8-aminoquinoline derivatives in liver-stage, but the C57BL/6 wild-type mice are more sensitive than C57BL/6 albino animals.

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LONG-TERM PROPHYLAXIS AND PHARMACOKINETIC EVALUATION OF A SINGLE INJECTION OF INTRAMUSCULAR DECOQUINATE IN MICE INFECTED WITH Plasmodium berghei SPOROZOITES
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This study was conducted to evaluate the prophylactic efficacy and pharmacokinetics of an intramuscular (IM) depot formulation of decoquinate (DQ) into mice infected with Plasmodium berghei sporozoites. Due to the poor bioavailability of oral DQ, a novel drug dispersion technique was utilized to create DQ microparticles suspended in an oily vehicle to retard drug release suitable for long-term malaria prophylaxis. To evaluate the depot formulation, pharmacokinetic studies in normal animals and antimalarial efficacy in liver-stage malaria mice were conducted following single IM-DQ injections at 60, 120 and 240 mg/kg for 2, 4, 6, or 8 weeks prior to infection with P. berghei sporozoites. The liver stage efficacy evaluation was monitored by using an in vivo imaging system (IVIS). Efficacy results showed that 100% of animals were protected from P. berghei infection after injection with a single dose of depot DQ at 120 and 240 mg/kg administered 8 weeks prior to sporozoite inoculation. The 120 mg/kg IM dose is the minimal prophylactic dose required to obtain causal prophylaxis of malaria sufficient for a period of 8 weeks. In addition, a significant increase in the elimination half-life of the depot DQ (1,484 hrs) was achieved compared to that of orally dosed plain DQ (8.3 hrs). The depot IM formulation with slow-release mode provided release of very low and constant drug concentrations in the plasma and liver resulting in a minimal inhibition concentration (MIC) at 5-6 ng/ml in plasma and 70-90 ng/ml in the liver. General safety and injection site tolerability were also assessed longitudinally to examine any issues with drug toxicity that may potentially preclude the use of this formulation in man. Modest body weight changes were found after IM dosing not only in the animals treated with IM-DQ, but also in mice treated with the vehicle control which may be a consequence of animal handling. Depot DQ injections up to a single intramuscular dose of 4,800 mg/kg, 40-fold higher than the dose required for treatment, were well tolerated in mice and final evaluations of toxicity will be conducted through histopathological monitoring. Further development of a depot IM-DQ formulation for malaria prophylaxis will be needed to deliver even longer periods of malaria prophylaxis protection beyond the 8 weeks achieved in this study.

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EXPERIMENTAL STUDY OF THE RELATIONSHIP BETWEEN Plasmodium GAMETOCYTE DENSITY AND INFECTION SUCCESS IN MOSQUITOES; IMPLICATIONS FOR THE EVALUATION OF MALARIA TRANSMISSION-BLOCKING INTERVENTIONS
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The evaluation of Plasmodium transmission blocking interventions (TBIs) such as vaccines or drugs to control malaria widely uses mosquito membrane-feeding assays. In these experiments, the intensity of Plasmodium infection within the anopheline vector is expected to affect the measured efficacy of the molecules to block transmission. Gametocyte density in the host blood is a broad determinant of the infection success in the mosquito; however, uncertain estimates of parasite densities and intrinsic characteristics of the infected blood can induce variability. To control this variation in TBI evaluation, a feasible method is to dilute infectious blood samples, ensuring that gametocyte numbers are directly comparable between parallel samples. To examine this in detail, we describe the effect of diluting Plasmodium gametocyte infected blood and the subsequent impact on mosquito infectivity and TBI efficacy, for two parasite-vector combinations: Plasmodium falciparum/Anopheles gambiae and Plasmodium berghei/Anopheles stephensi. We additionally examined how blood dilution influences the observed blocking activity of mAb 13.1 against Pbs28 of Plasmodium berghei. In the natural species combination (P. falciparum/An. Gambiae), blood dilution revealed a positive linear relationships between gametocyte density and oocyst load. Similar relationships was observed in the P. berghei / Anopholes stephensi system when using heat inactivated blood as a solvent, whereas diluting infected mouse blood with fresh uninfected blood substantially increases infectiousness. This suggests that highly infected mouse blood contains inhibitory factors or reduced blood moieties, which impedes infection and may lead to misinterpretation of TBI evaluation assays. In the lab-based system, the transmission blocking activity of mAb 13.1 was confirmed to be density-dependant. This data highlights the need to perform evaluations of TBI candidates carefully, at gametocyte densities and infection intensities that are broadly translatable.

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ANTIMALARIAL ACTIVITY OF NOVEL HARMINE-DERIVED HEAT SHOCK PROTEIN 90 INHIBITORS
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The emergence of multi-drug resistant Plasmodium falciparum strains poses a serious challenge in the control of malaria and could have catastrophic outcomes. This necessitates development of effective, safe and affordable new drugs. Previous studies have shown that the natural beta-carboline alkaloid compound, harmine, is a promising antimalarial targeting the P. falciparum heat shock-90 (PfHsp90) enzyme. In this study, we have developed a microwave-assisted synthetic route to generate analogs of harmine and have tested their antimalarial effect both in vitro and in vivo. Out of 45 harmine-derived compounds tested, two (17A and 21A) bound to PfHsp90 with EC50 values of 12.2 ± 2.3, and 23.1 ± 8.8μM, respectively. These compounds also inhibited P. falciparum W2 with IC50 values of 1.3 ± 0.027 and 1.4 ± 0.07μM, respectively. In vitro cytotoxicity assay showed that both compounds are not toxic. The efficacy of these compounds in vivo was assessed using P. berghei infection of BALB/c mice. Three daily injections of infected mice with 100μg/kg of
each of 17A and 21A showed significant reduction in parasitemia as
compared to the vehicle control. Compounds 17A and 21A resulted in
reduction of parasitemia by 51.5% and 56.1%, respectively. Mice treated
with 17A and 21A showed a median survival time of 11 and 14 days,
respectively while the vehicle control mice showed a median survival time
of only 8.5 days. Log-rank test indicated that the survival of mice treated
with 21A was significantly higher than vehicle control mice. In summary,
we have identified a novel, non-toxic harmine derivative able to bind
PfHsp90, inhibit P. falciparum W2 in vitro at micromolar concentrations
and reduce parasitemia in the P. berghei mouse model. 21A also improved
survival of infected mice, a trait not observed in the parent molecule.
This could be attributed to the changes made on harmine backbone. Further
dose-ranging studies of 21A are required coupled with development of
focused libraries based on the current lead structure.

DEVELOPMENT OF ANTIMALARIALS WITH SERCAP PROLIFE
FROM DOS DERIVED COMPOUNDS

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In the last decade, hundreds of small molecules with previously unknown
antimalarial activities were identified, but the drug discovery and
development pipeline still lacks compounds with non-erythrocyte stage
activity and target diversities. We screened and identified a novel series
of compounds derived from Diversity-Oriented Synthesis (DOS) with potent
activity against blood-stage Plasmodium falciparum. Importantly, the
compounds are potent against a panel of Plasmodium field isolates
with resistance to clinically relevant therapeutics, suggesting that these
compounds have a novel mechanism of action compared to current
therapeutics. The compounds are active in in vitro assays for liver-stage
parasites (P. berghei) and late-stage (IV & V) gametocytes (P. falciparum).
Intriguingly, the compounds also show in vitro activity against the liver
stage (small forms) of P. cynomolgi. Finally, compounds in this series
showed excellent in vivo efficacy in a P. berghei mouse model. This profile
is consistent with the target product profile envisioned for Single Exposure
Radical Cure and Prophylaxis (SERCap) for treatment of uncomplicated
malaria.

DEVELOPMENT OF A NOVEL PRECLINICAL ANTIMALARIAL
CANDIDATE

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Malaria is a devastating disease, leading to several hundred million clinical
cases and over 600,000 deaths each year. There is an urgent need to
develop new drugs to treat this disease, to counter resistance to current
drugs and to expand the range of clinical indications which can be tackled.
This includes the need for single-dose treatment, transmission blocking,
chemoprevention and treatment for relapsing malaria. Here we report
the discovery and development of a potential new antimalarial agent.
The starting point for this project was a phenotypic screen carried out
against Plasmodium falciparum at the University of Dundee, UK. Several
series were identified and one of these was optimized to a compound
which fulfilled the Medicines for Malaria Venture criteria for a late lead
compound. This compound was extensively profiled in a large number of
assays and has now been progressed into preclinical development
with the aim of entering human clinical trials. This pre-clinical candidate
shows promise as a possible single-dose treatment in combination with
another antimalarial and demonstrates both transmission blocking and
chemoprevention potential.

PROGRESS TOWARDS MALARIA ERADICATION: NEW
SCREENING ASSAYS AND NEW COMPOUNDS WITH ANTI-
GAMETOCYTE ACTIVITY

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Drugs able to inhibit Plasmodium falciparum gametocytes and thus
malaria transmission are strongly needed to achieve the goals of the malaria
eradication/eradication agenda. Here, we describe two microtiter
screening assays, based on different viability markers and read-outs: the
pLDH assay, based on the spectroscopic detection of the gametocyte
lactate dehydrogenase (pLDH) activity, and the LUC assay, performed
using a parasite strain which expresses a novel luciferase enzyme (LUC1-G)
under the control of a promoter specific for gametocytes. The assays
have been validated and show good Z’ factor and signal to background
ratio. Both assays have been used to screen the “MMV-Malaria box”
library. A primary screening at a single dose of 3.7 μM was performed
with the pLDH assay, measuring parasite viability at two different time
points: 72 h post-treatment (72 h assay) and 144 h. In the latter case,
the compounds were removed from the cultures, which were incubated
for further 72h (72+72h assay). The dual checkpoint assay allows the
identification of gametocytocidal compounds with fast versus slow speed
of action. Seven out of 400 Malaria box compounds inhibited gametocyte
viability by more than 50% already after the first 72h incubation; fifteen
compounds showed an activity higher than 75% at 144h. Based on the
results of the primary screening, thirty-six compounds with activity higher
than 50% at 144h were selected for dose-response experiments and
IC50 determination in both assays. Seven compounds showed an IC50
lower than 1 μM with both methods. A good correlation between the
IC50 results with the two assays was obtained. In conclusion, the pLDH and
LUC assays can be used as fast and cheap screening methods for the
identification of novel gametocytocidal compounds and to investigate their
mode of action (fast versus slow). The evaluation of potential transmission
blocking agents selected from other libraries or newly synthesized is on
going and results will be ready in the Fall of 2014.

PHOSPHOLIPASE A2 INHIBITION IN PLASMODIUM 
FALCIPARUM: A POTENTIAL NOVEL ANTIMALARIAL
STRATEGY

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Plasmodium falciparum causes millions of infection worldwide, including
severe disease, mainly in children residing in sub Saharan Africa. Currently,
artemisinin based combination therapies (ACT) are the treatment of
choice for the majority of malaria endemic countries. However, resistance

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to artemisinin has been reported, which may impact the future efficacy of ACTs. Thus, it is imperative to identify new drug candidates with antimalarial activity. We present data for a potentially novel antimalarial drug target. Phospholipases A2 (PLA2) hydrolyze phospholipids to lysophospholipids, fatty acids, and arachidonic acid, a precursor to leukotrienes and prostaglandin production, which are mediators of inflammation. This enzyme family includes secretory (sPLA2), calcium independent (iPLA2) and cytosolic PLA2 (cPLA2) members that carry out a range of functions, including antimicrobial activity, signaling and metabolism. We identified a putative PLA2 encoded in the P. falciparum genome. The open reading frame includes the GXSXG and DXG/A conserved catalytic motifs and its predicted molecular weight of 78kDa is consistent with either the iPLA2 or cPLA2 classification. To determine if PLA2 inhibition of P. falciparum alters parasite growth we carried out in vitro drug susceptibility assays against a panel of PLA2 inhibitors. We have found that AACOCF3, which inhibits cPLA2 has a 50% of inhibitory concentration (IC50) in multiple P. falciparum strains including, 3D7 (10.8 µM), Dd2 (15.2 µM) and HB3 (7.4 µM). PLA2 inhibitors against sPLA2 or against iPLA2, did not inhibit parasite growth. Detailed microscopy studies demonstrate that the antimalarial effect of AACOCF3 takes place during the trophozoite stage of the parasite’s life cycle. To determine the target pathway of pPLA2 we will carry out metabolic labelling with 13C-choline, demonstrate that the antimalarial effect of AACOCF3 takes place during the trophozoite stage of the parasite’s life cycle. To determine the target pathway of pPLA2 we will carry out metabolic labelling with 13C-choline, demonstrating that the antimalarial effect of AACOCF3 takes place during the trophozoite stage of the parasite’s life cycle.

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PHYTOMEDICINES FROM WARBURGIA UGANDENSIS AND ZANTHOXYLUM USAMBARENSE AGAINST PLASMODIUM KNOWLESI

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Malaria affects about 500 million people annually with a mortality of up to 2.7 million. The high cost of effective treatment hampers malaria control. In Africa, about 75% of the population either does not have access to or cannot afford conventional medicine and therefore traditional medicine for malaria treatment is an alternative, as reported previously. Warburgia ugandensis Sprague (Canellaceae) and Zanthoxylum usambarense (Engl.) Kokworo (Rutaceae) are commonly used as traditional medicine by many communities in Kenya (Njoroge and Bussmann, 2006). Both plants grow in both highland and lowland areas especially in forests around Nairobi, Masaai Mara, Samburu, Southwest of Mt. Kenya and Kakamega, as reported previously. Hot and cold decoctions from leaves and stem barks of Warburgia ugandensis are culturally used to treat tooth decay, asthma and bronchitis while Zanthoxylum usambarense is used to treat malaria, upper respiratory tract infections, tooth decay and sore gums. Phytochemical studies on Zanthoxylum usambarense revealed that it contains alkaloids of tetrahydropyrroleperine type, as reported previously. Moreover, its in vitro antimalarial activity against Plasmodium falciparum has been reported. The aim of this study was to determine the anti-plasmodial activities of extracts from Warburgia ugandensis and Zanthoxylum usambarense against the fifth human malaria parasite, Plasmodium knowlesi. Plasmodium knowlesi is widely distributed in parts of Kenya, Malaysia, the Philippines, Myanmar and Thailand, and can also be fatal, presenting an urgent need for more focused investigations on its control. Eight plant extracts were screened for in vitro anti-plasmodial activity against Plasmodium knowlesi, in a 96-well plate incubated at 37°C on a RPMI culture medium supplemented with Baboon serum. Inhibitory concentrations (IC50) values of between 3.14 and 75 µg/ml, up to 69% chemosuppression of parasites growth and over 80% survivorship of treated mice were observed. The two medicinal plants, Warburgia ugandensis and Zanthoxylum usambarense possess bioactive compounds against malaria parasites and could be exploited for further development into malaria therapy.
PROGRESS IN THE PRECLINICAL DEVELOPMENT OF ANTIMALARIAL DM1157

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We are developing a class of hybrid molecules made from a chloroquine-like moiety and a chemosensitizer (reversal agent) against chloroquine-resistance (CQR). These molecules have been shown to have low-nanomolar in vitro IC50 values against all strains of malaria tested so far, whether chloroquine-sensitive or -resistant, often surpassing the activity of even chloroquine against chloroquine-sensitive strains of P. falciparum. Here we report on the selection of a lead compound, DM1157, and its progress in the preclinical development pathway. This update will cover results from in vitro evaluations (including off-target activity), rodent toxicology and pharmacokinetics, and the initial second-species work. Also, aspects of the scale-up chemistry for GMP-production will be discussed.

TARGET IDENTIFICATION OF TWO CHEMICALLY DIVERSE DRUG-LIKE COMPOUNDS USING CHEMOGENOMICS

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We aim to identify the mode of action of the most prominent compounds from whole-cell Plasmodium falciparum screens of chemically diverse libraries. We have used a chemogenomic approach whereby resistant parasites are generated by in vitro selection under drug pressure and whole genome sequencing is employed to identify the genetic basis of resistance. Independent selections acquiring mutations in the same gene or pathway indicate candidate determinants of the resistance phenotype. Study compounds were selected based on availability, purity, potency in a multi-drug resistant strain, and lack of known mechanisms of action such as mitochondrial function and folate biosynthesis. To further eliminate overlap with known targets, we performed cross-resistance testing against drug resistant lines with well-characterized mutations in dihydroorotate dehydrogenase, heat shock protein 90, prolyl tRNA synthetase and PfATPase4. Two chemically distinct drug-like compounds, MMV006767 and MMV007564, did not exhibit cross-resistance in any of the assays employed indicating they inhibit novel targets. To gain more insight into the compounds’ mechanism of action, MMV006767- and MMV007564-resistant lines were generated in vitro by intermittent and continuous selection methods. Resistant parasites emerged with 3- to 12-fold EC50 shifts. Whole genome sequencing revealed that MMV007564-resistant lines from 3 independent selections have novel mutations in the Pf cysteine amino acid resistance locus (Pfcarr). PFCARL, a conserved protein with unknown function, has recently been shown to be the target of imidazolopiperazines – a class of compounds distinct from MMV007564. Given their diverse chemotypes, these results suggest that PFCARL can act as a common resistance mechanism in the parasite. Our findings may also reveal new structure-activity relationship for GNF156 – an imidazolopiperazine currently in Phase II clinical trials. Further characterization of the mode of action of MMV007564 as it relates to PFCARL and whole genome sequencing for MMV006767-resistant lines are underway.

INHIBITORS OF UBIQUITIN E3 LIGASE AS POTENTIAL NEW ANTIMALARIAL DRUG LEADS

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Malaria is a deadly disease caused by parasites of the genus Plasmodium and transmitted by female Anopheles mosquito. Malaria is curable if diagnosed early and treated appropriately. According to a recent WHO estimate, every year 219 million cases (range 154–289 million) and 660 000 deaths (range 490 000–836 000) occur every due to malaria. As the parasite has developed resistance for the most of the currently used antimalarial drugs, there is an urgent need to identify new molecular targets and to find new leads against those molecular targets. The ubiquitin/proteasome pathway is the principal system for degradation of proteins in eukaryotic. Ubiquitin is a highly conserved 76 amino acid polypeptide that covalently attaches to target proteins through the concerted action of three classes of enzymes: the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3). The endoplasmic reticulum associated degradation (ERAD) system of Plasmodium falciparum is essential for the survival and maintenance of the parasite. The ERAD system is composed mainly of three proteins: ubiquitin-activating E1 enzyme (UBA1), ubiquitin-conjugating E2 enzyme (UBC), and Plasmodium ubiquitin E3 ligase (HRD1). The protein encoded by PF14_0215, a putative E3 ubiquitin ligase, has been localized to reticular structures in the trophozoite and schizont stages of the parasite. In the late schizont stage PF14_0215 proteins reside within globular structures surrounding each budding merozoite. PF14_0215 E3 ubiquitin ligase resides in the ER membrane and is essential for the parasite survival. We screened a set of selected standard E3 ubiquitin ligase inhibitors in vitro against Plasmodium falciparum cultures. Among these, JNJ-26854165 and HLI 373 showed significant activity against both D6 and W2 strains of P. falciparum. The compounds were simultaneously tested against Vero cells for mammalian cell cytotoxicity. The active compounds JNJ-26854165 and HLI 373 were significantly less toxic to mammalian cells as compared to Plasmodium. Thus, these lead compounds showed a good selectivity as antimalarials. Further investigations on the E3 ligase inhibitors promise new understanding of the importance of E3 Ligase functions in the malaria parasite, and may provide new classes of antimalarial drug leads.

MAPPING OUT OF ANTIMALARIAL DRUGS ON STOCK AT THE MARKET IN A RURAL DISTRICTS OF GHANA

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Antimalarial drugs are a very important component of any policy for effective reduction of morbidity and mortality related to the malaria disease. The availability of efficacious and high quality antimalarials and their correct use can mitigate the risk of morbidity and mortality among the people of sub-Saharan Africa who have the highest risk of contracting and dying from malaria. Chemical (medicine) shops are major source of care for most developing countries where anti-malarial drugs can be purchase at the counter. The paper seeks to identify the different kinds of anti-malarial drugs on the market for malaria treatment in a rural district in Ghana. A structured questionnaire was used during two seasons (peak and low malaria transmission seasons) to collect information on anti-malarial drugs from all 58 chemical shops within the Dangme West district now (Shai Osudoku and Ningo Prampram districts). Pictures of the anti-
malarial drugs were taken, the active ingredients, and also the source of the drugs documented. GIS locations of the shops were also recorded to ascertain the proximity of the shops to households in the communities. Majority (72.0%) of the chemical and pharmacy shop owners are males. Only 7.0% of the shops are pharmacy while the remainder is licensed chemical shops. The total numbers of antimalarial drugs counted were forty nine (49). Among the stock, 4.2% were quinine, 31.9% of them were monotherapies such as artemether, Amodiaquine, Artesunate etc. Altogether, 59.4% of the artemisinin combination therapies (ACTs) were artesether + Lumefantrine, 25.0% were Artesunate + Amodiaquine. Other antimalarial observed were 9.4% Sulfadoxine + Pyrimethamine and 3.1% of of Artesunate + Sulfamethoxypyrazine + Pyrimethamine. About 47% of the anti-malarial drugs were pediatric formulations. GIS mapping shows that majority of the households are within a periphery of 5km to a chemical shop. The national antimalarial drug policy recommends the use of ACTs for malaria treatment however, all sorts of anti-malarial drugs which are not ACTs are in stock at the chemical shops in Ghana. Chemical shops are closer to households and play a very important role in the treatment of malaria hence there is the need to train chemical sellers to stock and administer the recommended antimalarials.

AN EFFICIENT PRIMARY HUMAN HEPATOCYTE CULTURE PLATFORM FOR ASSESSING DRUG AND VACCINE EFFICACY AGAINST PLASMODIUM PARASITES

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Malaria remains one of the world's most resilient public health problems, fighting the spread of this disease has been hampered due to a poor understanding of the parasite's unique biology and inadequate in vitro culture protocols for the liver stages of Plasmodium falciparum and P. vivax, two causative agents of malaria. Here we describe a novel dual-chambered microfluidic device designed to serve as an improved liver stage culture platform. Devices are manufactured from polydimethylsiloxane and feature a PDMS membrane with 10 µm pores separating two microfluidic channels. Primary human hepatocytes are concentrated and injected into a collagen-coated device channel to initiate culture. By incorporating in vivo-like cell confinement within an in vitro system, hepatocyte architecture is maintained; leading to production of albumin, clotting factor IX, and bile salts over three weeks of continuous culture. Optimized infection conditions with as few as 10,000 parasites yield several developing liver forms within each device. Arrays of devices, each containing dilutions of drugs or immune sera, form an inhibition of invasion and development assay useful for assessing the efficacy of novel compounds and vaccine targets. Future studies include high-resolution imaging of time-lapsed intracellular parasite development to better understand the parasite's liver forms and low-to-medium throughput drug screening.

EMERGING PIPERAQUINE RESISTANCE AND RISING MEFLOQUINE SUSCEPTIBILITY LINKED TO PLASMODIUM FALCIPARUM MULTIDRUG RESISTANCE GENE 1 (PFMDR1) GENOTYPES IN ISOLATES COLLECTED FROM CAMBODIA IN 2008-2013

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Growing reports of clinical failures with the current first-line artemisinin combination therapy (ACT) in Cambodia, dihydroartemisinin-piperine (DHA-PPQ), are alarming. We conducted ex vivo drug susceptibility testing of Plasmodium falciparum isolates to help inform public health policy in selecting an alternative ACT to replace DHA-PPQ in Cambodia. We performed histidine-rich protein-2 (HRP-2) ELISA ex vivo testing in a panel of antimalarial (DHA, artesunate, mefloquine, chloroquine, piperazine, lumefantrine, quinine, and atovaquone) and molecular marker genotyping of 753 isolates collected in 2008-2013 from western, northern, and southern Cambodia. We found temporal and regional trends in ex vivo susceptibility and pfmdr1 amplification results corresponding with the 2010 national malaria treatment guideline change replacing artesunate-mefloquine (AS-MQ) with DHA-PPQ. Northern isolates during 2010-2013 had a significant reduction in PPQ susceptibility with geometric IC50s more than doubling (12.8 to 29.6 nM), whereas MQ IC50s concomitantly decreased from 67.1 to 26 nM. Isolates with multiple pfmdr1 copies and the 184F mutation had significantly reduced susceptibility to DHA, AS, MQ, quinine, lumefantrine, and atovaquone, whereas isolates with a single pfmdr1 copy plus 184F mutation showed reduced susceptibility to chloroquine and PPQ. The frequency of northern isolates with pfmdr1 amplification declined over 2009-2013, corresponding with diminishing MQ use. We found a high level of chloroquine (CQ)-resistance, as indicated by all evaluable isolates being of the P. falciparum CQ resistance transporter (pfcrt) gene CVIET mutant haplotype. Nearly 20% of enrolled patients had plasma active against P. falciparum blood stages in an ex vivo bioasay indicating prior malaria drug use and correlating well with patient-reported malaria history. Overall, our findings suggest PPQ resistance is emerging, while MQ sensitivity is increasing and linked with pfmdr1 deamplification, and support reintroduction of artesunate-MQ in areas in Cambodia where DHA-PPQ is failing.
conditions are often investigated to minimize drug loss as part of analytical issues that are difficult to control in rural areas. At the analytical level, these conditions also affect the integrity of the samples. These are some examples of pre-analytical factors that need to be considered when collecting blood samples.

The storage conditions at the study site and conditions during transportation can affect the outcome of antimalarial drug measurements. DNA purification is genotyped with respect to twelve polymorphic microsatellite markers to determine multiplicity-of-infections in order to identify samples containing >1 parasite clone. Ninety one single nucleotide polymorphisms distributed across the P. falciparum genome were genotyped using the Sequenom platform as previously published. Effect of parasite genotype on clearance rates will be assessed by correlating the 91-locus genotypes to parasite clearance half-lives. Heterogeneity was assessed by comparing variance of parasite clearance within and between clonally identical parasites recovered in >2 subjects and phylogenetic analysis carried out to determine parasite relatedness. Parasite clearance half-life with a geometric mean of 2.6 h (1.19-4.70) was reported with the slower clearance patients showing >3.05h slope half-life. Of the 91 SNPs assessed, 85% (78) gave robust genotype data which were correlated with parasite clearance half-life to show a positive correlation. The results show that the prevalence of molecular markers of resistance of Plasmodium falciparum to SP and AQ is lower in areas with SMC. The quintuple mutation pfdhfr / pfmdr1 was not observed either in control areas or in SMC area after three years of implementation. However, the absolute prevalence of resistance markers was lower in areas with SMC. Mutations pfdhfr 164L to SP and AQ and ii) evaluate the impact of seasonal malaria chemoprevention on the acquisition of antibodies directed against malaria. To do this, blood samples on filter paper were made during cross-sectional surveys conducted after the implementation in three health districts in central Senegal in 2008, 2009 and 2010. We determined the mutation associated with resistance to SP (pfdhfr and pfmdr) and AQ (pfcr1 and pfmdr1) by PCR and secondly measuring the production of antibodies against antigens of MSP1 and AMA1 of P. falciparum. The results show that the prevalence of molecular markers of resistance that the triple mutation pfdhfr were high between 2008 and 2010. The quintuple mutation pfdhfr / pfmdr1 was not observed either in control areas or in SMC area after three years of implementation. However, the absolute prevalence of resistance markers was lower in areas with SMC. The quintuple mutation pfdhfr / pfmdr1 was not observed either in control areas or in SMC area after three years of implementation. However, the absolute prevalence of resistance markers was lower in areas with SMC. The quintuple mutation pfdhfr / pfmdr1 was not observed either in control areas or in SMC area after three years of implementation. However, the absolute prevalence of resistance markers was lower in areas with SMC. The quintuple mutation pfdhfr / pfmdr1 was not observed either in control areas or in SMC area after three years of implementation.
resistance transporter (Pfct), multidrug resistance protein (Pfmdr1) and the sodium/hydrogen exchanger gene (Pfneh1). In this study, we investigated the association between in vitro quinine susceptibility with genetic polymorphisms in Pfmdr1 codons 86 and 184, Pfct codon 76, and Pfneh1 ms4760 in field isolates from western (lowland and highlands) and coastal regions of Kenya. In vitro activity was assessed as the drug concentration that inhibits 50% of parasite growth (IC50). DNA was extracted and polymorphisms in Pfmdr1, Pfct and Pfneh1 genes were determined by sequencing. Associations between the in vitro QN sensitivity [phenotypic] and the polymorphisms of the Pfmdr1, Pfct and Pfneh1 gene [genotypic] were established. Data revealed there was significant association between polymorphisms in Pfmdr1-186Y, 184F and Pfct-76T whereas 74% of parasites susceptible to quinine carried the wildtype allele. Fifteen different profiles of ms4760 in Pfneh1 gene were observed, five of these had not been described. In addition, quinine IC50 of parasites with Pfneh1 ms4760 3 DNNND repeats was significantly higher compared to those with 1 or 2 repeats (p = 0.033 and p = 0.0043 respectively). The validity of these genetic markers is probably only relevant in the context of the genetic background of the isolates. Clinical efficacy studies are required to confirm the validity of these markers and the importance of parasite genetic background.

DOXYCYCLINE REDUCED IN VITRO SUSCEPTIBILITY IN PLASMODIUM FALCIPARUM KENYAN FIELD ISOLATES IS ASSOCIATED WITH PFTETQ KYNNNN SEQUENCE POLYMORPHISM

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Doxycycline is widely used for malaria prophylaxis by international tourists visiting malaria endemic countries. Its efficacy profile as an antimalarial in Kenya is not well defined due to limited available information regarding reference Plasmodium falciparum doxycycline molecular markers. We determined doxycycline in vitro susceptibility profiles in Kenyan isolates and further assessed their association with Plasmodium falciparum metabolite drug transporter and Plasmodium falciparum GTase tetQ protein gene polymorphisms from 2010 to 2013. In vitro susceptibility testing using SYBR green I dye was employed for determination of doxycycline 50% inhibitory concentrations. While quantitative real-time polymerase chain reaction (qPCR) was used to determine copy number variation in 96 Plasmodium falciparum Kenyan isolates. Direct sequencing was used for determination of the number of KYNNNN motif repeats within the PftetQ protein gene. There was a marked reduction in median doxycycline 50% inhibitory concentrations from 18,956nM [15,234-50,141] in 2010 to 3445nM [1,744-12,775] in 2013, indicative of increasing susceptibility to the drug. However 15% of the isolates with doxycycline IC50 above the resistance threshold of 35,000nM, had a higher odds of having <3 KYNNNN motif repeats polymorphism of Plasmodium falciparum GTase tetQ protein gene relative to those with IC50 < 35,000nM was (Odds ratio [OR], 15 [95% confidence interval (CI), 3.0-74.3; P < 0.0002). While the odds of having increased copy number of both Plasmodium falciparum GTase tetQ protein gene and Plasmodium falciparum metabolite drug transporter gene was (odds ratio [OR], 0.4100, 95% confidence interval [CI]) [0.04-3.7], P = 0.65) and OR, 0.205 [95% CI, 0.02-1.6]; P=0.172 respectively for the 96 samples. PftetQ KYNNNN polymorphism is associated with a reduced doxycycline susceptibility phenotype in Kenyan isolates.

USE OF MOBILE PHONE TECHNOLOGY FOR REAL-TIME REPORTING OF FEVER CASES AND MALARIA TREATMENT FAILURE IN AREAS OF DECLINING MALARIA TRANSMISSION IN TANGA, NORTHEASTERN TANZANIA: A PILOT STUDY

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Early detection and management of febrile illnesses need to be improved for communities to benefit from the declining trends of malaria. Capturing events on malaria, drug resistance or associated disease in real-time, and pipelining them to appropriate clinics and/or laboratories for proper management is essential for patient care and disease control. Currently mobile phone technology is common in developing world and most developing countries are using mobile phone technology to collect and transfer health information and services to remote populations. This study is assessing the applicability of mobile phone technology in facilitating detection and management of fever cases by village health workers (VHVs) in north-eastern Tanzania. This is a prospective pilot study involving three villages in Muheza district (Mambolo, Magoda and Mmapayu) and one dispensary of Magoda. VHVs are using mobile phone technology in surveillance of febrile episodes (axillary temperature ≥37.5°C or/and history of fever past 24hours) through active (ACD) and passive case detection (PCD). Cases found positive by malaria rapid diagnostic test (mRDT) are given first dose of artemether/lumefantrine (Alu) at the dispensary. VHVs visit each patient at home to supervise intake of remaining doses. In May 2013, the population was estimated to be 2934 individuals in 678 households. Between 15th November 2013 and 15th March 2014, a total of 528 febrile cases aged 6.5 months -94 year were attended. Most of the cases (82.3%) were detected through PCD; 21.0% were positive by mRDT and 19.3% by microscopy. Of those with malaria, 89.5% had completed one dispensary of Magoda. VHWs are using mobile phone technology in facilitating early detection and management of febrile illnesses by village health workers (VHVs) is assessing the applicability of mobile phone technology in facilitating early detection and management of febrile illnesses. VHVs are using mobile phone technology in surveillance of febrile episodes (axillary temperature ≥37.5°C or/and history of fever past 24hours) through active (ACD) and passive case detection (PCD). Cases found positive by malaria rapid diagnostic test (mRDT) are given first dose of artemether/lumefantrine (Alu) at the dispensary. VHVs visit each patient at home to supervise intake of remaining doses. In May 2013, the population was estimated to be 2934 individuals in 678 households. Between 15th November 2013 and 15th March 2014, a total of 528 febrile cases aged 6.5 months -94 year were attended. Most of the cases (82.3%) were detected through PCD; 21.0% were positive by mRDT and 19.3% by microscopy. Of those with malaria, 89.5% had completed one dispensary of Magoda. VHWs are using mobile phone technology in facilitating early detection and management of febrile illnesses. VHWs are using mobile phone technology in facilitating early detection and management of febrile illnesses. VHWs are using mobile phone technology in facilitating early detection and management of febrile illnesses.

ANALYSIS OF CANDIDATE GENETIC LOCI ASSOCIATED WITH DELAYED PARASITE CLEARANCE FOLLOWING TREATMENT WITH ARTEMISININ BASED COMBINATION THERAPY IN PLASMODIUM FALCIPARUM ISOLATES FROM WESTERN KENYA

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In a recent study conducted in South East Asia (SEA), regions on chromosomes 10, 13 and 14 of Plasmodium falciparum were identified that showed association with slow parasite clearance rates (CRs) when subjects were treated with artemisinin based combination therapy (ACT). We conducted a study to analyze chromosome 10, 13 and 14 regions as recently published to establish the genetic baseline of P. falciparum in western Kenya. Historical samples collected through surveillance studies from 1995-2013 and samples from an ACT efficacy clinical study conducted in western Kenya in 2013-2014 were analyzed. Parasite clearance rates for subjects in the ACT efficacy trial were calculated using the WWARN online parasite clearance estimator. All subjects from the ACT trials achieved parasite clearance within 42 hours of treatment, with a median CR constant of 0.27(range 0.15 - 0.58). More than 60% of the SNPs that were showed to be association with slow CR in SEA were present in samples from western Kenya. Interestingly however, the prevalence was highest in samples collected before the introduction of ACTs in Kenya, and decreased steadily over time. In the efficacy study, 24% of these SNPs were present. There was no increase in PfmDR copy
number observed. This data indicate unlike what was observed in SEA, these markers are not associated with ACT CRs, and are more likely associated with pressure from chloroquine which was removed from circulation at the time when reduction in the prevalence of the SNPs is observed.

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**PFCR HAPLOTYPE DIVERSITY IN CAMEROON AND EMERGENCE OF THE S(AGT)VMNT TYPE**

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Evolution and spread of chloroquine-resistant-malaria parasite *Plasmodium falciparum* have posed great challenge in malaria control efforts all over the malaria endemic countries of the globe. Although chloroquine (CQ) has been banned from national malaria control programs of many endemic countries; due to high efficacy, easy availability and affordability, CQ still continues to be non-officially used in many countries including Africa. The main objective of this study is to unravel distribution of different Pfcrf genotypes in central, littoral, eastern, southern and in international bordering part of Cameroon. This is because, (i) chloroquine (CQ)-resistant (CQR) malaria parasite *Plasmodium falciparum* is of wide occurrence in Cameroon, (ii) mutations in the 72nd - 76th amino acid positions of the Pfcr gene are known to confer resistance to CQ and (iii) only a single CQR haplotype (C72V73I74E75T76) has so far been reported in Cameroon. We have followed molecular approach with DNA sequencing of the 2nd exon of the Pfcr gene to identify Single Nucleotide Polymorphisms (SNPs) in 180 *P. falciparum* field isolates sampled in five different locations in Cameroon. The CQR-Pfcr PfV haplotype was mostly abundant, followed by the wild CVMNK haplotype. Five hitherto-unreported CQR-Pfcrf haplotypes were detected for the first time in Cameroonian *P. falciparum*, including the surprise appearance of the S(agt) VMNT haplotype. Observations on the high haplotype diversity of the CQR-Pfcrf haplotypes coupled with appearance of the S(agt)VMNT type is daunting and can pose a greater challenge to the malaria control program of Cameroon than before.

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**PARASITOLOGIC AND MOLECULAR ASSESSMENT OF EFFICACY OF SOME ANTIMALARIAL DRUGS USED DURING PREGNANCY IN LAGOS, NIGERIA**

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The prevention of malaria in pregnancy (MIP) reduces the adverse effect of malaria on both mother and foetus. Resistance to sulphadoxine-pyrimethamine (SP) has led to discontinuation of the use of SP in treatment of malaria, but used only in intermittent preventive treatment of malaria during pregnancy (IPTp). It is therefore essential to continuously monitor the efficacy of SP during pregnancy. Other non-recommended drugs but commonly used for antimalarial chemoprophylaxis during pregnancy (e.g. chloroquine and pyrimethamine) were assessed using molecular markers of resistance. Protective efficacy of SP in IPTp and the equivalence of monthly IPTp-SP to two-dose IPTp-SP was assessed in a longitudinal study from the second trimester to delivery in Lagos. A total of 1084 pregnant women were screened, but 259 were recruited (122 and 137 in two-dose and monthly-dose arms respectively) into the longitudinal study. The protective efficacy of SP was lower in both study arms (P=0.05): 98.3%, 99.1%, 100% and 100% at months 1,2,3 and 4 respectively in the two-dose arm versus 98.5%, 99.1%, 100% and 100% at months 1,2,3 and 4 respectively in the monthly-dose arm. The outcome of pregnancy (low birth weight and live births) was similar in the two study arms (P=0.05) irrespective of gravidity and age of the women. The frequency of Pfcr haplotypes were 24.1, 53.7% and 22.2% for CVMNK, CVIET and CVIET+CVMNK respectively. The dhfr haplotypes were 26.7%, 6.7% and 66.7% for ACICNVI, ACICNVI and ACIRNVI respectively. The pfmdr1 haplotypes were 53.6%, 17.9%, 21.4% and 7.1% for NYSND, YYSND, NFSND and YFSND respectively. SP was effective in IPTp and 2-dose was equivalent to monthly-dose SP regimen in Lagos. The high levels of resistance markers for pyrimethamine and chloroquine indicates that antimalarial treatment/chemoprophylaxis in pregnancy with either pyrimethamine or chloroquine may not be effective.

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**THERAPEUTIC EFFICACY AND SAFETY OF ARTEMETHER/ LUMEFANTRINE FOR TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA AND PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE**

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Following deployment of artemisinin based combination therapy (ACTs), regular testing for efficacy is recommended by the World Health Organisation (WHO). This study assessed the efficacy and safety of Artemether/Lumefantrine (AL) and parasite genetic factors associated with parasite clearance or treatment failure in an area where the transmission has significantly declined in recent years. This was an open-label, single-arm trial, involving 88 children (6 months to 10 years) with uncomplicated falciparum malaria attended at Mkuzi Health Centre in Muheza, Tanga . Follow-up was for 28 days and the primary end point was parasitological cure on day 28. Secondary end points included: haemoglobin improvement at day 28 and occurrence and severity of adverse events. Parasite sequencing is being performed at Sanger Institute, UK. Of the 163 patients screened, 88 were enrolled in the study. Geometric mean parasite density was 18,551 asexual parasites/µl (range: 256-200,000) and mean haemoglobin level was 10.4±1.8g/dL. Nine patients (10.2%) were lost during follow up and there was no early treatment failure. Before PCR correction; 78.4% (n=40) under-fives and 75.0% (n=21) children aged ≥5years had adequate clinical and parasitological response (ACPR) . Late clinical failure (LCF) was seen in 5.6% of under-fives (n=51) and 3.6% children aged ≥5years (n=28). Furthermore, 15.2% and 21.6% of the patients had late parasitological failure (LPF) among under-fives and children aged ≥5years respectively. After PCR correction, ACPR was 100% in both groups. Reported adverse events included abdominal pain (11.9%), cough (59.7%), diarrhea (1.5%), fever (23.8%), headache (1.5%), and skin rashes (1.5%). The association between treatment outcome and parasite genetic factors will be performed later. AL was safe and efficacious for the treatment of uncomplicated malaria. Since Mkuzi/ Muheza has been a hotspot of drug resistance in Tanzania, surveillance needs to be continued to detect future changes in parasite sensitivity to ACTs.
**310 INDEPENDENT EMERGENCE OF THE SUPER RESISTANCE-CONFLING MUTATION AT DHPS CODON 581 IN EAST AFRICAN PLASMODIUM FALCIPARUM POPULATIONS**

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Sulphadoxine-Pyrimethamine (SP) is no longer applied as treatment against malaria, due to a variety of mutations in Plasmodium falciparum populations conferring resistance to the drug. However, SP has been shown to still provide protection as preventive treatment against malaria. WHO now recommends SP in preventive treatment in pregnant women and in infants, as well as in combination with Amodiaquine for seasonal malaria chemoprophylaxis. Unfortunately, the parasites still harbor the resistance-conferring mutations, which in East Africa consist of a triple mutation in the PfDhps gene, and a double mutation in the PfDhps gene. Now it is being shown in East Africa that a third mutation has appeared in the PfDhps gene, terminating the efficacy of SP as preventive treatment. Emergence of this “super resistance”-confling mutation threatens the hitherto successful control measure of intermittent preventive treatment in pregnancy. In this study, we have analysed the origin of the “super resistant” triple mutant parasites in Ethiopia, Uganda and Tanzania, with regard to the previously established double mutants. We have applied microsatellite analysis of the genetic region flanking the PfDhps gene, to analyse the lineage of the double and triple mutants. In Ethiopia both double and triple mutants were derived from a single lineage which was distinct from those in Uganda and Tanzania. This correlates well with previous studies showing distinct parasite populations in Northeast and Southeast Africa. In Uganda and Tanzania, we also found triple mutants which were derived from the previously characterized Southeast African lineage. However, a novel microsatellite allele incorporated into the Tanzanian triple mutant lineage since 2004, exceeding in numbers the previously described Southeast lineage, illustrates the local expansion of a new triple mutant lineages. We conclude that the A581G mutation has occurred independently and multiple times on local PfDhps double mutant backgrounds.

**311 QUANTITATIVE ANALYSIS OF THE ANTIMALARIAL DRUG PYRANORIDINE USING DRIED BLOOD SPOTS**

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The fixed-dose antimalarial drug combination of artesunate and pyronaridine (PYRAMAX®) has recently been developed and introduced in the treatment of falciparum and vivax malaria. Pyronaridine have a long terminal elimination half-life and it is possible to detect and quantify the drug for several weeks after the last dose. To be able to measure pyronaridine from a small quantity of blood, a sensitive quantification method was developed for therapeutic drug monitoring and pharmacokinetic studies. Pyronaridine have a high blood-to-plasma ratio and this also makes whole blood the most suitable sample matrix. Therefore, a method to measure pyronaridine in dried blood spots was developed and validated. This method uses 50 µl of whole blood applied as blood spots on a filter paper to be dried at ambient temperature. The extraction process followed by separation and detection by electrospray LC-MS/MS will be presented as well as the stability tests of pyronaridine in dried blood spots. The developed method was validated according to the US FDA bioanalytical method validation guideline. Dried blood spots, as a sample collection technique, have become an increasingly useful tool in rural areas where transportation is problematic and where laboratory facilities are minimal. The accurate and sensitive method presented here requires a very small sample volume, which can be collected by finger or heel sticks. The work presented here may be particularly advantageous in studies involving small children, in whom it is ethically and practically impossible to collect large volumes of blood.

**312 THE EFFECT OF ANTIMALARIAL DRUGS ON INTRACELLULAR PH IN PLASMODIUM FALCIPARUM**

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The acidic digestive vacuole (DV) of Plasmodium falciparum is the site of action of several antimalarial drugs and its membrane contains transporters that are implicated in the regulation of drug resistance. The low pH of the DV lumen is necessary for hemoglobin degradation and the detoxification of the toxic byproduct heme and its maintenance is thus crucial to the survival of the parasite. The quinoline drugs chloroquine (CQ), mefloquine (MQ) and quinine (QN) have previously been shown to raise DV pH using spectrophotometry, however, no accurate quantitative measurements have been performed. In this study, CQ-resistant Dd2 and CQ-sensitive 3D7 parasites were transfected with a DNA construct encoding the ratiometric pH-sensitive green fluorescent protein pHluorin alone or pHluorin with a targeting peptide that mediates trafficking of the fluorescent protein to the DV. Quantitative pH determinations were carried out using confocal fluorescence microscopy of single live parasite-infected erythrocytes under physiological conditions. MQ had the strongest effect on DV pH at a concentration of 500 nM and caused the organellar pH to rise by 0.8 pH units, whereas CQ and QN addition resulted in a pH increase by 0.3 - 0.5 pH units. To test whether the acidification of the cytosol in response to CQ, MQ and QN may be due to H+ coupled drug export from the DV into the cytosol mediated by P. falciparum chloroquine resistance transporter (PICRT), the pH effects of PICRT inhibitor verapamil (VP) were measured in combination with a quinoline drug. VP prevented the cytosolic acidification caused by CQ and QN in Dd2 parasites, supporting the hypothesis that these drugs are expelled from the DV in sympot with protons in drug-resistant strains. In contrast, VP did not alter the pH effects of MQ, which is probably not transported by PICRT. The specific V-ATPase inhibitor concanamycin A at a concentration of 100 nM abolished the transvacuolar pH gradient between DV lumen and cytosol within 20 min, indicating that the V-ATPase might play a central role in maintaining this gradient.

**313 PREVALENCE OF DHFR AND DHPS MOLECULAR MARKERS IN PLASMODIUM FALCIPARUM PARASITES IN PREGNANT WOMEN OF NCHELENGE DISTRICT, NORTHERN ZAMBIA**

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Sulphadoxine pyrimethamine (SP) is the recommended drug for Intermittent Preventive Treatment in pregnancy (IPTp) in most African countries including Zambia. However, malaria is still one of the leading causes of morbidity and mortality in pregnant women despite reports of greater than 50% of women taking at least two doses of SP in IPTp. Studies have shown that resistance to SP is associated with mutations in the dhfr and dhps gene of the Plasmodium falciparum parasite. Few studies have been done to determine the prevalence of these mutations.
in parasites found in pregnant women in Zambia. This cross-sectional study was conducted in Nchelenge, Northern Zambia in February-April 2013. Nchelenge is a hyper-endemic area with estimated perennial malaria prevalence of 50%. Three Rural Health Centers were randomly selected and a census survey carried out at each health center. A questionnaire was administered and malaria testing done using RDT and microscopy, with collection of a dried blood spots. Parasite DNA was extracted from dried blood spots using the chexel method followed by nested PCR. Positive samples then underwent mutation specific enzyme restriction digestion. The overall results (n=385), showed a mean age of 25. The prevalence of malaria was 22%. Multivariate analysis showed that there was an association between malaria and anaemia (AOR: 2.94) and women aged >25 years old (AOR: 0.40) were less likely to have malaria. The prevalence of dhfr codon S108N was 94%; codon C59R was 93.4%; and codon A16N was 5.54% dhps codon 436 was present at 97% and no mutation was found on dhps codon 540. This study showed a high number of mutations in the dhfr and dhps genes than previously reported. The high malaria endemicity in the general population of this area may have contributed to the high prevalence of resistant parasites in pregnant women. Other studies have shown that in highly endemic areas resistant parasites tend to spread quickly. These findings call for close and more detailed monitoring and evaluation of IPTp in Zambia to ascertain its effectiveness.

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THE EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPPLICATED PLASMODIUM FALCIPARUM MALARIA IN THE GAMBIA: 2013 FOLLOW UP STUDY

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Artemisinin-based combination therapy (ACT) remains the main stay of case management of uncomplicated Plasmodium falciparum malaria. Because of increasing threat of artemisinin resistance, routine efficacy monitoring is recommended and World Health Organization guideline recommends a change in the choice of a first-line ACT when its treatment failure rate exceeds 10% in a 28 or 42-day in vivo therapeutic efficacy study. In 2008, The Gambia introduced artemether-lumefantrine (AL) for treatment of uncomplicated P. falciparum malaria and here, we report on the therapeutic efficacy monitoring of AL in the Western region of the country in the 2013 malaria transmission season. In a one-arm prospective study, 129 children aged >1 to 12 years with uncomplicated P. falciparum mono infection of between 1,000 to 100,000 asexual parasites/µl were treated with a weight-based oral AL and followed up for 28 days for clinical and parasitological response. Parasite density was determined using Giemsa stained thick blood films on day 0 and on days 1, 2, 3, 7, 14, 21, 28 or on any other day a child had symptoms of malaria. Filter paper spots were obtained during each of the follow up visits for polymerase chain reaction (PCR) analysis to determine if a recurring parasitemia was a re-infection or a recrudescence. Blood samples were obtained on day 0 and on whichever day a parasitemia >1,000 parasites/µl was detected for ex vivo parasite susceptibility drug testing. In the per protocol study population (n=100), PCR uncorrected results showed that proportion of children with adequate clinical and parasitological response by day 28 was 93% (93/100). Of these, 56% and 100% had complete parasite clearance by days 3 and 7 respectively. All the cases of parasitemia detected by day 3 were low grade (<1,000 parasites/µl). All the treatment failures (n=7) encountered were late parasitological failures between days 14 and 28. These results suggest that AL remains efficacious for the treatment of uncomplicated P. falciparum malaria in the Western region of The Gambia. A high proportion of low grade parasitemia by day 3 was however observed.

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MALARIA AND MIGRANTS IN THAILAND: TREATMENT-SEEKING (AND OTHER) BEHAVIORS

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Artemisinin-resistant malaria has emerged as an urgent concern in South East Asia. One strategy to address the problem is to improve the access of mobile and migrant populations (i.e. those considered at highest risk of malaria) to health services. This study sought to understand the knowledge, attitudes and practices (KAP) of different migrant groups in Thailand, in order to design strategies to increase migrants’ health access. In September 2013, 386 migrants who visited selected health facilities along the Thai-Myanmar border participated in a KAP retrospective study. The results showed that net usage among migrants who crossed borders daily was lower than migrants who stayed in Thailand for longer periods. Less than half (48.3%) of fever cases in the past 3 months sought treatment at public health facilities, even though 36.4% of those who were tested for malaria were positive. Lower net usage, as well as delayed treatment seeking for fever, was also reported among rubber plantation workers. New intervention packages (such as insecticide-treated net delivery and behaviour change communication delivered at official and unofficial border points) must be devised to reach migrants as well as those who work in rubber plantations, as both groups are more likely to sleep without mosquito nets, and delay seeking treatment.

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THERAPEUTIC EFFICACY OF CHLOROQUINE FOR THE TREATMENT OF PLASMODIUM VIVAX IN SOUTH ETHIOPIA

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The development and spread of chloroquine resistance Plasmodium vivax threatens the health of millions of people and poses a major challenge in the control of malaria. Monitoring of drug efficacy in two years interval is an important tool in establishing rational antimalarial drug policies. The current study assessed therapeutic efficacy of chloroquine for the treatment of Plasmodium vivax in South Ethiopia. A one-arm prospective evaluation of clinical and parasitological responses for uncomplicated microscopically confirmed vivax malaria was conducted to monitor the therapeutic efficacy of chloroquine from September to December 2012 in South Ethiopia. A total of 90 patients above 6 months and greater than 5kg with symptomatic, microscopically confirmed vivax malaria was conducted to monitor the therapeutic efficacy of chloroquine from September to December 2012 in South Ethiopia. A total of 90 patients above 6 months and greater than 5kg with symptomatic, microscopically confirmed, uncomplicated Pvivax and fulfill the inclusion criteria were selected and included. All eligible patients were treated with CQ and followed for 28 days. PCR was conducted to differentiate re-infection from recrudescence. Pmdr1 gene, the orthologue of pmdr1, were sequenced and analyzed for the presence of mutations at positions 976 and 1076. The results showed that net usage among migrants who crossed borders daily was lower than migrants who stayed in Thailand for longer periods. Less than half (48.3%) of fever cases in the past 3 months sought treatment at public health facilities, even though 36.4% of those who were tested for malaria were positive. Lower net usage, as well as delayed treatment seeking for fever, was also reported among rubber plantation workers. New intervention packages (such as insecticide-treated net delivery and behaviour change communication delivered at official and unofficial border points) must be devised to reach migrants as well as those who work in rubber plantations, as both groups are more likely to sleep without mosquito nets, and delay seeking treatment.
be due to parasite resistance to chloroquine. This finding alarms the need to launching a monitoring program of chloroquine for the treatment of *P. vivax*.

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**STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE SUGAR PHOSPHATASE PHAD1, A REGULATOR OF THE MEP PATHWAY FOR ISOPRENOID BIOSYNTHESIS IN *PLASMODIUM FALCIPARUM***

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The emergence of drug resistance to currently available antimalarial drugs is a major challenge in malaria control efforts, and thus, understanding the mechanisms that underlie drug resistance is of great importance. The methylerithritol phosphate (MEP) pathway for isoprenoid precursor biosynthesis is an attractive target for novel antimalarial drug development, and the small molecule compound fosmidomycin has been validated as a competitive inhibitor of the MEP pathway enzyme deoxyxylulose 5-phosphate (DXR). Fosmidomycin was employed in a forward genetics approach to gain understanding into regulation of the MEP pathway. Fosmidomycin-resistant *Plasmodium falciparum* strains were selected in *vitro*, and genetic analysis revealed that mutations in *PHAD1* are associated with fosmidomycin resistance. *PHAD1* is a previously uncharacterized enzyme with homology to haloacid dehalogenase-like sugar phosphatases. The crystal structure of *PHAD1* was solved in order to define the structural basis for loss-of-function mutations in *PHAD1*. The identified point mutations map to tightly packed hydrophobic inner core regions or catalytic regions of *PHAD1*, resulting in either protein misfolding or interference with substrate binding. Biochemical investigations show that *PHAD1* dephosphorylates a variety of sugar phosphate compounds, including intermediates of glycolysis, which feed into the MEP pathway. Several of these sugar phosphates have been co-crystallized with *PHAD1* in order to investigate the structural basis for its diverse substrate specificity. Finally, metabolic profiling reveals that fosmidomycin-resistant parasite strains lacking *PHAD1* have substantial increases in MEP pathway metabolites. Together, these results demonstrate that *PHAD1* regulates substrate availability to the MEP pathway and that loss of *PHAD1* function confers fosmidomycin resistance in *P. falciparum*.

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**DIVERSITY OF *PLASMODIUM MALARIA* AND MOLECULAR MARKERS OF SULPHADOXINE RESISTANCE IN TANZANIA**

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Interruption of antimalarial drug use and testing parasite isolates for drug resistance is critical to guiding antimalarial drug use and developing optimal strategies to control malaria. Molecular markers of sulphadoxine resistance were detected by nested-PCR followed by sequence specific oligonucleotide probes-enzyme-linked immunosorbent assay (SSOP-ELISA). A total of 466 (56.9%) patients were tested positive by mRDTs and 397 (48.5%) microscopy. Nachingwea had higher positivity rate compared to other sites, at 29.0% and 27.7% by mRDT and microscopy respectively. *Plasmodium falciparum* species was the predominant species at all sites while *P. ovale* was only detected at Muleba and Nachingwea. Prevalence of *P.falciparum* gametocytes was higher in Muleba (13.7%) followed by Nachingwea (7.2%) and the lowest was observed in Muheza (0%). Additionally, fever was a strong predictor of malaria parasite rates at Nachingwea (p<0.001). Prevalence of sulphadoxine resistance involving Pfahps codon 540E (96.6%) and 581G (53.4%) were higher in Muheza compared to the other sites. This study showed that Nachingwea had high malaria positivity rate compared to other sites. High Sulphadoxine resistance observed at Muheza could be attributed to local use of SP and it may spread to other areas. Further studies to monitor the spread of sulphadoxine resistance in other regions are warranted.

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**INVESTIGATION OF STRUCTURE-ACTIVITY RELATIONSHIPS OF ANTIMALARIAL DRUGS THROUGH NOVEL *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER (PFCRT) HAPLOTYPES**

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Mutation in the *Plasmodium falciparum* resistance chloroquine transporter (PfCRT) protein causes chloroquine resistance and alters susceptibility to various antimalarial drugs. Mutant PfCRT transports chloroquine out of the parasite digestive vacuole; however, the molecular details of drug-receptor interaction are poorly understood. The antiviral drug amantadine has moderate potency against *P. falciparum* and acts to reduce the level of chloroquine resistance determined by the pfcrt haplotype. In accordance, chloroquine-resistant parasites show enhanced sensitivity to amantadine. We used continuous, step-wise amantadine selection on three chloroquine-resistant lines of the *P. falciparum* 106/1 strain. Each of these lines contains a unique pfcrt allele differing only by a non-synonymous SNP in codon 76, resulting in PfCRT K76I, K76N or K76T, but otherwise contains an identical genetic background. Stepwise selection with amantadine resulted in parasite lines with six novel pfcrt alleles. Parasites showed resistance to amantadine, increased chloroquine sensitivity, and changes in susceptibility to other digestive vacuole-targeting drugs. The results shed light on new topographical regions of the PfCRT protein and amino acid residues involved in drug interactions and digestive vacuole morphology. Two additional amantadine-selected parasite clones, derived from 106/1 K76I and K76N, showed amantadine resistance and altered quinoline susceptibility without accompanying mutations in pfcrt. These parasite lines are currently under investigation. Our results increase the understanding of how digestive vacuole-targeting drugs interact with the PfCRT protein and explain patterns of cross-resistance in the malaria parasite.
INVESTIGATION OF THE THAI MULTIDRUG RESISTANT *PLASMODIUM FALCIPARUM* C2A STRAIN IN THE AOTUS MONKEY MODEL

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Antimalarial drug resistance is the biggest threat to the current malaria eradication agenda (MaLERA) based on oral artemisinin-based combination therapies (ACTs). ACTs are the standard of care for uncomplicated malaria, but reports of clinical treatment failures (defined as a reduced parasite clearance rate or persistence of parasites on the third day of treatment) of artesunate (AS) / mefloquine (MQ) combinations are rapidly emerging from South-East Asia. The Thai *Plasmodium falciparum* C2A clone is a Multiple Drug Resistant (MDR) isolate obtained from a patient in Thailand in 1992 before deployment of this ACT, first introduced in 1994 and adopted in 2005, though, artemisinins had been in use in western Cambodia since the late 70’s (Witkowski et al, 2013). In a previous in vitro adaptation study in Aotus monkeys, we observed that even in the absence of MQ pressure, C2A appeared to gradually lose its susceptibility to MQ as passage levels increased. In order to further characterize this observation we assessed the efficacy of MQ and AS alone or in combination in infected splenectomized Aotus monkeys. Our results confirmed the previous findings of resistance to oral MQ at 40 mg / Kg given once or to oral AS at 33 mg / Kg x 3 days alone or in combination with MQ at 40 mg / kg once. Strikingly, in the AS alone group, clearance occur in 1 / 2 animals on day 7 post-treatment (PT) but recrudescence on day 8 PT (slow clearance phenotype) and persisted in the other one until day 10 PT when it was finally rescued treated. As a comparison, AS at 20 mg / Kg orally x 3 days against infections of the Vietnam CQ resistant FVO strain, clears infection within 1-2 days PT in comparison, AS at 20 mg / Kg orally x 3 days against infections of the Vietnam CQ resistant FVO strain, clears infection within 1-2 days PT in spleen intact Aotus. Only when AS was administered IV at 20 mg / Kg x 3 days in combination with oral MQ at 40 mg / Kg, infections were cured in 4 / 5, but cleared and recrudescence in 1 / 5 rescue treated animals. Additional in vitro assays of C2A demonstrated reduced susceptibility to MQ, CQ, arteether, dihydro-artemisinin, and atovaquone-proguanil, artemether and AS compared to the sensitive D6 strain, the in vitro adapted TM90-C2A line or passage III Aotus adapted C2A when compared to the later monkey passage X. Analysis of drug resistance loci and copy number variation is underway to determine the genetic basis for the observed drug resistance profile. We anticipate that these studies will shed light on the early spread and evolution of multi drug resistance and reduced artemisinin susceptibility in South-East Asia.

ADDRESSING ARTEMISININ-RESISTANT MALARIA BY IDENTIFYING MALARIA HOTSPOTS IN CAMBODIA

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During the past decade Cambodia has made significant strides toward malaria reduction and now, elimination. The last remaining cases will be the hardest to reach, as they are located in remote border areas. Efforts are also threatened by the emergence of artemisinin-resistant malaria (ARM). The PMU/USAID Control and Prevention of Malaria (CAP-Malaria) Project developed a model to effectively identify malaria hotspots and treat remaining malaria cases in remote villages through household active case detection of ARM and a comprehensive response. Key strategies include individual index malaria case detection serving as the focal point for subsequent comprehensive response to surrounding areas. Village Malaria Workers (VMWs) are trained to diagnose malaria in their communities and take a blood smear. Following patient treatment through directly observed therapy (DOT), the VMW takes a second blood smear after 72 hours of the treatment initiation which is analyzed at a health center laboratory. Cases that are still positive on day-3, and therefore an early warning sign for ARM, receive further investigation and case management, including follow-up to day 28. In addition, 40-50 people surrounding the index case are screened for malaria regardless of symptoms and treated if infected, and any gaps in community coverage of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and malaria knowledge are addressed by a team including the VMW, health center staff, and district malaria officials. Between September 2010 and December 2013, 1,733 malaria patients were diagnosed with malaria by VMWs from 11 targeted health facilities in catchment areas where evidence of ARM has been found. Among the residents from four catchment areas, 1,585 *Plasmodium falciparum* and mixed infection malaria cases, 109 (7.7%) were positive on day-3. During the three year time period, however, the number of day-3 positive cases detected decreased, even while overall malaria enrolled cases increased. Potential reasons for the drop in day-3 positive cases could include the systematic DOT provided by the VMWs, ensuring treatment adherence and improved quality of the drug supply.
REAL-TIME PCR AND HIGH RESOLUTION MELTING (HRM) ANALYSIS OF POLYCLONAL PLASMODIUM FALCIPARUM INFECTIONS

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Recurrent Plasmodium falciparum parasitaemia in treated malaria patients results from either recrudescence of parasite clones which survived drug action, or acquisition of new clones that emerge from the liver during follow up after antimalarial treatment. One molecular method used to distinguish between the two is PCR-correction using size polymorphisms of various genes and microsatellites. The recurrent infection is commonly categorized by comparing the size of polymorphisms in genetic markers of the genes on enrolment samples (day-0) and on follow-up day on which parasite are detected by microscopy (day of parasitological failure). We have previously reported that by increasing the parasite genotyping to multiple time-points (day-0, day-1 and day-2), recrudescent clones are identified more often than by the conventional approach. However, most currently used gel-electrophoresis methods, particularly when multiple time-points are incorporated, are time-consuming, laborious and the interpretation of results can be ambiguous. The development of an alternative, rapid, simple and accurate method based on Real-time PCR and High Resolution Melting (HRM) analysis will be reported. Its usefulness for ex vivo drug sensitivity and complexity of infection studies will be discussed.

K13-PROPELLER, MAL10 AND MAL13 AND POLYMORPHISMS IN IN VIVO ARTEMISININ SUSCEPTIBLE PLASMODIUM FALCIPARUM PARASITES FROM BOUGOULA-HAMEAU, MALI

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Artemisinin resistance has been documented in South-East Asia and may already be spreading in that region. Molecular markers are important tools in monitoring the spread of antimalarial drug resistance. Recently, two SNPs on chromosomes 10 and 13 (MAL10-688956 and MAL13-1718319) were shown to be useful markers of delayed parasite clearance in surveillance for artemisinin resistance in South-East Asia. In addition, mutations in the PF3D7_1343700 kelch propeller domain (‘K13-propeller’) were associated with artemisinin resistance in vitro and in vivo. The prevalence of these molecular markers of artemisinin resistance is unknown in sub-Saharan Africa. We therefore extracted DNA from dried blood spots of pre-treatment falciparum malaria infections in Bougoula-Hameau, Mali where we recently showed that artemisinin monotherapy was highly efficacious with a median parasite elimination slope half-life of 2 hours. Polymorphisms of MAL10-688956 and MAL13-1718319 were genotyped by Nested PCR followed by restriction enzyme digestion with Nsil and MbsI, respectively. Mutations in K13-propeller were determined by direct sequencing of Nested PCR amplicons. One hundred children aged 1-10 years were included in this study. Sample processing and statistical analyses are underway and results will be presented at the meeting. This study will provide information on the prevalence of molecular markers of artemisinin resistance in a population of sub-Saharan African malaria parasites fully susceptible to artemisinin.

IDENTIFICATION OF MALARIA-INFECTIOUS INDIVIDUALS BY FIELD-BASED DIAGNOSTIC METHODS: LOOP-MEDIATED ISOThERMAL AMPLIFICATION AND LIgHT MICROSCOPY

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Malaria elimination strategies will require sensitive diagnostic tools capable of detecting most human falciparum infections in endemic communities. Malaria loop-mediated isothermal amplification (LAMP) is a field-friendly assay that has greater diagnostic accuracy compared to microscopy and consequently might have a key role in mass screening campaigns. Here, we assessed the infectiousness of 130 randomly-selected individuals living in a malaria endemic area of Burkina Faso by membrane feeding assays (N=307 assays) and related infectivity results to malaria infection status determined by two field-based methods (LAMP and light microscopy), and by quantitative-nucleic acid sequence-based amplification (QT-NASBA). In 72.0% of samples, Plasmodium falciparum infection was detected by LAMP. Parasites were identified by microscopy (sexual or sexual parasites) and 18s NASBA in 45.0% and 94.8% of samples, respectively. In 75.5% of feeding assays where at least one mosquito was infected, parasites were detected by LAMP, similar to light microscopy (74.4%). Our results confirm previous findings that LAMP is more sensitive than microscopy for detecting falciparum infection. A similar proportion of infectious individuals had parasites detected by LAMP and microscopy, suggesting that high parasite density, higher than microscopy detection threshold, is an important predictor of infectiousness. Further research is warranted to verify whether this pattern also occurs at low transmission intensities, where most infectious individuals have low-level infections.

UNEARTHING THE HIDDEN INTRICACIES BETWEEN FISHING AND MALARIA: A CASE STUDY OF RUSINGA ISLAND, WESTERN KENYA

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Establishment of underlying factors of malaria exposure remains key for identification of ideal preventive measures. It is apparent that malaria risk behaviours are rooted in wider aspects of local livelihoods, and socio-cultural beliefs and practices. This research work draws on empirical data derived from in-depth, one-to-one semi-structured interviews, focus groups and questionnaire administration to participants in villages in Rusinga Island to explore the nature of these practices and their potential impact on malaria exposure risk. Participants were fish crew, both men and women. By eliciting local understandings of malaria-related behaviours, we explore how malaria risks are played out in people’s everyday lives. Our findings reveal the problem of non-usage of bednets by community members resulting from their livelihood and socio-cultural practices and events. These practices contravene the consistent and sustained use of the bednet which are called for by public health policies. In particular, we try to explain how in reality livelihood activities and lifestyle play a role in exposure to malaria by Rusinga fish crew around dawn and dusk.
A COMMUNITY-BASED SURVEY ON KNOWLEDGE, ATTITUDE AND PRACTICE OF DIAGNOSIS AND TREATMENT FOR VIVAX MALARIA IN SOUTH BENGKULU AND MINAHASA DISTRICTS, INDONESIA

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Plasmodium vivax malaria globally threatened an estimated 2.49 billion people in 2010. In Indonesia, P. vivax is transmitted among over six million pregnancies and 130 million individuals, with overall prevalence of 3.84%. On the contrary to a conventional philosophy, growing evidence suggests that P. vivax demonstrate as debilitating illness and life-threatening for adults and children in eastern archipelago. Management of P. vivax malaria is challenging due to the presence of relapsing liver stage (hypnozoites), which can be killed by the only available drug, Primaquine (PQ) and administration with blood schizontocides acts as radical cure of vivax malaria. According to current recommendations, suspected fever cases should be confirmed by microscopy or rapid diagnostic tests (RDTs) prior to treatment. However, a vast majority of fever cases in Indonesia are clinically diagnosed and Artemisinin Combination Therapy (ACT) is not properly administered. PQ failure has been attributed to inadequate treatment regimen and dosage, and patients’ lack of compliance. Further, PQ can induce hemolytic toxicity in individuals who are deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD). Treatment and diagnostic practices essential for optimal PQ efficacy are inadequate in Indonesia, as indicated by high levels of drug resistance. To date research into malaria-related knowledge and behaviours has been widely conducted with household surveys. These measurements reveal information on determinants of malaria diagnosis and treatment processes. Cross sectional surveys were conducted between April and August, 2013. Three questionnaires were implemented in the household and primary health centers (PHCs). Total number of respondents was 562. Clinicians and laboratory technicians were queried in ten selected PHCs. Data was analyzed by STATA ver. 9. Overall, the processes involved in vivax malaria diagnosis and treatment must be evaluated in order to provide control programs with a “pulse of community. Most people stated an understanding about malaria, symptoms, severity level and consequence of untreated malaria. However, despite a call for malaria elimination programs, community awareness related to early diagnosis and effective treatment should be strengthened. Health providers require multi-faceted interventions to support better laboratory confirmation and treatment towards vivax malaria infections.

SINGLE DOSE OF PRIMAQUINE FOR MALARIA ELIMINATION IN A POPULATION WITH HIGH PREVALENCE OF G6PD DEFICIENCY

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Malaria caused by multi-drug resistant Plasmodium falciparum (including artemisinin resistance) is common in the population living along the Thai-Myanmar border. In the context of malaria elimination to combat the spread of artemisinin resistance, the Shoklo Malaria Research Unit has evaluated the safety of single dose of 0.25 mg base/kg of primaquine with an ACT in a population of over 2000 subjects. Blood collection for hemoglobin levels and sensitive detection of malaria parasite were performed every 3 months during one year in 4 different villages. G6PD was characterized by Fluorescent Spot Test in all study participants and genotyping performed for the most common local mutations in subjects with abnormal phenotype. Hemoglobin levels of subjects treated with PMQ before and after primaquine administration were analyzed. Results will be presented showing the effect of the single low dose primaquine in G6PD normal and deficient participants.

PROTEIN MICROARRAY ANALYSIS OF HUMAN ANTIBODY RESPONSES IN PLASMODIUM VIVAX RELAPSE VS. REINFECTION IN THE PERUVIAN AMAZON

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Accurate estimations of parasite prevalence, malaria disease incidence, and hence prioritization of control efforts require new, cost-effective, and field-deployable methods. Because determining the presence of malaria parasitemia is time-intensive, often insensitive, and expensive, interest has grown for using serological tools to monitor infection status and transmission dynamics. Because Plasmodium vivax is only available in relatively low parasitemia from humans and in limited quantities from non-human primates, microarray seroepidemiology using P. vivax asexual stage parasite lysates has only infrequently been done. The field activities of this study were carried out from 2005-2008 in the Santo Tomas, San Jose de Lupuna, and Padrecocha villages of the Peruvian Amazon. Genotyping of P. vivax isolates was performed using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the P. vivax merozoite surface protein-3alpha (PvMSP3α). Relapse was defined as identical RFLP patterns of the primary and subsequent infection parasites. Reinfection was defined as different RFLP patterns of the primary and subsequent infection parasites. Of 111 subjects, 96 had 2 episodes, 10 had 3 episodes, and 5 had 4 episodes over a 24-month follow-up period. Using PvMSP3α PCR-RFLP genotyping of the recurrent infections, 38 were classified as relapse; 88 were classified as reinfection; and 5 showed no RFLP band and thus were not classified. Using microarray analysis, the antigens differentially recognized by P. vivax-infected subjects were more likely to have significantly higher signal for subjects with PvMSP3α genotypes different than the previous infection than for subjects with PvMSP3α genotypes similar to the previous infection. Proteins recognized by Human IgG Antibodies are more likely to have amino acid changes than proteins coded by the genome as a whole. Proteins recognized by sera are more likely to have SNPs than the genome as a whole, suggesting selection arising from host-pathogen interactions, immune or otherwise. The protein microarray analysis results will provide tools for applying seroepidemiology to surveillance, control and elimination; establishing tools for decay kinetics.
EFFICACY OF INDOOR RESIDUAL SPRAYING (IRS) WITH ACTELLIC® 300CS IN SENEGAL

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In areas of low malaria transmission, such as western Senegal, where scaling up of control measures has been effective in reducing malaria incidence, additional measures are required to eliminate the disease. Targeting additional control measures to communities with persisting high transmission of malaria may be more effective than blanket control measures. As part of a cluster randomized trial of a targeted control strategy, efficacy of Actellic 300CS (pirimiphos methyl) for IRS delivered by district spray teams was evaluated. Hotspot villages were identified on the basis of the number of cases of malaria the previous year. In August 2013, IRS with Actellic300CS was delivered to all households in these villages by community health workers, trained and supervised by staff of the department of Public Health (Service National d’Hygiène). Efficacy was assessed in 4 villages over 4 months. In each village, 5 treated rooms were selected, and untreated rooms selected as controls. All fumigations were done on each of three walls per room to measure knock-down and 24-hour mortality of lab reared Anopheles gambiae and of wild mosquitoes locally caught. A household survey was conducted to assess completeness of coverage and acceptability. Results and conclusion: After two, three and four months the 24-hour mortality, adjusted for control mortality using Abbott’s formula, was 92% (95% CI 85%, 96%), 72% (63%, 80%), and 37% (29%, 45%) respectively. Sensitivity tests to different insecticides using local Anopheles showed 100% sensitivity to pirimiphos methyl in all four sites, and to bendiocarb and permethrin in three sites with lower sensitivity in one district, with resistance to DDT in all sites. These results show that IRS with Actellic 300CS was highly effective but the duration of efficacy was shorter than expected.

APPLICATION OF MOLECULAR BARCODE AND PRE-AMPLIFICATION TECHNIQUES IN TRACKING PLASMODIUM FALCIPARUM POPULATION STRUCTURE CHANGES DURING SCALE-UP MALARIA INTERVENTIONS IN SOUTHERN AFRICA

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Malaria is receding from many endemic countries in the wake of scaled-up interventions for possible elimination of the disease. However, the decline is heterogeneous within and between countries and the resilient scourge persists as low-grade submicroscopic infections among semi-immune members of the population. This transitioning epidemiology not only poses risk of possible resurgence typical for malaria, but also presents challenges for detection and tracking of intervention impact on the surviving parasite reservoir. Here we demonstrate application of molecular barcode and pre-amplification technology for the successful detection and documentation of Plasmodium falciparum population structure changes in relation to control interventions in areas of southern Africa. Dry blood spot (DBS) samples were collected through prospective cross-sectional surveys, between 2003 and 2013, in three areas of southern Africa exhibiting contrasting malaria epidemiology under control, namely Choma district in southern Zambia (successfully declining malaria), Mutasa district in eastern Zimbabwe (rebounding malaria) and Nchelenge district in northern Zambia bordering the Democratic Republic of Congo (persistently holoendemic malaria). Following DBS screening by realtime quantitative PCR (with preamplification for low-grade infections), P. falciparum infections were genotyped using a 24 SNP molecular barcode assay. Analysis of population structure across the 24 barcode markers demonstrated significant loss of genetic diversity and population structure decline for Choma (M-W U = 178.0, p = 0.023) but not for Mutasa (M-W U = 10, p = 0.487) or Nchelenge, where the genetic diversity was even higher than Mutasa (M-W U = 35.0, p = 0.029). Barcoding and pre-amplification techniques afford an instrumental tool for parasite identification, tracking and documenting the impact of interventions on parasite populations during malaria control and elimination programmes when parasitaemia is expected to decline to submicroscopic levels.

MALARIA AND HEALTH NEEDS ASSESSMENT AMONG MIGRANT FARM WORKERS IN AMHARA REGION, ETHIOPIA: A VENUE-BASED APPROACH

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Mobile populations present unique challenges to malaria control and elimination. Each year, around 350,000 people travel to areas of North Gondar Zone, Ethiopia to work on large-scale farms, doubling the population of areas with the heaviest malaria burden in Amhara Region. The farming season overlaps the major malaria season (September to December). Little is known about these migrant workers, including migration patterns, access to, and use of malaria prevention measures, housing accommodations, and health care seeking behavior. In order to assess these factors and prevalence of malaria and anemia, a venue-based survey of migrants who traveled for farm labor in two districts of North Gondar was conducted in July 2013 at the beginning of the farming season. A total of 605 migrants ≥18 years of age were recruited from three venue types: farms (58% of survey population), roads between farms (16%) and towns (26%). Most (73.1%) had started farm work at the time of survey and 99% were male. Mean age was 22.8 years, with 74.7% less than 25 years. Nearly all (95.8%) came from Amhara, with half (51.8%) of those coming from other districts in North Gondar. Most (77.4%) migrants arrived in June or July, and 46.3% intended to leave in September. Around half (52.1%) lived in temporary shelters, while 20.8% had no sleeping accommodations. Malaria was the leading identified health concern. LLINs were available to 12.0% of participants. Reported net use last night was 74.6% among those with access to a net and 8.8% overall. Plasmodium prevalence by RDT was 12.0%, with 9.6% Plasmodium falciparum, 1.7% P vivax, and 0.7% mixed infections. Anemia (Hb <13 mg/dl) was detected in 28.3% of migrants. Around one-third (30.3%) reported having fever within the past two weeks, of whom 31.3% sought treatment. Distance to a healthcare facility and cost were important reported barriers to seeking treatment. These results are being utilized to develop interventions tailored to this population.

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COMMUNITY PERCEPTIONS AND PRACTICES TOWARDS MALARIA CONTROL MEASURES IN RWANDA: A DESCRIPTIVE QUALITATIVE STUDY

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Long lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and malaria case treatment with artemisinin based combination therapy have been proved to reduce malaria significantly. Elimination requires consideration of other factors yet underexplored. Our objective was to explore behavioral and environmental predictors associated with malaria transmission, to draw operational recommendations. In December 2013, we conducted nine focus group discussions, in Ruhuha, Southern East of Rwanda with 81 participants. Various cooperatives members, community health workers, health care professionals, lay community, schools teachers, youth representatives, as well as local and religious leaders were included. Discussions were recorded, transcribed in English and coded using NVivo 10. Study protocol was approved by Rwanda National Ethics Committee. Malaria ranked first among the top five diseases in the area. High knowledge on transmission and symptoms was observed. The notion of malaria elimination by the community was acknowledged but challenges noted. A recent outbreak of bedbugs was found hindering the use of LLINs in addition to the hot weather characterizing the dry season. IRS was generally perceived as no longer killing insects including mosquitoes unlike spreads them. Seeking care at the health center as first choice was related with having a community based health insurance (CBHI) while buying medicines in pharmacies was commonly seen among those without a CBHI or who claim spending much time at the health center. A recent wealth categorization has had an impact on the amount to be contributed to get a CBHI. A large number of participants argued that the categorization was unfairly done and leading some families unable to afford the CBHI. In conclusion, joint efforts are needed to tackle issue of bedbugs for proper use of LLINs at household level. Further studies are needed to test the efficacy of IRS and look for appropriate chemicals if needed. Lastly, to uphold high coverage of CBHI, government is suggested to revise the wealth categorization.

EVALUATION OF THE LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION (LAMP) FOR MALARIA DIAGNOSE IN A PLASMODIUM VIVAX ENDEMIC SETTING

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Current malaria diagnostic tests, including microscopy and antigen-detecting rapid tests, cannot reliably detect low-density infections. Molecular methods such as polymerase chain reaction (PCR) are highly sensitive but remain too complex for field deployment. We assessed the field applicability of a new molecular assay for malaria diagnosis based on loop-mediated isothermal amplification (mLAMP) in Plasmodium vivax endemic setting of Colombia. A prospective cohort of patients was used to assess the diagnostic performance of the mLAMP. After signing the informed consent, volunteers were diagnosed using microscopy and LAMP at point of care (POC). To assess the field performance of mLAMP for detection of submicroscopic infections, a cross-sectional survey was conducted in 3 regions of Colombia with different epidemiological profiles. The mLAMP results were interpreted by visual reading under ultra violet light. Real time PCR and nested real time PCR in discordant samples was used as reference. LAMP sensibility and specificity was comparable to real time PCR for both Plasmodium vivax and P. falciparum detection at point of care diagnosis. 278 febrile patients were enrolled at Tierralta, Cordoba, the mLAMP sensitivity of for both P. falciparum and P. vivax was 100% and 90% respectively compared with PCR. All false-negative LAMP results involved samples of P. vivax with parasitaemia levels detectable by 3-well nested real time PCR but very low or undetectable by qPCR. In the cross-sectional, survey 980 volunteers in 10 sentinel sites at 3 different malaria endemic regions of Colombia were enrolled. LAMP detected 14.8 times more cases than microscopy with a prevalence across all sentinel sites of 6.02% (n=59) and 0.4% (n=4) respectively. Plasmodium falciparum infections accounted for 23.9% and P. vivax infections for 76.1%. No cases of mixed infections were identified by PCR. The proportion of asymptomatic infections among mLAMP confirmed cases was 98.0%. Malaria LAMP in a P. vivax low endemic setting achieved sensitivity similar to that of single-well nested PCR in a reference laboratory. LAMP showed detection limits comparable to PCR under minimum infrastructure condition under field conditions and dramatically increase the detection of asymptomatic malaria providing a new tool for diagnosis, surveillance, and screening in elimination strategies.

A NOVEL CROWD-SOURCING TECHNIQUE FOR PREDICTING DENSITIES AND DISTRIBUTION OF DISEASE-TRANSMITTING MOSQUITOES IN RURAL TANZANIA

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Lack of reliable techniques that can be used for large-scale programmatic monitoring of distribution and densities of disease-transmitting mosquitoes is a major challenge to public health authorities, especially in rural and remote communities where high-tech GIS and remote sensing facilities are not readily applicable for regular use. We developed and evaluated a new community-based participatory mapping approach that relies simply on the knowledge and experiences of residents to rapidly identify areas where disease-transmitting mosquitoes are most abundant. The method is proposed for use in spatial targeting of mosquito control interventions. Such simplified methodologies for mapping vector densities will be particularly necessary for optimal placement of new interventions to complement existing ones such as long-lasting insecticide treated nets (LLINs) for malaria control. This new crowd-sourcing technique consisted of 5 steps. We initially mapped three test villages comprehensively identify major land marks (step 1). We then selected 60 community members monthly, taught them basic map-reading and offered them gridded maps of their own villages so they could identify locations where they think mosquitoes are most abundant, by simply ranking the grids on scale of 1-5 (step 3). The data generated was interpolated in ArcGIS using inverse distance weighting method and classified to show places where people thought there were high, medium and low mosquito densities (step 4). Finally, mosquito sampling was done using an effective odor-baited sampling tool, to verify outdoor mosquito densities in locations pre-identified by community members as having high, medium and low mosquito densities, and to validate this crowd-based prediction method. Maps were derived from community knowledge and opinions on the mosquito density distributions. For twelve months in three villages, entomological surveys depicted the same vector densities and distribution pattern as the crowd-sourcing technique. This study thus provides evidence that we can rely on community knowledge and experience to identify suitable areas where mosquitoes are most abundant and where to locate outdoor complementary interventions. Such a method will be cheaper, quicker and easier even for planning and implementing large-scale vector control operations.
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EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL ACTIVITIES BY SIMULTANEOUS NATIONWIDE CROSS-SECTIONAL AND CASE-CONTROL SURVEYS IN MADAGASCAR

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The Malaria Control Program of Madagascar has set since 2007 an objective to pre-eliminate malaria. In order to help decision making to reach pre-elimination, we aimed at evaluating the actual effectiveness of malaria control activities. We conducted a nationwide survey in 2012-2013 in 62 sites representing all transmission patterns. This survey included (1) a cross-sectional study to measure the effectiveness of each control intervention on reducing the transmission, and (2) a concurrent case-control study to measure the effectiveness on reducing the morbidity.

We present here the results related to organized vector control activities, i.e. Long Lasting Insecticidal Nets (LLIN) distribution and Indoor Residual Spraying (IRS) campaigns. The cross-sectional survey included 15,734 individuals among which 3.7% had a positive Rapid Diagnostic Test (RDT). LLIN daily use was 52.3% in areas covered by universal distribution and IRS coverage was 64.8% in targeted areas. 818 uncomplicated clinical malaria cases were compared to 7,767 controls living in the same villages. Multilevel analysis of factors associated with a positive RDT or with the occurrence of an episode of non-complicated malaria revealed that LLIN daily use had a 45% protective effectiveness (PE) against infection (aOR 0.55 [95%CI 0.42, 0.72]) and a 48% PE against morbidity (aOR 0.52 [0.28, 0.96]). The PE of IRS was evaluated to be 23% against infection (aOR 0.77 [0.53, 1.13]) and 49% against morbidity (aOR 0.51 [0.39, 0.66]). In areas where both activities are implemented, coverage of LLIN was 21.3 percentage points lower than in areas where LLIN only were deployed. Combining IRS with LLIN provided almost no gain in preventing infection, but the PE of LLIN use against morbidity increased from 51% (aOR 0.49 [0.20, 1.20]) to 66% (aOR 0.34 [0.16, 0.74]) when IRS was added, although non significantly. Our results show that, taken separately, LLIN and IRS perform satisfactorily but that their concurrent use might have a limited benefit as compared with efforts to improve the coverage of a single intervention.

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IDENTIFICATION OF ASYMPTOMATIC MALARIA INFECTION IN BORDER CROSSING POPULATIONS IN CAMBODIA

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Cross-border population movement can hinder elimination of malaria by importing new infection, maintaining hot-spots of high transmission, and contributing to the development and spread of drug resistance. Cross-border movement is common in Cambodia yet there is limited surveillance at border points. To investigate malaria infection in border-crossing populations, three border crossing points were chosen as study sites, one from each of Cambodia’s borders with Thailand, Vietnam and Laos. From August 2013 to February 2014, 3,206 border crossers were tested for malaria (by RDT and PCR) and fever, and were interviewed regarding a priori risk factors for infection. Univariate and multivariate logistic regression were performed to investigate potential exposure variables against the outcome of malaria infection. Based on RDT analysis there were 103 cases of malaria (67 Pf, 34 Pv and 2 mixed) giving an overall prevalence of 3.21% (PCR prevalence and drug resistance analysis in progress and available April 2014). Prevalence differed between borderer sites (0.09% Thailand, 0.99% Vietnam and 8.04% Laos). Sixty-nine (67%) cases were asymptomatic. Main risk factors for infection determined by multivariate logistic regression were previous episode of malaria (OR 5.52, p<0.0001), forest-goers (OR 5.26, p<0.0001), <15 years old (OR 4.94, p=0.003), presence of fever (OR 4.03, p<0.0001), and working as security/armed forces (OR 3.11, p<0.02). Knowledge of two or more prevention methods gave a protective effect from infection (OR 0.45, p=0.008). The Lao border point had greater prevalence of forest-goers, high-risk occupations, previous malaria episodes, and low knowledge of prevention methods; leading to higher prevalence of malaria infection. Cross-border malaria will hinder elimination where border-crossing populations have high prevalence of risk factors for infection. Lessons learned from this project have set the basis for a comprehensive cross-border surveillance platform in the Greater Mekong Subregion. Similar border points should continue to be identified and targeted surveillance put in place for countries across the GMS where population movement is common.

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CHANGING TRENDS IN MALARIA AMONG MOZAMBIAN PREGNANT WOMEN: EFFECT ON ANTIMALARIAL IMMUNITY AND CLINICAL IMPACT OF PLASMODIUM FALCIPARUM INFECTIONS

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Understanding the determinants and consequences of malaria declines and resurgences as well as the timescales over which antimalarial immunity is gained and lost, has become a priority in the context of current goals of malaria elimination and eradication. Epidemiological changes provide a unique opportunity to investigate how anti-malarial immunity is lost following reductions in transmission and how this might impact disease burdens in potential resurgences. Here we present the results from a study that combines epidemiological and immunological description of malaria among pregnant women delivering between 2003 and 2012 at antenatal clinics in the Manhiça District hospital in Southern Mozambique. Plasmodium falciparum qPCR-positivity decreased from 33% in 2003 to 2% in 2010 (p<0.001) and increased to 6% in 2012 (p=0.026), with antimalarial IgGs mirroring these malaria trends. Parasite densities in peripheral blood and in the placenta were higher in 2010-12 than in 2003-5, whereas prevalence of peripheral infections that were submicroscopic followed an opposite trend. An attenuation of the parity effect on parasite densities and on IgGs against placental-type parasites was observed during 2010-12 compared to 2003-5. Malaria infection was associated with a larger reduction in maternal haemoglobin levels and in the birthweight during 2010-12 compared to 2003-5. These results suggest that a sustained reduction in the exposure to malaria parasites leads to a weakening of immune regulation of parasite densities that can increase the potential for occurrence of high-density infections and malaria-related harmful effects. These findings reinforce the importance of sustaining efforts when moving from control to elimination to avoid rebounds of malaria associated with the reduction in naturally acquired immunity.
PROGRESS TOWARD DEVELOPMENT OF A LOW DENSITY INFECTION-DETECTION TEST TO SUPPORT ACTIVE DETECT-AND-TREAT INTERVENTIONS AIMED AT REGIONAL MALARIA ELIMINATION

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As successful malaria control programs dramatically reduce malaria prevalence, strategic and programmatic changes are required to eliminate malaria transmission altogether. Development of new, game-changing, and possibly disruptive malaria-infection-detection technologies can enable more efficient elimination interventions in the most challenging malaria-endemic environments. For active detect-and-treat interventions, the essential product characteristics required are: low limit-of-detection, rapid turn-around times, ease of use, high sensitivity, portability, and appropriate pricing. PATH’s Diagnostics for Malaria Elimination Toward Eradication (DIAMETER) project is focused on bridging the gap between the rapidly evolving set of detect-and-treat tactics and the existing diagnostic capabilities. We aim to ensure that elimination interventions are not hampered by test performance. The goal of the DIAMETER project is to catalyze rapid access to the most cost-effective and temporally-effective elimination-infection-detection tools in the product development pipeline. Our phase I activities have produced an elimination market analysis, a six-country field investigation of user requirements, and a landscape of technologies in the product development pipeline. Our phase II activities focus on development of infection-detection tests (IDTs) for qualitative detection of low-density Plasmodium falciparum infections. We envision that IDTs will be used by malaria elimination programs to identify and treat subclinical, low-parasite-density populations that serve as reservoirs of the rapidly evolving set of detect-and-treat tactics and the existing diagnostic capabilities. We aim to ensure that elimination interventions are not hampered by test performance. The goal of the DIAMETER project is to catalyze rapid access to the most cost-effective and temporally-effective elimination-infection-detection tools in the product development pipeline. Our phase I activities have produced an elimination market analysis, a six-country field investigation of user requirements, and a landscape of technologies in the product development pipeline. Our phase II activities focus on development of infection-detection tests (IDTs) for qualitative detection of low-density Plasmodium falciparum infections. We envision that IDTs will be used by malaria elimination programs to identify and treat subclinical, low-parasite-density populations that serve as reservoirs of parasite biomass. We will present our phase I findings, the process used to develop the IDT target product profile, and the landscape of promising platform technologies that can be leveraged to achieve the required performance characteristics.

G6PD DEFICIENCY IN LATIN AMERICA: PREVALENCE, VARIANTS AND IMPLICATIONS FOR MALARIA ELIMINATION

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Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is the most prevalent human genetic disease worldwide. Predominance of Plasmodium vivax malaria in Latin America (LA) is especially relevant, since P. vivax radical cure requires the use of primaquine (PQ). It is important to consider that PQ may cause severe drug-induced hemolysis and may lead to acute intravascular haemolysis in G6PDd individuals. This could further hamper malaria control efforts. Despite clinical and epidemiological significance of G6PDd and malaria interaction, G6PDd prevalence has not been well measured in LA populations. A systematic review using existing bibliographic and biomedical databases was made to better understand the distribution of G6PDd in Latin America and the Caribbean. The review resulted in low prevalence rates in Argentina, Bolivia, Mexico, Peru and Uruguay. Studies performed in Curaçao, Ecuador, Jamaica, Saint Lucia, Suriname and Trinidad, as well as some surveys carried out in some areas of Brazil, Colombia and Cuba showed high prevalence (>10%) of G6PDd. G6PD encoded by the G6PD A202V mutation was the most broadly distributed across LA, being identified in 81.1% of deficient individuals surveyed. It was observed a virtual absence of G6PDd among Amerindians, suggesting that PQ use is safe in this population. In order to avoid unnecessary exposure to hemolysis inducing drugs, as PQ, it is crucial to develop a rapid and accurate G6PDd diagnostic test to be used in LA field conditions. Issues related to the radical cure of P. vivax infection and an eventual elimination of malaria in LA, such as massive drug treatment, must be linked to information about the genetic background of the population. Although considering the inexistence of multicenter studies with similar methodologies, it is possible to estimate roughly that G6PD deficiency in LA is a recognized phenomenon, however with the evidence that some populations are probably not affected, such as the indigenous.

QUALITY OF ARTEMISININ COMBINATION THERAPIES IN SUB SAHARIAN AFRICA AND CAMBODIA, ASSESSED USING LABORATORY ANALYTICAL TECHNIQUES

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Artemisinin combination therapies (ACTs) are recommended as first line treatment for malaria by the World Health Organisation (WHO) and officially implemented in 79 of 88 countries. Reports of ad hoc surveys from SE Asia showed that up to 50% of the artesunate monotheroy sold was falsified. Alarmingly the first case of falsified artesunate (that almost resulted in a fatality) and ACTs were also reported from Africa where the predominant parasite species is the potentially fatal Plasmodium falciparum. Resource poor countries do not have the technical (medicines control laboratories), financial, or human resources required to inspect and police the drug supply. The lack of reliable estimates of poor quality drug prevalence and its causes makes it difficult for national regulatory authorities to determine the need and scale of interventions to assure drug quality. We purchased and carried out qualitative and quantitative content analyses on over 10,500 ACTs in total from Cambodia, Ghana (Kintampo), Tanzania, Nigeria (Enugu and Ilorin) and Equatorial Guinea (Bioko Island) following varying sample collections methods to provide effective surveillance of ACTs available in a given geographical region. The percentage active pharmaceutical ingredient (% API), determined by laboratory chromatographic analysis, was used to classify the medicines as good quality (85% - 115%), as recommended by the United States Pharmacopeia for the analysis of single tablet samples. Poor quality samples included substandard samples (115% of both APIs), degraded samples (improper storage and/or transport) and falsified samples (absence of stated APIs). Most of the samples contained the stated APIs, with the exception of samples from Enugu (1% out of 2865), Ilorin (0.8% out of 1449) and Bioko (7.3% out of 683) which did not contained the stated APIs. Of concern are the monotherapy tablets on sale in countries with falsified ACTs. The results were disseminated to the country-specific ministry of health, as well as relevant manufacturers and logged on the WHO Rapid Alert System for the surveillance and monitoring of Substandard/Spurious/Falsely Labelled/ Falsified Counterfeit, SSFC, medicinal products.

SURVEILLANCE SYSTEMS TO FACILITATE MALARIA ELIMINATION

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Robust and responsive surveillance systems are critical for the success of malaria control and elimination. In elimination settings surveillance must be an intervention, where immediate action is taken in response to case
THE DIMINISHING RETURNS OF ATOVAQUONE-PROGUANIL FOR ELIMINATION OF PLASMODIUM FALCIPARUM MALARIA: MODELLING MASS DRUG ADMINISTRATION AND TREATMENT

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ARTEMISININ resistance is a major threat to current efforts to eliminate Plasmodium falciparum malaria which rely heavily on the continued efficacy of artemisinin combination therapies (ACT). It has been suggested ACTs should not be used in mass drug administration (MDA) in areas where artemisinin resistant P. falciparum is prevalent and that atovaquone-proguanil (A-P) might be a preferable alternative. However, a single point mutation in the cytochrome b gene confers high level resistance to atovaquone, which arises frequently during treatment and this would affect the potential efficacy of atovaquone-proguanil as a tool for elimination. A deterministic population level mathematical model was developed based on data from Cambodia to explore the possible effects of large-scale use of A-P compared to ACT for mass drug administration and/or treatment of P. falciparum malaria, with and without adjunctive primaquine (PQ), with the aim of local elimination. The model showed the initial efficacy of ACT and A-P for MDA to be similar. However, each round of A-P MDA resulted in rapid acquisition and spread of atovaquone resistance. Even a single round of MDA could compromise its efficacy sufficient to preclude its use for treatment or prophylaxis. A switch to A-P for treatment of symptomatic episodes resulted in a complete loss of efficacy in the population within 4-5 years of its introduction. For malaria elimination, A-P for MDA or treatment of symptomatic cases should be avoided. A combined strategy of ACT+PQ MDA, long-lasting insecticide treated bed nets and high coverage with ACT for treatment of symptomatic episodes would be preferable.
effective in any region, likely due to reservoirs of infection in older age-groups. Strengthening public facilities was ineffectual in contexts with strong private sector preference. The model outcomes demonstrate optimal packages of interventions at a national level are not always ideal interventions at a regional level. Improving the effectiveness of ACT delivery is highly dependent on local epidemiology, existing health systems provision and population preferences for either primary health facilities or private drug shops. Context-specific planning and consideration of local priorities e.g. reducing ACT wastage on NMFI versus reducing transmission, may improve progress towards international targets to decrease clinical disease and mortality, and in low transmission settings, to approach elimination.

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CONSEQUENCES OF THE TIMING OF GESTATIONAL ANEMIA ON NEWBORN’S HEMOGLOBIN CONCENTRATION IN A MALARIA ENDEMIC AREA

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Because of the high prevalence of gestational anaemia (GA) in developing countries, anaemia-related adverse effects on birth outcomes are expected to have an important public health impact. We studied the relationship between GA and newborn’s anaemia, taking into account the timing of GA within the MiPPAD trial of intermittent preventive treatment in pregnancy (IPTp) (http://clinicaltrials.gov/ct2/show/NCT00811421). Study design Prospective cohort of pregnant women followed up from early pregnancy until the time of delivery. Study site, population and procedures The study was conducted in the district of Allada (southern Benin) where malaria is perennial. The study population was composed of HIV negative women less than 28 weeks of gestational age and their newborns. Socio-demographic data were collected on inclusion (before first IPTP administration, IPTP1), clinical and biological data at each visit (IPTP1, second IPTP administration -IPTP2- and delivery). Children’s clinical and biological data were collected at birth. Statistical analysis Logistic and linear regressions were performed to evaluate at each time point the relation between maternal and newborns’ Hb levels. Direct and indirect associations between Hb levels in pregnancy and at birth, mediated by Hb levels at different times of pregnancy, were then tested using a path analysis. Newborn anaemia was common (63.5%) in the 862 analyzed mother-newborn pairs. Linear regression showed a significant association between maternal and newborns’ Hb levels at delivery only (P = 0.004). Path analyses confirmed the strong direct association between maternal Hb at delivery and newborns’ Hb, and also found an indirect effect of maternal Hb at IPT1 and IPT2 (P = 0.006 and P = 0.005). In conclusion, the strong association between mothers’ and newborns’ Hb status at delivery shows that late GA has important consequences on the baby. The indirect effect of Hb levels at earlier stages of pregnancy suggests that preventive measures such as IPTp and micronutrient supplemenations also play a role to lower the consequences of GA and should be reinforced.

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SPACETIME MAPPING OF MALARIA AND ITS CO-DISTRIBUTION WITH STUNTING AMONG CHILDREN BELOW FIVE YEARS IN SOMALIA USING BAYESIAN GEO-STATISTICAL APPROACH

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Several studies in developing countries have shown that malaria is associated with greater malnutrition morbidity and mortality and that there is a transient weight loss in young African children following a malaria attack. The majority of studies that have investigated the association of malaria and malnutrition were done in clinical settings and there are limited population-wide investigation of the co-epidemiology of malaria and malnutrition. In 2011, the rate of malnutrition in Somalia was cited to be the highest worldwide. Plasmodium falciparum prevalence in Somalia also varies from very low to moderate transmission. This study sought to map the distribution of malaria and stunting among children under the age of five years at similar spatial and temporal resolutions and determine their co-distribution using a large household cross-sectional survey data undertaken bi-annually from 2007 to 2010 in Somalia. We developed a Bayesian hierarchical space-time model through stochastic partial differential equation (SPDE) approach using R-INLA library to produce risk maps of malaria and stunting at 1 x 1 km spatial resolution and predict to each year of study from 2007 to 2010. To determine co-distribution of the two health issues, we developed a shared component model over space and time in a geo-statistical framework. We observed that the prevalence of malaria was high in the Central South zone with a prevalence of 30% followed by the Puntland, 15% and in Somaliland with less than 5%. This same pattern was observed in the rate of stunting where Central South zone had a mean rate of 30% followed by Puntland, 20% and lowest in Somaliland with mean rate of 18%. Results showed that the shared component distribution (representing social economic and environmental determinants) had a larger effect on malaria and malnutrition in the southern and central part of Somalia. Multivariate mapping models provide a better understanding of co-morbidity between health outcomes. In particular, the analyst of multiple disease outcomes can assess the underlying common and divergent spatial distributions of the diseases to optimally integrate disease management required to address the multiple burden of diseases.

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HOT SPOT OR NOT: A COMPARISON OF SPATIAL STATISTICAL METHODS TO PREDICT PROSPECTIVE MALARIA INFECTIONS


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Within affected communities, Plasmodium falciparum infections may be skewed in distribution such that single or small clusters of households consistently harbour a disproportionate number of infected individuals throughout the year. Identifying these hotspots of malaria transmission would permit targeting of interventions and a more rapid reduction in malaria burden across the whole community. This study set out to compare different statistical methods of hotspot detection using different indicators for prediction of infection the following year. Two full surveys of four villages in Tanzania were completed over consecutive years, 2010-2011. In both surveys, infection was assessed using nested polymerase chain reaction. In addition in 2010, serologic markers of exposure were assessed. Baseline clustering of infection and serological markers were assessed using three geospatial methods: spatial scan statistics, kernel analysis and weighted local prevalence analysis. Methods were compared in their ability to predict infection in the second year of the study using random effects logistic regression models, and comparisons of the area under the receiver operating curve (AUC) for each model. Sensitivity analysis was conducted to explore the effect of varying radius size for the kernel and weighted local prevalence methods and maximum population size for the spatial scan statistic. Guided by AUC values, the kernel method and spatial scan statistics appeared to be more predictive of infection in the following year. Hotspots of PCR-detected infection and seropositivity to AMA-1 were predictive of subsequent infection. For the kernel method, a 1km window was optimal. Similarly, allowing hotspots to contain up to 50% of the
population was a better predictor of infection in the second year using spatial scan statistics than smaller maximum population sizes. Clusters of AMA-1 seroprevalence or parasite prevalence that are predictive of infection a year later can be identified using geospatial models. Kernel smoothing using a 1 km window and spatial scan statistics both provided accurate prediction of future infection.

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FINE SCALE PARTICIPATORY MAPPING OF MALARIA INFECTION CLUSTERS BY RELATING ROUTINE DIAGNOSTIC RESULTS OF PATIENTS ATTENDING HEALTH FACILITIES TO THE NAMES OF THEIR LOCAL LEADERS IN URBAN DAR ES SALAAM, UNITED REPUBLIC OF TANZANIA

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Standard methods for assessing malaria infection burden such as cross-sectional and incidence cohort surveys are limited by sample size in terms of the sensitivity and the spatial resolution they can achieve, especially as transmission declines. This study investigated whether diagnostic data collected routinely at health facilities can provide an affordable alternative for identifying malaria transmission hot spots in urban Dar es Salaam, Tanzania. The study was performed in two adjacent wards of Buguruni and Vingunguti whose total population is 178,000. Anonymised, routinely collected clinical and test result data for patients tested for malaria were collected from June 2012 to January 2013 from a laboratory registry book at Buguruni Health Centre, located close to the boundary between the two wards. Additionally, patients were asked to provide the name of their local Ten Cell Unit Leader, the elected local representative responsible for residential housing clusters. Geographic coordinates of these local leaders’ houses were mapped with GPS receivers so that patients’ residential locations could be traced and mapped in the absence of further data or a residential address system. Geographic information systems and spatial scan statistics were deployed to detect clustering of malaria cases. Among 2,407 patients diagnosed for malaria, 1,941 (80.6%) provided the name of their respective local leader, 1,121 (57.7%) could successfully be traced to their residential location. Only 240 patients (21.4%) tested positive for malaria. Six geographic clusters of high malaria infections (hot spots) and four clusters of low malaria risk (cold spots) (p<0.05) were identified. Cluster radii for hot spots and cold spots varied from 0 to 276 meters, and 160 to 477 meters respectively. Participatory mapping by recording simple points of reference that community member can readily relate to (e.g. names of local leaders) during routine health facility visits can be used to map hot and cold spots of malaria infections on fine geographic scales as an affordable alternative.

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MALARIA CASE-FINDING AND TREATMENT STRATEGIES IN AN INTERNALLY DISPLACED PERSONS (IDP) CAMP IN THE DEMOCRATIC REPUBLIC OF CONGO

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Social upheaval and chronic human insecurity related to an internecine civil and international war in the Democratic Republic of Congo (DRC) have led to a 70% increase in mortality relative to pre-war levels, and large-scale population displacement. The principal causes of mortality are preventable and treatable infectious diseases such as malaria, and internally displaced persons (IDPs) in temporary shelters may be at elevated risk. Accurate case finding and treatment of malaria is a cornerstone of malaria control efforts; therefore, we explored several possible strategies for active case-finding and treatment in an IDP camp at Mugunga, in Eastern DRC. Beginning with a consecutive sample of 100 febrile patients under 5 years of age from the IDP camp presenting to a nearby clinic for management of a fever episode, we detected 19 cases of uncomplicated malaria diagnosed using HRP2-based rapid diagnostic test, who were then treated with artemisinin combination therapy (ACT). We engaged community health workers in the IDP camp to screen the household members of all 100 children for malaria. The median household size was 5 (range 3 to 9) and there was a median of 1 (range 1 to 2) children under 5 years of age per household, representing a total of 377 screened individuals. We detected 29 cases of malaria through this active case-finding procedure who had not presented for medical treatment, who were then treated with ACT. Fourteen cases were asymptomatic, while 1 participant reported fever, 6 headache, and 8 myalgia. Based on this data, we examined several hypothetical strategies for anti-malarial drug administration in this cohort including: [1] mass drug administration without screening; [2] screen all IDPs with RDT and treat positive cases; [3] screen selectively based on symptoms, then treat positive cases; [4] screen selectively based on household contacts of a child with uncomplicated malaria, then treat positive cases. Under these 4 strategies, the number of missed cases, unnecessary treatments, and cost per case detected and treated were: [1] 0, 348 (92%), $46; [2] 0, 0, $23; [3] 14 (49%), 0, $7; [4] 24 (83%), 0, $25. Although imperfect because nearly half of cases remain undetected, population-level symptom-based screening and treatment appears to be a low-cost strategy to detect and treat cases that might not otherwise present to local health clinics.

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MALARIA REPORTING THROUGH ELECTRONIC INTEGRATED DISEASES SURVEILLANCE AND RESPONSE (EIDSR) IN KAGERA REGION OF TANZANIA

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Malaria surveillance is an important component of malaria control program. Currently, malaria and other disease data are collected through a paper based system that faces challenges of delayed reporting and poor data quality. We describe the results of weekly reporting of health facility based malaria surveillance data through electronic Integrated Disease Surveillance and Response (IDSR) in the Kagera region. The Tanzania Ministry of Health and Social Welfare (MoHSW) and the Department of Computer Science - University of Dar es Salaam (UDSM) designed a system of data transfer through the use of mobile phone technology. A five day training of health facilities was undertaken in March 2013 on tallying of data from outpatient registers, recording of data on weekly reporting booklets, and electronic transmission of data. The key malaria indicators collected were: number of people tested for malaria; number tested positive; and number treated presumptively for malaria (clinical malaria cases). Malaria data were transmitted to the District Health Information System (DHIS2) on a weekly basis which enabled establishment of malaria early epidemic detection system (MEEDS), preparedness and response to unusual increase in malaria cases. A total of 289 health facilities in 8 districts of Kagera region participated in the training. In the first 10 weeks of implementation (week 12 to 20 of 2014), 87.6% of all expected reports were transmitted on time (by Monday 3pm). Over the same period, a total of 201,431 suspected malaria cases were reported of whom 184,191 (91.4%) were tested for malaria while 17,240 (8.6%) were clinical malaria cases. Malaria
positivity among those tested was 46.4%. Even at the very early stages of the system implementation, electronic disease surveillance has proven advantageous over the former paper based system. This data show an improvement in completeness and timeliness of reporting malaria illnesses, allowing the National Malaria Control Programme (NMCP) to better tailor its programmatic activities and potentially detect and respond to outbreaks in a more timely manner.

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GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM FROM WESTERN KENYA HIGHLAND AREAS PRONE MALARIA EPIDEMICS
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Transmission of Plasmodium falciparum malaria in the east African highlands is unstable and frequently epidemic, and recent analyses of globally sampled P. falciparum showed high levels of genetic diversity positively associated with variation in transmission intensity. In highland areas of unstable malaria transmission, parasite genotyping can provide valuable information on genotype-specific immunity, drug efficacy in the population, and potential etiologies of increased incidence. P. falciparum genotyping was conducted on filter paper blood samples from individuals with clinical malaria in two adjoining highland areas in western Kenya. Genotyping was performed at the antigen locus merozoite surface protein-2 (MSP-2), and on a panel of 12 microsatellite loci. At both sites, more than 70% of individuals had infection with >1 parasite genotype. Multiplicity of infection (MOI) was detected at similar frequencies by MSP-2 and microsatellite genotyping. However, microsatellite genotyping detected significantly greater parasite diversity than MSP-2 genotyping, indicating that microsatellite genotypes are more useful for interrogating parasite population diversity and structure. Neither MOI nor genetic diversity differed by age. All individuals with repeated symptomatic infections had infection with a new genotype rather than recrudescence, implying that treatments given to these individuals in an earlier episode of infection were effective and/or there was active immunity against earlier infections. There was also no difference in these metrics between the two areas, indicating a common P. falciparum life history and potentially the effectiveness of similar control measures.

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MOLECULAR MARKER TRENDS IN PLASMODIUM FALCIPARUM DIHYDROFOLATE REDUCTASE AND PLASMODIUM FALCIPARUM DIHYDROPROTOERATE SYNTHASE GENES IN KENYAN ISOLATES BETWEEN THE YEARS 2008 TO 2012
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Sulfadoxine-pyrimethamine (SP), an antifolate was replaced by artemether-lumefantrine as the first-line malaria drug treatment in Kenya in 2004 due of the wide spread of resistance. However, SP still remains the recommended drug for intermittent preventive treatment in pregnant women and infants (IPTp/P) owing to its safety profile. This study assessed the prevalence of mutations in dihydrofolate reductase (Pfdhfr) and dihydroprotoerotate synthase (Pfdhps) genes associated with SP resistance in samples collected in Kenya between 2008 and 2012. Field isolates collected from Kisumu, Kisii, Kericho and Malindi district hospitals were assessed for genetic polymorphism at various loci within the two genes by sequencing. Among the Pfdhfr mutations, codons N511, C59R, S108N showed highest prevalence in all the sites combined at 95.5%, 84.1% and 98.6% respectively. Pfdhfr S108N prevalence was highest in Kisii at 100%. A temporal trend analysis showed steady prevalence of mutations over time except for codon Pfdhps S581 which showed an increase in mixed genotypes. Triple Pfdhfr N511/C59R/S108N and double Pfdhps A437G/K540E had high prevalence rates of 86.56% and 87.86% respectively.

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ASSOCIATION BETWEEN LENGTH OF NIGHT TIME EXPOSURE AND PLASMODIUM FALCIPARUM MALARIA INFECTION RISK IN DAR ES SALAAM, TANZANIA
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Little is known about the role of time as a risk factor in areas that have various forms of malaria interventions. Socioeconomic demands, human behaviours and extended periods of outdoor activities are major contributing factors for malaria transmission in areas where other interventions like long lasting insecticidal nets (LLINs) are widely used. We investigated the associations between sleeping time and Plasmodium falciparum parasite prevalence (PfP) among residents in Dar es Salaam, Tanzania. From March 2009 to May 2012 a cross-sectional study was conducted among individuals aged 3-months to 98 years. A total of 4000 people were tested for malaria parasitemia by using rapid diagnostic test (RDT). Individual level information on length of time a person was potentially exposed to mosquitoes after 6:00pm (i.e. time before going inside and under a bed net), demography, house quality and bed-net use were also collected. Hierarchical model was used to assess malaria infection and identify time, social economic status (SES) and net use as risk factors. A total of 411 people (10%) surveyed tested positive for malaria parasitemia. People with outdoor exposure of 5 hours had the highest parasitemia prevalence of (Pfp=14.4%) followed by children of 0-5 years (Pfp=13%). People with the longest periods of time spent indoors (>5 hours) had the lowest prevalence (Pfp=6%). At least 76% of people surveyed slept under bed-net. Multivariate analysis showed a strong evidence that people spending 5 hours outdoors had higher infection prevalence than people spending less time outdoors (OR [95%CI]=2.32 [1.62-3.33] P<0.001). Interestingly, this study also determined that people who owned bed-nets were more likely to have malaria, compared to those without (OR=2.60 [1.76 - 3.84], P=0.04). In conclusion, the findings indicate an increased risk of malaria transmission for the part of population that spends most of the evening time outdoors in Dar Es Salaam because of either behaviours or socioeconomic activities. It is therefore important to plan interventions that can offer protection to this part of the population.

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INTERNALLY DISPLACED PERSONS (IDPS) ARE AT INCREASED RISK OF MALARIA RELATIVE TO NEIGHBORING VILLAGE CONTROLS
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Globally, 26 million internally displaced persons (IDPs) have been forced to flee their homes as a result of armed conflict (yet remain within their country of nationality). Civil and international war in the Democratic Republic of Congo (DRC) has generated tens of thousands of IDPs residing within temporary housing camps, at high risk of poor mental and physical health. Malaria is the chief cause of death among children in the area, but the burden of malaria has not been well described among children within IDP camps in the DRC. We conducted 2 surveys inWalikale district in Eastern DRC to compare the point-prevalence of P. falciparum antigenemia among (1) children residing in an IDP camp versus children from the surrounding village, and (2) febrile children presenting for medical care at a health clinic serving both the IDP camp and surrounding village. For the first community-based survey, a random sample of 200 temporary shelters
MALARIA PREVENTION IN VULNERABLE GROUPS NIGERIA, 2011-2012

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Malaria prevention is vital to its control. At least 80% of children less than 5 years (U5) and pregnant women (PW) sleep under long lasting insecticidal nets (LLIN) while all PW attending ante-natal clinics should receive at least two doses of Sulphadoxine-pyrimethamine for Intermittent Preventive Treatment (IPT). Even though there is gradual attempt to scale-up access to these interventions, little information is available about their current use. We analysed access to LLINs and IPT in Nigeria using secondary data. We collected national summary data on malaria preventive services from January 2011 to December 2012. Data on uptake of LLINs in U5 and PW, and uptake of IPT in PW from all reporting health facilities (HF) were extracted. Analysis was done by state using Microsoft Excel. In 2011, of the 2,741,485 PW who attended ANC in 96,418 HFs, 622,317 (22.7%) and 649,732 (23.7%) received LLIN and IPT respectively. In 2012, of the 3,392,363 PW who attended ANC in the 114,667 HFs, (9%) and (16.6%) received LLIN and IPT respectively. In 2011 and 2012 199289 (3.2%) of the 6,220,672 and 301896 (3.2%) of the 9,521,932 US seen received LLINs respectively. Despite increase in ANC attendance and reporting HF, access to LLIN and IPT remains low. In Nigeria, universal access to malaria preventive commodities and services has not yet been achieved in high risk groups. High antenatal clinic (ANC) attendance alone is not sufficient to ensure high IPT and LLIN coverage as the Roll Back Malaria target of 80% was not achieved. SP for IPT should always be made available at all HFs and health workers should be trained on the need to increase IPT coverage.

TEMPORAL ASSOCIATION BETWEEN FISHING ACTIVITIES AND MOSQUITO ACTIVITY PATTERNS IN RUSINGA ISLAND, WESTERN KENYA

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Fishing is a major source of livelihood for residents in Rusinga Island on the shores of Lake Victoria, western Kenya. This activity is conducted outdoors during the day but predominantly at night. Malaria is endemic in western Kenya and areas around the lake shore are associated with mosquito breeding habitats. The main objective of this study was to determine the association between fishing activities and mosquito activity patterns in a fishing beach in western Kenya. A longitudinal entomological survey on a fishing beach was conducted for a period of 5 months. Kolunga fishing beach was selected for this study as fishermen registered at this beach conduct fishing activities at night. Mosquitoes were captured using battery-powered MMX traps baited with a potent mosquito attractant. The traps were positioned approximately 10 metres inland from the shoreline. Hourly mosquito collections were conducted from 6pm till 7 am the next day. The number of people at the beach who conducted fishing activities was recorded at hourly intervals. A total of 1279 mosquitoes were collected, of which 93 were malaria vectors. Of the 93 malaria vectors collected, 28 (33.7%) were Anopheles gambiae s.l. while 65 (66.3%) were An. funestus. The peak mosquito biting activity for An. gambiae s.l. was 9 - 10pm and 3 - 4am while An. funestus was recorded as 9 - 10pm and 4 - 5am. The peaks in mosquito biting times coincided with fishermen’s activities at the fishing beach. In conclusion, individuals conducting fishing-related activities at the beach during the peak biting times are at high risk of receiving infectious mosquito bites.

MALARIA PREVALENCE, SPATIAL CLUSTERING AND RISK FACTORS IN A LOW ENDEMIC AREA OF EASTERN RWANDA: A CROSS SECTIONAL STUDY

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Rwanda reported significant reductions in malaria burden following scale up of control intervention from 2005 to 2010. This study sought to; measure malaria prevalence, describe spatial malaria clustering and investigate for malaria risk factors among health-centre-presumed malaria cases and their household members in Eastern Rwanda. A two-stage health centre and household-based survey was conducted in Ruhiha sector, Eastern Rwanda from April to October 2011. At the health centre, data, including malaria diagnosis and individual level malaria risk factors, was collected. At households of these Index cases, a follow-up survey, including malaria screening for all household members and collecting household level malaria risk factor data, was conducted. Malaria prevalence among health centre attendees was 22.8%. At the household level, 90 households (out of 520) had at least one malaria-infected member and the overall malaria prevalence for the 2634 household members screened was 5.1%. Among health centre attendees, the age group 5–15 years was significantly associated with an increased malaria risk and a reported ownership of ≥4 bednets was significantly associated with a reduced malaria risk. At the household level, age groups 5–15 and >15 years and being associated with a malaria positive index case were associated with an increased malaria risk, while an observed ownership of ≥4 bednets was associated with a malaria risk-protective effect. Significant spatial malaria clustering among household cases with clusters located close to water-based agro-ecosystems was observed. In conclusion, malaria prevalence was significantly higher among health centre attendees and their household members in an area with significant household spatial malaria clustering. Circle surveillance involving passive case finding at health centers and proactive case detection in households can be a powerful tool for identifying household level malaria burden, risk factors and clustering.
The effectiveness of bednet (LLIN) programs in Senegal is threatened by resistance to insecticides. The aim of this study was to determine the effectiveness of LLIN use by the individual and in the local community in reducing the incidence of malaria, in central Senegal. A matched case control study was conducted in rural parts of the district of Fatick, central Senegal, in an area covered by a demographic surveillance system (DSS). All confirmed cases of uncomplicated malaria presenting at health posts in the district between October 2011 and February 2012 were included in the study. For each case, one or two controls of the same age group, selected by random sampling from the DSS listing for the district, were recruited concurrently if a malaria rapid diagnostic test was negative. For cases and controls, and members of their households and of surrounding households, bednet use and approximate age was recorded after inspecting sleeping places. Information about bednet use was also obtained for all households in the district when visited in demographic surveillance rounds from January to December 2011. Conditional logistic regression was used to estimate the rate ratio associated with use of LLIN, and with the level of local coverage of LLINs in the community grouped by quintiles, adjusted for potential confounding factors including distance to health facility, socio-economic status, and distance to mosquito breeding sites. 540 malaria case-control pairs were recruited. Sleeping under an LLIN, and local coverage of LLINs, were independently associated with reduced malaria incidence. Malaria incidence for individuals in communities with coverage in the upper quintile (>81% of persons sleep under an LLIN) was 64% (95%CI 18%, 84%) lower than in communities in the lowest quintile (<50% LLIN coverage). In conclusion, LLINs remain effective in central Senegal but high coverage is required to maximize impact. LLIN distribution programs should target communities with low coverage.

Two separate hierarchical Bayesian models, using a flexible Gaussian process prior, were fitted to the matched falciparum and vivax data. Temporal variation was modelled by using a Poisson-Gamma mixture. The fitted relationships, along with appropriate uncertainty metrics, allows for estimates of clinical incidence of known confidence to be made from wherever P. falciparum or P. vivax prevalence data are available. This will allow for updated estimates of P. falciparum burden as well as novel measures of the global burden of P. vivax.

The knowledge, attitude and practice (KAP) towards malaria infection in the lowlands of the tropical Americas.

Quantifying of the number of malaria cases that occur annually in endemic countries is essential to national control program development and evaluation. Clinical incidence of malaria has proven to be difficult to enumerate. Strategies that adjust routine case reports or model incidence values using prevalence surveys have been applied to estimates of malaria as a whole and Plasmodium falciparum-specific incidence, but have yet to be developed for Plasmodium vivax. Infection prevalence, or parasite rate, is one of the most widely available metrics of malaria endemicity and is collected using simple methodologies. To help inform the number of clinical infections of P. falciparum and P. vivax per year, it is therefore useful to quantify the relationship between prevalence and clinical incidence for each parasite. Here we present updated methods to estimate P. falciparum incidence and a new equivalent model for P. vivax, developed to accommodate the unique epidemiological features of the parasite. A systematic search for active case detection surveys was performed. Recorded incidence values were then matched to measures parasite rates.
THE IMPACT OF URBANIZATION CLASSIFICATION AND POPULATION DENSITY ON CHILDHOOD PLASMODIUM FALCIPARUM PARASITE PREVALENCE RATES IN AFRICA

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Residents of urban areas have been shown to have lower rates of malaria infection compared to rural neighbors. There are, however, many challenges in defining urbanization. The transition from a rural settlement to one best described as urban does not follow a definite boundary. Defining the gradient between rural and urban settlements is an important factor in defining malaria prevalence patterns. In this study, we used boosted regression tree models to determine using whether (i) urbanization had a significant effect on malaria transmission and if this effect varied with urbanization definition used (ii) if population density had significant effect on malaria parasite prevalence and if so, could population density replace urban classifications in modeling malaria transmission patterns. The study focuses on 21 household surveys from the Demographic & Health Surveys (DHS) and Malaria Indicator Survey (MIS) conducted between 2006 and 2013 in 14 malaria endemic countries across Africa. Although analysis is expected to be complete by June 2014, preliminary results show that the BRT model with population density was found to perform significantly better (p-values <0.001) in predicting malaria risk when we compared the model's predictive performance to the other models with urbanization classification. However, adding urbanization to the BRT model with population density produced a better performing model implying there are aspects of the influence of urbanization on malaria prevalence that cannot be solely explained by population density or population distribution patterns.

THE EFFECTS OF MALARIA IN THE FIRST TRIMESTER MEASURED BY REPEATED FETAL CROWN RUMP LENGTH: AN OBSERVATIONAL STUDY

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Malaria in pregnancy causes low birth weight although the mechanism of this adverse effect is not characterized. At Shoklo Malaria Research Unit on the Thai-Myanmar border we have observed reduced fetal head diameter when women had malaria from < 14 to < 24 weeks of pregnancy. This investigation aims to determine if there is a reduction in growth with malaria infection in the first trimester (7-14 weeks) by using ultrasound confirmed fetal crown rump length measurement. Shoklo Malaria Research Unit on the Thai-Myanmar border has offered fetal biometry to all pregnant women since 2001 at their first antenatal visit to determine viability and gestation and the scan is repeated if necessary for ongoing care. The training manual and protocol for trans-abdominal CRL were from the British Medical Ultrasound society recommendations. Observed versus the expected increase in CRL measurements will be compared for women with malaria, women with fever from other causes and for women without malaria. Data collection is still in process but repeated CRL measurements are currently available for 1300 women of whom more than 100 have had malaria in the window of two CRL measurements. The final results will be available in May 2014.

AN ELITE INFECTION-CONTROL PHENOTYPE WITH IMMUNOLOGICAL CORRELATES IN A TANZANIAN BIRTH-COHORT EXPOSED TO INTENSE MALARIA TRANSMISSION

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Malaria incidence is highly heterogeneous even in hyperendemic areas, although no one has shown that innate or naturally acquired resistance can completely prevent infection. We examined immunoparasitological evidence for an elite infection-control phenotype from a birth-cohort followed 2002-2006 in Muheza, Tanzania, an area of intense transmission. Children (n=688) provided blood smears every 2 weeks during infancy and
ACTIVE CASE DETECTION IN MALARIA ELIMINATION SETTINGS: TIMELINESS AND COMPLETENESS OF MALARIA CASE NOTIFICATION IN ZANZIBAR

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Zanzibar Malaria Elimination Programme (ZAMEP) introduced malaria early epidemic detection system (MEEDS) in 2008 and malaria case notification (MCN), an active malaria surveillance system, in August 2012. MCN is an active surveillance system whereby individual malaria cases are transmitted in real time through the use of mobile phones and android tablets to a central database. We evaluated the timeliness of case notification after diagnosis and timeliness of case follow-up after notification. For MCN reporting, a massage for each case is sent from Health Facility to the server and the server generates an alert to the District Malaria Surveillance Officer's (DMSO) mobile phone and tablet. DMSO follows up notified cases to their households. All case-household members are tested for malaria using rapid diagnostic tests (mRDT). People with positive malaria test results are treated with artesine-based combination therapy (ACT). A total of 2,603 index malaria cases were notified in 2013 of which 75.8% were followed-up. Of the 1,974 cases followed-up notification after diagnosis was done within 24 hours in 50.6%, between 24 to 72 hours in 25.8%, while 23.8% of cases were notified after 72 hours. The median time from diagnosis to case notification was 1.2 days (interquartile range [IQR]=0.5 to 2.6). Household follow-up was undertaken within 48 hours in 54.2%, between 48 hours and 1 week in 21.5%, while 23.4% of cases were followed after more than 1 week. The median time to household follow-up was 3 days (IQR=1 to 8). A total of 1,812 (70% of those notified) had the household follow-up completed. Active case detection and treatment alongside other preventative interventions is likely to reduce malaria transmission and enhance malaria elimination efforts in Zanzibar. Following successful implementation of malaria case notification, ZAMEP has set performance standards for malaria case notification and case follow-up with a target of 90% of cases to be notified with 24 hours of diagnosis, 100% follow-up of all notified cases, and 90% of cases to be followed up within 48 hours of notification. Therefore more resources will be required to enable Zanzibar achieve these standards in order to make MCN more effective.

COMPARISON OF PFHRP2-BASED RDTs AND PCR IN AN AREA OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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Histidine-rich protein-2 (HRP-2)-based malaria rapid diagnostic tests (RDTs) are valuable in detecting Plasmodium falciparum infection; however, various factors can affect test validity. The test misses low level parasitaemia and will not detect non-Plasmodium falciparum species. More sensitive and species-specific tools such as PCR will become necessary to identify parasite reservoirs in elimination settings. To address this, HRP-2-based RDTs, pan Plasmodium species nested PCR and species-specific q-PCR were compared over a four-year period in a region of declining malaria transmission in southern Zambia. Cross-sectional surveys were conducted in Choma District, Zambia from 2008 to 2012. A total of 3,292 individuals were enrolled and tested with RDT and samples collected for microscopy and dried blood spots (DBS) with latter used for pan Plasmodium nested PCR. Species-specific q-PCR was performed on samples that tested positive

MAPPING OF ‘HOT SPOTS’ AND SIGNIFICANCE OF REACTIVE CASE DETECTION IN DETERMINING THE MALARIA BURDEN IN AN URBAN SETTING

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Malaria is a serious public health problem causing high morbidity and mortality in India. Chennai, a metropolitan city is endemic for malaria and the transmission is perennial with a peak between July and October. Plasmodium vivax is the predominant parasite species accounting for 93.6 to 99.8% of the prevalence in the city. The focus of national programme is to identify and treat the individuals presenting to the health care facility. In fact, human malaria parasite reservoir consists of both symptomatic and asymptomatic infections among people in a given area. It is well known that addressing the individuals who do not seek treatment and capturing asymptomatic subjects by active surveillance is essential to move forward for malaria elimination. As a part of the study of complex malaria in an urban setting, the objective was to estimate the burden of malaria (both symptomatic and asymptomatic) adopting reactive case detection strategy and finding spatial clustering of infections. Reactive case detection activity is initiated immediately in response to positive case reported at malaria clinic involving screening of all the household members of the index case. Individuals living in close proximity (<50m radius) to passively detected case and additionally households within 100m radius are surveyed for presence of fever, also randomly selected control households without any history of fever in previous 2 weeks are screened for presence of malaria parasites by microscopy, RDT and PCR. Coordinates of all the index case and enrolled households are captured by global positioning system (GPS) to provide information on spatial distribution of infections. Preliminary data from the ongoing study indicates reactive surveillance would help to identify malaria infection which otherwise would be missed by routine passive case detection. This strategy will help to identify the malaria reservoir which includes the asymptomatic individuals harbouring malarial parasites in the community and serve as vital information on the actual magnitude of the problem for the programme to tackle and undertake appropriate intervention measures to interrupt transmission.
either by microscopy, RDT or nested PCR. There were 12 individuals positive by microscopy (0.3%), 42 positive by RDT (1.3%) and 57 positive by nested PCR (1.7%). Thirty percent of 57 samples positive by nested PCR were also RDT positive. Eighty seven samples positive by either microscopy, RDT or nested PCR were also tested for the presence of Pfhrp gene. Of the 87, sixty one were positive by q-PCR for \textit{P. falciparum} (53% of which were also RDT positive) and 7 were positive by q-PCR for \textit{P. malariae}. Of the 7 positive for \textit{P. malariae}, five represented co-infection with \textit{P. falciparum}, two of which were also RDT positive. Mean copy number of Pfhrp for RDT-positive and negative samples was 1152 and 160, respectively and the difference was not statistically significant \([p=0.126]\) indicating that the RDT-negative, q-PCR-positive results samples were likely due to low parasite density and not Pfhrp gene deletions. Findings illustrate that individuals with low-level parasitaemia and non-falciparum malaria will not be identified by HRP-2 RDTs and the importance of more sensitive and specific diagnostics to achieve malaria elimination.
very few gametocytes just prior to the epidemic. Population stress as indicated by high grain prices due to a poor harvest caused by drought the previous year was a key risk factor for malaria epidemics. Although increased female anopheline survival due to increased humidity played an important part in the magnification of the epidemic, it does not explain its genesis. Current studies using large blood volume molecular methods in SE Asia indicate that a large number of asymptomatic individuals exist with very low falciparum parasitemia. Human population stress triggering a shift towards gametocytogenesis is hypothesized as the key initiation factor for malaria epidemics. Its evolutionary significance may be that it allows the parasite to match the tropical agricultural cycle. Public health programs will have to eliminate all parasites in asymptomatic persons probably by mass drug administration in order to eliminate the risk of malaria epidemics during war and natural disaster.

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SCHOOL-BASED COUNTRYWIDE SEROPREVALENCE SURVEY INDICATES SPATIAL HETEROGENEITY IN MALARIA TRANSMISSION IN THE GAMBIA

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As the geographical distribution of malaria transmission becomes progressively clustered, identifying such residual pockets of transmission is important for research and for targeting interventions. Malarial antibody-based surveillance is increasingly recognised as a valuable complement to classic methods for the detection of infection foci. The study presents serological evidence of transmission heterogeneity among school children in The Gambia, measured during the dry season. Healthy primary school children were randomly selected from 30 schools across the country and screened for malaria infection (microscopy) and antimalarial antibodies (MSP119). Antibody distribution was modelled using finite mixture models screened for malaria infection (microscopy) and antimalarial antibodies. The study presents serological evidence of transmission heterogeneity among school children in The Gambia, measured during the dry season. Healthy primary school children were randomly selected from 30 schools across the country and screened for malaria infection (microscopy) and antimalarial antibodies. The study presents serological evidence of transmission heterogeneity among school children in The Gambia, measured during the dry season. Healthy primary school children were randomly selected from 30 schools across the country and screened for malaria infection (microscopy) and antimalarial antibodies. The study presents serological evidence of transmission heterogeneity among school children in The Gambia, measured during the dry season. Healthy primary school children were randomly selected from 30 schools across the country and screened for malaria infection (microscopy) and antimalarial antibodies.

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MOLECULAR EPIDEMIOLOGY OF PLASMODIUM FALCIPARUM IN AREAS OF DIFFERENT MALARIA ENDEMISTRY - TANZANIA

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Malaria endemicity in sub Saharan Africa continues to decline in recent years as a result of disease intervention strategies. While symptomatic malaria is recognized and treated, asymptomatic infections become increasingly important for interrupting transmission as they contribute substantially the infective reservoir to mosquitoes. To determine the asymptomatic carriage in urban and rural settings in Tanzania, crosssectional community surveys were conducted using highly sensitive molecular tools. A total of 440 finger prick blood samples were collected in Dar es Salaam (DSM, urban malaria with low transmission), and 506 samples from coastal Rufiji, (high malaria transmission). Plasmodium falciparum (Pf) was detected by mRDT (PanPf), light microscopy (LM) and quantitative PCR (qPCR) or qRT-PCR targeting the S-type 185 rRNA genes or transcripts of A-type 18s rRNA respectively. Pf gametocytes were detected by light microscopy and qRT-PCR targeting transcripts of gametocyte specific marker pfs25. Pf prevalence was 73.9%, 49.4%, 47.43% and 25.3% by qRT-PCR, qPCR, RDT and LM in the coastal area (high transmission); while in DSM (low transmission) prevalence was 10.4% 5.23%, 1.59 and 3.41%, respectively. Comparing the two endemic areas, we observed up to 10-fold differences in prevalence rates by the three detection methods. Gametocyte prevalence was 0.23% by LM and 0.9% by qRT-PCR in DSM. In high transmission gametocyte prevalence was 10 fold higher than in low transmission (2.57% by LM and 43.7 % by qRT-PCR). The huge difference in prevalence observed in rural Tanzania argues for reliable molecular tools in malaria surveillance, since 50% of infections would remain undetected by microscopy diagnostics. Such precise molecular data on asymptomatic carriage seems instrumental for planning of malaria interventions. Gametocyte prevalence was about 50% of asexual prevalence at high endemic settings and mirrored asexual prevalence rates. Thus, the difficult and costly molecular detection of gametocytes seems dispensable from surveillance.

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A MULTI-PATHOGEN SPATIO-TEMPORAL MODEL TO PREDICT INCIDENCE OF Rarer DISEASES

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The quantification of spatial and temporal patterns of disease risk is often hampered by substantial noise associated with small population sizes, particularly for rarer diseases. Here, we propose a novel Hierarchical Bayesian model that relies on spatial and temporal correlation, as well as between-pathogen correlation, to improve the characterization of disease risk. We illustrate this model with malaria incidence data from the Brazilian Amazon collected from 2004 to 2008 by the government surveillance system, totaling an area of 3.6 million km2 and 2.4 million malaria cases. In this region, Plasmodium falciparum is much rarer than P. vivax, making it hard to estimate P. falciparum disease risk. However, there is greater public health concern regarding P. falciparum because it tends to result in more adverse health outcomes. Preliminary findings reveal striking geographical differences in disease risk for both pathogens, which seem to be strongly influenced by forest cover. Our model also reveals a strong correlation between P. vivax and P. falciparum malaria incidence, which suggests that P. vivax data can help in inferring P. falciparum risk in a way that is above and beyond the information contained in our covariates. We anticipate conducting a 10-fold cross-validation exercise by August 2014 to better assess the relative importance of temporal, spatial, and between-pathogen correlation in improving predictions of P. falciparum disease risk. The modeling framework proposed here can be valuable for inferring disease risk of a rarer (but more severe) disease based on a more common disease. Finally, although geospatial models have been extensively used to create interpolated surfaces of disease risk, our preliminary malaria results suggest that the uncertainty associated with these surfaces is large and that relatively few areas have significantly high disease risk (hotspots). This finding is important from a public health perspective because it reveals the critical role of uncertainty in determining priority areas for malaria interventions.
A COUNTRYWIDE CROSS-SECTIONAL SURVEY OF THE PREVALENCE OF ASYMPTOMATIC PLASMODIUM FALCIPARUM INFECTION IN THE GAMBIA

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Malaria indicators have decreased substantially in The Gambia with pre-elimination status targeted for 2015. Achieving this goal requires in-depth understanding of the burden of asymptomatic Plasmodium falciparum parasitaemia (APFP) as asymptomatic carriers can maintain or re-initiate transmission. A cross-sectional survey was conducted to determine the prevalence of APFP in the Gambia, describe its heterogeneity and its associated risk factors. A cross-sectional survey was conducted in 36 villages in six geographical locations in November 2012 during the peak of malaria transmission. 350 persons across all age groups were randomly selected in each village (whole populations for villages <350). Finger-prick blood sample were collected for microscopy, species-specific PCR and haemoglobin estimation. Information on bed net use and previous antimalarial treatment were also collected. Data were analysed for the prevalence of APFP by village; summary statistics and factors associated with APFP are presented. Of the 9094 participants enrolled; 59.6% females and 27.2% were aged <5 years. The median (IQR) age and mean (±SD) haemoglobin were 12 (5, 28) years and 11.7 (±2.3) g/dl, respectively. The prevalence of APFP was 16.01% with marked heterogeneity between villages ranging from 1.6% to 49.1%. The mean parasite density was 4329.77/µl. 95% of participants owned bed nets, 78.8% reported indoor residual spraying (IRS). The odds of APFP were higher in children aged 5-15 years (OR1.93; 95% CI: 1.63 -2.30) and adults (OR1.62; 95% CI: 1.35 -1.95) relative to those aged < 5 years. Participants with severe anemia had twice the odds of APFP (OR 2.76; 95% CI: 2.01 - 3.79). Other predictors associated with increased odds of APFP were; axillary temperature, sleeping on verandas at night and IRS. APFP is substantially high in the villages in the eastern region of the country despite high coverage of bed nets and use of IRS. Any intervention aiming at interrupting transmission should target asymptomatic carriers as they represent a substantial reservoir of infection.

HOUSEHOLD HEALTH CARE-SEEKING COSTS: EXPERIENCES FROM A RANDOMIZED, CONTROLLED TRIAL OF COMMUNITY-BASED MALARIA AND PNEUMONIA TREATMENT AMONG UNDER-FIVES IN EASTERN UGANDA

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Home and community-based combined treatment of malaria and pneumonia has been promoted in Uganda since mid 2011. The combined treatment is justified given the considerable overlap between the symptoms of malaria and pneumonia among infants. There is limited evidence about the extent to which community-based care reduces health care-seeking costs at the household level in rural and urban settings. This paper assesses the rural-urban differences in direct and indirect costs of seeking care from formal health facilities compared to community medicine distributors (CMDs). Exit interviews were conducted for 282 (159 rural and 123 urban) caregivers of children below five years who had received treatment for fever-related illnesses at selected health centers in Iganga and Mayuge districts. Data on the direct and indirect costs incurred while seeking care at the health center visited were obtained. Using another tool, household level direct and indirect costs of seeking care from CMDs were collected from a total of 470 caregivers (304 rural and 166 urban). Costs incurred at health facilities were then compared with costs of seeking care from CMDs. Household direct costs of seeking care from health facilities were significantly higher for urban-based caregivers than the rural (median cost = US$0.42 for urban and zero for rural; p<0.0001). The same is true for seeking care from CMDs (p=0.0038). Overall, caregivers travelled for an average of 75 min to reach health centers and spent an average of 80 min at the health center while receiving treatment. However, households in rural areas travelled for a significantly longer time (p<0.001) to reach health care facilities than the urban-based caregivers. Besides travelling longer distances, rural caregivers spent 150 min seeking care from formal health facilities compared to community medicine distributors (CMDs). In conclusion, time and monetary savings for seeking care from CMDs are significantly larger for rural than urban households. Thus, home and community-based treatment of child febrile illnesses is much more cost-saving for rural poor communities, who would spend more time travelling to health facilities - which time could be re-directed to productive and income-generating activities.
HIGH GENETIC DIVERSITY IN PLASMODIUM POPULATIONS IN AREAS OF LOW TRANSMISSION: A COMPARATIVE STUDY OF COLOMBIAN POPULATIONS

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The success of malaria programs is measured by the spatial scales at which disease burden is reduced or eliminated. Consequently, spatial patterns of transmission are of particular interest. In this study, we describe changes in the population structure of Plasmodium vivax (Pv) and P. falciparum (Pf) among endemic areas of Colombia with different transmission intensities. In 2012, some representative areas like Buenaventura (Valle) reported 979 cases in 2012, whereas Tumaco (Nariño) and Tierralta (Cordoba) reported 1,475 and 7,482 respectively. These areas display differences in the relative importance of Pf and Pv (e.g. Valle Pv 90%, Tierralta Pv 92%, and Tumaco Pv 6.9%). We analyzed a total of 550 samples, including both Pv (358) and Pf (192) from these areas using a set of unlinked microsatellite loci. We found different pattern of population structure in each parasite. Using Structure v2.3.3, two clusters were identified for Pf and 9 for Pv. We estimated a total of 37 haplotypes for Pf (Tierralta: 6, Tumaco: 33), sharing only 2 between populations, indicating limited gene flow between these areas. The heterozygosity for Pf was higher in Tumaco (He: 0.871) than in Tierralta (He: 0.453). In contrast, the genetic diversity was very similar and high in the three Pv populations (Buenaventura: He: 0.976, Tierralta He: 0.985 and Tumaco He: 0.979). A total of 195 haplotypes were found in Pv and all were private haplotypes (Buenaventura: 20; Tierralta: 131 and Tumaco: 44) indicating strong population structure. Overall, these set of microsatellites allowed us the identification of locally related, but geographically distinct genotypes that potentially can be tracked in time and space. These characteristics makes possible their use in molecular surveillance (e.g. separating local cases from migrants) in the context of control and elimination.

PLASMODIUM VIVAX MOLECULAR PREVALENCE IN THE SAHEL REGION OF MALI

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The prevalence of Plasmodium vivax infection is traditionally reported as low in West and Central Africa because of the high prevalence of the Duffy-negative antigen (Fya-b-). Recent studies have found cases of P. vivax infection in Duffy negative people, and sporozoites of P. vivax have also been found in the salivary glands of Anopheles mosquitoes in endemic areas inhabited by a predominantly Duffy-negative population. In these regions, a Duffy-positive minority may serve as a reservoir of P. vivax. Infection of Duffy-negative individuals may occur, with selection of parasites capable of invading erythrocytes lacking the Duffy antigen. The potential presence of P. vivax infection in a Duffy-negative population in Africa may be a significant public health problem in the era of malaria elimination/eradication, where strategies are mainly directed against Plasmodium falciparum malaria. We hypothesized that there is endemic circulation of P. vivax in our study population in Bandiagara, Mali and that the prevalence of vivax malaria is underestimated. Blood samples were collected from 300 children aged 0-6 years during quarterly surveys in Bandiagara, Mali. Polymerase chain reaction diagnosis of P. vivax was carried out using DNA samples extracted from filter paper. We will report the prevalence of P. vivax infection at five quarterly time points from 2009 to 2010, with confirmatory testing with microsatellites. In addition, we will characterize the Duffy antigen status in each child that was P. vivax positive, and we will assess the presence of P. vivax sporozoites in mosquitoes caught in the study areas.

SEROLOGICAL DYNAMICS OF MULTIPLE PLASMODIUM SPECIES CO-INFECTIONS IN A HUMAN POPULATION

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Malaria is an important parasitic disease posing major public health challenges in developing countries. Infection with malaria parasites elicits immune responses, and after drug treatment antibodies to Plasmodium-specific antigens are often detectable in serum. The dynamics of antibody responses to Plasmodium spp. co-infection in populations from Zimbabwe where malaria is endemic were determined by ELISA. Antibody (Ab) responses to the C-terminal region of merozoite surface protein 1 (MSP 1a) were determined for all four human Plasmodium species. A panel of other antigens, derived from MSPs of P. falciparum, were used to determine the serological complexity of each individual’s recent P. falciparum exposure. Preliminary analysis of this data shows that IgG responses to MSP 1a of P. falciparum (PIMSP 1a) were the most frequently observed, followed by responses to P. malariae and P. ovale, with responses to P. vivax MSP 1a rarely detected. Interestingly, all sera positive for P. malariae MSP 1a were also positive for PIMSP 1a, indicating that co-infection with these two species is a common occurrence, and that monospecies P. malariae infections are rare. Competition ELISA showed that Abs to PIMSP 1a and/or PmMSP 1a did not cross-react with each other, indicating Ab specificity. Serum samples with antibody reactivities to PIMSP 1a often also showed reactivity to PmMSP2A and PmMSP2B derived antigens, but the patterns of reactivity with these polymorphic antigens varied markedly between individuals. This study reinforces the importance of diagnosis not only the presence of Plasmodium parasites, but also the species of Plasmodium infecting a patient for optimal treatment of malaria co-infections. Further understanding of the dynamics of multiple Plasmodium species infection is important, as co-infections may exacerbate the pathology of the disease or alternately decrease the severity of clinical episodes. Measurement of species-specific antibody responses may provide new insights into the extent (or indeed existence) of cross-species immunity that exists in individuals infected with multiple malaria parasite species.
relapsed infection can lead to activation of memory T cells pool which might bring up protective immunity. This study aims to characterize natural immune responses in acute P. vivax infected patients living in a sole P. vivax infection cohort in Central China. We conducted the cross-sectional immune-phenotypic analysis from three recruitments: patients infected with P. vivax, malaria-immune and malaria-naïve controls. Using flow cytometry, we showed memory T cells were elevated in blood during acute infection. The level of γδ T cells was two fold higher than that of naïve controls. This suggested that two populations, memory and γδ T cells, responded specifically to the P. vivax parasites. On contrary, B, NK and NKT cells were decreased during acute infection. In addition, regulatory T cells were reduced, suggesting the non-immune suppressive role of P. vivax parasites. Interestingly, P. falciparum antigens cross-stimulated T cells obtained from these P. vivax-infected patients. These results provided a further insight into interaction between P. vivax parasites and host cell-mediated immunity in the exclusive P. vivax endemic area that could be important for future development of a successful vaccine designation.

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**IMPACT OF AGE OF FIRST EXPOSURE TO PLASMODIUM FALCIPARUM ON ANTIBODY RESPONSES TO MALARIA IN CHILDREN: A RANDOMIZED, CONTROLLED TRIAL IN MOZAMBIQUE**

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Background: Malaria continues to be an important cause of morbidity and death among children younger than 5 years old. Nevertheless, the impact of the age of first Plasmodium falciparum infection on the rate of acquisition of immunity to malaria and on the immune correlates of protection has proven difficult to elucidate. Previous studies have explored in-depth the contribution of age and exposure in the development of protective immune response. Such that their findings suggested that more a mature immune systems like those of older children should benefit the development of protective immune systems. Moreover, previous work hypothesized a key age period where exposure to malaria parasite should occur in a way that would exalt the development of naturally acquired immune (NAI) response without jeopardize the health of infants from endemic areas. The notable need of discerning the exact period for adequate development of NAI was the rational for the present study.

Methods: Participants (n=349) were enrolled at birth to one of three groups: late exposure, early exposure and control group, and were followed up for malaria morbidity and immunological analyses at birth, 2.5, 5.5, 10.5, 15 and 24 months of age. Total IgG, IgG subclasses and IgM responses to MSP-119, AMA-1, and EBA-175 were measured by ELISA, and IgG against variant antigens on the surface of infected erythrocytes by flow cytometry. Factors affecting antibody responses in relation to chemoprophylaxis and malaria incidence were evaluated. Results: Generally, antibody responses did not vary significantly between exposure groups except for levels of IgM to EBA-175, and seropositivity of IgG1 and IgG3 to MSP-119. Previous and current malaria infections were strongly associated with increased IgG against MSP-119, EBA-175 and AMA-1 (p<0.0001). After adjusting for exposure, only higher levels of anti-AMA1 IgG were significantly associated with reduced clinical malaria incidence (IRR 0.67, p=0.0178). Conclusions: Overall, the age of first P. falciparum infection did not influence the magnitude and breadth of IgG responses, but previous exposure was critical for antibody acquisition. IgG responses to EBA-175 were the strongest correlate of protection against clinical malaria.
merozoite phagocytosis as a correlate of acquired immunity and clinical protection against *P. falciparum* malaria, and demonstrates that using a geographically diverse parasite strains can significantly influence the statistical associations detected.

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**DENDRITIC CELLS SUBSETS MEDIATED IMMUNE RESPONSE DURING *PLASMODIUM BERGHEI* ANKA AND *P. YOELII* INFECTION**

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Immune response responsible for the induction and regulation of this response is poorly understood during malaria infection. As immunity is initiated by dendritic cells (DCs), we compared their phenotype and function during *Plasmodium berghei* ANKA (PbA) and *P. yoelii* 17XL (Pyoelii) infection in Swiss mice individually. PbA infected mice developed a greater number of myeloid and mature DCs on 8 dpi spleen, which were fully functional and capable of secreting IL-12. In contrast, nonlethal *P. yoelii* infected mice produced more plasmacytoid and less mature DCs, resulting in higher levels of IL-10. The mice infected with PbA resulted in an increase in expression of stimulatory (MHCII) and co-stimulatory (CD80 CD86 and CD40) molecules, whereas opposite result was observed during *P. yoelii* infection. Correlating with expression of splenic DCs subsets from PbA and *P. yoelii* infected spleen, our immunoblot assays confirmed the difference in expression of FoxP3, IL-17, TGF-β and IL-6. Thus, results from this study indicate that the subset, the phenotype and the type of inflammatory and anti-inflammatory signals of splenic DCs are critical factors responsible for the irregularity in the ability to induce or regulate Th1 immune responses on host pathogenesis during malaria infection.

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**PROTECTIVE IMMUNITY AGAINST BLOOD-STAGE MALARIA INDUCED BY VACCINATION WITH *PLASMODIUM MIF***

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A major obstacle to a malaria vaccine is the inability of the host to establish an effective memory T cell response. All *Plasmodium* species encode a close ortholog of the innate cytokine macrophage migration inhibitory factor, termed PMIF, which regulates the IL-12/IFNγ response during blood-stage infection to promote the differentiation and exhaustion of effector T cells and reduce the development of memory precursor CD4 T cells. We hypothesized that PMIF may be an attractive vaccine target since its neutralization may reduce deleterious cytokine expression and increase protective memory T cell responses. Using a novel, non-viral self-amplifying RNA vaccine, mice (BALB/c) immunized with PMIF and infected with blood-stage *P. berghei* showed reduced parasitemia and a 37% prolongation in mean survival time when compared to a group vaccinated with control antigen (p=0.0016). This effect was associated with a greater number of antigen-experienced CD4 T cells and an 80% increase in CD62LIL7R+ memory T cells (Tmem). Notably, PMIF-immunized mice that were infected, cured with chloroquine, and re-challenged with *P. berghei* showed 50% expansion of their *Plasmodium*-responsive Tmem populations and complete protection from re-infection when compared to a control vaccine group. Protection in this model of malaria infection also was associated with a 50% increase in plasmablasts (CD19*β*220*CD138*IlgD) and a 15-fold higher titer of an anti-*Plasmodium* antibody response (IgG2a and IgM). An enhancement in both protective humoral and cellular immunity could be demonstrated by PMIF immunization. Passive transfer of immune IgG from infected and cured mice conferred 33% (p=0.0001) protection in lethal cerebral malaria (*Pberghei* infected C57BL/6 mice) and adoptive transfer of CD4 T cells from PMIF-vaccinated mice into naive congenic BALB/c mice conferred complete protection (p<0.0001) against subsequent infection. These data suggest that targeting an active mechanism by which *Plasmodia* interfere with immunologic memory holds promise for a novel vaccine approach.

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**DIFFERENT LATE STAGES OF *PLASMODIUM FALCIPARUM* STIMULATE IFN-Γ PRODUCTION IN CULTURED PBMCs FROM SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS BY ELISPOT**

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*Plasmodium* infections trigger complex immune reactions from their hosts against several life stages of the parasite. These immune responses are highly variable, depending on age, genetics, and the condition of the disease in the host as well as species and strain of parasite. Interferon (IFN)-gamma ELISPOT was used to monitor the magnitude of cellular immune response to *P. falciparum* antigens. Production of IFN-γ from cryopreserved PBMCs collected by leukapheresis procedure from Asymptomatic (Asym), Symptomatic (Sym) individuals infected with *P falciparum* and Endemic Controls (C) was measured against different antigens (Ag): Hemozoin (Hz) and DNAse-treated Hz and synchronized-purified parasites at different stages (mature trophozoites, schizonts in developing and mature) from referential (TG) and native strain (F06) of *P. falciparum* parasites cultured *in vitro*. In the Sym group we observed an increased number of Spot Forming Cells (SFC) when we stimulate with stages of developing and mature schizonts; being this response more increased with the reference strain compared to the native strain. A lower response was observed when PBMCs from these individuals was stimulated by mature trophozoite. The Asym individuals showed increased response of IFN-γ when stimulated with the native strain of *P. falciparum*, and this response was higher when PBMCs were exposed to mature stage parasites (trophozoites or schizonts). The responses in the three groups were greater when the PBMCs were stimulated with any stage of the parasite in compare to the response of the stimulation with the hemozoin. The response with this last antigen (Hz) was high in the purified hemozoin of referential strain and such response was greater in the Sym group compared to the Asym and control groups. DNAse-treated Hz presented a lower IFN-γ SFC in all groups. In conclusion; based on these results and experimental conditions, it seems that PBMCs could be stimulated by different developing stages of schizonts of *P. falciparum* in ex-vivo experiments, however, in Asym individuals the response seems to be higher when PBMCs are exposed to parasites from native strain in terms of IFN-γ response.
EPITOPE MAPPING OF PLASMODIUM FALCIPARUM APICAL MEMBRANE ANTIGEN 1 USING AN E. COLI AUTOTRANSPORTER SYSTEM ON HIGH-THROUGHPUT PROTEIN MICROARRAYS

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Despite extreme diversity, apical membrane antigen 1 (AMA1) is a leading blood stage vaccine candidate antigen in part because anti-AMA1 antibodies inhibit erythrocyte invasion in vitro. Although the protein is known to be immunogenic, there is limited information on which epitopes of AMA1 are associated with cross-protective antibody responses. Autotransporters are virulence factors of gram negative bacteria that can be modified to become bacterial expression systems with advantages over other systems such as phage display. Autotransporters are easily manipulated in the laboratory using standard cloning techniques, and they allow the presentation of relatively large epitopes. To identify cross-reactive and cross-protective AMA1 epitopes, we engineered EspP, an E. coli autotransporter, to display regions of AMA1 and variants of those regions. Sera from rabbits immunized with 3D7- and FVO- AMA1 ectodomains and cross-protective AMA1 epitopes, we engineered EspP, an E. coli autotransporter, to display regions of AMA1 and variants of those regions. Sera from rabbits immunized with 3D7- and FVO- AMA1 autotransporters. When p-distance and Grantham variation scores were calculated by comparing rabbit immunogen/AMA1-autotransporter sequence pairs, we found that sera were cross-reactive with heterologous haplotype constructs when p-distance and Grantham variation scores were smaller. We optimized expression and purification of AMA1-autotransporters for use in high throughput protein microarrays. We will present results of protein microarray reactivity with human sera from children and adults pre- and post- malaria season and pre- and post- receipt of AMA1 vaccine FMP2.1AS02A. Our hypothesis is that seroreactivity to AMA1-autotransporters will be higher in adults than in children and increase in magnitude and breadth over the course of a malaria season, reflecting acquisition of immunity to AMA1 epitopes and ultimately allowing for the identification of cross reactive epitopes. A protein expression system that can readily express conformational peptides in a variety of sizes will be helpful in determining AMA1 epitopes important to a broadly protective AMA1-based malaria vaccine.

PLASMODIUM FALCIPARUM, BUT NOT P. VIVAX, CAN INDUCE ERYTHROCYTIC APOPTOSIS

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Apoptosis can occur in red blood cells (RBC) and this process could play a role in the pathogenesis of many diseases. In malaria it is well known the participation of parasitized RBC in anemia and thrombotic processes. However, non-parasitized RBC (npRBC) apoptosis could amplify the hematologic disorders associated to malaria. In fact, in experimental malaria, increased levels of apoptosis were observed in npRBC during Plasmodium yoelii infection, but in human malaria erythrocytic apoptosis has never been studied. The present study was performed to investigate if npRBC apoptosis also occurs in P. vivax and P. falciparum infections. Apoptosis of npRBC was evaluated in blood samples of P. vivax malaria patients and clinical health individuals living in Manaus, Brazil, both ex vivo and after incubation of RBC for 24h. Additionally, it was tested the capacity of P. vivax or P. falciparum plasma patients to induce in vitro apoptosis of normal RBC from a clinical health individual living in Rio de Janeiro, a non-endemic malaria region. Apoptosis was detected by flow cytometry using annexin V staining. Contrasting with experimental malaria that significantly increase the levels of apoptotic npRBC both ex vivo and after 24h of incubation, no significant alteration on apoptotic npRBC rates was detected in P. vivax infected patients, when compared with non-infected control individuals. Similar results were observed when plasma of these P. vivax patients was incubated with normal RBC. Conversely, plasma from P. falciparum-infected subjects induced significant apoptosis of these cells. It was concluded that apoptosis of normal RBC can be induced by plasma from individuals with P. falciparum (but not with P. vivax) malaria. This finding could reflect the existence of erythrocytic apoptosis during the course of falciparum malaria that could contribute to the pathogenesis of hematological and vascular complications associated to P. falciparum malaria.

STUDY ON ASSOCIATION BETWEEN GENETIC POLYMORPHISMS OF TUMOR NECROSIS FACTOR-A, INTERLEUKIN-10, INTERFERON-IFN, AND MALARIA VIVAX IN BRAZIL

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Malaria is a major cause of morbidity and mortality in many tropical and subtropical countries. In Brazil, the Plasmodium vivax has been the most prevalent species, accounting for approximately 83% of malaria cases in the Brazilian amazon region. Despite of the clinical descriptions of the disease caused by P. vivax is well described, standards regarding humoral and cellular immune response, as well as the pattern of cytokines are scarce and not fully understood. Polymorphisms in genes cd28, ifnγ, tnfrα and il10, that are encoder molecules that interact in modulating pathways of the cellular and humoral immune response, may influence the resistance or susceptibility to malaria. We had analyzed 90 blood samples from patients with vivax malaria diagnosed by molecular and non-molecular techniques and 51 from non-malarial and all from goianésia of pará city, pará state, brazil. Polymorphisms in genes IL10 (-819 c>t, -592 c>a), ifnγ (-183 g>t), tnfα (-372g>a), and cd28 (-372g>a, +17t>c) were analyzed by PCR-RFLP. All subjects were genotyped with 48 ancestry informative insertion-deletion polymorphisms to determine the proportion of African, European and Amerindian ancestry to avoid bias due to differences in ancestry contributions in malaria and non-malaria groups. We used the Fisher exact test to measure association between genotypes and malaria infection. All polymorphisms tested were in hardy-weinberg equilibrium. The African, European and native American admixture did not differ among cases and controls. No significant association was found between the polymorphisms tested and vivax malaria and non-malarial individuals. P-values in co-dominant, dominant and recessive models were also calculated and no significant association was found. These findings make us to believe that the analyzed polymorphisms are not associated with susceptibility or resistance with vivax malaria in the studied population the results will be finalized by june 2016.
CONCURRENT MALARIA PARASITES AND CHIKUNGUNYA VIRUS CO-INFECTION ABATES NEUROPATHOLOGY OF EXPERIMENTAL CEREBRAL MALARIA

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Malaria and Chikungunya fever (CHIKF) are arthropod borne diseases that share the same geographical distributions and recent reports in human shows that co-infections do occur. With the increase in the spread of Chikungunya virus (CHIKV) in the tropics, the potential risk of co-infection in malaria patients is heightened. However, little is known if malaria-associated pathologies are modified during co-infections. In this project, we studied the effect of the viral infection on the pathologies in non-lethal and experimental cerebral malaria (ECM) using Plasmodium yoelii c1.7x (Py17x) and Plasmodium berghei ANKA (PbA) respectively. No effect was observed in sequential or concurrent co-infection of CHIKV and Py17x. Interestingly, although no effect was seen in sequential co-infection of CHIKV and PbA, protective effects were observed during concurrent co-infection. Concurrent co-infection reduced ECM mortality with little effects on the parasitaemia. We found that the reduction of ECM were associated with reduced parasite load in the brain, improved blood-brain-barrier integrity, reduced pathogenic T cells in the brain and reduced cross-presentation of pathogenic epitopes by brain endothelial cells. Leukocytes profiling in the spleen early in the infection suggests that the lack of pathogenic T cells in the brain is not a result of T cells induction but of migration. Taken together, this study demonstrates that host pathogenic immune response towards Plasmodium parasites was altered during CHIKV co-infection leading to reduced neuropathology.

IL4 GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH PLASMODIUM VIVAX MALARIA IN BRAZIL

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Interleukin 4 (IL-4) is an anti-inflammatory cytokine, which regulates balance between Th1 and Th2 immune response, immunoglobulin class switching and humoral immunity. The present study investigated the influence of polymorphisms in IL-4 gene related to the immune system of patients with malaria caused by Plasmodium vivax in Brazilian endemic area. A total of 83 individuals infected by P. vivax were genotyped by pcr/ rflp for two (-590 c/t, -33 c/t) single nucleotide polymorphisms (snps) and the intron 3 vntrr polymorphism pcr method in IL4 gene. The density of parasitaemia in the infected individuals was recorded and expressed as the number of asexual P. vivax per microliter of blood assuming a count of 100 fields per slide. The serum levels of IL-4 were determined by enzyme-linked immunoassay. The parasitemia on thick blood films ranged from 1 to 70. The parasitemia on thick blood films ranged from 5 to 15,000 parasites/mm3. There were no statistically significant differences either in the intron 3 vntrr polymorphism pcr method in IL4 gene. However, the proportion of the no severe malaria vivax.

POTENTIAL IMPACT OF INTERMITTENT PREVENTIVE TREATMENT (IPT) ON THE ACQUISITION OF ANTIBODIES TO MALARIA ANTIGENS GLURP-R0 AND AMA-1 IN SENEGALESE CHILDREN

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Intermittent preventive treatment of infants and children (IPTi/IPTc), currently implemented in several sub-Saharan countries with endemic malaria, may disturb natural acquisition of malaria immunity in infants and children. This study aimed to determine the possible impact of IPTi/IPTc on IgG responses to Glutamate-rich Protein-R0 (GLURP-R0) and Apical Membrane Antigen 1 (AMA1) antigens, as markers of acquisition of malaria immunity in Senegalese children living in Saraya district, where IPTc has been implemented since 2007 (IPT-+), and in Tambacounda district without IPTc (IPT-). sera from 372 randomly selected blood samples (186 from IPT+ and 186 from IPT- communities) blotted onto filter paper, were obtained tree years after IPT was introduced in IPT+ communities and tested by ELISA for IgG response to AMA1 and GLURP-R0 antigens. Malaria prevalence by microscopy was 7.5% (176/2353) and 10.1% (81/804) in the IPT+ and IPT- districts respectively. For both antigens, total IgG prevalence and concentrations were higher in IPT- than in the IPT+ group. Among P. falciparum negatives samples; the mean antibody response (OD) against GLURP-R0 was significantly higher (0.089 vs. 0.025 OD) in the IPT- as compared to IPT+ group. Likewise, for the Plasmodium falciparum positives samples, the IgG response was significantly higher in IPT- group (GLURP-R0=0.258, AMA-1=0.089) compared to IPT+ group (GLURP-R0=0.128, AMA-1=0.043). The low level of IgG antibody response to AMA-1 and GLURP-R0 antigens observed in the IPT+ group indicate that the IPT strategy could delay acquisition of antimalarial immunity. However, other factors could also explain these findings such as marginal differences in the intensity of malaria transmission in the two localities and/or genetic differences between the two populations in their response to the antigens.

REGULATORY T CELLS AND IL-10 KINETICS DURING ACUTE UNCOMPLICATED MALARIA AND CONVALESCENCE IN MALAWIAN CHILDREN RESIDING IN HIGH AND LOW MALARIA TRANSMISSION AREAS

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Malaria still infects most Malawian children causing mortality in about 5% of those infected living in both high and low transmission areas. Several factors contribute towards why some children develop the more severe form of malaria which causes death one of which is the host’s own immune response to the infection. Regulatory T cells (Tregs) are known to play some role in negating immune-related pathology but also
The CTLA-4 snps may be associated with malaria vivax in other endemic areas, but it appears to have no such effect in this studied population. The study also highlights the importance of conducting genetic association studies in different ethnic populations.

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ACQUIRED IMMUNITY AND RISK OF RECURRENT FOLLOWING RADICAL TREATMENT AGAINST PLASMODIUM VIVAX

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Plasmodium vivax recurrence after supervised Primaquine/Choloquine (CQ/ PQ) radical cure is due to interactions between parasite and host factors controlling recrudescence, relapse or re-infection. Since immunity to symptomatic malaria is primarily mediated by antibodies, we hypothesized that high antibody titers against merozoite surface proteins (MSP) during acute malaria infection would have decreased risk of recurrence following radical cure. A clinical trial aimed at assessing the efficacy of three different primaquine regimes in the Peruvian Amazon enrolled 540 acute P. vivax monoinfections between 2006-8. Patients were followed for 210 days after treatment and 90 recurrent cases were identified while 395 individuals did not experience malaria recurrence. Antibodies against selected merozoite proteins were measured using ELISA assays and six putatively neutral microsatellite markers was genotyped to distinguish homologues vs. heterologous recurrence. The relationship between age-dependent antibody responses during acute infection and the risk of recurrence in this population was analyzed using survival analysis. The median titers of anti-P. vivax MSP-1 IgG antibodies (PvMSP-1 IgG) was 3.5 times higher among recurrent cases versus recurrence-free individuals (p<0.001). Only 2% (4/194) of subjects in the two upper antibody level quintiles experienced recurrence while 36% (69/194) and 17% (17/97) had recurrences in the second/third and lower quintile, respectively. This effect was confirmed by Cox hazard regression analysis and was found to be independent of patient age, parasitemia, PQ dose and other covariates likely to influence recurrence rate. Our findings suggest that the intensity of anti-PvMSP-1 antibodies during acute infection is strongly associated with symptomatic recurrence independently of the dose of primaquine used in the radical cure treatment.

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ACUTE PLASMODIUM FALCIPARUM MALARIA EXPANDS T FOLLICULAR HELPER CELLS IN MALIAN CHILDREN

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Immunity to clinical malaria is only acquired after years of repeated Plasmodium falciparum infections in children living in malaria endemic areas. Antibodies have been shown to be protective against clinical malaria, but the acquisition and maintenance of long-lived plasma cells and memory B cells in response to P. falciparum appear inefficient in response to natural infection. The generation of long-lived plasma cells and memory B cells requires the function of follicular helper CD4+ T (Tfh) cells in germinal center reactions. To better understand the cellular basis of the inefficient acquisition of protective antibody immunity in malaria, we conducted a longitudinal study to determine whether Tfh cells are generated in response to natural P. falciparum infections in children. We found that Tfh cells as defined as CD4+ , CD45R0+ , CXCR5+ , PD1+ and CXCR3−, increase in the peripheral circulation of children during acute P. falciparum malaria compared to the children’s pre-infection baselines. ICOS, a costimulator that is essential for Tfh development and function, was markedly up-regulated during infection suggesting that these Tfh may...
be highly functional. Work is in progress to directly measure the capacity of these T<sub>eff</sub> cells to provide help to memory B cells in vitro. In addition we are examining whether the magnitude and quality of malaria-induced T<sub>eff</sub> correlate with the magnitude and quality of P. falciparum-specific antibody responses and clinical malaria outcomes.

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**A CROSS-SECTIONAL SURVEY OF ANTIBODY RESPONSES TO PLASMODIUM FALCIPARUM ANTIGENS IN A REGION OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA**

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Malaria transmission has declined in southern Zambia during the past decade. With reduced transmission, malaria reservoirs become increasing difficult to identify using conventional diagnostic methods such as rapid diagnostic test (RDT). Serology could be a useful tool to detect areas with ongoing transmission. Households were randomly selected using satellite imagery and participants were enrolled into a cross-sectional survey in 2012. A blood sample was collected by finger prick for RDT and dried blood spots (DBS) following written, informed consent. The DBS were collected on Whatman, Protein Saver card 903 and dried overnight. The DBS were later assayed for immunoglobulin G (IgG) antibodies to P. falciparum whole asexual stage lysate using an enzyme immunoassay (EIA). The threshold for seropositivity was determined using serum from US residents never exposed to malaria (which was defined as three standard deviations from ten samples). In 2012, 686 DBS were collected and analysed. The parasite prevalence by RDT was 0.2%. The mean EIA optical density (OD) values were 0.65, 0.71, 0.83, 0.90 and 1.1 for age less than 5 years, 5-10 years, 10-15 years, 15-20 years and older than 20 years, respectively. Similarly, seropositivity increased with age and was 12%, 22%, 45%, 57% and 75% for age less than 5 years, 5-10 years, 10-15 years, 15-20 years and older than 20 years, respectively. The low seropositivity and mean EIA OD values in younger age groups are consistent with a reduction in exposure to P. falciparum. The household locations of seropositive children younger than five years of age were mapped using ArcGIS to identify areas of malaria transmission. Serology can complement information obtained from RDTs to identify hot spots of recent malaria transmission.

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**ELEVATED LEVELS OF IL17 AND ITS ASSOCIATION WITH LOW HAEMOGLOBIN IN SEMI-IMMUNE MICE INFECTED WITH PLASMODIUM BERGhei ANKA**

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Malaria anaemia is still a major public health problem and its pathogenesis still unclear. Interestingly, the progression of anaemia is at relatively low parasitaemia with some mortality in the semi-immune individuals in the endemic areas. A recent study has observed variation in IL17 levels in different mouse strains, thus implicating autoimmune mechanism in RBC loss due to Plasmodium infection. The objective of this study was to evaluate IL17 levels in anaemic condition and to determine if genetic factors are involved. To address this objective, we crossed two mouse strains (Balb/c of low parasitaemia) and CBA (moderately high parasitaemic) to get the progeny, called the F1. Balb/c (8), CBA (8) and F1 (12) were taken through 6-7 cycles of infection (with Plasmodium berghei ANKA) and treatment (with chloroquine/pyrénomethamine) to generate semi-immune status. Parasitaemia and haematological parameters were monitored. Kinetics of antibody production, cytokine levels (in serum and cultured supernatant of stimulated spleen cells) and CD4+CD25+T regulatory cells were evaluated by ELISA, bead-based multiplex assay kit and FACS respectively; at days 0, 16, 28 for Balb/c and F1, and days 0, 8 and 12 for CBA. Similar survival (57%), mean %Hb loss (45%) and mean parasitaemia (5%) was observed in Balb/c and F1, while 0% survival, mean %Hb loss (80%) and mean parasitaemia of 15% was observed in CBA. IgG subtypes were two times higher in Balb/c and F1 than CBA. While IL1a, IL4, IL10, IL12a, IFNγ and TNFα were similar in the three mice strains, IL17 was three times higher in Balb/c and F1 than CBA. Maximum cytokine level was observed at D16 (point at which recovery from parasitaemia occurs, with lowest Hb) in the Balb/c and F1. CD4+CD25+ Treg cells in CBA were lower than those of Balb/c and F1. In conclusion, autoimmune is implicated in Hb loss due to high IL17 levels and may be controlled by a genetic factor. Further studies of F2 between the F1 and Balb/c will be informative to evaluate if these genes are segregated or further apportion.

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**THE MALARIA VACCINE CANDIDATE GMZ2 ELICITS FUNCTIONAL ANTIBODIES IN INDIVIDUALS FROM MALARIA-ENDEMIC AND NON-ENDEMIC AREAS**

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GMZ2 is a hybrid protein consisting of the N-terminal region of the glutamate-rich protein fused in frame to the C-terminal region of merozoite surface protein 3 (MSP3). GMZ2 formulated in Al(OH)<sub>3</sub> has been tested in 3 published phase 1 clinical trials. The GMZ2/alum formulation showed good safety, tolerability, and immunogenicity, but whether antibodies elicited by vaccination are functional is not known. Serum samples prior to vaccination and 4 weeks after the last vaccination from the 3 clinical trials were used to perform a comparative assessment of biological active against Plasmodium falciparum. We showed that the maximum level of immunoglobulin G (IgG) antibodies obtained by GMZ2 vaccination is independent of ethnicity, time under malaria-exposure, and vaccine dose and that GMZ2 elicits high levels of functionally active IgG antibodies. Both, malaria-naive adults and malaria-exposed preschool children elicit vaccine-specific antibodies with broad inhibitory activity against geographically diverse P. falciparum isolates. Peptide-mapping studies of IgG subclass responses identified IgG3 against a peptide derived from MSP3 as the strongest predictor of antibody-dependent cellular inhibition. These findings suggest that GMZ2 adjuvanted in Al(OH)<sub>3</sub> elicits high levels of specific and functional antibodies with the capacity to control parasite multiplication.

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**FREQUENCY AND PHENOTYPE OF Γ+ T CELL SUBSETS IN AN AREA OF HIGH MALARIA TRANSMISSION INTENSITY**

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Plasmodium falciparum infection is thought to induce potent immunoregulatory responses, but the precise mechanisms in humans are unclear. Prior studies have shown that a subset of γδ lymphocytes, γΔ+ T cells, become rapidly activated upon stimulation with P. falciparum antigens, suggestive of an innate-like immune response. We have found that repeated exposure to malaria is associated with loss and dysfunction of γΔ+ T cells in 4-year-old children living in a high endemicity setting. To test the hypothesis that repeated exposure to malaria also leads to increased expression of immunoregulatory markers on this γδ subset,
we measured the frequency and phenotype of γδ T cell subsets in children and their caregivers (n=171) enrolled in a longitudinal cohort study in Nagongera, a rural area in Eastern Uganda with high perennial transmission intensity (EIR 315). We measured frequencies of Vδ1+ and Vδ2+ T cells and evaluated cell surface expression of Tim-3, PD1, ILT2, CD57, CD56, CD16, and NKG2A by multiparameter flow cytometry using freshly obtained peripheral blood mononuclear cells. The mean age of children in the cohort was 6.6 yrs (range 10 months-11 years, n=128), and was 37 yrs in their caregivers (range 20.2-67.7 years, n=43). The mean incidence of malaria was 4 episodes ppy in children (range 0-11 ppy) and 1 episode ppy in their caregivers (range 0-5 ppy). In multivariate linear regression, we observed a significant inverse association between the frequency of Vδ2+ T cells and the prior incidence of malaria (P<0.001), independent of age. We further found significantly increased expression of Tim-3, CD57, NKG2A, and CD16 on Vδ2+ T cells with increasing age. Our findings are consistent with the hypothesis that, in high endemicity settings, cumulative exposure to malaria may be associated with increased expression of regulatory markers on Vδ2+ T cells. These findings have important implications in understanding the immunopathogenesis of malaria in childhood.

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THE ASSOCIATION BETWEEN MATERNAL HOOKWORM INFECTION IN PREGNANCY AND ANTIMALARIAL ANTIBODY RESPONSES IN THE OFFSPRING

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Several studies have shown that helminth infections have adverse effects on malaria. However, the immunological mechanism by which helminths modify the host response to malaria is uncertain. In a trial of anthelmintics in 2, 507 pregnant women [ISRCTN32849447], maternal hookworm infection was associated with an increased risk of clinical and asymptomatic malaria in their offsprings. We hypothesised that helminth infection in pregnancy modifies the fetus’ initial response to malaria and, consequently, their antimarial immune responses in early childhood. Stored blood samples from children of study women were tested for IgG antibody concentrations (µg/ml) to Apical Membrane Antigen-1 (AMA1) and Merozoite Surface Protein-1 (MSP1) by enzyme immunoassay (EIA). Overall, antibody concentrations to anti-AMA1 and anti-MSP1 were significantly higher (p<0.001) among children with asymptomatic parasitaemia compared to children without parasitaemia. However, at the age of one year, parasitaemic children of hookworm infected mothers had a significantly lower concentration of anti-AMA1 antibody (mean log10 (95% CI): 3.51 (3.03-4.07)) compared with parasitaemic children of infected mothers (4.07 (3.58-4.63)), (p for interaction = 0.003). Similarly at five years of age, parasitaemic children of hookworm infected mothers had a significantly lower concentration of anti-AMA1 antibody (4.48 (3.67-5.48)) compared with parasitaemic children of uninfected mothers (5.84 (5.01-6.80)), (p for interaction = 0.01). Maternal hookworm infection did not modify anti-MSP1 responses in children. These results highlight the importance of controlling for helminth co-infections on antimarial immune responses, and could subsequently lead to improved results for the malaria vaccine effort.

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PHAGOCYTOSIS AND OPSONIZATION OF PLASMODIUM FALCIPARUM GAMETOCYTES

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During natural infection malaria parasites are injected into the bloodstream of a human host by the bite of an infected female Anopheles mosquito. Immune responses are generated against various life cycle stages of the parasite that have important roles in resistance to malaria and in reducing natural transmission. A better understanding of the early immune response against mature gametocyte stage of the parasite will inform transmission blocking vaccine strategies. The asexual and sexual erythrocytic life cycle forms of Plasmodium are stages that pass through humans into Anopheles mosquitoes during a blood meal. The sexual stages develop into competent parasites in the mosquitoes and are later injected back into a human host to complete a transmission cycle. There is a fairly good understanding of the adaptive cellular and humoral immune responses, but little is clearly known about the innate immune response. Several published studies suggest that phagocytosis and opsonization of asexual, immature sexual and free merozoite stages occur. We hypothesize that circulating mature gametocytes are phagocytosed directly or after opsonization with specific antibodies present in immune sera from people with previous exposure to malaria. We evaluated phagocytosis of various developmental forms of erythrocytic stage parasites in the absence of immune sera, and opsonization in the presence of immune sera. Results focusing on the uptake of mature gametocytes by phagocytic cells in order to differentiate the opsonic and phagocytic responses will be presented. Identification of phagocytic receptors and targets of opsonizing antibodies will enhance understanding of the role of phagocytosis in the clearance of circulating mature gametocytes, and the link to humoral arm of adaptive immunity. Phagocytosis and opsonization mediated immune activation together with presentation of gametocyte specific antigens will contribute to natural transmission blocking immunity. Specific strategies may also be developed to induce similar antibodies by vaccines aimed at overall transmission reduction.

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ASYMPTOMATIC MULTICLONAL PLASMODIUM FALCIPARUM INFECTIONS CARRIED THROUGH THE DRY SEASON PREDICT PROTECTION AGAINST CLINICAL MALARIA IN THE FOLLOWING HIGH TRANSMISSION SEASON

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Immunity to the antigenically diverse Plasmodium falciparum parasite is acquired gradually after repeated exposure. Infections often consist of several concurrent parasite clones and the diversity of infections in asymptomatic individuals has predicted a reduced risk of malaria during follow up in high transmission settings. In areas of continuous transmission, the number of clones might be a marker of exposure and it is difficult to distinguish between persistent and transient infections. Therefore, we assessed the genetic diversity of P. falciparum infections in a longitudinally followed cohort in an area of highly demarcated seasonal malaria transmission in Mali. During the one-year study period, cases of febrile malaria were detected by passive surveillance among 225 individuals aged 2-25 years of age.. Genotyping of the highly polymorphic merozoite surface protein 2 gene (MSP2) was performed on blood samples collected at five cross-sectional surveys. The genetic diversity was highest in children 5-10 years old and increased during the peak of transmission in all age groups. Detection of multiclonal infections before the rainy season...
conferred a decreased risk of malaria, compared to being parasite negative or having one clone detected, when assessing time to first malaria episode. The results suggest that persistent multiclonal infections carried through the dry season protect against subsequent clinical malaria, probably by maintaining otherwise waning protective immune responses.

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IDENTIFYING MRNA TARGETS OF PLASMODIUM FALCIPARUM RNA BINDING PROTEINS USING DNA MICROARRAY

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Although resistance has rendered the once standard antimalarial Pyrimethamine ineffective against Plasmodium falciparum across the world, it is hoped that new compounds can be developed to combat the pyrimethamine resistant strains of P. falciparum. Although much is known about the drug-resistant variant enzymes in terms of their structures and biochemical characteristics, little is known of how the DHFR-TS enzyme is regulated in the parasite cell. Through greater understanding of the cellular regulation, antifolates could be employed in a more effective manner, e.g. avoiding the known phenomenon of drug-induced expression of DHFR-TS. Intriguingly, DHFR-TS has nucleic acid binding activity in vitro, and is thought to be involved with post-transcriptional regulation of gene expression, including itself. In an attempt to characterize its putative mRNA targets and identify conserved regulatory elements amongst the targets, we use the recombinant DHFR-TS to separate its targets from total parasite RNA. The selected RNAs are then interrogated by DNA microarray analysis and identified en masse. In order to validate the approach, the RNA targets of the P. falciparum Bruno-like putative RNA binding proteins will be characterized initially and compared by bioinformatics with known Bruno-like mRNA targets in other species.

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RAPID GENE REPLACEMENTS IN PLASMODIUM FALCIPARUM USING A NEW APPLICATION OF ATTP X ATTB TECHNOLOGY

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Allelic exchange transfection in Plasmodium falciparum is an arduous, low efficiency process that often takes 6 - 12 months; many experiments do not work and are presumed to fail because the desired changes may be lethal to the haploid blood-stage parasite. We have found that Bxb1 integrase mediated attP x attB technology could be successfully used to overcome limitations in manipulating the endogenous copy of clag3, recently shown to be essential for parasite nutrient acquisition. The attB sequence was successfully introduced into a native clag3 intron by conventional allelic exchange transfection. Introduction of this sequence did not adversely affect intron splicing and yielded an intact, unmodified protein. Subsequent transfection with attP-containing plasmid that carries desired downstream changes in the clag3 gene yielded rapid introduction of desired site-specific integrants that came up as early as 3 weeks in culture. The major limitation of this strategy of introducing attB into an intronic sequence is that nearly half of the genes in the Plasmodium genome are devoid of introns. To overcome this limitation, we are adapting this strategy to study the function of endogenous copies of nonintronic genes such as Dynamin-2 (PfDyn2), Cardiolipin synthase (PfCLS) and MSP7. We are introducing an intron that contains the attB sequence, into the coding (exon) regions of PfDyn2, PfCLS and MSP7. For integration into the ORF of PfDyn2, we have made a construct that comprises a 2 Kb gene fragment from the 3’ end of PfDyn2, which incorporates a 200bp intron that includes a central 40bp attB site. The distal 800bp of the 2 kb construct has been re-coded (using E.coli codon frequencies) to ensure integration occurs upstream of the intron. A similar approach has been followed for making the PfCLS construct. Transfection studies are underway, and upon successful introduction of introns with the attB sequence into the coding regions, we will be able to rapidly manipulate the PfDyn2, PfCLS and MSP7 loci by Bxb1 integrase mediated, rapid site specific recombination of attP-containing constructs into the attB sequence. This technology will help us manipulate endogenous copies of nonintronic genes and is highly cost-effective. It has the potential to open up new avenues for investigations of malaria parasite molecular biology.

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GENETIC CHARACTERISTICS OF PARASITES IN PREGNANCY-ASSOCIATED MALARIA IN COLOMBIA

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This study wanted to analyze the genetic diversity and frequency of resistant-sensitive genotypes of Plasmodium falciparum and P. vivax isolated from peripheral and placental blood of pregnant women, and non-pregnant population of Colombia, as well as to explore associations between the plasmodial infection in peripheral and placental blood with placental histology, maternal anaemia and low birth weight. Study populations: a) 96 pregnant women at delivery, independently of malaria symptoms; b) 130 patients with acute malaria (about half per each species), corresponding to 50 pregnant women attending antenatal care, 40 non pregnant women and 40 men. Plasmodial infection was detected by thick smear and quantitative real time PCR (qPCR). Parasite genotypes in compartments (periphery vs. placenta) and populations (pregnant vs. non pregnant) were compared using five molecular markers per each species, which were analyzed by nested PCR and capillary electrophoresis. Point mutations in the genes dhfr, dhrf and mdr1 of both species were analyzed by direct sequencing. In pregnant women at delivery, qPCR detected significantly more infections (130/288) than microscopy (20/288); 65% of women had at least one positive compartment and the placenta exhibited the highest frequency of infection (57%). P. falciparum was detected in 63% (82/130) of infections at delivery and most of them (65%; 84/130) had <2 DNA copies/µL. Both P. vivax and P. falciparum microscopic and submicroscopic placental infections were associated with villitis and intervillitis. Placental malaria infections were not associated with maternal anaemia or low birth weight. P. falciparum placental isolates had significant genetic differentiation compared to peripheral isolates from pregnant and non-pregnant patients with acute malaria. On the contrary, all the P. vivax isolates were genetically similar independently of the population or compartment. More than 90% of the analyzed isolates of both species had the triple mutation genotype in genes dhfr-dhps associated with sulfadoxine/pyrimethamine resistance. All P. falciparum isolates had the mutation B6Y in Pfmdr1, while only one P. vivax isolate had the double mutation 976F-1076L in Pfdmr1, associated with chloroquine resistance.
ANTI-PLASMODIAL PROPERTIES OF SOME SELECTED GHANAIAN MEDICINAL PLANTS; IDENTIFICATION OF NOVEL ACTIVE COMPOUNDS AGAINST PLASMODIUM FALCIPARUM

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Malaria, caused by Plasmodium spp has been considered as a major disease of public health importance affecting multitudes of people worldwide, particularly in the tropics and sub-tropics. Due to increasing drug resistant parasites to existing drugs, development of new anti-malarial drugs are eagerly awaited. In Africa, there is extensive use of traditional medicinal plants for treatment of various diseases. Many research studies into medicinal plants have shown their significant potentials for anti-plasmodial properties but a few of the active ingredients have been studied. The present study aimed at screening selected medicinal plants used in Ghana for activity against Plasmodium falciparum to determine their active compounds. A high-throughput 96 wells flow-cytometry screening system was established using the method reported by Smilkstein and others, with modification. 50% Et-OH crude extracts of medicinal plants were prepared and applied to synchronized P. falciparum (3D7 strains) culture and FACS analysis was carried out to determine the IC50 of the extracts. Although screening is still ongoing, one active crude extract, JINCO8L, was found possessing activities against P. falciparum and identified two novel compounds from this extract, compound #1 and #2. These compounds have significantly high anti-P. falciparum activities with IC50 values of 3.89 µM and 1.52 µM respectively. Novel compounds identified in this study could be candidates to develop new chemotherapy for malaria. Furthermore, our high-throughput FACS screening system could be useful tool for malaria drug assay.

PRODUCTION AND CHARACTERIZATION OF A LIBRARY OF FULL-LENGTH PLASMODIUM VIVAX MEROZOITE PROTEINS

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A vaccine targeting the illness-inducing blood stage of parasite development is an essential component of any worldwide malaria eradication campaign, but major gaps in our understanding of Plasmodium vivax biology, including the protein-protein interactions that mediate erythrocyte invasion, hinder the search for an effective vaccine. Only a single parasite ligand-host receptor interaction is presently known, that between P. vivax Duffy Binding Protein (PvDBP) and Duffy Antigen Receptor for Chemokines (DARC), and strain-specific immune responses to PvDBP make this antigen a challenging vaccine target. We are carrying out a comprehensive study of P. vivax proteins that mediate erythrocyte binding and invasion in order to identify additional vaccine candidates. As a first step, we produced a library of 39 full-length recombinant P. vivax proteins to test for erythrocyte binding and immunoreactivity. To our knowledge, this represents the largest full-length P. vivax antigen set ever assembled. Candidates were selected based on predicted localization to the merozoite surface or invasive secretory organelles, and on homology to P. falciparum vaccine candidates. 37/39 P. vivax recombinant proteins were expressed in the HEK293E cell system, which has been successfully used for expression of full-length P. falciparum invasion ligands such as PfRH5. Known or predicted functions, such as the interaction between merozoite surface proteins Pv12 and Pv41, were confirmed and several novel parasite protein-protein interactions were identified. Pilot immunoreactivity screens using sera from Cambodian patients with P. vivax malaria showed that IgG variously recognize the majority of antigens tested. The large-scale initial screenings of this library will be presented through protein expression, protein interaction and seroreactivity data, as well as immunoepidemiological studies.

GENETIC VARIABILITY AND POPULATION STRUCTURE OF KENYAN PLASMODIUM FALCIPARUM ISOLATES

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Malaria parasite genetic variability and population structure varies among areas of differing endemicity and are key factors in malaria control strategies. Unlike previously used antigen-encoding loci under strong selection, Plasmodium falciparum microsatellite markers are an attractive target for population structure studies. In Kenya, molecular studies on drug resistant malaria and antigenic molecules have been inadequate in providing accurate genetic profiles of parasite populations in the country. Understanding the genetic structure of malaria parasites is essential to predict how fast phenotypes of interest, such as novel antigenic variants or drug resistant alleles, originate and spread in populations. Twelve polymorphic P. falciparum microsatellite loci were genotyped in 250 parasite isolates from five locations in Kenya using capillary electrophoresis. Analysis of the generated fragments was performed to determine proportions of mixed genotype infections, genotype diversity among isolates, multilocus standardized index of association, and inter-population differentiation. The data revealed dramatic differences in parasite population structure in different geographical locations. An 80% multiplicity of infection was detected in parasites circulating in Kenya. Samples from Kisumu, a high malaria endemic region had a high diversity (He = 0.73) revealing genetic crossing. Results have shown a P. falciparum population structure as well as significant linkage disequilibrium in Kenyan parasites. Regional diversity was observed in range of population structures. These results could be related to geographic differentiation and flow of parasites between sites. These data reveal a range of population structures within a single pathogen species and suggest intimate links between patterns of epidemiology and genetic structure.

GENETIC DIVERSITY OF PLASMODIUM VIVAX IN AREAS OF HIGH RISK OF MALARIA IN CÓRDOBA-COLOMBIA

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The aim of this work was to study the genetic diversity of natural populations of Plasmodium vivax in areas of high malaria risk in Córdoba-Colombia. Molecular confirmation of infection by P. vivax DNA extraction using Chelex-100 was made, thereafter a nested PCR protocol was used to amplify the gene encoding 185 ribosomal small subunit (ssARN) P. vivax and P. falciparum. A sample confirmed as P. vivax underwent a PCR-RFLP for Pvmsp - 3α, gene, restriction enzymes used were Alu I and Hha I, which generated an electrophoretic profile that identified different haplotypes of the Pvmsp - 3α gene. Of the 125 samples analyzed by nested PCR for Pvmsp - 3α gene, 116 successfully amplified. The size of the PCR products allowed to demonstrate the movement of three different genotypes for the Pvmsp - 3α gene: A (1900 bp), type B (1500 bp) and type C (1100 bp), being the most frequent genotype A (88%). 97.4 % (113 /116) of the samples showed simple infections and 2.6% (3/116) polyclonal infections, two by types A and B and one by types A and C. Besides finding a band is reported in the electrophoretic profile of the amplification products Pvmsp - 3α gene with an approximate size of 800 bp which does not correspond with the sizes reported to date, which may represent a new allele of Pvmsp-3α gene however, these results should be interpreted cautiously, pending the results of sequencing. Digestion of PCR products obtained for the Pvmsp - 3α gene with enzyme Alu I showed ten different restriction patterns while with the enzyme Hha I produced nine. The results of
the restriction enzyme digestion of the 113 samples tested revealed that 40/113 (35.3%) and 47/113 (41.6%) of these samples showed polyclonal infections when they are digested with the enzyme Alu I and Hha I, respectively. There is a high degree of genetic variability in P. vivax parasite population circulating in the department of Córdoba.

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THE ROLE OF TEMPORALLY REGULATED RNA-BINDING PROTEINS IN PLASMODIUM INFECTION

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Translational repression of specific mRNAs allows the temporal modulation of gene expression by Plasmodium species. This mechanism enables regulated preparation for the vector/host and host/vector transmission events and perhaps the infection of a new red blood cell. To further our understanding of this mechanism, we have employed genetic manipulation of P. yoelii, a rodent-infectious parasite, to investigate RNA-binding proteins that may play critical roles in this process. Our previous studies have shown that PuF2, a member of the Pumilio-FBF family of RNA-binding proteins, plays an integral role in maintaining the infectivity of salivary gland sporozoites, as well as regulating RNA homeostasis and translational repression. In the absence of PuF2, salivary gland sporozoites become less infectious with prolonged salivary gland residence time, and undergo premature developmental changes, resembling liver stage parasites while still in the salivary gland. In the absence of PuF2, the transcript abundance of ALBA4 increases by approximately 120-fold.

Proteins containing the ALBA domain (acetylation lowers binding affinity) are functionally important in several parasite species, including Leishmania, Trypanosoma, and Plasmodium, where it has been shown to bind DNA and RNA. Because of their ability to bind RNA, we are interested in the potential role of ALBA proteins in translational repression. In the human-infectious species Plasmodium falciparum, ALBA4 protein localization changes from perinuclear to cytoplasmic during blood stage progression. In P. yoelii, we also have evidence for cytoplasmic localization of ALBA4 in blood stage schizonts as seen by live fluorescence microscopy. To fully understand the implications of this finding, we are extending these studies by genetically deleting ALBA4 to determine its essentiality, and examining ALBA4's expression profile in other life cycle stages to determine its function and importance.

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GENETIC VARIATIONS OF PLASMODIUM VIVAX IN THE PERUVIAN AMAZON: DIVERSITY ASSOCIATED WITH POPULATION GENETICS AND VACCINE CANDIDATES GENES AGAINST MALARIA

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Knowledge of both genetic variability of Plasmodium vivax and polymorphism of its main vaccine candidate genes is of great importance to complement the strategies of control, treatment and development of vaccines against this parasite. The current study had two main objectives, first, to determine the genetic variability of P. vivax in the Peruvian Amazon among years 2006-2009 using the merozoite surface protein gene (PvMSP-3α) as a molecular marker, and second, to determine the genetic polymorphism of both the Circumsporozoite protein (PvCSP) and major transmission blocking vaccine candidates, Pxs25 and Pxs28. A total of 1780 samples were obtained from the communities of Santo Tomas, San Jose de Lupuna, Padrecocha and Mazan. By PCR, we found 3 variants of PvMSP-3α gene named type A of 1.9 kb (93.4%), type B of 1.5 kb (6.1%) and type C of 1.2 kb (0.3%); besides, a mixture of two types of amplified fragments (A/B or A/C) were showed in 0.2% of the samples. Also by PCR-RFLP, we identified 11 different alleles of PvMSP-3α. To know the Circumsporozoite protein genetic polymorphism, 42 samples were analyzed; we found 11 types of PxCSP which were composed of 5 different nonapeptides, all belonging to VK210 type. On the other hand, the genetic polymorphism of Pxs25 and Pxs28 were studied by sequencing 51 and 49 samples, respectively, each result were compared to reference strain Sal I. For Pxs25, a non synonymous mutation (Q87K[cag/aac] was identified giving two haplotypes. For Pxs28, four non synonymous mutation were reported (M52L[agt/ctt], D87N[gat/aat], N110Y[aat/ta] y T140S[acc/agc]) resulting on four new haplotypes. All Pxs25 haplotypes and just one Pxs 28 haplotype were reported for all the studied Peruvian Amazon Communities. Our results confirm there are different genotypes of P. vivax in the Peruvian Amazon and that polymorphism of major transmission blocking vaccine candidates, Pxs25 and Pxs28, is more limited than polymorphism of PxCSP; the implications of these genetic diversity in vaccine development should be studied.

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INHIBITION OF RETICULOCYTE MATURATION TO INCREASE PLASMODIUM VIVAX PROLIFERATION IN VITRO

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Plasmodium vivax is restricted to the reticulocyte for both growth and invasion. One of the major obstacles to successful continuous in vitro culture of P. vivax is that reticulocytes rapidly mature to erythrocytes in vitro. This steady maturation effectively dilutes the culture, as constant replenishment of reticulocytes is essential for sustained parasite propagation. We hypothesize that inhibition of reticulocyte maturation would increase the number of viable cells for invasion and would enhance P. vivax proliferation overall. Reticulocyte maturation involves hallmark cellular processes: (i) membrane remodeling and (ii) autophagy of organelles, which can be targeted by small molecules. We initially undertook a small-scale screen to show that reticulocyte maturation can be inhibited and the inhibited cells can support parasite invasion and growth. On going research includes a large-scale screen with a panel of bioactive small compounds and a genetic approach using RNAi with cultured reticulocytes from hematopoietic stem cells.

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PLASMODIUM VIVAX: A GENOTYPIC INTERPRETATIONAL PROBLEM

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It is conventionally assumed that in the absence of either reinfection, or recrudescence following persistence of merozoites in the bloodstream, the hypnozoite stage (this term was coined by me 3.5 decades ago) is the source of recurrent Plasmodium vivax malaria, including recurrences caused by parasites that are genetically similar to those which were responsible for the initial clinical manifestations. How frequently (if ever) this is the case, though, is uncertain, partly because hypnozoites are never, as far as is known, generated by the prior blood-stage infection. Consequently, the source of homologous recurrences is on genetic grounds conceptually not easily ascribable to hypnozoites. This parasite form seems to be directly sporozoite-derived. If so, it has yet to be shown that genotypically homologous sporozoites inoculated by the mosquito can behave in two different ways, i.e. involving some sporozoites initiating early hepatic schizogony but others becoming dormant as hypnozoites. This might indeed happen, of course. However, there are
also other reasons (partly resulting from recent research findings) for asking the question: “Could it normally or sometimes be non-hepatic, non-bloodstream, quiescent parasites (as opposed to hypnozoites) that give rise to recurrent homologous \textit{P. vivax} malarial episodes?” Attention will be drawn via the poster to both new and hitherto overlooked, indirect evidence for this possibility (if not probability). That aside, uncertainty concerning whether \textit{P. vivax} recurrences have a dual origin (sometimes hypnozoites and other times non-hypnozoites) could complicate molecular identification of drug-resistant parasites, for reasons that are perhaps not readily apparent.

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HEMIE-INDUCED ENDOTHELIAL CELL APOPTOSIS IS MEDIATED BY MULTIPLE SIGNALING PATHWAYS

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Recent studies in our laboratory and by others demonstrate the increasing importance of elevated serum concentrations of free heme, a by-product of parasite proliferation, as a key inducer of inflammation and damage to the host microvascular endothelium as well as the mortality associated with fatal cerebral malaria (CM). Previous results indicate that the cytotoxic effect of artemisinin is mediated by free radicals followed by alkylation of malarial proteins1-3. The endoperoxide bridge, the active moiety of artemisinin derivatives (ART), is cleaved in the presence of ferrous iron generating reactive oxygen species (ROS) and superoxide anions4,5. However, the emergence of resistance to artemisinin in \textit{P. falciparum} underscores the need for new insights into the molecular mechanisms of anti-malarial activity of artemisinin. Here we show that artesunate (ART) functions by causing DNA double strand breaks in malaria parasites in a pharmacologically relevant dose and time-dependent manner. We found that DNA damage induced by ART was accompanied by an increase in intracellular ROS in the parasites. Presence of mannitol, a ROS scavenger, during the first 60 minutes reversed the cytotoxic effect of ART and reduced DNA damage with minimal effect on parasite growth. Accumulation of DNA damage resulting from ROS was accompanied by an anti-parasite effect, suggesting a causal relationship between ROS, DNA damage and parasite death. Furthermore, we show that modulating glutathione (GSH) levels impacts ROS and DNA damage induced by ART. Finally, we also show that ART-induced ROS production involves a potential role for NADPH oxidase, an enzyme involved in the production of superoxide anions. Our results provide novel insights into the molecular mechanisms underlying the anti-malarial activity of artemisinin. These studies will provide enhanced understanding of the molecular mechanism involved in resistance to artemisinin and help in the design of new anti-malarials against the most virulent \textit{Plasmodium} species.

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QUANTITATIVE EXPRESSION OF MCHERRY-LUCIFERASE IN ALL LIFE CYCLE STAGES OF \textit{PLASMODIUM FALCIPLAYER}

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Live cell imaging and sensitive quantitative assays are valuable tools in the study of \textit{Plasmodium falciparum} life cycle, especially for stages in the mosquito vector and in the liver. The objective of the project is to generate a stable \textit{P. falciparum} line with high levels of luciferase and mCherry expression for drug discovery in liver stages or other life cycle stages. Since the emergence of GFP several fluorescent proteins have been employed for live imaging of \textit{Plasmodium} parasites. Relative to GFP, mCherry has improved photosensitivity and less background fluorescence, resulting in better signal-to-noise ratios. In contrast, luciferase provides a more sensitive measure in quantitative assays and as consequence has been widely used for high throughput screening (HTS) in drug discovery.

To create stable transgenic parasites expressing mCherry-luciferase we used the TTA-specific transposon \textit{piggyBac} genetic engineering of \textit{P. falciparum}. Luciferase and PbdHFR3$^*$ UTR gene was amplified from existing expression cassette and inserted it into a \textit{mCherry-hDHFR piggyBac} vector \textit{pl-BacII-bEDMH} to obtain \textit{pl-BACII-bEDMH-Luc} plasmid vector. The vector was designed to express both mCherry and luciferase driven by \textit{P. berghei} EF1α, which is a constitutive promoter active in all developmental stages. Promega Luciferase Reporter System was used to detect luciferase activity and mCherry was checked by live cell imaging. Seven clones carrying integrated transposon reporter cassettes were confirmed.

Theses integrated clones express high level of luciferase and mCherry signals through all stage of parasite blood cycle. The clone PFK7FG4 was confirmed to infect mosquito, form sporozoites and express both luciferase and mCherry in mosquito stage. The luciferase-mCherry expressing cassette was successfully integrated in the genome of \textit{P. falciparum} K7 by \textit{piggyBac} transposon system. In blood cycle and mosquito stage, a stable high level expressing luciferase and mCherry reporter gene \textit{P. falciparum} parasite line-PFK7FG4 has been developed. PFK7FG4 can be used for parasite visualization and quantitative analysis. It may offer more efficient drug assays for vector and liver stage studies of \textit{P. falciparum}.
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**INTRAGENIC PROMOTER HAPLOTYPES OF IL-10 AND TNF-α INFLUENCE SUSCEPTIBILITY TO PLASMODIUM FALCIPARUM-INDUCED SEVERE MALARIAL ANEMIA**

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Plasmodium falciparum associated severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dL] is a leading cause of morbidity and mortality in African children. Examination of the host immune response and underlying genotypic traits that condition SMA can offer an improved understanding of malaria pathogenesis. Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) serve as early response signaling molecules that activate the innate immune response. Discordantly, anti-inflammatory mediators, such as interleukin-10 (IL-10) act as secondary modulators to regulate and balance the pro-inflammatory response. Dysregulation of both TNF-α and IL-10 have been shown to influence clinical outcomes in human malarial infections. To investigate the role of polymorphic variation in the IL-10 and TNF-α genes on susceptibility to SMA, haplotypes were constructed using six promoter variants [IL-10 -627A/C, IL-10 -854C/T, IL-10 -1117A/G, TNF-α -238G/A, TNF-α -308G/A and TNF-α -1031T/C]. Selection of variants was based on minor allelic distribution of >10% in African populations and potential transcription factor binding elements. Acutely infected children (n=1220, aged 3-36 mos.) from western Kenya were investigated in this study. Logistic regression was performed [controlling for age, gender, nutritional status, co-infections (HIV-1, bacteremia), G6PD deficiency, alpha-thalassemia and Hbs]. These analyses revealed that presence of the IL-10-627AC/-854CT genotypes were associated with protection against SMA (average OR, 0.68; 95% CI, 0.47-0.91; P<0.05), whereas the TNF-α -308GG genotype enhanced susceptibility to SMA (OR, 1.23; 95% CI, 1.08-1.54; P<0.05). Similarly, several extended haplotypes between the two promoters (i.e., list) were associated with enhanced susceptibility to SMA (average OR 1.39; 95% CI 1.02-1.94; P<0.05), while others (list) showed protection against SMA (average OR 0.88; 95% CI 0.35-0.97; P<0.05). Taken together, these results demonstrate that promoter variation in IL-10 and TNF-α are important in conditioning susceptibility to SMA.

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**SCHOOL-BASED TYPHOID VACCINATION PROGRAM IN KARACHI: POLICY IMPLICATIONS FOR TYPHOID CONTROL**

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Population-based surveillance conducted from 2002-2003 as part of the Diseases of the Most Impoverished (DOMI) Program indicated high burden of typhoid fever in the slums of Karachi-Pakistan. Incidence of typhoid fever was 573 per 100,000 persons per year in children aged 2-4 years and 423 per 100,000 in children aged 5-15 years. Adding to the available evidence providence for information for decision making, all parties were provided with multiple informational tools on typhoid fever and vaccination campaign. In order to ensure quality, clarity and relevance of messages, formative research was also conducted prior to social mobilization activities. Vaccination campaigns were conducted according to international guidelines. Approximately 120,000 school and madrasah going children were given typhoid vaccine. AEFI cases were reported and documented among children who were vaccinated accounting for about 0.28% of children vaccinated. 30 % increase in vaccination coverage was found in Jamshed Town as compared to Gulshan Town as result of implementation of modified communication strategy. The initial phase of the project has sensitized the population for Typhoid prevention. The collaborative efforts of the use of typhoid vaccine in the schools of Karachi added to existing information on the safety and feasibility of injectable typhoid vaccine. A routine vaccination program in schools will increase the use of the vaccine for prevention of typhoid. Policy advocacy is need of hour for inclusion of Typhoid vaccine in routine EPI program.
GUT HOMING MUCOSAL RESPONSE TO ORAL VACCINES IN INFANTS: A COMPARISON BETWEEN UNDERNOURISHED AND WELL NOURISHED

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Oral polio vaccine (OPV) and rotavirus vaccines are less effective in children in the developing world. The reasons for this poor response to these oral vaccines are not well understood. In the present study we investigated the association of malnutrition with the mucosal response to Polio and Rotavirus vaccine in infants from an urban slum of Kolkata, India. An ELISPOT assay was utilized to measure Polio and Rotavirus vaccines induced immune response. The assay was used for simultaneous detection of mucosal derived gut homing IgA producing antibody secreting cells (ASCs) expressing mucosal integrin α4β7 against Polio Virus 1, 2 and 3 and Rotavirus. Systemic responses to Poliovirus vaccines were also measured by positive selection of ASCs expressing HLA-DR and CD19 antigen. HLA-DR and CD19 markers are expressed during maturation and development of B-cells. In infants 4 fold rise in gut mucosal ASC count were observed to Polio vaccine. The percentage of subject with 4 fold rise in ASC count to Polio type 1, 2 and 3 were 50%, 30% and 50% respectively. In the case of Rotavirus vaccine the 4 fold rise in gut mucosal ASC count was observed in approximately 26% of subjects. Infants with moderate to severe malnutrition (HAZ<-2) compared to better-nourished infants had significantly lower polio type 1 (Number of Positive respondents in HAZ -2, P=0.01) and type 3 (Number of Positive respondents in HAZ -2, P=0.03) ASCs response. However, in case of polio type 2 (Number of Positive respondents in HAZ -2, P=0.25) and rotavirus vaccine (Number of Positive respondents in HAZ <2versus HAZ> -2, P=0.47), no association of vaccine response and malnutrition was observed. The ELISPOT data suggested a poor mucosal response to oral vaccines in infants. The mucosal immune response to Polio vaccine was affected by malnutrition. However, there was no effect of malnutrition on Rotavirus vaccination in this population at the measured time points. This study has also demonstrated the role of ELISPOT in measuring mucosal response to oral vaccines. However, this data needs to be correlated with other vaccine measures to determine whether these blood ASC responses could provide proxy markers of the mucosal immune response to oral vaccine.

DIARRHEAGENIC ESCHERICHIA COLI IN MOTHER-CHILD PAIRS IN ILE-IFE, SOUTHWESTERN NIGERIA

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Diarrhoeagenic Escherichia coli are among the most common bacterial causes of morbidity and mortality particularly in young children. These pathogens are not sought routinely and capacity in detection is still grossly limited in developing countries. We investigated the distribution and dissemination of diarrhoeagenic E. coli (DEC) in children paired with their mothers in a Nigerian community. A total of 252 stool samples obtained from 126 children with diarrhea paired with their mothers at a state hospital in Ile-Ife, Nigeria were screened for DEC by multiplex polymerase chain reaction. DEC were identified in specimens from 35.7% of individuals. The most common DEC pathotype in children with diarrhoea as well as their mothers was shiga toxin producing E. coli (STEC) (42, 16.7%). Identical pathotypes were found in 13 (10.3%) of the mother-child pairs. These consisted of enterotoxigenic E. coli (ETEC) (5, 4.0%), enteroaggregative E. coli (EAEC) (5, 4.0%) and STEC (3, 2.4%). The DEC isolates were commonly resistant to ampicillin (121, 96.8%), sulphomamide (118, 94.4%), tetracycline (119, 95.2%), streptomycin (115, 92.2%) and trimethoprim (113, 90.4%), but less commonly resistant to ciprofloxacin (9, 7.2%). This study suggests that healthy mothers may be asymptomatic reservoirs of multiply resistant strains of E. coli that are pathogenic in their children.

POPULATION-BASED INCIDENCE OF ROTAVIRUS AMONG CHILDREN AGED < 5 YEARS IN THE CATCHMENT AREAS OF TWO DIARRHEA SURVEILLANCE HOSPITALS IN DHAKA, BANGLADESH

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Rotavirus is the most common cause of severe diarrhea in children worldwide. It is a vaccine-preventable disease. However, introduction of a vaccine in a resource constrained country like Bangladesh depends on its cost-effectiveness which, in turn, depends on incidence. icddr,b conducts diarrhea surveillance in its Dhaka and Mirpur hospital, but hospital-based incidence underestimates the real burden because many patients seek treatment elsewhere. We estimated the incidence of rotavirus among children aged < 5 years in the catchment area of Dhaka and Mirpur hospital by adjusting the hospital-based incidence by the proportion of severe diarrheal children in the hospital catchment areas who were admitted to surveillance hospitals. In Dhaka Hospital every 50th admitted patient and in Mirpur Hospital every 10th admitted patient was enrolled in surveillance and tested for rotavirus using a stool enzyme immunoassay. We extrapolated the total number of rotavirus cases in surveillance hospitals among all children admitted from the hospital catchment areas. We defined the catchment areas of the surveillance hospitals as those neighborhoods where more than two-thirds of admitted patients resided. To estimate the proportion of severe diarrhea cases among children aged < 5 years in the hospital catchment area who were admitted to surveillance hospitals, we conducted a house-to-house survey in randomly selected areas. We considered diarrhea in the preceding 12 months to be severe if a child with diarrhea was admitted to a healthcare facility, or received intravenous rehydration, or died following frequent loose or watery stools. Among the surveillance enrolled children aged < 5 years from January 2011 through December 2013, 1,247 (42%) in Dhaka Hospital and 626 (33%) in Mirpur Hospital were positive for rotavirus. According to survey in hospital catchment areas, the proportion of severe diarrheal children who were admitted to surveillance hospitals was 0.69 (95% CI: 0.62-0.75) in Dhaka Hospital and 0.77 (0.70-0.83) in Mirpur Hospital catchment areas. The population-based incidence of rotavirus among children aged < 5 years was estimated to be 28 (95% CI: 25-31) in Dhaka Hospital and 38 (95% CI: 35-42) in Mirpur Hospital catchment areas per 1,000 population. This study provides a credible estimate of rotavirus incidence in Dhaka, which can be used to assess the cost effectiveness of rotavirus prevention activities including vaccination.
INCIDENCE AND SEVERITY OF TRAVELER'S DIARRHEA AMONG STUDENTS AT A SPANISH LANGUAGE SCHOOL IN CUSCO, PERU

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Travelers’ diarrhea (TD) is the most common illness among travelers to low and middle income countries, yet there are few prospective incidence studies evaluating the full spectrum of disease. To evaluate the incidence and causes of TD, we conducted a prospective cohort study among travelers in Cusco, Peru from June 2012 to January 2014. Participants provided 1 stool sample at enrollment, then weekly, and when sick with diarrhea. Two definitions of diarrhea were used: 1) the WHO definition of 3 or more loose stools in a 24 hour period and 2) any self-reported diarrhea by participants. Subjects reporting diarrhea were evaluated daily to record signs and symptoms. In total, 249 participants were enrolled accounting for 663 observed patient-weeks. Participants were 65% female with a mean age of 28.1 years (SD 11.4 years) and represented 22 different countries principally from North America and Europe. Of the 711 samples collected, 168 (24%) represent diarrheal episodes and 543 (76%) represent asymptomatic baseline or follow-up samples. We recorded 174 episodes of diarrhea representing 131 (53%) participants and an incidence rate of 1.14 episodes per patient per month, or a 26% chance of having diarrhea each week while in Cusco. Seventy two percent of diarrhea episodes met the WHO definition, however 28% still self-reported problems with diarrhea. Those meeting the WHO definition lasted on average, 1.18 days longer (95% CI 0.53-1.82) than those not meeting the definition. However, subjects in both groups lost a workday (45% WHO; 37% non-WHO) and experienced abdominal pain (6% WHO; 8% non-WHO), excessive thirst (43% WHO; 40% non-WHO), decreased urination (18% WHO; 15% non-WHO) and dark urine (19% WHO; 20% non-WHO) each observation day. Non-WHO cases were more likely to report nausea (OR: 2.54; 95% CI: 1.29, 5.02) and bloating (OR: 2.02; 95% CI: 1.03, 3.96) per observation day. In conclusion, the WHO definition misses 14% of TD episodes which still have similar symptoms and impact among travelers. A better diarrhea definition for travelers should be considered.

PERSISTENT TYPHOID FEVER EPIDEMICS IN INTERNALLY DISPLACED PERSONS CAMPS IN UGANDA

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Typhoid fever has continued to cause recurrent epidemics in some districts in Uganda from 2009 to date. According to the epidemiological data from the Ugandan Ministry of Health (MOH) from 2009 to 2013, there has been an increase in the number reported cases of typhoid fever particularly from districts with people living in internally displaced peoples camps (IDPs) where some patients are even admitted with complications such as intestinal perforations. The factors leading to this increase have not yet been established and worse still, is the fact that the reported typhoid fever cases were diagnosed using the Widal test which is non-specific.
and may lead to over diagnosis of the disease. The study therefore was to attempt to establish factors leading to the increase of typhoid fever cases and to confirm all such reported cases with culture methods. The specific objectives were to study the socio-demographic profiles of the residents of the four most affected districts, their environmental sanitation situations as well as the water sources used. Both Widal and culture methods were used to establish the typhoid fever causative organisms, establish the carrier status of recovered cases, and establish antibiotic sensitivity of the salmonella organisms isolated. A survey was conducted to study the situations of residents of four camps in the four districts. Follow-up interviews and Focus group discussions were carried out. Observations of the homesteads and latrines for sanitation and hygiene were also conducted. Water samples from sources and households were analysed for faecal contamination. Similarly blood samples obtained from admitted patients and previously confirmed cases who had recovered from typhoid fever were cultured and the isolated salmonella typhi organisms, tested for antibiotic sensitivity. The main findings why typhoid fever is recurrent in four camps in the four districts included: poor hygienic practices, poor sanitation. Low latrine coverage (40%), unsafe water sources, contaminated with faeces (both E. coli and Salmonella typhi organisms were isolated. Salmonella typhi was isolated from 27/81(33%) patients by blood culture, while 58/81(71.6%) were positive with the Widal test. 76% was isolated from 27/81(33%) patients by E. coli and cipro. carrier status was 36%.

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#### SPATIOTEMPORAL TRENDS AND IMPORTANT LOCAL INFLUENCES ON HOSPITALIZED PEDIATRIC DIARRHEA IN HO CHI MINH CITY, VIETNAM

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1Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, 2Princeton University, Princeton, NJ, United States, 3Children’s Hospital 1, Ho Chi Minh City, Vietnam, 4Children’s Hospital 2, Ho Chi Minh City, Vietnam, 5Hospital for Tropical Disease, Ho Chi Minh City, Vietnam, 6Diarrheal disease in Ho Chi Minh City (HCMC), Vietnam remains a significant cause of morbidity in young children. Distinct seasonal patterns are present in diarrheal disease reporting to hospital in this setting, with large peaks occurring in the colder and drier months. The reasons for these trends are unclear. In order to explain these observed patterns, we analyzed a set of hospitalized pediatric diarrheal admissions from three large hospitals in HCMC from 2005-2010. We developed a mixed effects model to explore important influences in reported diarrhea, examining time series trends and associations with a variety of environmental covariates including flood level, temperature and rainfall. Additionally, we used Empirical Bayesian Kriging to map smoothed rates of reported diarrhea stratified by season and age-group to investigate the spatiotemporal distribution of reported diarrheal cases across HCMC. Our results highlight the continuing burden of pediatric diarrheal disease in this setting: of ~480,000 hospital admissions, gastroenteritis was the second most common reason for hospitalization in children under 16, accounting for 12% of all admissions. From our mixed effects model, we found a strong epidemic component that was spatially synchronized across HCMC, suggesting citywide epidemics, probably due to viral pathogens. We speculate that these patterns are due to the entrance of new seasonal variants or related to the seasonal birthrate across the city. Although several crucial limitations are present in such a dataset, the results still provide insight for public health practitioners and clinicians working in HCMC, Vietnam and may also reflect trends present in other industrializing, densely populated settings.

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#### HOST GENE POLYMORPHISMS AND SUSCEPTIBILITY TO BACTERIAL ENTEROPATHOGENS IN CHILDREN LIVING IN GAMBIA AND KENYA

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The Global Enteric Multicenter Study (GEMS) is a prospective collaborative study to determine the pathogen specific, diarrhea associated attributable disease burden of diarrhea in Africa and Asia in children less than 5 years of age. Using samples from children that participated in this case-control study and were seen at in the GEMS clinics in The Gambia and Kenya, we conducted a host gene candidate study that examined the association of the host DNA single nucleotide polymorphisms (SNPs) and specific bacterial enteropathogens causing diarrhea. Cases of diarrhea were matched with healthy controls from the same area. Stool was collected and examined comprehensively for the presence of enteropathogens and host DNA polymorphisms. We studied de-identified fecal DNA for the presence of SNPs (N=144) in 26 genes that code for host proteins involved in pathogen attachment, inflammation, innate and acquired immune responses to enteropathogens. We analyzed the distribution of host genotypes and compared cases vs. controls according to enteropathogens identified. Comparisons were made using SNPTAs software following an additive model. A mixed logistic regression model was used to adjust for potential confounding factors including site, age, weight for height and weight for age scores. Microbiological and genotype data were available in 1,164 subjects. Diarrhea was associated with 6 SNPs located in SELPLG, CDSS, LPLUNC, IL12 B, and CORO1C. In The Gambia, diarrhea due to Shigella was associated with a SNP in SELPLG, diarrhea from enterotoxigenic E. coli was associated with SNPs in DAF (CDSS), LPLUNC, SELPLG, diarrhea from enterotaggregative E. coli was associated with SNPs in CORO1C. In Kenya, diarrhea from enterotaggregative E.coli was associated with SNPs in IL12B and CORO1C. Distinct SNPs were associated with pathogen specific diarrhea in children under 5 years of age living in two African countries.

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#### GUT MICROBIAL SUCCESSION AFTER ENTEROTOXIGENIC ESCHERICHIA COLI INFECTION IN BANGLADESH

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Enterotoxigenic Escherichia coli (ETEC) is a common cause of bacterial enteritis in the developing world. Recent human and animal studies
suggest that alterations in commensal flora influence recovery from enteric infections. We examined serial rectal swabs from patients after ETEC infection to observe microbial succession during the recovery period. DNA was extracted from rectal swabs and analyzed using 16S ribosomal RNA gene sequencing. Over a three-month follow-up period, 75 samples were analyzed from 18 individuals in Dhaka, Bangladesh. To simplify the analysis of these complex communities, we investigated bacterial dynamics at the genus level. The bacterial family including E. coli species was most dominant on the day of patient enrollment when ETEC stool culture was positive. The day after antibiotic treatment for ETEC, bacteria including skin flora such as Gram positive organisms were most dominant, with a decrease in bacteria from the family including E. coli species. Compared to day 10 of follow up, the skin flora group was predominant on Day 2 (Day 2, 7/16 44% versus Day 10, 0/17, P=0.003 by Fisher’s exact test). On day 10 of follow up, anaerobic flora such as Prevotella species and other common enteric anaerobes were predominant (Day 10 12/17 71%, Day 2 4/16 25%, P=0.011 by Fisher’s exact test). The predominant bacterial populations on Day 10 and Day 30 were similar to gut microbiome studies in healthy controls in the developing world. The gut microbiome following ETEC infection rapidly recovers to baseline after a dramatic perturbation and progression through distinct phases of recovery. Understanding the pattern of microbial succession in recovery from enteric disease is a foundation for studying factors of susceptibility and protection from disease and could potentially lead to interventions to hasten recovery from infection.

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MEMORY B CELL RESPONSES TO VIBRIO CHOLEREA O1 O-SPECIFIC POLYSACCHARIDE (OSP) IN PATIENTS WITH CHOLERA IN DHAKA, BANGLADESH

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Cholera is a severe, dehydrating diarrheal illness that in the last two decades has been caused largely by Vibrio cholerae O1. There is a growing body of evidence that protective immunity against cholera targets lipopolysaccharide (LPS), especially the O-specific polysaccharide (OSP) component of LPS. Immunity following wild-type cholera persists for at least 3 to 10 years, even in young children. Immunity following oral cholera vaccination is of lesser magnitude and shorter duration, especially in young children under the age of five years. Following wild-type cholera, protection against cholera persists despite decreases in effector immune responses, suggesting that memory responses may play a critical role in mediating long-term protection against cholera. We took advantage of the recently described ability to purify V. cholerae O1 OSP, and assessed memory B cell responses targeting OSP in patients recovering from wild type cholera in Bangladesh. The Ogawa serotype was the primary circulating serotype at the time of this study, and we assessed immune responses out to 6 months after presentation with illness. We found that serum OSP IgA and IgG responses were evident within 7 days of presentation, and that these values decreased toward baseline over the follow-up period. We found that IgA and IgG memory B cell responses targeting OSP were also present within 30 days of presentation, and persisted through the follow-up period. When we analyzed the data by age of patients, we found IgG and IgA memory responses targeting OSP were detectable in adults (>18 years of age, n=15), older children (6-17 years of age, n=20) and children (2-5 years of age, n=11). Our results suggest that memory B cell responses following wild-type cholera are induced, that they persist in time, and that these responses are comparable among adults, older children, and younger children in this cholera endemic area. These results suggest that memory B cell responses targeting OSP could play a role in mediating long-term immunity against cholera.

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ASSESSING SERUM LEPTIN LEVELS IN CHILDREN HOSPITALIZED WITH CHOLERA IN BANGLADESH

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Cholera is a severe watery diarrhea caused by the noninvasive gram-negative bacterium Vibrio cholerae. Leptin is a critical hormone in human metabolism that plays a role in developing adequate immune responses. Malnourished children with low leptin levels have reduced T cell responses. In order to evaluate the potential role of leptin during cholera, we measured leptin levels on day 2, 7 and 30 in cholera patients 5 years of age and younger at the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh. We compared these levels with respect to age, gender and nutritionally-matched healthy controls, as well as day 180 levels within the cholera patients themselves. We found that patients at the acute stage of cholera had significantly lower serum leptin levels than matched healthy controls, and that these acute-phase leptin levels rose through convalescence. We also evaluated the association between leptin on day 2 and immunological responses that developed at later time points to cholera toxin B subunit (CtxB), a T cell-dependent antigen, and V. cholerae lipopolysaccharide (LPS), a T cell-independent antigen. We found a significant association of acute phase leptin levels to immune responses to CtxB. Our results suggest that leptin may play a role during cholera, especially in maturation of immune responses to T cell-dependent antigens. Our results also suggest that malnourished children that have low leptin levels may be impaired in their ability to develop such responses.

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CAMPYLOBACTER JEJUNI CAPSULAR TYPE DISTRIBUTION IN THE AMAZON AND COASTAL REGIONS IN PERU

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The enteric pathogen Campylobacter jejuni constitutes one of the major causatives agents of diarrhea worldwide. C. jejuni expresses 47 different capsule polysaccharides (CPS) and constitutes a major factor in C. jejuni-mediated pathogenesis. Recent published findings demonstrate a CPS-mutant diphtheria toxin conjugate vaccine as protective against CPS-homologous C. jejuni infection in an Aotus nancymaae non-human primate diarrheal model. World-wide surveillance describing CPS type distribution is necessary to determine regional vaccine efficacy of CPS-specific vaccines against C. jejuni. To this end, two regionally distinct pediatric prospective community based studies were considered to evaluate CPS distribution in Peru. Santa Clara, a rural community located in the Peruvian Amazon was compared to Pampas de San Juan, a shanty town located in the peripheral area of Lima. Both populations were analyzed by a multiplex PCR capable to detect the 47 CPS types of C. jejuni. Santa Clara study included 131 symptomatic and asymptomatic individuals of which 201 Campylobacter jejuni isolates were acquired from stool samples. The Pampas de San Juan study included 95 samples obtained from 51 symptomatic and asymptomatic children. Only 7% of the isolates from Santa Clara were untypeable by this technique. The most prevalent CPS types detected from Santa Clara were HS5/HS17 (15%), HS15 (13%) and HS3 complex (12%). In Pampas de San Juan, a higher percentage of isolates, 25%, were untypeable by this technique while HS15 (9%), HS4 complex A (7%) and HS41 (6%) were the most astmh.org
prevalent CPS types. In addition, tracking of CPS types in individuals over time demonstrate different CPS types present from C. jejuni re-infection suggesting a possible acquired immunity to distinct CPS. The results herein describe differential CPS type distribution in two distinct regions of Peru, with H5B/H517 (15%) and H515 (9%) being the most prevalent in Santa Clara and Pampas de San Juan, respectively.

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A HIGH PREVALENCE OF HYDATIDOSIS IN SLAUGHTERED GOATS AT A SOUTHWESTERN ABATTOIR, NIGERIA: IMPLICATIONS ON LIVESTOCK PRODUCTION AND HUMAN HEALTH

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Hydatid disease is an important zoonosis globally particularly in developing countries like Nigeria where backyard slaughter is rampant and carcass wastes are easily accessible to scavenging dogs. Adult goats raised on an extensive system of management slaughtered at a tropical abattoir in south-western Nigeria were inspected for the presence of hydatid cysts. In all, a total of 2,140 goats were slaughtered from which 214 were randomly selected and inspected over the study period. The results show a prevalence of 53.27% with 50.88%, 38.6%, 6.14% and 4.39%, respectively located in the lungs, liver, heart and intestines. In addition, 33.33% of the hydatid cysts were fertile with a significantly higher proportion (51.72%) from the lungs. Our findings reveal a high prevalence of hydatidosis in slaughtered goats in south-western Nigeria and this portends serious economic implications on livestock production through reduced productivity and even death. The prevalence of fertile cysts obtained in this study suggests a high potential for human infection with hydatidosis through contacts with exposed dogs or consuming water contaminated with infected dogs faeces. The current results suggest that a thorough investigation that leads to a disease control strategy is required to reduce the economic and public health consequences of hydatidosis.

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KAP (KNOWLEDGE, ATTITUDE AND PRACTICE) OF ECHINOCOCCOSIS - FIRST REPORT FROM CENTRAL SUDAN

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In Sudan, Echinococcosis is a chronic neglected disease caused by Echinococcus granulosus (EC). It affects animals and humans can be accidentally infected through direct contact with dogs or ingestion of contaminated water or food. Studies have shown infection rate as follows: Dogs 50-70%, camels 35%, sheep/goats and cattle 10-11%, where as humans in central Sudan 0.3-1.0%. Hydatid cyst in humans might lead to serious complications if left untreated. Treatment is expensive and sometime is cumbersome where advanced surgery can be needed. The study area is known by its large livestock with camels preponderance and sometime is cumbersome where advanced surgery can be needed. The study area is known by its large livestock with camels preponderance and where previous surveys in dogs, camels and human has been performed. Screening in humans has led to successful medical treatment only, using portable ultrasound in all asymptomatic cases. In Sudan regarding Human EC genotyping, G6 (camel strain) is the cause for almost all human cases collected. Therefore, the objective of this study is to explore knowledge, attitude and practice (KAP) of people living in three villages in Tambool area, central of Sudan. A cross sectional study using close ended structured questionnaire covering the thematic areas of Hydatid KAP was administered to 312 household (full coverage). The data was entered and analyzed by SPSS. Results showed that participants mean age was 37.5 ±16.3 years. Substantial number of the respondents (73.7%) never heard about the disease. Only 26% heard about it, however they reflect low knowledge about cause, mode of transmission, prevention and control of Echinococcosis. In terms of attitude, half of the participants who heard about the disease believe Hydatid cyst patients should be isolated. This might indicate potential stigma. Participants practice showed some behavioral risk as 81.7% slaughter animals inside home without veterinary supervision and 70 % throw the offal outside home where stray dogs eat it. In conclusion, the study mapped the baseline for KAP of the community of the study area. It revealed that the population had poor knowledge and attitude. Their practices might help circulating the parasite in the area. Consequently health education is highly needed to prevent and control the parasite and disease.

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OVALBUMIN-INDUCED AIRWAY INFLAMMATION IS REDUCED BY ECHINOCOCUS GRANULOSUS INFECTION WHICH DOWN-REGULATED IL17 IN MICE

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Cystic echinococcosis (CE) is a cosmopolitan zoonosis caused by the larval stage of the dog tapeworm Echinococcus granulosus. The infection induces a polarized T-helper type 2 (Th2) response in its intermediate hosts. Here, we examined the effects of E. granulosus infection on mouse ovalbumin (OVA)-induced asthma. BALB/c mice were intraperitoneally transplanted with 50 small Eg cysts cultured in vitro 3 months prior to being sensitized and challenged with OVA. The mice with cysts transplanted harbored an average of 34.8 cysts per mouse, with a mean diameter of 4.4 mm. Histological staining of lung tissues showed that E. granulosus infection significantly reduced the severity of OVA-induced airway inflammation including reduction of eosinophil cell accumulation and mucus production. Airway hyperresponsiveness of OVA-challenged mice after E. granulosus infection was significantly suppressed as compared to the OVA-only challenged mice. The infection significantly increased both Th1 and Th2 cytokine expression in PBMC and also in lung tissue, but significantly down-regulated IL-17a expression. When the mice after infection were challenged with OVA, the expressions of IFN-γ, IL-2, IL-4 and IL-5 in lung were down-regulated, whereas, IL-2 and IFN-γ were expressed in high level. In conclusion, E. granulosus infection markedly reduced the severity of OVA-induced airway inflammation. Down-regulation of Th17 responses in lung tissue may be a potential therapeutic treatment against allergic asthma.
RIEC: THE FIRST REGISTRY OF CYSTIC ECHINOCOCCOSIS, FROM ITALIAN TO EUROPEAN

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Cystic Echinococcosis (CE) is endemic in Eastern and Southern Europe, Italy included. However, its real burden is largely unknown due to the lack of efficient reporting systems designed to take into account the peculiar features of this disease. In Italy, only a yearly summary of regional data is required by the health authorities and the prevalence and incidence of human CE are hugely underestimated because only hospitalized cases are registered, while the majority of CE cases are diagnosed and managed on an outpatient basis. Furthermore, no official data are transmitted to European authorities. The neglect of CE also results in general lack of knowledge on its diagnosis and clinical management outside referral centres, with consequent heterogeneity in clinical practices and often unnecessary procedures with associated risks and costs. In 2012 the Istituto Superiore di Sanità (ISS - Italian National Health Institute - Rome) in collaboration with the University of Pavia, WHO Collaborative Centre for the Clinical management of Cystic Echinococcosis, implemented the Italian Registry of Cystic Echinococcosis (RIEC). This is a prospective multicenter registry of CE patients visited from January 2012 in Italian health centres that adhered voluntarily. RIEC is accessible on the website of ISS since October 2012 with the aims of: indicating the burden of CE in Italy; bringing the importance of this infection to the attention of health authorities; encouraging public health policies geared toward its control; stimulating research on CE. Moreover, it provides an useful tool for patients follow-up and evaluation of therapeutic interventions. As of February 2014, 346 patients were enrolled in 11 centres, figures largely outnumbering the national reports of many endemic European countries. We will discuss updated results and challenges of RIEC, that is the outnumbering the national reports of many endemic European countries.

TRANSCRIPTITIONAL PROFILES OF PROTOSCOLECES OF ECHINOCOCCUS GRANULOSUS IN RESPONSE TO TGF-ß REVEAL INCREASED EXPRESSION OF GENES INVOLVED IN GROWTH OR DEVELOPMENT

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TGF-ß is a crucial cytokine participate in the interplay between the intermediate host and helminthes. TGF-ß receptors were discovered in many cestode, and could bind the human TGF-ß. However, the function of host TGF-ß on the Echinococcus is still not elucidated, and this paper aim to explore the question at transcription level. Microarray analysis was used to investigate differential expression genes in protoscolices of Echinococcus granulosus cultured in the presence or absence of human TGF-ß at different time points (4h, 8h and 24h) in vitro. A total of 523 genes were up- or down-regulated in response to TGF-ß, compared with control group, 390 genes were up-regulated and 47 genes were down-regulated at 8h, and 376 genes were up-regulated and 19 genes were down-regulated at 12h, including 310 differential genes were regulated at both time point. Gene ontology (GO) analysis showed that the biological process of the up-regulated genes in protoscolex were predominantly involved in DNA packaging, nucleosome assembly, chromatin assembly, etc. And the cellular component gene were located in cell nucleus. TGF-ß appeared to promote growth or development of the protoscolex by up-regulated the gene related with mitosis. In addition, the study also indicated that TGF-ß has a multiple influence on the protoscolex, as reflected in the increased stimulation of gene expression of the ErbB signaling pathway, MAPK signaling pathway, Notch signaling pathway and VEGF signaling pathway.

IDENTIFICATION OF FUNCTIONAL MITOGEN-ACTIVATED PROTEIN KINASE KINASES (MAPKK) HOMOLOGUES FROM ECHINOCOCCUS GRANULOSUS: PROTOSCOLICIDAL ACTIVITY OF MAPK SIGNALING PATHWAY INHIBITORS

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Cystic echinococcosis (CE) treatment urgently needs a new drug. Understanding molecular regulation is essential for identification of drug targets. In the present study, we identified two new members of the MAPKKs, MEK3/6 and MEK1/2 homologues (termed as EgMKK1 and EgMKK2, respectively) from Echinococcus granulosus, the causative agent from CE. Both EgMKK1 and EgMKK2 were co-expressed in the larval stages. EgMKK1 encoded protein was localized in the sub tegumental and tegumental layer and EgMKK2 was localized in the tegumental and sucker of the parasite. As shown by yeast two-hybrid and Co-immunoprecipitation analysis, EgMKK1 strongly interacted with the p38-like MAP kinase Egp38. EgMKK2, on the other hand, not only interacted with a member of the parasite's 14-3-3 protein family, but also with the Erk-like MAP kinase EgERK. Recombinant EgMKK1 and EgMKK2 displayed kinase activity towards myelin basic protein substrate. When Sorafenib Tosylate (Bay 43-9006), an inhibitor of MAPKKK (Raf-1/B-Raf), and U0126 and PD184352, inhibitors of MAPKK (MEK1/2), were added to the medium for in vitro cultivation of E. granulosus protoscoleces, these inhibitors resulted in a marked dephosphorylation of EgERK for 20 and 30 days, then had actually an inhibitory and cytolytic effect on the larval stage of the parasite. In addition, Sorafenib Tosylate killed protoscoleces more effectively. In vitro culture of protoscoleces in the presence of 25 µM Sorafenib Tosylate or 100 µM U0126 for a period of 30 days was parasiticidal, as determined by murine bioassays, while treatment with 100 µM PD184352 was not. Our study indicates that small inhibitors of MAPK signaling pathway may be potential drugs for the treatment of CE.

IDENTIFICATION OF FUNCTIONAL SMOAD8 AND SMOAD4 HOMOLOGUES FROM ECHINOCOCCUS GRANULOSUS: PROTOSCOLICIDAL ACTIVITY OF TGF-ß/BMP SIGNALING PATHWAY INHIBITORS

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Cystic echinococcosis (CE) treatment urgently needs a new drug. Understanding molecular regulation is essential for identification of drug targets. In the present study, we identified two new members of the Smad proteins, Smad8 and Smad4 homologues (termed as EgSmad8 and EgSmad4, respectively) from Echinococcus granulosus, the causative
agent from CE. Both EgSmad8 and EgSmad4 were co-expressed in the larval stages and their encoded proteins were localized in the subterminal and tegumental layer of the parasite. As shown by yeast two-hybrid and pull-down analysis, EgSmad8 displayed a positive binding interaction with EgSmad4 and a previously identified EgTβR I. In addition, EgSmad8 localized in the nuclei of Mv1Lu cells (mink lung epithelial cells) upon treatment with human TGF-β1 or human BMP2, indicating that translocation of EgSmad8 into nuclei depends upon signals initiated by the ligands of human origin. When LDN193189, an inhibitor of TGF-β/BMP type receptors, and SIS3, an inhibitor of R-Smads, were added to the medium for in vitro cultivation of E. granulosus protoscoleces, these inhibitors had actually an inhibitory and cytolytic effect on the larval stage of the parasite. Our study indicates that small inhibitors of TGF/BMP signaling pathway may be potential drugs for the treatment of CE.

### NEW ULTRASONOGRAPHIC CLASSIFICATION OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

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Ultrasonography provides one of the most important diagnostic tools in suspected alveolar echinococcosis. The aim of the study was to establish a new sonographic classification based on a large patient population with confirmed hepatic alveolar echinococcosis. In 225 patients, ultrason angography of liver lesions due to an alveolar echinococcosis was retrospectively examined. The findings were grouped into the new classification scheme. The following classification have been established: storm and hail pattern, pseudo hemangioma-like pattern, ossification pattern and metastasis-like pattern. The respective classification patterns are demonstrated by imaging examples. The proposed ultrasonographic classification improves the diagnosis of hepatic alveolar echinococcosis. This makes it possible to interpret different clinical courses better and helps in the context of scientific studies to improve the comparability of ultrasonographic findings.

### HYDATID DISEASE IN IRAQ: A RETROSPECTIVE STUDY OF 1,980 PATIENTS IN BAGHDAD

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Human hydatid disease caused by Echinococcus granulosus has been endemic in Iraq for many years. Migration of people and intermediate hosts over time can impact the distribution and epidemiology of this disease, especially in developing countries and in times of conflict. Observations made on the clinical services that care for hydatid patients in Baghdad posed the question of whether there is an age-related association with the location of cysts in various organs. Therefore to address this question we conducted a retrospective chart review of 1,980 patients with hydatid cyst admitted to three major hospitals in Baghdad, Iraq. In particular we noted the age at which the patient presented and the location of hydatid cysts diagnosed radiographically or surgically. The majority of patients with hydatid disease of the body were between 20 and 40 years old. In our series, we observed that abdominal hydatid was seen in 66.7%, liver (54%), spleen (8%), pancreas (2.5%), chest (29%), lungs (30%), pleura (0.2%), pericardium (0.1%), mediastinum (0.4%), bone and spine (30-39 years). Brain and orbit disease was most common in the 5-19 year old age group. These observations suggest that several possible explanations for the majority of brain/orbit disease in young people. The age and/or immune status when hydatid disease is acquired by humans may impact the ultimate location of cysts in the body. These observations may lead to new insights into the pathogenesis, diagnosis and treatment of human hydatid disease.

### PREDICTION AND IDENTIFICATION OF ANTIGENIC EPITOPES IN EG95 OF ECHINOCOCCUS GRANULOSUS

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This study was undertaken 1) to predict the T and B union epitope of the Eg95 antigen of Echinococcus granulosus, 2) to obtain the different peptide fragment of T-B union epitope via Phage display technology, 3) to identify and screen the peptide fragment via Western Blot and ELISA, and 4) to provide the basis for the T and B union epitope vaccine's manufacture of Echinococcosis. Online networks BCPred and LEPS predicted the B cell epitope of Eg95 antigen. Using SYPEITHI software predicted the MHC-I nonamer T cell epitope of HLAA 0201 and H2-Db. To analyze the secondary structure, transmembrane structure, B cell epitope, T cell epitope of humans and mice. Finally, determine the area of B cell and T cell union epitope. Using DNAman software design primers, amplified amino acid sequence of T-B cell union epitope, then construct the gene and M13KE Phag carrier using T4 Ligase. Constructed M13KE/Eg95-1 M13KE/Eg95-2 and M13KE/Eg95-3 plasmid then transformed into E.coli ER2738 respectively, identified the correct sequence using PCR. Purified the recombinant phage by PEG/NaCl way of precipitation. Identified the expression level of recombinant protein rPIII by SDS-PAGE electrophoresis analysis. Prepared the rEG95 patient serum and antiserum as antibody, confirmed the correctness of epitope peptide antigen by Western Blot. Finally, compared with epitope peptide antigen's reactivity by ELISA, screened and confirmed better of antigen epitope peptide as candidate epitopes of epitope vaccine of Echinococciosis. Predicted the advantage of Eg95 antigen epitope by line through using the bioinformatics method, and confirmed 3 T-B union epitope. To successfully cloned and constructed the prokaryotic eg95-3 expression plasmid M13KE/Eg95-1 M13KE/Eg95-2 and M13KE/Eg95-3 plasmid and transformed into E.coli ER2738 respectively, identified the correct sequence using PCR. Purified the recombinant phage by PEG/NaCl way of precipitation. SDS-PAGE assay showed that Eg95-1, Eg95-2, and Eg95-3 protein III was successfully expressed. Echinococcosis patient and anti-rEG95 serum can identify the 3 T-B union epitope, but the signal intensity was differences.

### HUMAN CYSTIC ECHINOCOCCOSIS IN SUDAN: A SILENT HEALTH THREAT

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Cystic echinococcosis (CE) is a zoonosis caused by cestodes of the genus Echinococcus. The adult tapeworms are intestinal parasites of dogs and some other carnivores which require a larval tissue stage (hydatid cyst) in other animal species (intermediate hosts). This larva usually grows in the form of a large, fluid-filled cyst (“cystic echinococcosis”), which can affect many organs, most often liver and lungs. In humans, CE causes significant morbidity, largely depending on the size and localization of the cyst(s). Treatment requires abdominal or pulmonary surgery or other invasive procedures (percutaneous treatment techniques) in combination with
prolonged chemotherapy, which is costly and often not available in remote areas. The economic burden of CE is caused by human mortality and disability. The transmission of the parasite to humans is facilitated by the presence of dogs and livestock in conjunction with slaughtering practices (feeding of raw offal to dogs) and poor hygiene. CE has a particular impact in developing countries and in terms of global disease burden (in DALYs), it is close to African trypanosomosis and schistosomosis, zoonoses which receive far more attention in terms of research and control. Prevalence levels of CE can be >5% in the nomadic or semi-nomadic ethnicities of northern Kenya or southern Sudan and yet not exactly determined in other parts. The access to therapy for this particular part of the population is usually sporadic, and established procedures for treatment are not always feasible under the local conditions. Preventive programs are difficult to implement and sustain over sufficiently long periods due to the lack of infrastructure and/or financial resources. It is important to have a clear picture about the disease in the country to enable its control regardless of all these difficulties, thus study aims at highlighting the prevalence, diagnosis and distribution of cystic echinococcosis in patients distributed in different parts of the Sudan since the disease was first reported in 1908.

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TH1 /TH2 AND TH17/TREG IMMUNE RESPONSE IMBALANCE IN PATIENTS WITH HEPATIC ALVEOLAR ECHINOCOCCOSIS

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Alveolar echinococcosis (AE), caused by the larval stage of echinococcus multilocularis (E.m), continues to be a worldwide public health problem. During the infection, E.m interacts with and modulate host’s immune system for its successful survival. Herein, we have comprehensively studied T cell subsets immune response profile in patients with AE. Total number of 55 subjects were enrolled and divided into three groups: AE group, (n=15), AE with albendazole treatment (AE+ABZ, n=14), and healthy controls (HC, n=26). Th17 and Treg cells frequencies in peripheral blood mononuclear cells (PBMCs) were measured by flow cytometry. The mRNA expression levels of T-bet, GATA3, RORç, and FoxP3 were measured by RT-PCR. Plasma level of IFN-γ (Th1), IL-5 and IL-6 (Th2), IL-17A, IL-17F, and IL-23 (Th17); and IL-10 (Treg) were detected by ELISA. Result showed that the Th17 cell frequency significantly decreased in AE group, however it is remarkably increased in AE+ABZ group than that in HC group; T-bet mRNA expression in AE group is lower than HC group, while, higher in AE+ABZ group; GATA3 mRNA expression is slightly increased in AE group, however, it is significantly decreased in AE + ABZ group; RORç mRNA expression level decreased in both group than that in HC group, moreover, a slight increase showed in AE +ABZ group than AE group; FoxP3 mRNA expression level slightly increased in AE group than HC group, however, it is decreased in AE + ABZ group. Plasma level of IFN-γ slightly decreased in AE, and increased in AE + ABZ group compared to HC group; Levels of plasma IL-5 and IL-6 slightly increased in AE group, whereas, it decreased in AE +ABZ group than that in HC group; IL-17A, IL-17F, and IL-23 significantly increased in both group than that in HC group, however, the levels are lower in AE + ABZ group; Plasma levels of IL-10 are increased in both AE and AE + ABZ group, however, the level was lower in AE +ABZ group. Our results demonstrated that there is Th1 / Th2, and Th17 / Treg immune response imbalance exist during the infection, this imbalance may play a potential role in parasite immune evasion and immune-pathogenesis of hepatic alveolar echinococcosis.

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EXPRESSION OF TOLL-LIKE RECEPTOR 2, 4 AND 7 IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH HEPATIC CYSTIC AND ALVEOLAR ECHINOCOCCOSIS

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This study aims at to explore expression of Toll-like receptors (TLRs) and related cytokines in patients with cystic echinococcosis (CE) and alveolar echinococcosis (AE). 55 subjects were enrolled and divided into three groups: AE group (N=15), CE group (N=14), and healthy controls (HC, N=26). The mRNA expression levels of TLR2, TLR4 and TLR7 in peripheral blood mononuclear cells (PBMCs) were measured by using real-time fluorescent quantitative reverse-transcription polymerase chain reaction (RT-PCR). Plasma levels of INF-γ, IL-5, IL-6, IL-10 and IL-23 were detected by using ELISA. Values are expressed as mean ± SD and the data were analyzed by ANOVA. If significance was found, Newman-Keuls test was performed for post-hoc analysis to detect the difference among groups. TLR2 mRNA expression was significantly increased in the CE and AE groups compared to the HC group (P<0.05). TLR4 mRNA expression was higher in the CE and AE groups and statistical significance was only shown in CE group (P<0.05). However, TLR7 mRNA expression was remarkably decreased both in CE and AE groups with statistical significance. Plasma levels of IL-10 and IL-23 in patients with CE and AE were significantly higher than those in controls (P<0.05). Levels of serum IL-5 and IL-6 CE and AE group were higher than those in HC group with no statistical significance (P>0.05), IFN-γ level was slightly decreased in AE group, oppositely, increased in CE group with no statistical significance. Expression pattern of TLR 2, 4 and 7 in PBMCs in patients with AE and CE might be involved in the cytokine modulation, which allowed the parasite to escape, which seems to be stronger in AE, host immune-surveillance and promoted chronic infection.

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NEUROCYSTICERCOSIS: AN UNKNOWN, A FORGOTTEN, OR A NEGLECTED PARASITIC ZOONOSIS IN NIGERIA?

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Epilepsy or seizure is among the most common neurological disorders all over the world, including Nigeria and the aetiology may vary. However, the International League Against Epilepsy recognizes neurocysticercosis as a growing problem in many tropical countries and as a leading cause of epilepsy and seizures. Neurocysticercosis (NCC) is an infection of the central nervous system (CNS) caused by the metacestode stage (Cysticercus cellulosae) of the pig tapeworm of man Taenia solium. It is probably the most common helminth parasite incriminated in central nervous system parasitic infections in human beings. T. solium taeniasis and therefore, NCC are common in many of the world’s poorer countries especially where the environmental hygiene is poor and families raise free-roaming pigs that have easy access to and consume human faeces. In Nigeria, T. solium cysticercosis is common in pigs but there is little information on human T. solium taeniasis and virtually none on human T. solium cysticercosis (ocular- and neuro-cysticercosis). Conversely, epilepsy is the commonest neurological condition diagnosed in adults in most Teaching Hospitals in Nigeria but whether it could be attributed to neurocysticercosis is not clear. This paper examines neurocysticercosis vis a vis epilepsy and seizures and draws attention to this important zoonosis which it seems in Nigeria is an unknown, a neglected or a completely forgotten major causative agent of neurological conditions.
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RE-EVALUATION OF PORCINE CYSTICERCOSIS IN NSUCCA AREA OF ENUGU STATE, NIGERIA

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The prevalence of porcine cysticercosis in Nsukka Area of Enugu State, Nigeria was re-evaluated between February and June, 2013, using a structured questionnaire and post-mortem examination of slaughtered pig carcasses at Ibagwa, Orba and Nsukka slaughter slabs. Questionnaires were distributed to willing butchers and pig marketers and completed copies retrieved and analysed. The slabs were visited every other week and pig carcasses examined in accordance with standard meat inspection procedures. Brieﬂy, carcasses were examined visually under natural light and palpated before longitudinal incisions were made on the heart, tongue, masseter, neck, intercostal, shoulder and thigh muscles and examined. Carcasses containing cysticerci were recorded as positive for Cysticercus cellulosae. The sex, age and breed of all animals examined were determined. Results showed cysticercosis prevalence of 3.3%, 4.3% and 0% for Ibagwa, Orba and Nsukka slaughter slabs respectively and an overall prevalence of 2.4% for the three study areas during the period. Moreover, the type of pig husbandry practiced was the most important factor inﬂuencing the prevalence of the infection. Analysis of the questionnaire responses showed that the majority of the respondents were not aware of the zoonotic implication of porcine cysticercosis irrespective of their educational background and that epilepsy in the family could be associated with the infection in man. It is concluded that Taenia solium cysticercosis remains endemic in Nsukka area of Enugu State, Nigeria and poses a major health hazard that must be addressed by coordinated control programs.

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EVALUATION OF TRICLABENDAZOLE AGAINST TAENIA SOLIUM METACESTODE IN NATURALLY INFECTED PIGS

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Taenia solium cysticercosis is an important zoonotic disease in developing countries. Many studies have been conducted to evaluate the efficacy of different antiparasitic drugs against the larval stage (cysticercus) of T. solium. Currently, oxfendazole (OFZ) is the best drug against porcine cysticercosis, with excellent efficacy using a single oral dose. On the other hand, triclabendazole (TCZ) is the most common drug used to treat livestock helminthiases. Therefore, the aim of this study was to evaluate the efficacy of TCZ in naturally cysticercosis-infected pigs. Eighteen pigs were divided into 3 groups of 6 individuals each. The groups were treated as follows: a first group was treated orally with TCZ at a single dose of 30 mg/kg of body weight, the second group was treated orally with OFZ at a single dose of 30 mg/kg of body weight and the third group was left untreated (control). All animals were kept under the same management conditions. The pigs were humanely killed 17 weeks post-treatment and the number of surviving cysts in muscles was assessed. Cysts in pigs treated with TCZ had a normal appearance, not different from the control group (p>0.05). All pigs treated with OFZ had only degenerated cysts in their carcasses (p<0.05 for parasiticidal efficacy compared with the control group). TCZ is not efficacious to treat porcine cysticercosis.

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TAENIA SOLIUM GENOTYPES IN A RURAL COMMUNITY: RELATIONSHIP BETWEEN PORCINE CYSTICERCOSIS AND HUMAN TAENIOSIS

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Taenia solium is a cestode that infects pigs and humans in different regions of the world and is cause of neurocysticercosis in humans. The understanding of the association between the genetic variability of the parasite with geographic origin and phenotypic characteristics like infectivity, pathogenicity and response to treatment, will be a critical tool in the efforts to prevent and control this disease. After analyzing the entire T. solium genome, we demonstrated that microsatellite markers are widely distributed throughout of the genome. In addition these markers have shown to have enough variation to differentiate strains. The objective of this study is to use microsatellite markers and to compare the genetic proﬁles between cysts recovered from naturally infected pigs and tapeworms from human hosts in a rural community in Northern Peru. We performed a cross sectional study where four tapeworms were genotyped and showed different proﬁles. All pigs in the community were screened for cysticercosis, using the tongue test. We identiﬁed 8 cysticercosis-tongue-positive pigs. Animals were humanely sacriﬁced and 30 individually cysts were recovered from the carcass of each pig. Cysts were genotyped with the same microsatellite markers and compared to the genotypes of the previously identiﬁed tapeworms. We found genetic variation among this population. Two pigs had about 30% of cysts with a genotype that exactly matched to the tapeworm corresponding to the geographically closest human host. Interestingly, one pig showed 50% of cysts with a genotype different to any of the tested tapeworms. We propose microsatellites as promising genetic markers with which it would be possible to study the genetic epidemiology of this disease, in particular the transmission from tapeworm carrier to pig and the reintroduction of foreign cysts/tapeworms into a community. We are conducting additional studies to verify the source of genetic variation of microsatellites in T. solium cysts.

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PURIFICATION OF VESICULAR FLUID ANTIGEN OF CYSTICERCUS TAENIA SOLIUM, FOR HUMAN DIAGNOSIS IN THE ANDEAN TRAPEZIUM AND UNASUR COUNTRIES

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Neurocysticercosis (NCC) is a neglected parasitic disease and a leading cause of seizures and epilepsy in developing countries. Serological assays detecting T. solium antigens complement standard imaging techniques to establish NCC diagnosis. Crude antigens extracted from the vesicular fluid of T. solium cysticerci (Tsol/VFag) have been used for this purpose; however, the availability of technology and cost required to purify is limited in developing countries. We compared and evaluated four puriﬁcation methods to obtain T. solium antigens for serological assays. Cysticerci were collected from different endemic areas of Peru and Tsol VFag were puriﬁed using the following methods: i) ammonium sulphate, ii) lentil-lectin sepharose (affinity chromatography), iii) sephadex G-75, and iv) electro-elution. The analytic sensitivity and speciﬁcity were evaluated using patient serum from individuals with NCC and other parasitosis. The crude antigenic protein concentration was 3.6μg/μL which is within the range as suggested by CDC and also SDS-PAGE electrophoresis revealed seven glycoproteins (GP50, GP42-39, GP24, GP21, GP18, GP14 and GP13kDa) with aﬃnity/lentil-lectin method. Our research identiﬁed eight
bands diagnostics with affinity/lentil-lectin method (GP35, 31, 24, 23, 18, 17, 14 and 13kDa.), while the other three methods detected different fractions of those proteins (ammonium sulphate: no band, electro-elution: 6 bands of 97-GP12kDa, and sephadex G-75: 17 bands of 100-GP6kDa). Western blot using purified glycoprotein (affinity/lentil-lectin method) identified 50/50 patients with NCC (100% sensitivity), 50/50 healthy control individuals (100% specificity), 20 individuals with other parasitosis (Hymenolepis nana, Echinococcus granulosus, Fasciola hepatica) give cross-reacted with the GP42-39kDa protein. Affinity chromatography (lentil-lectin sepharose) is the method of choice to purify glycoproteins from 

\textit{T. solium} cysterci for the development of a reliable and affordable immunodiagnostic kit for NCC which can be applied in the Andean trapezium and UNASUR countries.

**454 EPILEPSY AND CYSTICERCOSIS IN THE HIGHLANDS OF MADAGASCAR: IS SEROLOGY RELEVANT?**

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In sub-Saharan Africa epilepsy reflects complicated delivery and sequelae of infections during childhood. However in rural countries like Madagascar the profile of epilepsy is mostly unknown. In the same time, Madagascar is one of the hotspot of cysticercosis and its burden during epilepsy is unknown. To describe this profile and relation with cysticercosis a survey was conducted on epileptics in 40 dispensaries of the Moramanga district, a rural area and in two private hospitals in Antsirabe (a small town near the capital). Patients older than 5 years were recruited after at least two unexplained seizures. For each patient recruited in dispensaries a control subject matched on age was recruited in household randomly selected in the same village. Socioeconomic and clinical data were collected. Serology for cysticercosis was conducted using glycosylated crude proteins of the cysticerco in ELISA and western blot. A total of 247 subjects were investigated (125 epileptics and 122 controls). No type of seizure was associated with positive serology for cysticercosis. Seroprevalance was the same for epileptics and controls (49.5% / 50.4%). It was higher in rural than in urban area, and for female than for male. However epileptics have higher ELISA mean OD than controls (0.461 vs 0.26), and younger epileptics have higher OD than older ones. In univariate analysis higher prevalence of cysticercosis in epileptics was associated with the number of children in the household and the use of backyard wells for water supply, but not with the presence of pigs. In multivariate analysis age, gender, urban/rural habitat and presence of pigs were retained to explain epilepsy but not seropositivity for cysticercosis. For seropositivity only the absence of toilets and the number of children in the household were retained. Over all poor access to sanitation and the number of children in the household were the major parameters associated with cysticercosis, undoubtedly in relation with taeniasis. However in this area, serology for cysticercosis is not relevant for diagnostic.

**455 MONOClonal antibodies for detection of parasite antigens in body fluids from patients with neurocysticercosis**

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Neurocysticercosis (NCC), an infection of the brain by 

\textit{T. solium} cysts, represents the most common cause of adult-onset epilepsy in developing countries. Serologic diagnosis has historically been based on detection of antibodies to 

\textit{T. solium} in serum, but antigen (Ag) detection assays using T. saginata cross reactive monoclonal antibodies (MBAs) have been applied to serum and body fluids more recently to identify individuals with live parasites. We generated and characterized 22 murine monoclonal antibodies against 

\textit{T. solium} cysts in BALB/c mice using whole cyst (WA), vesicular fluid and excretion/secretion Ags of 

\textit{T. solium} cysts as immunogens. Using these MBAs in an enzyme-linked immunosorbent assay (ELISA) format, we developed an Ag capture assay for antigen detection in body fluids. Only one of 20 MBAs tested demonstrated weak cross-reactivity to Echinococcus granulosus Ags. Localization of the target Ags on the parasite was performed by immunofluorescence and immunohistochemistry on histological sections of muscle cysts from naturally infected pigs showing reactivity to different anatomical locations on the parasite: 1 MAb was reactive to the neck and fluid spaces, 4 to the neck alone, 9 to the cyst wall and neck, and 6 to the cyst wall, neck and fluid space. Pooled samples of serum and urine from patients with NCC and negative controls were evaluated by the Ag capture ELISA, using the MBAs as a capture antibody and a polyclonal rabbit anti-

\textit{T. solium} WA antibody for detection. ELISA results show that 7 MBAs could detect antigens in serum and 3 in urine samples. Reactivity of these MBAs expressed as normalized ratios of optical densities (OD positive control/OD negative control) show that 3 MBAs had ratios >20 and 4 between 5-20. These results suggest that these monoclonal antibodies have potential utility for the diagnosis and follow up of treatment in NCC patients. Use of MBAs for detection of different parasite Ag in the same patients may provide insight into the kinetics of antigen release in relation to parasite damage during treatment in NCC.

**456 GENOTYPING OF TAENIA SOLIUM**

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Human taeniasis/cysticercosis due to 

\textit{Taenia solium} remains a serious public health problem particularly in developing countries. Genetic variability among 

\textit{T. solium} is limited because the nature of the adult tapeworm. The objective of the present study was to develop a genetic tool to genotype tapeworm isolates. The internal transcribed spacers (ITS1 and ITS2) including the 5.8S gene of the ribosomal genes were amplified by PCR. The amplified products were then subjected to restriction enzyme analysis (REA). Restriction products were further analyzed by gel electrophoresis, different genotypes were analyzed and compared using PAUP 4 and MEGA 4 softwares. A total of 78 tapeworm isolates were analyzed, two distinct genotypes were observed when Alu I and Msp I were used, while two others genotypes were observed when Hae III and Mbo I were employed. Analysis of ribosomal spacer sequences might be an important tool to differentiate 

\textit{T. solium} isolates.
EVALUATION OF A MONOCLONAL ANTIBODY TO DETECT OF URINE ANTIGENS FOR THE DIAGNOSIS OF NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), a zoonotic disease caused by the larval stage of the parasite Taenia solium, is considered the main cause of acquired epilepsy around the world. Computed tomography (CT) and Magnetic resonance imaging (MRI) are the standard neuroradiographic techniques for the diagnosis of NCC. Diagnosis of NCC is supported by immunological testing, through antibody detection by Enzyme-linked immunoelectrotransfer blot (EITB) assay, and recently by antigen detection using an enzyme-linked immunosorbent assay (ELISA). Detection of antigens either in serum, cerebrospinal fluid or urine may be an indicator of active infection. In this study an ELISA was developed using both monoclonal (for detection) and rabbit polyclonal antibodies (for capture) raised against a crude T. solium cyst extract, and evaluated for its capacity to detect antigens in urine samples of patients with NCC. Urine samples from 108 patients with diverse types of neurocysticercosis (n=61) and from individuals negative for NCC by CT scan and EITB (n=47) were processed by Ag-ELISA. Fifty-three of 61 NCC positive samples were Ag-ELISA positive. All patients with subarachnoid NCC were Ag-ELISA positive. Among 8 individuals with NCC who tested negative by the Ag-ELISA, 3 patients presented only viable cysts with no inflammation (2-3 cysts). All NCC negative patients gave a negative result by the Ag-ELISA test. Overall the Ag-ELISA test demonstrated 87% sensitivity and 100% specificity. Additional modification of antibodies such as conjugation of MABs to biotin might further improve sensitivity of the test.

PRESENTATION AND MEDICAL TREATMENT OF SPINAL NEUROCYSTICERCOSIS

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Spinal cord involvement is noted in about 1-3% of cases of neurocysticercosis (NCC) patients. Leptomeningeal spinal NCC, evident in about 80% of spinal cases, usually presents as an extension of the subarachnoid disease of the brain (SANCC). The cysts are thought to migrate downward from the basilar cisterns eventually become fixed at one level and can be accompanied by inflammation resulting in arachnoiditis and dural thickening. Surgical treatment is most commonly reported in the literature. This is a large series of medically managed spinal NCC cases. We performed a retrospective review of spinal NCC seen in Jacobi Medical Center's Tropical Medicine Center between the years 2000 and 2014. Eight cases were identified. The median age was 35.5 (range 28-65), 7 males and 7 were born in Latin America and median time from immigration was 8 years (1-18). Six patients had concurrent SANCC of the brain, 1 had a 4th ventricular cyst and 1 was a primary spinal case. Location of lesions was: cervical (62.5%), thoracic (37.5%), lumbar (75%). Four had cysts and all had arachnoiditis and hydrocephalus at presentation. Symptoms in descending order of frequency were: headache (75%), seizures (50%), radicular pain (37.5%), extremity weakness (37.5%), and back pain (25%). The median time for treatment with albendazole and steroids was 58 weeks (12-157). Aseptic meningitis was seen in 62.5% of the patients. At the end of treatment only 1 resolved completely, the other 7 had never clumping and of these 5 had enhancement. One patient continued to have cysts and he was actively being treated. Only half improved their clinically. Spinal NCC is commonly associated with SANCC and requires prolonged medical therapy. In this series the clinical presentation was due to both brain and spinal involvement. Patients presenting with aseptic meningitis with hydrocephalus from an endemic region should be evaluated for both spinal and SANCC. Radiographic improvement was seen, but residual nerve clumping may account for the persistent clinical symptoms.
BIOMARKER-BASED ASSOCIATION MODELS FOR PREDICTING COGNITION DEFICITS IN CASSAVA CYANOCENIC POISONING

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While risk factors for konzo are known, determinants of cognitive impairment in konzo-affected children remain unknown. We anchored cognitive performance (KABC-II scores) to serum levels of free-thyroxine (free-T4), thyroid-stimulating hormone (TSH), albumin, and motor proficiency (BOT-2 scores) in 40 children including 21 with konzo (median age: 9 years) and 19 without konzo (median age: 8 years). A multiple regression model was used to determine variables associated with changes in KABC-II scores. The results were: Age (-: -0.818, 95%CI: -1.48, -0.152) (p=0.018), gender (-: -5.72, 95% CI: -9.87, -1.57 for females) (p=0.009), BOT-2 score (-: 0.390, 95% CI: 0.113, 0.667) (p=0.008), and free-T4 (-: 0.81, 95% CI: 0.009, 3.74) (p=0.049) explained 61.1% of variation in KABC-II scores. Subclinical hypothyroidism was not associated with poor cognition. A crude association was found between serum albumin and KABC-II scores (-: 1.26, 95% CI: 0.136, 2.39) (p=0.029). On spot urinary thiocyanate reached 688 µmol/l in children without konzo and 1032 µmol/l in those with konzo. In conclusion, female gender and low serum albumin are risk factors common to cognitive and proportionally associated motor deficits in children exposed to cassava cyanogens. The two types of deficits may share common mechanisms.

EASE OF USE AND SAFETY OF A CONICAL CUP BLOOD TRANSFER DEVICE FOR USE WITH RAPID DIAGNOSTIC TESTS FOR HUMAN AFRICAN TRYPANOSOMIASIS (HAT)

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Rapid diagnostic tests (RDTs) are increasingly being used for screening and diagnosis of infectious diseases in remote settings. Appropriate devices to safely transfer fixed amounts of blood from finger pricks to RDTs pose a significant challenge. Recently, a 5-µL inverted cup was evaluated and found to be the most acceptable to health workers (HWs) in terms of safety, ease of use and accuracy of volume transferred when used with malaria RDTs. The success of this device and its subsequent uptake by various manufacturers led to the development of a 23-µL conical cup for RDTs requiring higher volumes of blood. This study compares this conical cup with a plastic pipette when used for screening for Human African Trypanosomiasis (HAT) infection with the SD BIOLINE HAT RDT in Yumbe and Arua districts in Northwestern Uganda. After a half-day of training, conical cups and plastic pipettes were given to 49 HWs who used them for testing of suspect patients who voluntarily accepted to participate in the study. Each HW used the blood transfer devices with at least 10 patients in the field. Questionnaires and focused group discussions were used to gather information on ease of use, blood safety and acceptability. Preliminary results show that HWs generally preferred the conical cup over the plastic pipette. A higher rate of successful blood transfers and fewer occurrences of blood safety issues were observed when using the conical cup. Results of this study not only demonstrate the ease of use of a new blood transfer device for RDTs for HAT and other diseases such as HIV, but also raise awareness about the need for further improvements in BTDs for the use of RDTs on hands of health workers in remote areas. Additional and more detailed results will be presented during the meeting. Final results will be available by the end of April.

THE EFFECT OF MAGNESIUM SULPHATE ON AUTONOMIC DYSREGULATION IN ENTEROVIRUS 71 RELATED HAND FOOT AND MOUTH DISEASE IN VIETNAMESE CHILDREN

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Enterovirus 71 (EV71) associated hand foot and mouth disease (HFMD) has emerged across much of Asia as a serious infection of young children during the last decade. HFMD predominantly affects children under 5 years of age, with a clinical spectrum varying from a relatively mild self-limited febrile illness with a rash, to fatal cardiopulmonary collapse. An outbreak of HFMD in 2011 in southern Vietnam resulted in over 110,000 reported cases and 116 deaths, and epidemiological studies from neighboring China report over 7 million HFMD cases between 2008 to 2012, with 2,457 deaths. Autonomic nervous system (ANS) dysregulation is considered as the main indicator of progression to severe disease, initially manifesting with hypertension, and later progressing to cardiopulmonary failure and death in a small proportion of cases. Although lacking a formal evidence base, current guidelines in many Asian countries suggest milrinone as the first choice for management of ANS related hypertension in patients with severe HFMD. Based on clinical experience and evidence from a randomized controlled trial magnesium sulphate (MgSO4) has become the mainstay of treatment for ANS dysregulation in patients with tetanus in many centres. We describe a case series involving 10 EV71 confirmed HFMD cases with ANS dysregulation managed at HTD over a 4 month period in early 2012. Magnesium sulphate was added when hypertension remained poorly controlled despite high dose milrinone (up to 0.75 ug/kg/minute), and in all cases the blood pressure reduced within 30-60 minutes and remained stable subsequently on a continuous magnesium infusion for 48-72 hours. No patient required hemofiltration, although 2 of 10 cases were ventilated because of respiratory distress. Brain MRI was performed later in 4 of these cases and in the 2 children with neurological sequelae abnormalities were found, involving the medulla in both cases, and with extensive atrophic changes in one child. These promising initial results are currently being investigated in a formal randomized controlled trial of magnesium sulphate versus placebo in Vietnamese children with severe HFMD and signs of ANS dysregulation.
conducted a clinic based retrospective cohort study that involves review of data from Pre-ART adult clients ≥15 years) register and patient treatment card number 2 (CTC2 Card) at three CTCs in Morogoro from July, 2010 to July 2011. Pre ART clients who were not in care at their original sites at 1 year of follow up were traced through home based care volunteers and phone calls. Relative risk was then calculated using Epi info statistical soft ware.A total of 351 CTC clients were enrolled between July 2010 and July 2011. Most enrolled clients were ART eligible 161(45.9%) of whom 92(57.1%) were initiated on ART. Despite being ART eligible 69 (42.9%) of enrolled clients were not Initiated on ART. Mortality among those not initiated ART was 47 (17.6%) of whom 30 (63.8%) were ART eligible. Majority 259 (66.7%) of enrolled clients had no ART start Status, of the 259 clients status of 25(9.6%) could not be determined until the end of the study. 67 (25.9%) were confirmed not to be in care at one year of follow up due to different reasons such as death, opt out and some were reached and promised to return to care. 67 (25.95) were pre ART and still attending at their original clinics whereby 80 (30.9) were in care with other providers. Being enrolled in care at an advanced disease stage, paying for transport to go to the clinic and stigma were risk factors for Pre ART attrition. Attrition due to mortality is high among Pre ART clients who are enrolled in CTC while already in advanced disease stage. Attrition from clinic is higher than attrition from care. Self stigma and advanced disease stage were pre ART attrition risk factors. Status disclosure and sensitization to reduce stigma as well as Strengthening of CTC to improve linkage and referrals between CTC and other clinics as well as prioritizing ART initiation among clients who are ART eligible is recommended.

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COMMON CLINICAL COMPLICATIONS OF HUMAN MONKEYPOX INFECTION AT THE GENERAL HOSPITAL OF KOLE, IN DEMOCRATIC REPUBLIC OF CONGO


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Monkeypox (MPX) virus is an orthopoxvirus that is endemic in central and West Africa, causing a smallpox-like disease ranging from subclinical to severe cases. With the cessation of smallpox vaccination in 1982, rural populations in central Africa were increasingly susceptible to MPX infection due to waning herd immunity to poxviruses. We have observed an increase in severe clinical manifestations of MPX in both children and adults, leading to more frequent clinical complications. Most of these complications are moderate requiring nurse care and prolongation of the hospitalization, however, severe complications can be fatal (death, miscarriage) or lead to grave sequels (blindness), albeit rarely. To describe frequent and severe clinical complications observed in human subjects with MPX infection at the Kole General Hospital, one of the most remote Ministry of Health Hospital in the rain forest of the Sankuru District, in Democratic Republic of Congo (DRC) where the majority of the population relies on bush meat as a principal source of protein. From 2007 to 2011, we conducted an observational study of 229 cases of human MPX infection in order to describe its clinical and biological characteristic as it occurs in the DRC. Clinical signs, symptoms, and laboratory results were recorded during their hospitalization. Clinical complications observed during this study were identified and recorded. Miscarriage was the most common complication in infected pregnant women (3/4) with evidence of fetal contamination, followed by secondary dermatitis on infected skin lesions (30/229), death (4/229), abscess of cervical lymph node (6/229), keratitis (3/229), staphyloma occurring approximately 20 months later after the onset of keratitis, and caseation of eye lesions (1/229) in confluent lesions spreading in the sclera. Clinical complications in human MPX infection are common in DRC, which is likely attributable to the decline of cross-protective immunity after cessation of smallpox vaccination and the subsequent increase in the susceptible population in endemic regions.

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INTRAVENOUS ARTUSENATE VS. QUININE IN THE TREATMENT OF SEVERE MALARIA IN LIBERIAN CHILDREN

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Liberia is a West African Country whose health care system is recovering from years of civil war. Liberia is endemic for malaria and recent reports estimate the malaria prevalence to be as high as 32% in children under 5 years of age. Recent data collected at JFK Medical Center, the national referral hospital, shows that 42% of pediatric admissions are for the treatment of severe malaria. The goal of this study was to compare the outcomes of children admitted with severe malaria and treated with either quinine or artesunate, to establish the optimal treatment regimen in this setting. We studied children age 5 and under who were admitted to JFKMC during the time period from May 2013 through October 2013 with a diagnosis of severe malaria. 92 children were treated with artesunate (2.4 mg/kg/dose IV q 12 x 4 doses) and 73 children were treated with quinine (15mg/kg/dose IV q12 x 3 doses). We measured time to symptom resolution, length of hospital stay and clearance of parasitemia for each treatment arm. Of the 165 study participants, the average age was 27 months; 43% were female, 57% were male, and 48% used bednets regularly. 39% had at least one prior episode of malaria. Children treated with artesunate cleared their symptoms sooner (2.08 days) and had shorter hospitalizations (3.56 days) than their counterparts treated with quinine (cleared symptoms in 2.32 days and hospitalized an average of 3.67 days). A larger number of children treated with artesunate had persistent parasitemia on hospital day 3 (6.5%) versus those children treated with quinine (1.3%). These results align well with prior reports of the superiority of parenteral artesunate over quinine for the treatment of severe malaria in children in Sub Saharan Africa. Future studies include following children post hospital discharge to examine clearance of parasites after completion of outpatient regimen, and screening for drug resistance mutations in this population.

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IMPLEMENTATION AND EVALUATION OF A RAPID ASSESSMENT CLINIC FOR FEBRILE RETURNED TRAVELERS IN AMBULATORY TROPICAL MEDICINE

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Fever in the returned traveler is a medical emergency warranting prompt medical attention to exclude potentially life-threatening illnesses such as malaria. However, prolonged evaluation in the Emergency Room (ER) may not be required for all patients. As a quality improvement initiative, we designed and implemented an algorithm for rapid assessment of febrile travelers (RAFT) in an ambulatory tropical medicine clinic. Criteria for referral to the RAFT clinic include: presentation to the ER, history of and/or documented fever, and travel to the tropics or sub-tropics within the past year. Exclusion criteria include a diagnosis of Plasmodium falciparum malaria and fulfillment of other admission criteria such as unstable vital signs or significant laboratory derangements. We performed a time series analysis pre- and post-implementation of the clinic, with primary outcome of time to definitive tropical medicine consultation. Secondary outcome measures include number of ER visits averted for repeat malaria testing.
and number of ER visits averted for definitive management of an infectious illness. Interim analysis 2-weeks post-implementation indicate a mean time to RAFT clinic assessment of 1.29 days (range 0.2 days) compared to 5.44 days (range 0.26 days) prior to implementation (p=0.15). A total of 7 patients were referred to the RAFT clinic over 2 weeks, thus averting 7 repeat ER visits for follow-up malaria screening. Of 7 RAFT patients, 4 (57%) had an infectious illness that required specific therapy, thus, 4 “call-backs” to the ER over 2 weeks were averted. Extrapolated over 1 year, our interim results suggest that implementation of a RAFT clinic can aver 182 repeat ER visits for follow-up malaria screening, and 104 ER “call-backs” for management of a positive infectious work-up. In addition to provision of more timely care to ambulatory febrile returned travelers, we have demonstrated at our interim analysis that implementation of a RAFT clinic in an urban tertiary care setting can reduce ER visits by 5.5 per week.

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**COST EFFECTIVENESS OF A POINT OF CARE TEST FOR SEPSIS AMONG PATIENTS WITH FEBRILE ILLNESS IN LOW RESOURCE SETTINGS**

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Bacterial sepsis is an important cause of mortality in low- and middle-income countries, yet distinguishing patients with sepsis from others remains a challenge. Currently management decisions are based on clinical assessment using algorithms such as Integrated Management of Adolescent and Adult Illness (IMAI). Efforts to develop and evaluate point-of-care (POC) diagnostic tests for sepsis to guide decisions on the use of antimicrobials are underway. To establish the minimum performance characteristics of such a test, we varied the characteristics of a hypothetical POC test for sepsis required for it to be cost effective. We applied a decision tree model to a population of febrile patients presenting at the district hospital level in a low-resource setting. We compared existing clinical assessment algorithms against the POC test. Patient survival was the outcome of interest. Costs and performance characteristics for the POC test were benchmarked against existing malaria rapid diagnostic tests. Prevalence of bacterial sepsis among febrile patients and outcome data were informed by the literature. We used a case fatality probability of 20% for appropriately treated sepsis and of 50% for inappropriately treated sepsis. A 13.4% prevalence of sepsis among febrile patients seen at the district hospital was assumed. Based on a clinical assessment for sepsis with the established sensitivity of 83% and specificity of 62%, we found a POC test for sepsis with a specificity of 94% and a sensitivity of 83% was cost effective, resulting in parity or equivalence in survival but costing US$2.05 less per live saved than clinical assessment. A POC test with sensitivity and specificity of 100%, equivalent to the best malaria rapid diagnostic tests, yielded an incremental cost effectiveness ratio that compared performance of antimicrobials are underway. To establish the minimum performance characteristics of such a test, we varied the characteristics of a hypothetical POC test for sepsis required for it to be cost effective. We applied a decision tree model to a population of febrile patients presenting at the district hospital level in a low-resource setting. We compared existing clinical assessment algorithms against the POC test. Patient survival was the outcome of interest. Costs and performance characteristics for the POC test were benchmarked against existing malaria rapid diagnostic tests. Prevalence of bacterial sepsis among febrile patients and outcome data were informed by the literature. We used a case fatality probability of 20% for appropriately treated sepsis and of 50% for inappropriately treated sepsis. A 13.4% prevalence of sepsis among febrile patients seen at the district hospital was assumed. Based on a clinical assessment for sepsis with the established sensitivity of 83% and specificity of 62%, we found a POC test for sepsis with a specificity of 94% and a sensitivity of 83% was cost effective, resulting in parity or equivalence in survival but costing US$2.05 less per live saved than clinical assessment. A POC test with sensitivity and specificity of 100%, equivalent to the best malaria rapid diagnostic tests, yielded an incremental cost effectiveness ratio that showed strong dominance, that is being both cheaper and more effective.

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**HIGH INCIDENCE OF SNAKE BITES IN THE UPPER EAST REGION OF GHANA: A CLARION CALL FOR IMPROVEMENT IN SNAKE ENVENOMING OUTCOMES**

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Snake bites and snake envenomning are a common emergency problem in many rural health centres in sub-Saharan Africa. Although this phenomenon is not peculiar to sub-Saharan countries due to the presence of poisonous snakes on every continent and in almost every country, the incidence of snake bites is higher in the inter-tropical world than the temperate regions. Records on snake bites are minimal and scanty and the statistics may be misleading. This study sought to estimate the incidence of snake bites and mortality rates in a savannah farming area in Ghana. A review of records on snake bites in the Regional Hospital, a referral facility of the Upper East Region of Ghana for the year 2013. The Region has a population of approximately, 1,084,621. A total of 146 persons (male: 68/146 (46.6%); female: 78/146 (53.4%)) with snake bites were referred to the hospital out of a total of 94,707 OPD cases for year 2013. The overall snake bites incidence in the region for the year was 13.4 per 100,000 persons. A mortality rate of 7.5% (11/146) was reported (54.5% (6/11) females; 45.5% (5/11) males). 83.2% (129/146) of the snake bites were recorded in the peak farming season of March-August, which is also the major raining season. This study shows a high incidence of snake bites and relatively high mortality. This calls for efficient health care delivery for snake bite victims to improve snake envenoming outcomes.

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**TRAVEL MEDICINE SPECIALIZATION REDUCES MEDICAL ERRORS: CHLOROQUINE PRESCRIPTIONS SIGNAL A BROADER PROBLEM**

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There has yet to be a systematic analysis of antimalarial prescription errors in the United States. Utilizing the U.S. Military Health System’s (MHS) outpatient patient medical records, all chloroquine (CQ) prescriptions from military facilities to the pediatric patients during the period 2006 to 2010 were reviewed. Adherence to destination specific prescribing guidelines from the CDC and MHS was assessed. Records from 547 encounters were reviewed, yielding 440 with destination information. Results were stratified to compare travel medicine clinics vs. primary care clinics. Travel medicine clinics in the MHS are operated by pediatric/adult infectious disease, preventive medicine and allergy & immunology specialties, all others were considered non-travel medicine clinics. Errors were categorized as either contraindicated (CI) for areas with CQ resistance or not indicated (NI) for areas without a recommendation for chemophrophylaxis. Travel Clinics provided 42% (n=185) of patient care. No CI errors were made at travel clinics. Non-travel clinics made CI errors in 6.3% (n=16) (p=0.001) of prescriptions. The overall rate of CI errors was 3.6%. Overall error rates (CI+ NI) for travel clinics was 6.5% (n=12) and non-travel clinics was 11.4% (n=29) (p=0.08) for a weighted average of 9.3% based on proportions of total chloroquine prescriptions. Travel Medicine specialty clinics have significantly lower rates of prescribing errors that place patients at risk for malaria. CQ-NI risks are common across clinic types and offer little to no benefit to the patient and exposes them to increased risk for adverse drug effects. These results suggest other aspects of care may also show disparities in quality of care. Strategies to improve travel medicine care, particularly among non-specialists are needed.

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**HIGH PREVALENCE OF HYPOGLYCEMIA AMONG PEDIATRIC PATIENTS ADMITTED TO A RURAL MOZAMBIAN HOSPITAL AND RISK FACTORS FOR DEATH**

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Hypoglycaemia is a common and life-threatening problem in African children, and is associated with a wide variety of diseases. We describe the prevalence and incidence of hypoglycaemia among admitted African children, establishing risk factors for a poor outcome. We retrospectively reviewed 13 years of systematic clinical data collected through a morbidity surveillance system ongoing in a rural Mozambican hospital. Data from all paediatric admissions were analysed, and independent risk factors assessed for 1) Hypoglycaemia and 2) death in children with
hypoglycaemia. Additionally, we used a glucose sensor to continuously monitor interstitial glucose levels (every 5 minutes) during the first 72 hours of admission for a subset of malaria patients. From January 2001 to December 2013, 45,593 children <15 years had a glycaemia determination performed on admission. 1477 children (3.2%) presented hypoglycaemia (glycaemia <3mmol/L), of which about 2/3 (917) with levels < 2.5mmol/L. Independent risk factors for hypoglycaemia on admission included being male (OR 1.17; p=0.033); having respiratory distress (OR 1.30; p=0.001), anorexia (OR 2.60; p<0.001), a positive blood culture (OR 1.69; p<0.001), malaria (OR 1.41; p=0.001), being a newborn (OR 2.50; p<0.001) or being severely malnourished (WAZ<3SD; OR 1.28; p=0.006). Hypoglycaemic children were significantly more likely to die than normoglycaemic ones (OR 6.8; p<0.001), with an associated CFR of 19.4% (245/1266).

Independent risk factors for death among hypoglycaemic children included having respiratory distress (OR 2.75; p<0.001), anorexia (OR 3.55; p=0.015) and being severely malnourished (OR 5.07; p=0.002).

We also aim to present data on continuous monitoring of hypoglycaemia (first 72 hours) for 80 malaria patients, which suggests that up to a third of admitted children have at least one hypoglycaemia episode detected through continuous monitoring. Hypoglycaemia remains a frequent and hazardous condition for African children, not only on admission but also throughout hospitalization, with many episodes remaining undetected. Symptoms, signs or conditions found to be associated with an increased risk of hypoglycaemia should trigger the verification of glycaemia, and the implementation of life-saving corrective measures.

TRAVEL RELATED VACCINATION COVERAGE RATES IN A COHORT OF DEPARTMENT OF DEFENSE BENEFICIARIES SEEKING PRE-TRAVEL MEDICAL CONSULTATION

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Vaccine-preventable diseases are significant threats to international travelers. We evaluated the rates of travel-related vaccinations in Department of Defense beneficiaries seeking care at military travel clinics who enrolled in the TravMil cohort. 1323 patients were enrolled at 3 military travel clinics; 321 (24%) were active duty personnel, and 1002 (76%) were retirees or dependents. The most common destinations for travel were South/Central America and the Caribbean (31%) followed by Southeast/North Asia (28%) and Africa (28%). The median time between pre-travel consultation and departure was 26 days. Active duty personnel had higher immunization rates prior to travel when compared to retired military and dependents (hepatitis A: 97% vs 66%; hepatitis B: 80% vs 45%; Td/Tdap: 97% vs 74%; influenza: 90% vs 67% (p<0.05 for all)).

The typhoid vaccine was the most common vaccine prescribed; 462 of 478 travelers to high-risk regions in Asia (97%) had current vaccines or were newly vaccinated. 26% (89/343) of at-risk travelers to destinations with endemic Japanese encephalitis (JE) did not receive vaccination, most often due to insufficient time to complete the series in 53% (47/89) of cases. Yellow fever vaccination was not prescribed in 14% (68/482) of patients traveling to endemic areas either due to low risk of exposure or presence of relative contraindications. 97% (156/161) of travelers to meningococcus-endemic regions of Africa received appropriate meningococcal vaccinations. The rabies vaccine was prescribed for 72 (5%) participants, 20 of whom were traveling for > 28 days. No vaccine-preventable diseases were identified in participants during post-travel follow-up visits. Travelers should be encouraged to schedule pre-travel visits at least 6 weeks prior to departure to allow for sufficient time to complete vaccination series such as JE or rabies. The pre-travel visit is a good opportunity for providing routine vaccines to retirees and dependents.

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POTENCY OF POLYVALENT ANTI-SNAKE VENOM SERUM AGAINST VIPERS AND ELAPIDS VENOMS IN GHANA

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Parenteral preparations “Inoserp Pan-Africa” (from Veteria Labs, Mexico for Inssan Biopharma, Spain) for activity against venoms of some snakes common in Ghana was evaluated for safety, potency and sterility. In vitro demonstration of antibody/toxin reactivity using immunological methods and determination of effective neutralization doses, as well as, in vivo demonstration of biological activity by neutralization of snake venom toxins in laboratory mice (ICR strain) were carried out. Results of the experiments revealed that samples of the product submitted for testing are sterile and contains no contaminating microbes. The product contains antibodies that are capable of binding toxins in the venom of the representative Elapidae and Viperidae snake species tested namely Naja nigricollis (Spitting cobra), Bitis arietans (Puff adder) and Echis ocellatus (Saw-scaled viper). The product was thus found to contain antibodies that could neutralize both Elapidae and Viperidae venoms in vivo and thus may therefore be used clinically as post-exposure prophylaxis in snake bite victims.

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COST EFFECTIVENESS OF SURVEILLANCE FOR BLOODSTREAM INFECTIONS FOR SEPSIS MANAGEMENT IN LOW RESOURCE SETTINGS

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Bacterial sepsis is a leading cause of mortality among febrile patients in low- and middle-income countries, but blood culture services are not widely available. Consequently, empiric antimicrobial management of suspected bloodstream infection is based on generic guidelines that are rarely informed by local data on etiology and patterns of antimicrobial resistance. To evaluate the cost-effectiveness of surveillance for bloodstream infections to inform empiric management of suspected sepsis in low-resource areas, we compared costs and outcomes of generic antimicrobial management with management informed by local data on etiology and patterns of antimicrobial resistance. We applied a decision tree model to a hypothetical population of febrile patients presenting at the district hospital level in Africa. Focusing on patient survival as the outcome of interest, we used a probability of death of 20.0% for appropriately treated sepsis and 50.0% for inappropriately treated sepsis. Laboratory and treatment cost data were obtained from Tanzania and costs of antimicrobials were derived from World Health Organization data. Costs were inflated to 2011 U.S dollars. Based on the literature, a 13.4% prevalence of sepsis among febrile patients was assumed. Using susceptibility patterns from bloodstream infection studies in Africa obtained by systematic review, we estimated that 44.0% of organisms were covered by a regimen tailored to local etiology and patterns of antimicrobial resistance of bloodstream infections whereas 12.0% of organisms were covered by a generic regimen. We found that the evidence-based regimen saved an additional 1,067 lives per 100,000 persons with fever at a cost of US$43.14 per life saved. Based on World Health Organization recommendations that interventions below a threshold of US$5,705 per Disability-Adjusted Life Year avoided should be pursued, our findings indicate that routine surveillance for bloodstream infections is likely a cost-effective service in the African context.
RESULTS OF BASELINE OPHTHALMOLOGICAL EXAMINATIONS CONDUCTED IN ONCHOCERCA VOLVULUS INFECTED PARTICIPANTS FROM ONCHOCERCIASIS MESO- AND HYPERENDEMIC AREAS IN NORTH-EAST DRC AND GHANA IN A STUDY OF THE EFFECTS OF A SINGLE DOSE OF 8 MG MOXIDECTIN VS. IVERMECTIN

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A study comparing the safety and efficacy of a single dose of 8 mg moxidectin vs. a single standard dose of ivermectin (150 µg/kg) treated 762 males and 411 females, including 79 children of 12-17 years, with ≥10 microfilaria/mg skin in Nord-Ituri (DRC, N=460), Nord-Kivu (DRC, N=472) and Nkwanta North district (Ghana, N=241). Ophthalmological pre-treatment examinations included: detailed history, visual acuity, anterior segment examination with up to x25 slit lamp, dilated fundus examination by direct and indirect ophthalmoscopy, color vision, intraocular pressure, visual field with FDT perimetry, ocular mobility, pupillary reflex, external ocular structures, and number of dead and live microfilaria in the anterior chamber and the cornea. Medical histories were to be reported into the data base if, in the investigator’s judgement, they were relevant for assessing post-treatment effects. They were coded according to the Medical Dictionary for Regulatory Activities. All oculcar examination results were entered into the data base and their severity graded according to the Onchocerciasis Chemotherapy Research Center Common Toxicity Criteria [grade 0 (normal) - grade 4] or for events not included in these criteria, by method specific grading systems, NCI CTC version 2 or a generic grading system. Substantial variations between the three sites were observed in the percentages of participants with grade 3 or 4 abnormalities in visual acuity and visual field (range 6-16% and 21-40%, respectively). Different types of abnormalities of the anterior and posterior segments were diagnosed in 0-11% and 0-15% of participants,
rerespectively. Ocular symptoms were reported for 37-75% of participants. The most frequent symptoms (beyond those related to vision itself) reported by the participants or detected at the baseline examination were eye pruritus (6.6-40.2%), blurred vision (6.1-30.3%), corneal disorders (0-8.0%), eye pain (2.5-6.6%), conjunctivitis (0-1.9%).

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**DELAY IN HANSEN’S DISEASE DIAGNOSIS IN THE UNITED STATES**

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The burden of Hansen’s disease (HD) (leprosy) has declined worldwide but it remains endemic in many less-developed countries. In developed countries, HD is rare, mostly associated with immigrants from endemic areas. In the United States, 72% of 213 HD cases reported in 2009 were foreign born. Untreated HD can progress to a severely debilitating disease, involving functional loss of extremities and disability. Risk of impairment increases with longer delay between onset of symptoms and disease diagnosis. We identified risk factors associated with longer interval between HD symptom onset and diagnosis in the United States. HD cases registered with the National Hansen’s Disease Program and diagnosed during 2000-2010 (n=1,241) were included in the analysis. Cox proportional hazards models were used to assess median times between symptom onset and diagnosis. Foreign-born persons whose symptoms began before their arrival in the United States had a longer delay in diagnosis than U.S-born and foreign-born cases with onset after arrival (3.3 years vs. 1 year, p<0.05). U.S-born patients residing in states with an HD specialized ambulatory care clinic experienced a shorter delay to diagnosis, compared with those with no clinic nearby (0.9 vs. 2.3 years) (p<0.05). Proximity to a HD clinic did not affect delay to diagnosis among the foreign born. Among the U.S-born delay in diagnosis was significantly (p<0.05) longer for those whose symptoms began when they were 30-44 years old (2.3 years) compared to other age groups. For the foreign-born the longest delay (3 years) was for those with onset with onset at ≤15 years old (3 years). Better understanding of the reasons for delayed diagnosis is needed, particularly for younger foreign-born patients and for those with symptom onset before U.S. arrival. The role of enhanced HD screening and education during immigration-related medical examination needs to be assessed. For the U.S-born, improved access to HD clinics should be explored. Increased HD awareness and outreach to physicians are also needed, especially in states without HD clinics and in areas with large foreign-born populations.

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**PHYSIOLOGICAL ROLE AND CHEMOTHERAPEUTIC POTENTIAL OF THE ECYSONE RECEPTOR HOMOLOGUE OF THE HUMAN PARASITE BRUGIA MALAYI**

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A homologue of the ecysone receptor (EcR), a master regulator of development in insects has previously been identified and shown to be responsive to 20-OH ecysone in transfected Brugia malayi. As the EcR is not found in vertebrate animals, it and the regulatory pathways it controls represent an attractive potential chemotherapeutic target. To further delineate the physiological role that the EcR plays in filarial parasites, adult female parasites were treated with 20-OH ecysone in culture and microfilarial output and embryograms monitored in treated and control parasites. Females treated with 20-OH ecysone produced significantly more microfilaria that control worms, implicating the EcR in regulation of microfilarial development. RNAsq of the transcripts of adult females treated with 20- OH ecysone was also conducted to observe changes in gene expression. RNAsq identified 67 genes whose expression was genes significantly up-regulated in the treated parasites compared to untreated controls. A mammalian two hybrid system was used develop a high throughput assay to identify agonists and antagonists of the filarial EcR. This assay was based upon a mammalian two hybrid system involving BmECR Gal4 and RXR VP16 fusion constructs and a GAL4 luciferase reporter. The fusion constructs heterodimerize and when bound to the cognate ligand, activates transcription of the luciferase reporter. On induction with 20-OH ecysone, transactivation of the luciferase gene was seen in triply transfected mammalian cells, with a signal to noise ratio of roughly 6 and Z’ of the assay is 0.7. This assay will be employed to screen natural product libraries and a collection of ecysone analogs in the near future.

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**INVESTIGATING ONCHOCERCA VOLVULUS - WOLBACHIA BACTERIAL ENDOSYMBIOMTS COPY NUMBER VARIATIONS AFTER IVERMECTIN TREATMENT**

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Onchocerca volvulus - causative agent of onchocerciasis - harbour Wolbachia bacterial endosymbionts (wOv), whose activities are vital for worm survival. For instance, doxycycline treatment caused parasite mortality due to wOv death. In addition, co-evolution wOv and worm genomes, and lateral gene transfers have been reported. Despite this survival benefit, “un-interrupted” worm autophagy mechanisms regulate wOv copy numbers to achieve a homeostatic balance. Here, we investigate whether interruptions in worm biology, such as ivermectin (IVM) treatment, can lead to changes in wOv copy number. We used a relative real time quantitative PCR assay to measure wOv copy number variations (CNV) between IVM treated and naive worms. The Wolbachia surface protein gene (wsp) for wOv and the glutathione reductase gene (gr) for worm are single copy, therefore gene copy numbers were equivalent to genome copies. In all, 143 adult parasites (34 naive and 109 treated), sampled from Ghana, were assayed. Treated parasites had significantly reduced variance of wsp/gr ratio compared to naive ones (P = 0.001). Also, treated parasites had a lower median wsp/gr ratio (4.25, S.E. = 2.31) than naive (10.36, S.E = 10.11), though difference not statistically significant (P = 0.531). These results may be indicative of IVM treatment influencing the worm wOv copy numbers.

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**RAPID, SENSITIVE DETECTION OF FILARIAL DNA WITH MINIMAL EQUIPMENT**

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Currently, molecular xenomonitoring efforts for lymphatic filariasis (LF) rely on the PCR or RT-PCR-based detection of Brugia malayi, Brugia timori and Wuchereria bancrofti. Typically, extraction of DNA from infected mosquitoes is performed using the column-based DNeasy Blood and Tissue Kit from Qiaogen. However, this extraction is both time consuming and expensive, and the diagnostic testing which follows requires expensive thermal cyclers or Real-Time PCR instruments. Such expenses make these diagnostic techniques impractical for laboratories within many endemic areas. Accordingly, in such locations, there exists a substantial need for an inexpensive, equipment-minimizing diagnostic option. In this work, we evaluate a crude, NaOH extraction method for the isolation of template DNA that minimizes the presence of inhibitors, in conjunction with an isothermal DNA amplification method that requires minimal laboratory equipment. This simple extraction reduces the total cost of the DNA isolation by 50%. We show that for pools of up to 25 mosquitoes, spiked with one infected B. malayi or W. bancroftri L3 worm, detection
by real-time PCR, and thDA isothermal amplification are equally sensitive using both NaOH extraction, and Qiagen-based extraction methods. Furthermore, as a portable and simple diagnostic assay, thDA isothermal amplification can be coupled with HybrDdetect® test strips, enabling detection of amplified B. malayi DNA with equal sensitivity to gel electrophoresis product visualization. By eliminating the need for expensive equipment without compromising sensitivity, this assay and product detection combination provides a diagnostic alternative for endemic locations lacking the means to perform the molecular assays currently employed for xenomonitoring purposes.

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APPLICATION OF RECOMBINANT HUMAN ANTIBODY FOR VALIDATION AND QUALITY CONTROL OF SEROLOGICAL ASSAYS IN SUPPORT OF ONCHOCERCIASIS ELIMINATION PROGRAMS

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Monitoring progress toward onchoceriosis or river blindness elimination has largely relied on the detection of microfilariae in skin snips of infected patients, a technique that presents challenges: acceptability, timing with mass drug administration (MDA) interventions, and sensitivity when the disease burden is low. The detection of antibodies to Onchocerca volvulus (Ov)-specific antigens provides an alternative and proven approach that requires only capillary blood from a finger stick and where the sensitivity of antibody detection is not dependent on timing with recent MDA. One challenge with serological assays is the availability of a reliable and consistent source of positive control. To address this issue, a recombinant, monoclonal human IgG4 antibody was developed to the antigen Ov16. With anti-Ov16 IgG4 ELISA-based serological assays already widely used to confirm elimination in the Americas and parts of Africa, the recombinant Ov16-specific human IgG4 antibody could be used for standard curves on ELISA-based assays and as a quality control reagent for an Ov16 rapid diagnostic test (RDT). The performance of two ELISA tests for anti-Ov16 human IgG4 was assessed when using the recombinant IgG4 antibody, plasma samples, and dried blood spots. The data demonstrates that use of a horseradish peroxidase-based (HRP) ELISA results in a lower limit of detection than the currently used alkaline-phosphatase-based ELISA format. A dried-down format of the recombinant antibody was developed and used under field conditions without refrigeration as a quality control for Ov16 RDTs. Stability was demonstrated for over 15 weeks, unrefrigerated. The recombinant human anti-Ov16 IgG4 antibody will be useful for inter-laboratory validation of ELISA assays and as a quality control reagent for RDTs at different points of the supply chain from manufacturer to field use.

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EXPRESSION PATTERNS OF NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) IN BRUGIA MALAYI Filarial Worms

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Nematode nicotinic acetylcholine receptors (nAChRs) are the targets for a number of anthelmintics including levamisole, pyrantel, morantel and oxantel. The levamisole AChR type is composed of five subunits, Cel-unc-29, Cel-unc-38, Cel-unc-63, Cel-lev-1 and Cel-lev-8 in C. elegans. Nine nAChRs are present in the Brugia malayi genome including orthologues of Cel-unc-29, Cel-unc-38, and Cel-unc-63. We studied expression these genes by qRT-PCR in B. malayi adult female, male, and microfilariae. Six of eight nAChRs genes studied were differentially expressed. Four were more highly expressed in males, 1 in females, and 1 in microfilariae. We performed in situ hybridization with CRNA probes to localize expression of five B. malayi nAChRs genes in adult worms. Most of them had similar expression patterns with signals in developing embryos, spermatogonia, the uterine wall, vas deferens, and lateral chords. For example, Bm1_35890, an orthologue of cel-unc-29 encoding the subunit of levamisole-sensitive receptors that had equal expression in male and female worms by qRT-PCR, was strongly expressed in both male and female worms. In females, strong expression signals were detected in the ovary, developing embryos and lateral hypodermal chords, with moderate expression in the uterus wall adjacent to stretched microfilariae. Expression signals in males were strong in spermagonia and in the wall of vas deferens. Expression patterns for the novel gene Bm1_48815 (with no orthologue in other nematodes and equal expression in males and females) were similar to those of Bm1_35890. Anti-peptide antibody to Bm1_48815 bound to the same tissues that were labeled by in situ hybridization, but the antibody also bound strongly to body muscle in both male and female worms. Increasing evidence suggests nAChRs may play important developmental roles as the receptors are expressed early during embryogenesis in vertebrates. The expression patterns of these genes suggest that they are involved in reproduction, and this may explain the effect of drugs that target nAChR on reproduction in filarial worms.

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HIGH CONTENT IMAGING: MORE THAN A PRETTY PICTURE

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The A-WOL consortium aims to find a novel macrofilaricidal drug to treat the debilitating diseases lymphatic filariasis and onchocerciasis, through targeting the Wolbachia bacteria that reside within these parasitic nematodes. Typically, screening chemical libraries directly against parasitic nematodes is cumbersome, low throughput and relies on animal reservoirs. By focusing on the endosymbiont, we have been able to utilise an insect cell-based screening approach that originally relied upon a quantitative polymerase chain reaction readout, but now employs a High Content Imaging readout running on the Perkin Elmer Operetta® platform. This assay uses texture analysis of cells stained with SYTO®11 (fluorescent DNA stain) as a direct measure of bacterial load and allows the consortium to screen up to 10x 384 well plates per day, a radical increase in throughput from the qPCR screen. Further to its use as a screening tool, the Operetta® is also being used for more fundamental biological investigations such as experiments surrounding the infection dynamics within host cells and, as part of a separate project aiming to create a novel cell-based screen for discovery of a macrofilaricide, the development of a filarial nematode cell line. The nature of the anti-Wolbachia screening approach will be presented in addition to preliminary data from other High Content Imaging investigations surrounding Wolbachia, host cells and cell line development.
A•WOL MACROFILARICIDAL DRUG DISCOVERY AND DEVELOPMENT - OPTIMIZATION OF ANTI-WOLBACHIA EFFICACY

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There is an urgent need to develop a novel treatment for filariasis, and targeting Wolbachia provides safe macrofilaricidal activity with superior therapeutic outcomes compared to standard anti-filarial treatments. The Anti-Wolbachia (A-WOL) Consortium has developed both in vitro and in vivo assays, to screen chemical libraries for anti-Wolbachia activity. The outputs from the A-WOL program are now being pursued as part of A-WOL II Macrofilaricide Drug Discovery & Development programs. Screening of >10000 compounds from the BioFocus library and chemoinformatic analysis have generated six independent lead series chemotypes with the potential to enter a medicinal chemistry "hit-to-lead" and lead optimization program. A-WOL Drug Discovery is now progressing these lead series through a rigorous lead optimisation and candidate selection process, using iterative cycles of medicinal chemistry and biological testing in order to deliver at least one novel pre-clinical candidate and a chemically distinct back-up, aligned with our Target Product Profiles for an anti-Wolbachia macrofilaricide. In addition, ongoing screening of large diversity-based libraries (150-500k compounds) aims to provide additional, chemically diverse hits, with one-order improvement in absolute potency or significant shortening of treatment time, in order to expand the structural diversity of anti-Wolbachia chemotypes. A-WOL Drug Development is optimising regimens of anti-Wolbachia monotherapy and combination treatment of registered anti-Wolbachia and anti-filarial drugs in vivo using an adult Brugia malayi mouse model. This efficacy testing is driven by a rational PK/PD modelling approach which supports dosage regimens, in order to identify the best treatment regimens to test in field trials.

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THE DEPLETION OF WOLBACHIA FROM BRUGIA MALAYI MICROFILARIAE AND SUBSEQUENT EFFECT ON THE DEVELOPMENT OF INFECTIVE LARVAE IN AEDES AEGYPTI

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The filarial nematode Brugia malayi is a human pathogen that harvests the bacterial endosymbiont, Wolbachia. Tetracycline treatment causes the depletion of Wolbachia leading to infertility and early death in the adult worm. If tetracycline treatment is ongoing at the time of infection, development from the L3 stage to adult is inhibited. The depletion of Wolbachia in microfilariae (Mf) has been shown to affect development to L3 infective larvae in the intermediate host Litonosoides sigmodontis. To assess the effect of tetracycline treatment on B. malayi microfilariae and development to the L3 infective stage within the mosquito, Aedes aegypti, infected gerbils (Meriones unguiculatus) were treated for 2, 4 and 6 weeks. At each time-point, Mf were extracted and fed to mosquitoes and the subsequent development to L3 larvae was assessed versus controls. To further understand the relationship between Wolbachia and L3 development, live Mf were directly visualised within the mosquito midgut and also proteomic analysis was conducted of secretory/excretory products from treated and control Mf. The results show the significant effect of Wolbachia depletion on the ability of Mf to progress to L3 stage and treated Mf are unable to escape the mosquito midgut. The retardation of L3 development by tetracycline may have an important additional blocking effect on the transmission of filarial parasites and understanding the mechanism by which this occurs may offer further insight into the important role Wolbachia play in each of the life-cycle stages of B. malayi.

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MICRONNAS IN PARASITIC NEMATODES - DEFINING A FUNCTION

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We previously identified a large number of microRNAs in the filarial nematode Brugia species and in Haemonchus contortus, a sheep nematode related to human hookworms. Recent experiments have sought to define the function of two microRNAs, Bpa-mir-5364 and Hco-mir-5352. The Brugia miRNA was selected for study on the basis of an intriguing expression pattern, being ~x12-fold increased between vector derived L3 and L3 recovered at 24 h post-infection. This miRNA is a novel member of the let-7 family. Using target prediction programs combined with a comparative genomics approach we sought to identify predicted miRNA targets, and selected three of these for further analysis. We established a dual luciferase assay in transfected HEK cells to examine the relationship between Bpa-mir-5364 and the 3’UTR of the predicted targets and used anti-sense oligonucleotides to attempt to inhibit Bpa-mir-5364 in vitro. For H. contortus, we focused on a single miRNA that is parasite-specific and only found in nematode parasites that live in the gastrointestinal tract (irrespective of nematode clade). Hco-mir-5352 is one of a cluster of four microRNAs that is conserved in a number of gastrointestinal nematodes. We could detect Hco-mir-5352 in adult worm excretory-secretory products and in tissues from infected sheep, indicating that it may be released by the parasite in vivo and thus could interact with a host mRNA. A predicted target of Hco-mir-5352 is mammalian CD69 and we have used the dual luciferase assay in an attempt to confirm this relationship. An improved understanding of the function of parasite microRNAs casts novel light upon the intricacies of the host-parasite relationship.

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DIRECT VEGF-SPECIFIC ANTI-ANGIOGENIC ACTIVITIES OF THE ANTI-WOLBACHIA DRUGS, DOXYCYCLINE AND MINOCYCLINE, IN AN IN VITRO MICROVASCULAR BLOOD AND LYMPHATIC ENDOTHELIAL CELL CULTURE SYSTEM

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Lymphatic filarial infection (LF) is the cause of elephantiasis and hydrocele lymphoedemas (LE). LE is initiated as a result of episodic inflammatory damage and remodelling of parasitized lymphatics. Heightened inflammatory responses in LE patients are associated with pro-angiogenic/lymphangiogenic molecules in circulation, such as vascular endothelial growth factors (VEGFs), which suggests inflammatory angiogenesis is relevant in the pathophysiology of LF. Current mass drug administration treatments for LF have mainly microfilaricidal activities and do not help alleviate the suffering of current LE patients. As such, there is an unmet need to identify new therapeutics to help reduce LF morbidity. Encouragingly, in two recent trials, 6-week doxycycline therapy was identified to improve LE grade after 12–24 months. The anti-morbidity mode of action (MoA) of doxycycline has not been defined. LE grade is improved both in doxycycline treated patients with and without active LF infection, suggesting a separate MoA to that of targeting the filarial inflammatory endosymbiont, Wolbachia. We developed an in-vitro blood and lymphatic endothelial cell culture system to assess the VEGF-specific anti-angiogenic activities of the anti-Wolbachia compounds, doxycycline and minocycline. Utilising a 96 well/200 µL based Operetta™ (Perkin Elmer) fluorescent bio-imaging system we have stimulated the proliferation of
human adult dermal microvascular blood endothelial cells (HMVECd) and equivalent lymphatic endothelial cells (HMVECdl), using VEGFs targeting VEGF receptors 1-3. Titration of both doxycycline and minocycline toward physiological plasma levels have demonstrated a dose-dependent reduction in both VEGF-specific HMVECd and HMVECdl proliferation. This data supports a direct anti-angiogenic MoA of second-generation tetracyclines in use for the treatment of LF, which may target pathological inflammatory VEGF processes. The bio-imaging platform has been validated for onward screening to identify new candidate compounds with VEGF-specific anti-angiogenic activities.

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ONCHOCERCIASIS: TRANSCRIPTOMIC AND PROTEOMIC APPROACHES FOR IDENTIFYING BIOMARKERS ASSOCIATED WITH THE PRESENCE OF VIVABLE ADULT FEMALE PARASITES FOR POST-CONTROL SURVEILLANCE
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Control of onchocerciasis or ‘river blindness’ has had considerable success in Africa. As efforts shift from control to disease elimination better tools are needed to identify viable adult females (OvAF) for the “end game” needs. The goal of this project is to identify host- and/or OvAF-specific biomarkers for use in rapid field-friendly tests during post-control surveillance programs. By taking advantage of the recently sequenced genome and assembly of Onchocerca volvulus (~97.4Mb) organized by optical mapping into chromosomes, we have overlaid RNAseq analyses of the important mammalian stages of O. volvulus (Ov) L3, adult males, adult females and microfilariae. Of the predicted 12994 genes, a total of 12416 transcripts were identified from all stages, with over 9600 adult female transcripts. Analysis of transcripts across all the stages resulted in the identification of 75 select gene products that were abundant in OvAF and absent or minimally expressed in other stages. In parallel, shotgun proteomics of the somatic extracts from each of the Ov mammalian stages (including excretory-secretory proteins of OvAF) are in progress using UPLC MS/AMS (Thermo Easy nLC 1000 UPLC coupled to Thermo Q-exactive quadrupole-Orbitrap mass spectrometer). Of even more interest is the proteomic analysis of O. volvulus infected human sera in which hundreds of Ov-specific proteins have been identified, of which four are among the female-specific proteins found during the transcriptomic analysis. Recombinant antigens and monospecific polyclonal antibodies are being made to test the validity of these targets as biomarkers for use in post-control surveillance assay systems.

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THE MUTUALISTIC SYMBIOSIS OF WOLBACHIA AND THE FILARIAL NEMATODE BRUGIA MALAYI - UNRAVELLING THE PROTEOME AND TRANSCRIPTOME
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The parasitic nematode Brugia malayi is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. B. malayi exists in a mutualistic symbiotic relationship with the α-proteobacterium Wolbachia. We have applied a global proteomic profiling approach to investigate the molecular basis of this symbiosis. Adult female B. malayi in the mammalian host Meriones unguiculatus were sampled at multiple time-points post-antibiotic depletion. Deep proteome mining combined with high-resolution mass spectrometry was used for comprehensive proteome profiling of Wolbachia/worm at these selected time-points. Wolbachia/worm ratios were also monitored by qPCR. Using a combination of extensive peptide pre-fractionation and established proteomic workflows we observed improved proteome coverage by an increase in peptide/protein identification. Such proteomic approaches coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome profiling. Proteins of interest from this initial global proteomic ‘screen’ have been further investigated using a targeted proteomics approach. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic Wolbachia/B. malayi symbiosis.

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IDENTIFICATION OF CHEMOSENSORY RESPONSES IN THE FILARIAL WORM, BRUGIA MALAYI
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Lymphatic filariasis (LF) is a disease caused by mosquito-borne filarial nematodes including Wuchereria bancrofti and Brugia malayi. Although over 120 million people suffer from this disfiguring disease, chemotherapeutic options for LF are limited to three drugs: diethylcarbamazine citrate, albendazole and ivermectin. The threat of drug resistance combined with the inefficacy of these drugs against adult parasites highlights the need for new anthelmintic drugs. Chemosensation is an essential behavior used by multi-cellular organisms to interact with the environment. In the free-living nematode, Caenorhabditis elegans, chemosensation plays a crucial role in development, avoiding noxious conditions and finding food and mates. In parasitic nematodes, chemosensation is thought to play a critical role in host-seeking and host-invasion behaviors making genes involved in this system attractive targets for drug or vaccine development. However, little is known about the chemosensory system in filarial parasites of animals. In this study, we sought to elucidate chemosensory-induced behavior in B. malayi during the infectious L3 stage of the parasite. Scanning electron microscopy revealed that amphids, the major chemosensory organs of nematodes, are present in both juvenile and adult stages of B. malayi. In addition, orthologues of several genes known to be involved in chemosensory behavior in C. elegans were identified in B. malayi based on sequence homology. Finally, we identified over 10 chemical compounds that were either attractive or repellent to B. malayi L3 stage parasites using chemotaxis assays. This research is the first study to demonstrate that B. malayi has a responsive chemosensory pathway. In addition, the results obtained indicate that the chemosensory response in B. malayi plays an important role in host-seeking and host-invasion behavior, and therefore is a prime candidate for chemotherapeutic intervention.

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GENOMICS OF WUCHERERIA BANCROFTI FROM FOUR ENDEMIC REGIONS OF INFECTIVITY: HAITI, KENYA, MALI AND PAPUA NEW GUINEA
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Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and...
characterization of putative Brugia malayi acetylcholine-gated chloride channels

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The neglected tropical disease, lymphatic filariasis (LF), affects over a million people and an even greater number of individuals are at risk for this disease in endemic tropical regions. The filarial nematodes, Wuchereria bancrofti, Brugia malayi and Brugia timori, can cause severe lymphedema leading to gross disfigurement in the infected human host. Mass Drug Administration (MDA) programs deliver chemotherapeutics diethylcarbamazine, ivermectin, and albendazole, annually to endemic areas to manage and treat this disease. Despite success with MDA, growing concerns of resistance combined with the inefficacy of these drugs against the adult stages of the parasites emphasizes the need for novel and broadly effective antifilarial chemotherapeutics. Cholinergic neuro transmission is a proven source of effective anti-nematodal drug targets. Although much is known about Na+ conducting nicotinic acetylcholine receptors, the family of acetylcholine-gated chloride channels (ACC) is poorly understood. We used reverse genetics to investigate Brugia malayi acetylcholine chloride channels (BACCs), which are a novel family of ACCs. Transcriptomics, homology based searches, and 5’ and 3’ RACE-PCR identified 8 putative BACCs. qRT-PCR results revealed that three BACCs, BACC-1, BLGC-46, and BLGC-47, are expressed in both juvenile and adult stages. Results from in situ hybridization demonstrate that BACCs are distinctly localized. We are further probing the potential function of BACC-1, BLGC-46, and BLGC-47 by using RNA interference and microfluidics assays. Our results show that BACCs have similar characteristics to other ACCs thus these genes are prime candidates for further investigation as novel chemotherapeutic targets.
ECONOMIC COSTS AND BENEFITS OF MORBIDITY MANAGEMENT AND DISABILITY PREVENTION FOR LYMPHATIC FILARIASIS

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Lymphatic filariasis (LF) is endemic in 73 countries, with 120 million people infected, of whom 40 million suffer serious disability, including lymphedema of the legs (15 million), arms, and breasts; hydrocele in 25 million men; and acute attacks of fever and disabling pain that last several days, termed adenolymphangitis (ADL), which contribute to worsening lymphedema and substantial productivity loss. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has two goals: interrupting LF transmission by 2020 and caring for people already infected through morbidity management and disability prevention (MMDP). While 53 countries have ongoing mass drug administration, only 27 had begun MMDP by 2013, in part, due to the perceived high cost and low return on investment for MMDP. To address this concern, we estimate costs and benefits of MMDP for the global population affected and at risk over the lifetime for affected cohorts. MMDP changes the distribution of disability and productivity over time. Actual program costs are based on 3 delivery modes: community-based care (CBC), referral to primary care clinics, and specialty clinics. We calculate societal costs of untreated morbidity due to acute attacks, chronic lymphedema and hydrocele, consequent lost wages, and other costs. In each country with cost comparisons, research to date suggests societal cost of untreated LF far exceeds cost of MMDP. In India, patients enrolled in CBC morbidity management averaged 29 fewer lost work days per year. In Haiti, ADL was reduced substantially after simple training in self-care. A program in Togo, after 3 years of MMDP, reported stabilization of lymphedema stage and slight decrease in ADL. In Ghana, patients reported substantial improvement in work capacity after hydrocele surgery. We will combine results from these and other countries and adjust costs and benefits to each context, to estimate savings in direct costs, productivity loss, and marginalization that provide economic rationale in addition to the ethical mandate for MMDP, the second pillar of GPELF.

ORAL FLUBENDAZOLE: A POTENTIALLY USEFUL MACROFILARICIDE

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The search for a macrofilaricide that can enhance the goal of elimination of filarial infections, and the diseases they cause, is a current and relevant goal. The most attractive approach to macrofilaricide discovery is to re-purpose agents already in use in humans. High amongst these potential candidates is flubendazole, a drug that was shown in a screening survey in the early 1980s to be effective against Onchocerca volvulus in humans but was difficult to administer due to the properties of the formulation used. The need to reformulate this agent to overcome the issue of insolubility and make it suitable for use as an oral administration was a goal regarded as important for field program use of this anthelmintic in filariasis. We will discuss here results obtained with a new formulation of flubendazole that achieves, after a single oral doses of 2-6 mg/kg, a plasma concentration of between 1-3µg/ml with a peak plasma level at 3-5 hr which decreases to control levels by 12-20hr in rodents. Using the Litomosoides sigmodontis model in gerbils, we have achieved >90% killing of adult worms when the animals were assessed 9 weeks after treatment with 6 mg/kg of the oral formulation of flubendazole given daily for 5 days. In vitro incubation of Brugia sp. with pharmacologically-relevant concentrations of flubendazole caused alterations in the internal organs of the adult worms. Histological observations in both filarial species exposed to flubendazole in vitro and in vivo showed that several structures in the worms were affected by the drug; the early developing forms in the uterus were the most affected component, and hypodermal cells were also significantly damaged when worms were incubated at concentrations of flubendazole equivalent to the plasma levels obtained after oral dosing. The beneficial effect of treatment with oral flubendazole on host tissue pathology induced by the presence of the parasite was marked when comparing untreated animals to flubendazole-treated animals 9 weeks after dosing. The degree of tissue and organ changes were scored subjectively, and the histopathology occurring examined and scored histologically. The very significant reduction in pathology in animals treated with 6 mg/kg flubendazole suggests that the death of adult worms induced by this drug is not likely to invoke severe adverse events in the host. Our study shows that oral flubendazole can safely kill the majority of adult filariae in this experimental model.

LOA LOA IN A CONGOLESE REFUGEE WOMAN IN MINNESOTA

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An estimated 13 million people are living with the neglected tropical disease Loiasis. The Chrysops deerfly vector can be found in rainforest canopies of Central and West Africa. Loa loa microfilaria migrate into subcutaneous tissue, maturing into adult worms, mating, and producing more microfilaria for more than a decade in a human host. This could cause a refugee to experience intermittent symptoms for many years after resettlement to the US. Calabar nodules (pruritic, non-tender skin swellings) and white worms moving across the visual field should lead to the search for Loa loa. A 49 year old Congolese refugee presented with 2 days of a “needly” sensation in her right eye after 6 days of left eye pain with peri orbital swelling. She had temple pain and intense pruritis in her palms and soles. She reported numerous episodes of migratory pruritic swellings on her left forearm and foot and a sensation of threads moving across her vision in the previous 2 years. While living near Kinshasa 9 years earlier, she was treated with an herbal poultice that allowed tiny white worms to be removed from her eyes. Her husband has been treated for onchocerciasis in the past. An NIH onchocerciasis antibody test was negative, and a slit-lamp exam found no evidence of coinfection. Her filarial serologies were consistent with a past or acute filarial infection. She was treated with 3 weeks of DEC, causing only mild nausea. Her eosinophilia decreased from an AEC of 800 to 200 after treatment. There is a 50% chance that this course will have treated her filarial infection definitively. This woman suffered from Loiasis, an illness that can plague patients with intermittent symptoms for over a decade. Although short-term travelers are rarely infected, the current influx of Congolese refugees to the US heightens the importance of Loiasis awareness. As onchocerciasis can be endemic in the same regions, coinfection must be considered when selecting treatment for Loiasis to avoid the severe Mazzotti inflammatory reaction that occurs when given DEC while infected with onchocerciasis.
ONCHOCERCA LUPI: AN EMERGING ZOONOSIS IN NORTHERN EUROPE AND THE UNITED STATES?
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The geographical distribution of cases caused by Onchocerca lupi, a common parasitic worm in wolves, has widened during the last decade. Moving from Southern European countries such as Greece and Turkey, an increasing number of cases have begun to emerge in Northern Europe and the Americas. Common arthropod vectors for O. lupi include blackflies (e.g., Simulium yahense and midges (e.g., Culicoides spp.). O. lupi commonly causes ocular problems such as conjunctivitis, photophobia, and excessive lacrimation. In recent years, however, clinicians have identified cases that have involved extra-ocular sites such as the spinal canal. As of 2014, more than sixty cases of O. lupi, ocular and otherwise, have been identified throughout Europe and the United States. This presentation will examine not only the global epidemiology of Onchocerca lupi but potential surveillance measures for this emerging zoonosis.

OPTIMIZATION OF THE ACANTHACHEILONEMA VITEAE LIFE CYCLE FOR INTENSIVE PRODUCTION
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The filarial nematode Acanthachelonema viteae is of great interest to the filariasis community because it lacks the endosymbiotic Wolbachia bacterium found in several filarial parasites of humans. The NIH/NIAID Filariasis Research Reagent Resource Center (FR3) began distributing A. viteae to its users in 2011. The FR3 strain of A. viteae and of its tick vector Ornithodoros tartakowskyi were originally acquired from TRS Laboratories and the life cycle is currently being optimized for intensive production to meet increasing demand. Variables targeted in this optimization include 1) the microfilariaemia of gerbils used for infecting ticks, 2) the life stage and sex of ticks used for infections, 3) the number of subcutaneous injections used to infect hamsters with third-stage infective larvae (L3), and 4) the medium used for subcutaneous injection of gerbils with L3. By feeding only adult stage ticks on gerbils selected for high microfilaraemia we increased our yields from 13 L3/tick (n=497) to 126 L3/tick (n=107 ticks). Similarly, the average recovery of adult A. viteae from hamsters has risen from 51 ± 45 (n=13) to 90 ± 48 (n=22), partially due to the finding that worms isolated in Hank’s Balanced Salt Solution (HBSS) produce patent infections whereas those isolated in RPMI 1640 do not. Methods to infect ticks with A. viteae via artificial membrane feeding and inoculation are currently being developed, and a recent trial resulted in recovery of 208 L3/tick inoculated by enema with microfilaricemic gerbil blood (n=4 ticks). The increased recovery of A. viteae from host animals has allowed the FR3 to meet higher demand from the filariasis research community for all life stages of the worm.

ACTIVATING AUTOPHAGY AS A NOVEL ANTI-WOLBACHIA MODE OF ACTION FOR MACROFILARICIDAL DRUG DISCOVERY
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River blindness (Onchocerca volvulus) and elephantiasis (Wuchereria bancrofti and Brugia malayi) affect over 150 million people in more than 80 countries, with a further 1 billion at risk of infection. Each of these nematode parasites has evolved a mutualistic association with the bacterial endosymbiont Wolbachia. Depletion of Wolbachia with the antibiotic doxycycline arrests development, fertility and viability and delivers potent macrofilaricidal efficacy in clinical trials. In order to identify alternative anti-Wolbachia drugs with a more rapid activity we have exploited the host nematodes immune regulation of Wolbachia populations through autophagy to discover drugs with a wolbachiacidal mode of action. We have screened libraries of 100 autophagy inducing drugs and compounds against B. malayi. Selected ‘hits’ are then screened against transgenic C. elegans and human embryonic kidney (HEK) cells expressing a fluorescent autophagy marker, ATG8 to identify drugs, which are selectively more potent against nematode versus human autophagy activation. Hits ranked by relative nematode potency are progressed through the A-WOL drug discovery and development screening pipeline to identify pre-clinical lead candidates and optimized combinations of anti-Wolbachia drugs to reduce treatment timeframes.
Development of a small animal model of onchocerciasis for the screening of macrofilaricidal drugs

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Onchocerciasis affects >37 million people with approximately 800,000 suffering visual impairment (river blindness). Development of macrofilaricides for onchocerciasis is hampered by the lack of a facile animal model. Our laboratories and others have demonstrated protracted survival of non-murine filariae in immune-compromised mice. Therefore we tested whether cattle-sourced Onchocerca could persist in Severe-Combined Immuno Deficient (SCID) mice. O. ochengi modules were harvested from the skins of infected cattle while infective L3 were produced from blackflies fed on cattle in Cameroon. Motile male O. ochengi were isolated following culture of disrupted onchocercomas, prior to surgical implantation into the peritoneal cavity of SCID mice. Viable macrofilariae were recovered in 100% of recipients assessed between 1-5 weeks post implant (mean survival=49.22±5.08, n=22). Therefore, the implant model was assessed for suitability to test for efficacy of direct-anti-nematodicidal drugs using the standard macrofilaricide, flubendazole suspension (FBZ). FBZ or vehicle control (VC) was administered parenterally (10mg/kg sc, +5 days) into groups of SCID recipients (n=7-8) of 15 male O. ochengi. FBZ induced an almost total macrofilaricidal response assessed 4-5 weeks after implant (mean survival FBZ=1.67±1.09 vs VC=43.81%±11.44, p=0.0089). O. ochengi L3 larvae were also inoculated into SCID mice. However, only 1 viable larva could be recovered after 7-14 days following sc or ip injection of between 40-100 L3 (n=26). In conclusion, we have developed a small animal model of onchocerciasis using adult O. ochengi parasites that is sufficiently robust to commence screening of macrofilaricidal candidates. We are currently validating this model for additional screening of anti-Wolbachia candidates. Further, we have commenced development of a murine onchocercoma xenograft model to evaluate whether male and female macrofilariae and released microfilariae can survive in SCID recipients for periods necessary to further validate efficacy of macrofilaricidal drugs.

A laser diffraction assay for low resource quantification of microfilarial burden

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Efforts to eliminate lymphatic filariasis and to control onchocerciasis by mass drug administration (MDA) in West and Central Africa have been hampered by the risk of severe adverse reactions among persons harboring high-level onchoceriasis infections (>8,000 microfilariae per mL of blood). A test-and-NOT-treatment strategy has been proposed for excluding persons with high-level onchoceriasis from MDA. This will require low-resource, real-time, point-of-care diagnostics that can be used at the time of MDA to identify persons with high-level onchoceriasis and exclude them from treatment. Using Caenorhabditis elegans L1 larvae as surrogates for microfilariae, we have developed a low-resource laser diffraction assay for microfilarial quantification. The prototype apparatus is composed of a low-power red laser (similar to a laser pointer) directed through a sample chamber (cuvette, flow cell, or capillary tube) towards a photodiode connected to a laptop-powered oscilloscope. Preliminary studies show that the assay is capable of quantifying worm concentrations between 500 and 50,000 L1 larvae/mL. Assay sensitivity is dependent on depth of the sample chamber: a 1 cm chamber depth gives maximum sensitivity while a 0.05 cm depth gives lower sensitivity but greater precision. Cellular blood components interfere with the diffraction assay, and low-resource methods to separate larvae from blood components prior to quantification are being developed. These preliminary data suggest that a portable, battery-powered, low cost laser-diffraction device may be capable of rapid, point-of-care quantification of microfilarial burden in the setting of MDA.
specific to the antigen Ov16 has been developed and will be manufactured by Standard Diagnostics, Inc. Prior to commercial availability, the test has been evaluated in the PATH laboratory and in rural settings in Togo with 1,500 participants during routine Ov epidemiological surveillance activities. In the laboratory setting, our data indicate that the RDT has a sensitivity of 89% and a specificity of 99%, when compared to an Ov16-specific ELISA. The read window, an important indicator of test result stability, is highly consistent over a 24-hour time period. We will also present the performance of the RDT relative to the Ov16 ELISA and sensitivity as compared to skin-snip derived microfilaria status when tested in real-world settings in Togo that include factors such as exposure to high temperature and humidity. Finally, we describe possible facilitators and barriers for integration of the test into routine surveillance practices. Our data verify the performance of the test in field settings prior to a commercial launch of the Ov16-based RDT and informs how it should be used in current onchocerciasis control and elimination efforts.

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SURVIVOR COPYCAT BEHAVIORS AND AUTOCHTHONOUS HELMINTHIASES IN THE U.S.

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Popular Survivor TV programs glamorize rugged outdoorsmen surviving wilderness adventures by seeking natural sustenance. To identify risk factors for autochthonous helminthiasis resulting from Survivor copycat behaviors, Internet searches identified 54 cases of helminthiasis transmitted by the raw consumption of animals recommended as safe, natural foods by Survivor series programming during the period, 1984-2013. Cases were defined by positive microscopic, serologic, or molecular diagnostics. Continuous variables were analyzed for significant differences by unpaired t-tests; categorical variables were analyzed by chi squares. Statistical significance was indicated by p-values ≤0.05. 38 autochthonous cases of neuroangiostrongyliasis (NAS) with eosinophilic meningoencephalitis and 16 cases of autochthonous paragonimiasis (PG) with hemorrhagic pneumonitis were reported following consumption of raw intermediate hosts infected by causative parasites, the rat lungworm (Angiostrongylus cantonensis) and the American lung fluke (Paragonimus kellicotti) respectively. The mean age of NAS cases was 21.5 years; most cases (93%) were reported consuming raw snails or frogs or eating unwashed greens harboring infective larval snails (P = 0.003). The mean age of the PG patients was 27.3 years; most cases (93%) were reported in males (P < 0.0001) from Missouri, most of whom (67%) had consumed raw crayfish (P < 0.0001), often while intoxicated (47%) on camping, paddling, or floating trips within the Mississippi River Drainage Basin (73%, P = 0.028). There was one death in a 71-year-old male with PG. The most significant risk factors for autochthonous helminthiasis from Survivor copycat behaviors included male gender; and consumption of raw, wild animals in parasite-endemic regions, especially while intoxicated outdoors. Recommended preventive-behavior interventions included proper food preparation of self-harvested wild animals, wilderness survival training, and alcohol avoidance during outdoor recreation.

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EPIDEMIOLOGY OF SOIL TRANSMITTED HELMINTHS AND PLASMODIUM FALCIPARUM AMONG SCHOOL CHILDREN IN BUMULA DISTRICT, WESTERN KENYA

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Soil-transmitted helminthiasis and malaria continue to be important health problems among Kenyan school children, many of whom are at risk of coinfection. It has been suggested that the immune response evoked by helminth infections may modify immune responses to plasmodia species and consequently alter risk of infection; however previous studies have been inconclusive. As part of baseline activities of a current trial investigating deworming impact on risk of clinical malaria among school children in Bumula District in western Kenya (ClinicalTrials.gov: NCT01658774), a cross-sectional survey was conducted to investigate the prevalence, intensity and geographic distribution of soil-transmitted helminth (STH) and Plasmodium falciparum infections among 4,842 school aged children in purposively selected 22 primary schools. Single stool samples were collected and examined in duplicate for STH (hookworm, Ascaris lumbricoides and Trichuris trichiura) using the Kato Katz method. A finger-prick blood sample was taken and examined for P. falciparum by expert microscopy. Overall, 23.4% of the children were infected with P. falciparum and 26.5% with one or more STH species, with hookworm (16.4%) and A. lumbricoides (14.3%). The prevalence of STH and P. falciparum infections varied significantly by age group and sex and by geographical location. After adjusting for age, sex and clustering within schools, the risk of P. falciparum infection was higher among children with any STH infection (odds ratios= 1.5, 95% confidence intervals [CI] (1-1.4), p=0.03), although there was no significant association between P. falciparum and individual STH species. Interestingly however, P. falciparum infection was positively associated with medium to high intensity A. lumbricoides infections (odds ratios= 1.5, 95% CI (1.1-2.1), p=0.006). Coinfection with P. falciparum and STH is common among school children in western Kenya, supporting the need for integrated helminth and malaria control. There is also a need to investigate the consequences of deworming on the risk of malaria.

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PHYTOCHEMICAL, EFFICACY AND SAFETY PROFILES OF EMBELIA SCHIMPERI VATKE

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Emblica schimperi fruits are among the most common traditionally used agents to treat parasitic / helminthes infections in Ethiopia. Despite the plants widespread use, scientific evidence on constituents responsible for the biological activity, efficacy and safety is not well documented. A 96 well micro titer pale assay technique was used to evaluate the in vitro anthelmintic properties of crude hydro alcohol extract and solvent fractions of E. schimperi fruits against hookworm and strongylodeis spp. The in vivo anthelmintic activity of the extract was determined in albino mice experimentally infested with Hymenolopis nana worms. The percent killing activity of the crude extract was from 92-100% when tested at a concentration of range of 50-400mg/ml against hook worm and Strongylodeis spp. The percent motility inhibition against hookworm larvae was from 66.6-86.6% when fractions were tested at a concentration of astmh.org
THE EFFICACY OF ALBENDAZOLE AND LEVAMISOLE DRUG COMBINATION IN INDIVIDUALS WITH REDUCED EFFICACY FOR SINGLE-DOSE ALBENDAZOLE TREATMENT AGAINST HOOKWORM INFECTIONS

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The Soil-Transmitted Helminth (STH) infections control programme has been on-going in Ghana since 2000 with mass drug administration (MDA) of albendazole (ALB) to school children. However, our research revealed, 100% cure rate with albendazole-levamisole (ALB-LEV) combination in hookworm population responding sub-optimally to ALB treatment in four endemic communities in the Northern region of Ghana. This was an eighteen months longitudinal study. A total of 421 school children between the ages of 2-17 years were randomly selected from four endemic communities in Kpandae district of the Northern region of Ghana. One hundred and three (103) patients positive for hookworm were categorised into four groups: Control, ALB only, LEV only and ALB - LEV combination with 26 patients in each group. Coprological assessment for parasites was based on the Kato-Katz technique. Parasites recorded were hookworm (Ancylostoma doudenale and Necator americanus), Trichuris trichiura, Hymenolepis nana, and Taenia sp. in the communities selected. Overall, the highest cure rate after 21 days of treatment was recorded for LEV-ALB treatment (100%) followed by LEV alone (92.31%) and ALB alone (88.46) whiles the Control was zero. Also, faecal egg count reduction rate was 100% (LEV-ALB), 96.15% (LEV alone), 91.84 (ALB alone) and 9% for Control.Cumulative re-infection rates were 12, 32 and 48% and 23, 50 and 69% respectively for LEV-ALB, LEV alone, ALB alone and ALB alone at 6 months and one year after treatments. Hookworm still remains a significant public health burden in Africa. The result shows a significant efficacy for LEV - ALB combination in hookworm population with reduced ALB treatment (100% FEC) after 21 days treatment and therefore can be used to augment ALB for clearing possible resistant parasites of hookworm infections. All parasitic samples are currently being analysed to identify polymorphism related to benzimidazole resistance in hookworms.

MOLECULAR DIAGNOSIS OF SOIL-TRANSMITTED HELMINTH INFECTIONS USING A NOVEL ISOTHERMAL AMPLIFICATION METHOD

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Ascariasis, Trichuriasis, and Hookworms are the three main soil-transmitted helminth infections, causing human morbidity in tropical areas of the world. Diagnosis of human soil-transmitted helminths (STHs) relies on microscopy to identify eggs in the feces. The precise detection of STHs by microscopy requires training and is labor intensive and can lead to false negative results. As a result of the limitations of microscopic methods, it was of interest to develop new methods to identify different STH species. Accurate diagnostic is not only essential for epidemiological studies but is vital for monitoring the possible emergence of drug resistance and for the development of new anthelmintics since the efficacy of different drugs shows variation among the species. For a variety of infections, studies have shown that molecular diagnostic tools can be more reliable and sensitive. Therefore, development of a simple, rapid and sensitive molecular tool for STH species diagnosis is desirable. We developed novel diagnostic assays based on the Smart Amplification method (SmartAmp) to detect STH infections under isothermal condition. This isothermal method uses asymmetric primer design to prevent non specific amplification. Control plasmids were employed to develop and optimize the assays. The assays were applied to analyze fecal samples using species-specific primer sets. Real-time PCR monitoring of the amplification was achieved within 20-40 min with complete suppression of the background amplification. SmartAmp assays were developed for diagnosis of STH infections and the reliability of the method was validated using the conventional PCR method.

IDENTIFICATION AND CHARACTERIZATION OF THE IMMUNOMODULATORY MOLECULE(S) IN EXCRETORY-SECRETORY (ES) PRODUCTS DERIVED FROM THE GASTROINTESTINAL NEMATODE HELIGMOSOMOIDES POLYGYRUS

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Heligmosomoides polygyrus (Hp) is a murine gastrointestinal (GI) nematode used as a model of helminth parasites that infect humans and livestock. We previously showed that Hp-derived excretory-secretory products (UES) potently suppress effector CD4+ T cell responses to an unrelated antigen by modulating the antigen presenting function of dendritic cells (DC) to induce an anti-inflammatory network involving regulatory T cells. Interrogation of mass spectrometry (MS) data from 1D-SDS PAGE and LC-MS/MS analysis of UES with transcriptomic information indicated that Hp contains over 200 proteins, including several with known immunomodulatory effects. To better define the immunomodulatory molecules(s) in UES, we used biochemical approaches and assessed the suppressive activity of the separated fractions by measuring IL-12p70 secretion by bone marrow-derived DC (BMDC) pre-treated with HP fractions prior to stimulation with the TLR9 ligand CpG-ODN. First, we performed size exclusion chromatography to separate HP proteins based on their molecular weight and identified 12 fractions that suppressed DC secretion of IL-12p70 in response to CpG-ODN. The suppressive fractions were pooled and subjected to further separation by anion-exchange chromatography followed by testing on CpG-ODN-stimulated BMDC. We identified 3 fractions that suppressed IL-12p70 secretion. Analysis of these fractions by LC-MS/MS identified 21 candidate proteins. To further narrow down the immunomodulatory candidate(s), HP was resolved by cation-exchange chromatography. Only one fraction
Dexamethasone could decrease the expression of IL-5, IL-6, IL-10 and TNF-α inhibiting MMP-2, 9 and Th2 inflammatory cytokines expressions.

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Dexamethasone Downregulated the Expression of Matrix Metalloproteinase 2, 9 and Th2 Cytokines in Mice with Eosinophilic Meningitis Caused by Angiostrongylus Cantonensis Infection

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Angiostrongylus cantonensis, also known as the rat lungworm, is the major cause of eosinophilic meningitis in the Pacific Islands and Southeast Asia. Rats serve as the definitive host of the nematode. Humans are infected incidentally and lead to eosinophilic meningitis. Previous BALB/c animal study had demonstrated increased matrix metalloproteinase 9 (MMP-9) expressions and blood brain barrier damage in mice infected with A. cantonensis. Steroids have been shown to be one of the effective treatment options for eosinophilic meningitis caused by A. cantonensis infection. However, the mechanism of how steroids can influence on eosinophilic meningitis is still unclear. We hypothesize that the beneficial effect of steroid on eosinophilic meningitis are mediated by decrease MMP and cytokynes expressions. In a BALB/c mice model, mice were orally infected with 40 A. cantonensis L3 via an orogastric tube and were sacrificed every week for 3 consecutive weeks after infection until the end of the study. Dexamethasone was injected via the intra-peritoneal route from 7th days of infection until the end of the study. Evans blue method was used to measure the blood brain barrier changes and the serum/CSF and brain homogenates expression of MMP-2, 9 and cytokynes were analyzed by gelatin zymography, western blot, ELISA and reverse transcriptase polymerase chain reaction (RT-PCR) respectively. There were an increased MMP-2 and MMP-9 expressions in CSF and brain homogenates following 2-3 weeks of infection. Dexamethasone administration could down-regulate the expression of MMPs. These changes were parallel to the blood brain barrier disruption as evidence by the Evans blue extravasation following infections. Furthermore, the brain homogenates cytokynes , such as IL-5, IL-10 and INF-γ were also elevated following 2-3 weeks of infection. Dexamethasone could decrease the expression of IL-5, IL-6, IL-10 and TNF-α by using ELISA. The brain homogenates mRNA expressions of IL-5, IL-6, INF-γ and TNF-α were decreased gradually by RT-PCR. All of these findings suggested that the Th2 cytokynes play an important role in mice with eosinophilic meningitis caused by A. cantonensis infection and provided the evidences supporting steroid effects on A. cantonensis infection by inhibiting MMP-2, 9 and Th2 inflammatory cytokines expressions.
tested compounds from plants for their anthelmintic activity against the adult stage of the hookworm, Ankylostoma ceylanicum. The present study screened extracts from five plant species and chromatographically-enriched fractions of the most active one. These plants were collected from the western United States. Extracts from two of the plants namely Dalea ornata and Oemlia cerasiformis showed anthelmintic activity (mortality and/or reduced motility) of their crude extracts and enriched fractions against A. ceylanicum. Associated worm mortality rates ranged from 25% to 100% after 120 hours of incubation with the test compounds. Three concentrations of the compounds were tested (10, 50, 10 mg/mL). Our study revealed a dose-dependent activity where the lowest concentration (10 mg/mL) achieved 100% mortality 120 hours post exposure while the same activity level was obtained at 48 hours with 100 mg/mL. Our data show that plants represent a potentially untapped source of effective anthelmintic molecules. Studies are underway with the aim of purifying and testing active components of the extracts ex vivo and in vivo. The anthelmintic activity of these compounds in the animal model of the disease will be evaluated using clinical, parasitological and immunological parameters such as weight gain, anemia, egg output, worm burden, immune cell proliferation indices, and immune cell population types and sizes by flow cytometry.

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CYTOKINE EXPRESSION PROFILES AND IMMUNITY TO MEASLES VACCINATION IN KENYAN CHILDREN WITH HELMINTH INFECTION

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Helminths are among the most prevalent infections in resource-limited countries, with an estimated one billion school-age children worldwide living in areas at high risk for helminth transmission. Children are particularly susceptible to high-burden infections, which can result in derangements in host cytokine expression. These derangements, in turn, lead to both immunosuppression and pathologic inflammation in the host. The disruption of cytokine signaling by helminths has been proposed to impair the ability of children to mount protective immune responses to vaccination. We are currently enrolling children in a cross-sectional study to test the hypothesis that helminth infection is associated with a modified Th2 cytokine response, which is permissive for chronic helminth infection and reflected by an elevated IgG4/IgE ratio. Furthermore, we will explicitly test the prediction that helminth infection is associated with impaired vaccine-induced immune responses, specifically for measles vaccination. This study is nested within a large pediatric surveillance cohort at three clinical sites in Western Kenya. Plasma and stool samples are collected at the time of enrollment from eligible children aged 1 through 5 years with documented measles vaccination. Shortly after collection, stool samples are tested by Kato-Katz and formol-ether concentration methods to diagnose helminth infection and quantify parasite burden. Plasma from children with and without helminth infection will be analyzed by ELISA to compare serum levels of Th1 and Th2 cytokines, as well as titers of IgE, IgG4, and anti-measles antibodies. Preliminary enrollment demonstrates a helminth infection prevalence of approximately 25% in our Western Kenya cohort. Improving our understanding of helminth-mediated immune dysfunction will facilitate the rational design of both anti-helminth vaccines and novel adjuvants to enhance vaccine efficacy in helminth-endemic areas.

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TOXOCARIASIS IN NORTH AMERICA: A SYSTEMATIC REVIEW

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Toxocariasis is an important neglected tropical disease that can manifest as visceral or ocular larva migrans, or covert toxocariasis. All three forms pose a significant public health problem in areas of high prevalence. To determine the burden of toxocariasis in North America we conducted a systematic review of the literature following PRISMA guidelines. We found 18 articles with original prevalence, incidence, or case data for toxocariasis. Of these articles, 5 reported data for Canada, 8 reported data for the United States, and 5 reported data for Mexico. One article reported only prevalence data, no articles reported incidence data, 5 articles reported only affected cases, and 12 articles reported both prevalence data and number of affected cases. 63% of articles with seroprevalence estimates for the United States tested blood samples collected during the 1988-1994 National Health and Nutrition Examination Survey. The most commonly cited risk factors for toxocariasis included male sex, pet ownership, particularly if the animals are allowed to live outdoors and eat other animals or otherwise consume unconventional pet food, African American race, age less than 18, low level of education, poverty, foreign birth, living in the southern United States, and playing at parks or in sandboxes where dogs and cats have defecated. Of the studies that reported prevalence data by sex 73% found a statistically significant greater prevalence in males compared to females, while 27% of studies found no significant differences between genders. No studies found females to have significantly higher prevalence of Toxocara infection than males. Further research is needed to determine the true current burden of toxocariasis in North America, however the prevalence estimates gathered in this review suggest that the burden of disease is significant.

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THE ROLE OF SOIL-TRANSMITTED HELMINTH INTENSITY AND CO-INFECTION IN THE SPATIOTEMPORAL VARIATION OF CHILDHOOD ANEMIA: RESULTS OF A 5-YEAR MDA PROGRAM IN BURUNDI

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We investigated the role of Ascaris lumbricoides, Trichuris trichiura and hookworm infection and co-infections in the anaemia burden in school age children as a result of a 5-year mass drug administration (MDA) program in Burundi. The aim of the study was to investigate for the first time the impact of the MDA program on the spatiotemporal variation in anaemia burden in the country. Longitudinal parasitological data was collected from 40,553 children from 2007 to 2011 in 31 schools in Burundi. These data included faecal egg counts for A. lumbricoides, T. trichiura and hookworm, co-infections with these parasites and blood iron levels, age, sex, weight and height. Locational data included the GPS coordinates of the schools, annual mean land surface temperature, NDVI, precipitation and the distance to perennial water bodies. Spatiotemporal Bayesian geostatistical models of anaemia were built adjusting for age, sex, helminth intensity and co-infections, malnutrition and physical environment factors associated with the geographical location of the school. Our results indicate that the geographical distribution of the prevalence of anaemia did not change significantly during the 5-years. Our results indicate an association between the spatiotemporal variation in anaemia burden in children in Burundi and STH intensity and co-infections and environmental
conditions. Our study demonstrates the important role of malnutrition in anaemia burden over the five year period. The findings of this study suggest that MDA has played an important role in the control of anaemia in school-age children in Burundi. The results also suggest the need to integrate nutritional interventions to the current MDA programme to achieve the desired level of anaemia reduction in Burundi. Our maps can be useful to guide future MDA campaigns to achieve this reduction.

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DOMESTIC ANIMALS MECHANICALLY TRANSMIT NECTATOR AMERICANUS HOOKWORM TO HUMANS

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Necator americanus hookworm infections cause serious impairment in millions of children across the tropics. While humans are the only definitive host of Necator americanus, epidemiological evidence from South America, Africa, and Asia suggest that risk for hookworm infection is increased by contact with dogs and pigs. Building on experiments showing that N. americanus eggs can survive passage in the pig digestive tract, we demonstrate that pigs and dogs in twelve rural communities in central Ghana harbor and excrete viable human hookworm eggs in their stool. Using logistic and ordinal regressions, we show that pig ownership and the proportion of households owning dogs in one’s village significantly predicted hookworm prevalence and infection intensity. In these communities, 67% of dog feces and 55% of pig feces contain hookworm eggs. Using PCR, we confirmed a majority of collected animal stools contained N. americanus. Further, infective third stage hookworm larvae developed from all of the samples (n=41), demonstrating egg viability after natural passage through an animal’s digestive tract. The presence of high rates of antibodies to Toxocara canis in their serum suggests humans in these communities are frequently exposed to helminth parasites through contact with dogs. We introduce a new hookworm transmission model that indicates that animal-mediated transmission of hookworm eggs may play an important role in human disease. Hookworm control strategies embracing the ‘One Health’ concept by interrupting hookworm transmission from animals should be developed.

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A PARADIGM FOR STUDYING ANTHELMINTIC COMBINATIONS IN VIVO

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Soil-transmitted helminth (STH) nematode parasites (hookworms, Ascaris and Trichuris) are key contributors to morbidity and poverty worldwide. Few anthelmintics are available for treatment, and only anthelmintic, albendazole, is commonly used in mass drug administrations, even though there are more than 1 billion people are infected. New anthelmintics and treatment strategies are greatly needed, in particular as albendazole resistance is inevitable given its current method of usage and as commonly occurs with veterinary use of this drug. Our group has identified Cry5B made by Bacillus thuringiensis as a promising new anthelmintic for treating STH parasites. Apart from developing Cry5B as a much needed new class of anthelmintic, we are also interested in preserving the potency of Cry5B and other anthelmintics as much as possible—i.e., preventing resistance. To achieve this aim, combination therapies with anthelmintics is an excellent approach. The challenge with anthelmintic combinations is defining a good combination and at what ratio drugs can be productively combined. Although we have published combination studies using the nematode Caenorhabditis elegans, it was not clear how these translate in vivo in infected mammals. Surprisingly, little work has been done to define the characteristics of anthelmintic combinations in vivo. By using the hookworm (Ancylostoma ceylanicum) infections in hamster, we establish a new and powerful in vivo paradigm for studying anthelmintics combinations—defining not only how well two drugs combine but also providing some optimization of the ratio for combinations. Here, we will update you our latest results of the anthelmintic combinations with Cry5B and other anthelmintics, e.g., tribendimidine. We show that, like in C. elegans, these two drugs can be combined to give powerful effects in vivo. Work on other in vivo combinations is also proceeding. Our paradigm here provides a powerful means on how to examine and demonstrate the anthelmintic combination therapies.

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THE ROLE OF SOIL-TRANSMITTED HELMINTH INFECTIONS IN THE GEOGRAPHIC RISK OF FUNCTIONAL ILLITERACY OF SCHOOL-AGED CHILDREN IN THE PHILIPPINES: SPATIAL VARIATION AND NUMBERS AT RISK

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We aimed to understand the contribution that soil-transmitted helminth (STH) infections make to functional literacy status of school-aged children in the Philippines and investigated, for the first time, the geographical distribution of prevalence of functional literacy indicators adjusting for STH infections. STH infection data were collected from 29,919 individuals during the most recent National Schistosomiasis Survey conducted in 2005 to 2007 in 177 locations throughout the Philippines. Data were also provided by the National Statistics Office on three functional literacy indicators (i.e. ability to compute, read and comprehend) of 19,673 school-aged children in the Philippines. Our study was conducted in two phases: first, Bayesian geostatistical models were developed to predict the complete geographical distribution of Ascaris lumbricoides, Trichuris trichiura and hookworm co-infection and intensity of infection across the Filipino population. Second, Bayesian geostatistical models were built for each of the functional literacy indicators, adjusting for the location and the predicted co-infection and intensity of A. lumbricoides, T. trichiura and hookworm generated during the first phase. Our results indicate that A. lumbricoides and T. trichiura co-infection is an important contributor to the spatial variation of functional illiteracy of school-aged children. We identified significant spatial heterogeneity in functional literacy indicators between regions of the Philippines and remarkable spatial variation in functional literacy indicators within different regions of the Philippines accounted by STH co-infection and intensity of infection. Our results demonstrate the important role of STH co-infection and intensity of infection in the geographical variation of prevalence of functional illiteracy in the Philippines. The findings of this study can be useful to guide the Integrated Helminth Control Program to improve the health and functional literacy in the Philippines by reducing STH infection levels in school-aged children.
mixed feeding practice. In conclusion, pressure from family/neighbours to practice mixed feeding should be discouraged. Incorporation of family members into programs promoting safer infant feeding options in mothers living with HIV/AIDS and male involvement is imperative. Keywords: infant food, HIV, Women, Nigeria

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EVALUATION OF THE IMPACT OF HIV INFECTION ON THE DIGESTIVE FLORA OF CHILDREN

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The recurrent bacterial infections and affections observed in HIV positive infants could be explained by the imbalance in their digestive flora. The study aims at evaluating the impact of HIV infection on the digestive flora of infants aged 3–24 months, since there have been no studies addressing this issue in Cameroon. A cross-sectional and case-control study was carried out in two hospitals in Cameroon. Stool sample was collected from each of the proxy-consented HIV positive and HIV negative children. These stools were cultured using aerobic, strict anaerobic, 10% CO2, and microaerophilic conditions. Identification of the bacteria species were done by biochemical characterization. Out of the 80 children enrolled for the study, 33 (41.25%) were HIV positive children and 47 (58.75%) were HIV negative children. 15 different types of bacteria species were isolated from HIV positive infants with high presence of Lactobacillus spp. (96.97%), Bifidobacterium spp. (84.85%) and Streptococcus spp. (81.81%). Opportunistic bacteria like Shigella spp. (24.24%), Staphylococcus aureus (15.15%), Klebsiella spp. (12.12%), Acinetobacter spp. (84.85%) and Proteus spp. (96.97%) were also identified. Statistically, Clostridium spp. (p=0.009), Shigella spp. (p=0.002), Enterococcus spp. (p=0.006) Staphylococcus aureus (p=0.015) and Streptococcus spp. (p=0.015) were significantly more present in HIV positive infants than in HIV negative infants. Bacteria species like Proteus spp. produced high presence of bacteria like Staphylococcus aureus were isolated only in HIV positive infants and absent in HIV negative children giving a frequency rate of 24.2%. HIV positive infants at stage 3 and 4 harbored more opportunistic bacteria. We remarked the imbalance in bacteria flora of HIV infected infant’s harbouring quantitatively more isolated bacteria than in HIV non-infected children. Systematic stool culture would significantly benefit for the follow-up of HIV infected children to reduce the risk of recurrent bacteria infection.

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A THEORETICAL PERSPECTIVE OF THE LIVED EXPERIENCES OF HIV INFECTED MOTHERS TOWARDS ADMINISTERING DAILY COTRIMOXAZOLE PROPHYLAXIS TO THEIR HIV EXPOSED BUT NOT INFECTED CHILDREN IN MALAWI

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Although new HIV infections among adults in sub Saharan Africa have declined by 34% since 2001, the epidemic continues to disproportionately affect this region bearing 70% of all new infections in 2013. However, the sustained scale up of interventions for prevention of mother to child transmission of HIV has led to fewer new HIV infections in children. The World Health Organization recommends that all HIV exposed infants be started on cotrimoxazole prophylaxis (CPT) until breastfeeding is stopped and HIV infection is excluded to provide adequate prevention against early opportunistic infections. Adherence may be challenging when CPT is given to very small infants who need to take it for long periods of time. Previous studies have only focused on adherence to PMTCT services hence it is
important to build a grounded theory of the social and cultural influences of CPT adherence in this group. Three focus group discussions and seventeen in depth interviews were conducted with HIV infected mothers administering daily CPT to their HIV exposed but uninfected infants in a semi urban district in Malawi. A thorough literature search supplemented the concepts that emerged from the data. Findings We propose a fusion of the ecological theory of perception and the Health Belief Model to explain adherence to CPT in this study. The women themselves, their families, the communities in which they live and the health care system relate and influence each other through social interaction which play a role in influencing actions either positively or negatively. Motivators such as a supportive family help someone to take a positive action and factors such as shortage of drugs at the health facility act as deterrents. Interestingly, the data in this study demonstrates that despite negative influences that might arise at any level, the determination of the individual to take a health related action and the realisation that the recommended action will prevent any negative outcomes motivated the mothers to believe that they could successfully do something to prevent any negative health outcomes on their children. In conclusion, these findings could be used to design individualised interventions on HIV disease counselling as well as importance of treatment adherence in infants. The most currently used theory of Behavioural Learning lacks this capacity as it ignores effects such as past behaviour, habits and lack of acceptance of one’s diagnosis.

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KNOWLEDGE, ATTITUDE AND PRACTICES OF SEXUALLY TRANSMITTED INFECTIONS AMONG WOMEN OF REPRODUCTIVE AGE LIVING IN KATANGA SLUM KAMPALA, UGANDA

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Sexually transmitted infections (STIs) still stand as one of the commonest health problems affecting women of reproductive age especially in slums. These have further been identified as cofactors of HIV transmission, multiple complications such as, abortion, infertility and ectopic pregnancy among others. The burden of STIs continues to remain high in Uganda at 69%. In order to design appropriate preventive measures, there is need to establish the profile of knowledge, attitudes and practices of sexually transmitted infections among susceptible populations such as women of reproductive age living in slums like Katanga in Kampala Uganda. This was a descriptive cross-sectional study with 339 participants. A consecutive sampling method was done. Using a standardized questionnaire, woman in Katanga slum who met the eligibility criteria were interviewed and data collected from them. Data was coded, cleaned, transcribed and double entered using EPI-data 3.1 and analyzed using SPSS 17.0. Coded data was summarized using frequencies for categorical data and medians for continuous data. In this study 76.7% knew what STIs are with 41.9% giving Syphilis, Candida, HIV and gonorrhoea as examples. Most of the participants (99.1%) were between the age of 25-49 years with the majority (31%) being between 25-29 years. The commonest symptoms known to the participants were genital itching (59.2%) and genital rash (14.5%). Only 2.9% did not know about the predisposing factors for STIs, however most mentioned multiple partners (63.7%) and unprotected sex (50.7%). Only 51% could identify STIs by the signs and symptoms with 71.9% knowing that they are treatable and curable. 40% of the participants lacked knowledge on the effects and complications of STIs on their health and body. Although knowledge on methods of prevention was high (92.3%), it was not followed by appropriate behavioural patterns since 18.8% were found positive for STIs using the syndromic approach and 82% mentioned having suffered from STIs in the past 06 months more than once. The level of knowledge about STIs and their prevention is not matched by sexual behavioural patterns. Many women in Katanga slum still don’t practice the appropriate preventive measures for STIs.

There is a need to lay strategy on how the preventive measures which are well known by this vulnerable population can actually be effectively adopted and practiced.

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CROSS SECTIONAL ASSESSMENT ON THE PREVALENCE OF HIV AMONG DOTS CLIENTS IN ADDIS ABABA ADMINISTRATIVE REGION 2011-2012

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TB and HIV are the major public health problems in Addis Ababa, considering this fact, Health institutional based cross-sectional study was undertaken in Addis Ababa administrative region from September 2011 to February 2012. The objective of the study was to determine HIV prevalence among registered tuberculosis patients in Addis Ababa public DOTS clinics. The HIV antibody was determined using a single ELSA technique. A total of 417 tuberculosis patients in 26 Dots (intensive care) clinics aged 16 years and above was enrolled in the study. The overall HIV prevalence rate among tuberculosis patients was 32.5%. The highest rate was observed in the age group 30 to 39 years. Almost equal proportions of male 49.3% were found to be HIV sero positive compared to the females 50.7%. Being unmarried was found to be associated with HIV positive test result (p<0.005). Those divorced and widow/widower patients had high proportion of HIV positive. Daily labourers and patients who are living alone were found to be significantly infected with HIV (p<0.001). The HIV positive rate was higher among pulmonary tuberculosis patients compared to extra-pulmonary tuberculosis cases. Smaer positive pulmonary patients 25% were found to be significantly associated with HIV sero positive test result compared with smear negative pulmonary tuberculosis cases 15.6%. It was concluded that HIV infection is highly prevalent among smear positive TB, higher schooling, daily labourers, young age group, unmarried TB-clients, people who had history of STD, history of multiple sexual partners. Finally, additional qualitative supportive study on KAP on TB patients towards HIV/AIDS is recommended.

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MYCOBACTERIUM TUBERCULOSIS-SPECIFIC CD8+T CELL RECALL IN CONVALESCING TB SUBJECTS WITH HIV CO-INFECTION

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Memory T cell populations recover following phase I chemotherapy for tuberculosis (TB) and augment the effectiveness of antibiotics during the continuation phase of treatment. For those with human immunodeficiency virus (HIV), the CD8+ T cells may have an especially important role in host defense to Mycobacterium tuberculosis (M.tb) as CD4+T cell function and/or numbers decline. Here we performed a preliminary study to investigate the impact of HIV infection status on CD8+ T cell effector function during the convalescent TB period. Peripheral blood samples from convalescent HIV+ and HIV- TB subjects were used to determine CD4+ T cell count and monitor antigen-specific CD8+ T cell activation of effector function (lymphoproliferation, IFN-γ, granulysin) in response to M.tb antigen. Our preliminary results suggest that HIV co-infection is associated with moderate suppression of the M.tb-specific memory CD8+ T cell compartment in many subjects convalescent for TB. Interestingly, highly activated CD8+ T cells were observed in recall experiments using peripheral blood from several HIV+ subjects that had low (<200 cells/mm3) CD4+ T cell counts. Further investigation may provide important information for development of novel approaches to target M.tb-specific CD8+T cell memory to protect against TB in HIV-endemic regions.
PREVALENCE AND RISK FACTORS OF PULMONARY TUBERCULOSIS AMONG HIV/AIDS PATIENTS IN IHIALA, ANAMBRA STATE, NIGERIA

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The prevalence and risk factors of pulmonary tuberculosis among HIV/AIDS patients were determined in this study. A cross-sectional study design was adopted and hospital records from 2007 to 2011 of 375 HIV/AIDS patients attending Highly Active Antiretroviral Therapy (HAART) Centre of Our Lady of Lourdes Hospital Ihiala, Anambra, Nigeria, were reviewed. A standard proforma was used to generate data on other risk factors and diagnostic records. The prevalence rate of PTB among HIV/AIDS patients was 6.1%. The mean age, BMI and CD4 levels were 35.53 years (SD 9.37), 22.22Kg/m² (SD 2.23) and 429.85cells/mm³ (SD 268.41) respectively. Pulmonary TB prevalence was highest among HIV/AIDS patients aged 30-39 years (60.9%), married patients (60.9%), patients with normal BMI (100%) and patients with CD4 levels <200cells/mm³ (60.9%). Lower CD4 levels <200cells/mm³ (P<0.0001), age 30-40 years (P<0.019) and marital status (being married, P<.005), respectively, were significantly associated with the occurrence of PTB among HIV/AIDS patients in this study. Co-treatment of TB/HIV/AIDS (DOTS-HAART), lifestyle modification and education to minimize exposures to risk factors should be scaled up and encouraged.

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THE IMPACT OF NUTRITIONAL STATUS ON FIRST LINE HAART FAILURE IN HIV-INFECTED CHILDREN IN CAMBODIA

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While the impact of antiretroviral treatment on growth in children is well established, the influence of prior nutritional status on the response to treatment is not well known. The objective of this study was to assess impact of nutritional status on treatment failure in HIV infected children receiving first line highly active antiretroviral therapy (1st line HAART). Nutritional status was assessed by height-for-age, weight-for-age and weight-for-height Z scores using the 2006 World Health Organization growth reference. CD4 cells counts and viral load (VL) were measured every 6 months or when CD4 drop-down has been observed, consequently minimally twice per years. Laboratory parameters and genotypic resistance tests were performed in case of clinical or immunological failure by National Paediatric Hospital laboratory affiliated to Institute Pasteur Cambodia, Phnom Penh. First line therapy was consisted of Zidovudine + Lamivudine + Nevirapine or Efavirenz in those with concomitant tuberculosis infection. In our study group of HIV infected children treated with HAART (n=98) the median Z score for HAZ, WAZ, BAZ at baseline of HAART was -3.3 (IQR = -4.6/-2), -3.4 (IQR= -4.1/-1.4), -1.4 (IQR= -2.3/0.4) and decreased during study period -2 (IQR= -2.7/-1.5), -2.1 (IQR= -2.8/1.5), -0.9 (IQR= -1.8/0.2) respectively. Concerning risk factors for treatment failure, we compared 77 (79%) children on 1st line HAART with 21 (21%) children with treatment failure (treated with second line regiments). Low CD4 cell percentage (<5%) and wasting (WAZ > -3) at the baseline were not significantly associated with treatment failure. In our study, baseline poor nutritional status was not associated with failure of 1st line HAART among HIV-infected Cambodian children. All of our children were placed in 2 orphanages with good nutrition (five times a day) on inpatient basis, which may contribute to low failure rate. We advise the development of further studies to assess the nutritional status of children with HIV/AIDS using anthropometric measurements.

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DECREASING OCCURRENCE OF BACTERIAL SEXUALLY TRANSMITTED DISEASES AFTER INTRODUCTION OF VOLUNTARY COUNSELING AND TESTING HIV-PROGRAM IN ELDORET, KENYA IN 2009-2013

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Incidence of bacterial sexually transmitted diseases (STD) correlates with HIV as well with Hepatitis B and C and introduction within integrated program usually has impact on multiple diseases. The aim of this research is to assess the impact of community based integrated health program focused on HIV/AIDS and tuberculosis (TB) on the occurrence of bacterial STD in rural community of about 50 000 inhabitants in north Eldoret, Kenya, in area of HIV prevalence of 8-14 %. Among all outpatient department (OPD) visits during last 4 years (2009-2013) in Bl. Ladislau Battthyany-Strattmann Clinic serving for about 50 000 people, incidence of STD and HIV in infections cases was assessed and correlated. HIV VCT program has been established in 2008 as VCT center next to the clinic with one VCT counselor and 1 trained nurse. All VCT/HIV program and all OPD visits for STD were recorded monthly. Syphilis and gonorrhea were evaluated as STD. Among 42711 OPD visits in last 4 years, STD was diagnosed in 1446 patients (3.39 %) and HIV in 462 patients (1.1 %). However, 10 years ago, when the clinic started its work, HIV prevalence in males was 8.6 % and in females 11.9 %. Dramatic decrease of HIV was correlated with sustained decrease of bacterial STD’s (syphilis, gonorrhoea). While in 2009, 505 cases of bacterial STD and 110 new cases of HIV were detected, 421 STD’s were diagnosed in 2010, 176 in 2011 and 201 in 2012 and 148 in 2013 were recorded followed decrease of HIV from 110 in 2009 to 53 in 2013. Unfortunately, proportion of adults with HIV and STD was decreasing in 2009-2013, more then 3-fold, while pediatric STD in children <5 years increased from 0 % to 1 % in 2009 , 3 % and 6% in 2010 and in 2013, respectively. Integrated HIV/STD community program led to more 3.3 - fold decrease of STD ad 2.1-fold decrease of AIDS in rural community of Eldoret after 5 years of the introduction of VCT. Moreover, increasing prevalence STD in children <5 years of age is of great concern.

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BACTERIAL INFECTION AND MALARIA IN HIV POSITIVE CHILDREN ADMITTED TO HOSPITAL IN NORTHEAST TANZANIA; RESULTS OF A ONE-YEAR OBSERVATIONAL STUDY

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Over three million children under 15 are now infected with HIV in sub-Saharan Africa, with profound health consequences. Both HIV infection, HIV treatment and malaria infection affect the incidence of invasive bacterial disease in children and the range of causative organisms, yet few studies have provided these data systematically. We present data from an observational study of paediatric admissions between June 2006 and July 2007 to a district hospital serving a rural population in northeast Tanzania. Children aged 2 months to 13 years admitted to the hospital during study hours with fever, or history of fever, were considered eligible.
Enrolled children underwent a standard clinical examination and panel of diagnostic blood tests including a research blood slide and rapid-test for malaria, blood culture and HIV serology. Children aged less than 18 months with positive serology were tested for HIV-1 RNA. CD4 T-cell counts were performed by flow cytometry on-site. Results from this study have been published, but data pertaining to the range infections in HIV positive patients are presented here for the first time. 3,704/4,334 (85.5%) of those children admitted during study hours were eligible, consented and enrolled. The data on 3,639/3704 (98.2%) were complete. HIV infection was diagnosed in 142/3,639 (3.9%). The most common clinical diagnosis in children admitted with HIV was very severe pneumonia (33/142 (23.2%). In HIV positive children the prevalence of Plasmodium falciparum by blood slide was significantly lower (48/142, 33.8%) than in HIV negative (2,147/3,497, 61.4%, p<0.001), though the median parasite density in HIV positive (22,731 parasites/µl) and HIV negative children (36,758 parasites/µl) were similar (p=0.128, Wilcoxon rank-sum).

HIV positive children had a higher prevalence of invasive bacterial disease (27/142, 19.0%) than HIV negative children (314/3,497, 9.0%, p<0.001). In children negative for HIV the most common bacterial isolate was non-typhi Salmonella, in HIV positive children Streptococcus pneumoniae was the most common isolate. HIV was implicated in over 1 in 8 inpatient deaths. We were unable to show such an association between HIV infection and severe malaria and malarial death in this population.

PUBLIC-PRIVATE DIAGNOSTICS AND REFERRAL SERVICES FOR HIV/TB CO-INFECTED PATIENTS: A SYSTEMATIC REVIEW

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In 2012, there were an estimated 8.6 million new infections and 1.3 million deaths due to tuberculosis (1). Tuberculosis is the second leading cause of death due to infectious diseases worldwide (2, 3), and is the leading cause of death among people living with HIV (3). The successful integration of tuberculosis and HIV services in both the public and private sector is essential to the fight against the dual burden of TB and HIV. The WHO Stop TB department has called for a collaboration of public and private healthcare providers (often referred to as public private mix [PPM]) to maximize TB/HIV integrative services while minimizing costs. Many challenges remain for engaging all care providers into integrated HIV/TB services, particularly in regards to co-current diagnostics and referrals. HIV patients must be screened for tuberculosis, and tuberculosis patients must be screened for HIV. An efficacious public private partnership has the potential to integrate tuberculosis and HIV diagnostics and referral services to improve health outcomes and reduce patient costs. This study was undertaken to determine the frequency of public-private diagnostic and referral services for HIV/TB co-infected patients in high burden countries? Using several electronic databases and WHO indicators, we will conduct a systematic review to assess the degree of integration of various public-private partnership models for co-current HIV/TB diagnostics and referral services. We would like to evaluate the efficacy of various PPM models using published indicators of successful integration of services, and discuss the subsequent policy implications of our findings.

PREVALENCE OF HIV/AIDS AND TOXOPLASMOSIS IN NIGERIA

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Human Immunodeficiency Virus (HIV) has been infecting people all over the world at an alarming rate and giving rise to full blown AIDS ( Acquired Immune Deficiency Syndrome). Its prevalence in Nigeria is a concern. It is at present believed to infect 3.1% of a population of 150 million. Associated with HIV/AIDS is opportunistic infections. Some of these are cryptosporidiosis, Pneumocystis carinii infection and Toxoplasmosis.

Toxoplasmosis is one of the most serious opportunistic infections in HIV/AIDS patients that lead them to their early demise. For HIV/AIDS patients, cerebral toxoplasmosis causes encephalitis which worsens the patient’s condition. Human beings get infected by eating raw or undercooked meats containing Toxoplasma gondii cysts. Such meats come mainly from pigs, sheep and goats, especially the last two which Nigerians eat a lot and most commonly during festivities like Christmas celebration, birthday celebration, Muslim festivals, etc and are sold commercially. There is scarcity of information about co-infection of HIV/AIDS and Toxoplasmosis in Nigeria. A review of available literature on the prevalence of toxoplasmosis in humans in Nigeria shows that there are about 17 reports between 1960 and 2010. Of these only three show co-infection of HIV/AIDS and toxoplasmosis. Even so, the infection rate is fairly high (38.8%/ 219 and 43.4%/60 and a positive case report). There is a need in Nigeria to step-up activities involving the screening of HIV/AIDS patients for toxoplasmosis and treating those found infected as there is treatment for toxoplasmosis. This will prolong the lives of patients.

POVERTY, FOOD INSECURITY AND LOW BODY MASS INDEX, ARE ASSOCIATED WITH POOR QUALITY OF LIFE FOR PEOPLE LIVING WITH HIV IN RURAL HAITI

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Poverty, food insecurity, and HIV/AIDS often co-occur and have a synergistic negative effect on health. However, the impact of these factors on quality of life (QOL) has only recently begun to be explored for people living with HIV (PLWHIV) in resource-poor settings. This study investigates the relationships between poverty, food insecurity, body mass index (BMI), and QOL in a cohort of 524 HIV-positive patients in rural Haiti. Multivariate logistic regression models were fitted to identify predictors of high QOL based on a survey containing a validated poverty score, the Household Food Insecurity Access Scale, and three domains of the AIDS Clinical Trials Group Health-Related Quality of Life Scale: general health, physical functioning, and role functioning. Of 489 respondents, 246 (50%) reported severe food insecurity and 428 (88%) reported some food insecurity. Mean QOL scores were 45 (Health), 62 (Physical), and 65 (Role), out of 100. Not being severely food insecure (OR=0.453, p=0.035; OR=0.387, p=0.008), having less poverty (OR=0.957, p<0.001; OR=0.969, p=0.005), and having a higher BMI (OR=1.1, p=0.002; OR=1.08, p=0.007) were each independently associated with higher health and physical QOL. Having access to food from a garden (OR=1.79, p=0.05; OR=2.01, p=0.016) and younger age (OR=0.978, p=0.039; OR=0.97, p=0.005) were associated with higher physical and role functioning QOL. Food insecurity is highly prevalent at 88% in this population of PLWHIV in rural Haiti. This population’s mean QOL scores were also substantially lower than scores reported from other cohorts of PLWHIV in resource-poor settings. Food insecurity, low BMI, and poverty are independently associated with poor QOL in PLWHIV and must be addressed as an integral component of comprehensive HIV programs in this setting.
EVIDENCE OF A DISTINCT PROFILE OF METALLOPROTEINASES 2 AND 9 AND THEIR INHIBITORS IN CARDIAC REMODELING OF CHAGAS DISEASE

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Dilated chronic cardiomyopathy (DCC) in Chagas disease is associated with myocardial remodeling and interstitial fibrosis, resulting in significant extracellular matrix (ECM) modifications. ECM remodeling is regulated by proteolytic enzymes such as matrix metalloproteinases (MMPs). The main objective of this study was to evaluate the involvement of MMPs 2 and 9 and their inhibitors (TIMPs) in indeterminate (IND) and cardiac (CARD) clinical forms of Chagas disease. We evaluated, for the first time, the serum levels of MMPs 2 and 9 and TIMPs 1 and 2, as well as the main cell sources of MMPs 2 and 9 in the peripheral blood from patients presenting IND or CARD clinical forms of Chagas disease. Our results showed that MMP-9 serum levels are associated with the severity of Chagas disease. The serum levels of TIMP-1 were not different between the studied groups; however the serum levels of TIMP-2 were higher in CARD group. The correlation analysis showed a possible specificity of TIMP-1 for MMP-9. The analysis of MMPs production by T lymphocytes showed that CD8+ T cells are the main source of both MMP-2 and MMP-9 molecules. Using a new 3-dimensional model of fibrosis we also observed that serum from patients with Chagas disease induced an increase in the extracellular matrix components in cardiac spheroids obtained from mice cardiomyocytes. Furthermore, MMP-2 and MMP-9 have shown different profile of correlation with matrix proteins (laminin and fibronectin) and extracellular matrix components in cardiac spheroids obtained from mice cardiomyocytes. Our results suggest that MMP-2 and MMP-9 show distinct activities in Chagas disease pathogenesis. While MMP-9 seems to be involved with the inflammation and cardiac remodeling of Chagas disease, MMP-2 does not show any correlation with inflammatory molecules. In conclusion, our data stress the involvement of MMP-9, and not of MMP-2, in heart disease and, for the first time, its participation in chagasic cardiomyopathy. These data are innovative and represent an advance in the knowledge of the mechanisms involved in the establishment/maintenance of the Chagas heart disease pathology.
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3D MODELS OF SAND FLY SALIVARY PROTEINS

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Sand flies (Diptera: Phlebotominae) are vectors of Leishmania (Trypanosomatidae), the causative agents of cutaneous and visceral Leishmaniasis. During the blood feeding, sand fly females inject saliva into the host skin to overcome host haemostatic mechanism. Many proteins with distinct functions were found in cDNA library of sand fly salivary glands. 3D models of salivary proteins would allow us to better understand the protein function in antihaemostatic and immune responses. Our study was focused on yellow-related proteins, the high affinity binders of the host prohemostatic and proinflammatory biogenic amines such as a vascoconstrictor serotonin. In Lutzomyia longipalpis, it has been shown that salivary yellow-related proteins bind serotonin into their binding pocket located in the central part of the molecule, thereby hamper its physiological function and allow blood feeding of sand fly females. We took advantage of the known 3D structure of L. longipalpis yellow-related protein. The predicted 3D structures showed interspecies variability in the amino acid residues inside the binding pocket. For example, the bond of serotonin is probably stronger in P. orientalis yellow-related protein PorMsp24 than in 3Q6K of L. longipalpis due to the substitution of phenylalanine for glutamine and the new possible hydrogen bond between serotonin and glutamine. Such substitutions may affect the binding ability of yellow-related proteins. In L. longipalpis, the binding ability differs also between the yellow-related protein variants within the species. Since there are several yellow-related proteins in each sand fly species, we constructed 3D models to predict also the interspecies variability in serotonin binding ability. Yellow-related proteins are also highly antigenic for hosts and are considered as candidates for transmission blocking vaccine against Leishmaniasis, which means the need to know their exact function and structure. 3D models of sand fly salivary yellow-related proteins were constructed in MODELLER and visualised in The PyMOL Molecular Graphics System.

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SPONTANEOUS HEART DISEASE IN THE ADULT CHIMPANZEE: THE ROLE OF TRYPANOSOMA CRUZI

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Heart disease is the leading cause of death in the United States, and kills over 650,000 people annually. A high incidence of heart disease, especially idiopathic cardiomyopathy, is seen in chimpanzees and is the leading cause of death in chimpanzees at the Southwest National Primate Research Center (Seiler 2009). The high prevalence of heart disease-related mortality in captive colonies makes chimpanzees a valuable animal model for studying heart disease. The aim of this study was to retrospectively examine potential contributing factors and causes of heart disease in chimpanzees with emphasis on Trypanosoma cruzi (T. cruzi). We reviewed necropsy reports of adult chimpanzees that died with heart disease as a histopathological finding. We examined age, sex and cause of death. The overall prevalence of heart disease in chimpanzees was 67.81% (Seiler 2009). Of these, 30.12% have myocarditis and the remainder died of cardiomyopathy. We divided the animals into two groups based clinical findings and histopathological characteristics. Group1: myocarditis and Group2: cardiomyopathy. Myocarditis was indicated by lymphocytic infiltration of the myocardium. Cardiomyopathy was characterized by diffuse myocardial fibrosis with minimal inflammation. By simple PCR, we tested 78 heart tissue samples from chimpanzees in these groups for the presence of T. cruzi DNA. Of the chimpanzees that died of heart disease, only 19% tested positive for the presence of T. cruzi. Of all of the animals tested, the myocarditis group was 23% positive for T. cruzi while 18% of the cardiomyopathy group tested positive. Combined, the percentage that tested positive was 19% (15/78). In some tissues (2/35), T. cruzi microorganisms were observed. The cause of heart disease in these animals is still under investigation. Based on these results, T. cruzi is one of the causes of heart disease in the chimpanzees at SNPRC, however it is not the main cause.

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INFECTION BY LEISHMANIA BRAZILIENSIS IS ALTERED IN U937 DERIVED MACROPHAGES EXPRESSING EXOGENOUS GFP

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Gene knock-down mediated by RNA interference is increasingly used during the study of host-parasite interaction. Often exogenous gene expression such as GFP is used as a reporter during functional assays. The aim of this work was to evaluate exogenous gfp as a positive control in order to verify RNAi mediated gene silencing, in the context of U937 derived macrophages infected by Leishmania braziliensis. Parental cell line U937 was genetically modified via lentiviral transduction with a construct encoding for constitutive expression of GFP, generating the cell line U937-GFP. In turn this cell line was transduced with vectors encoding for a shRNA targeting GFP or a non-relevant shRNA as negative control, to generate the cell lines U937-GFP/shRNA-GFP and U937-GFP/shRNA-NR, respectively. The three U937 derived cell lines were characterized in terms of GFP expression by Western blot, as well as their ability to be infected by Leishmania braziliensis. One-way ANOVA and Sidak test were applied for statistical analysis using STATA 13 software. Results showed a reduction of 88.9% in GFP levels in the cell line U937-GFP/shRNA-GFP while there was no change in GFP expression in the negative control U937-GFP/shRNA-NR. In functional terms, the U937-GFP cell line showed reduced infection rate as compared with the parental cell line U937. Interestingly, this infection parameter was reconstituted to parental levels, in the U937-GFP/shRNA-GFP cell line, but remained low in the negative control U937-GFP/shRNA-NR still expressing GFP. This suggests that expression of GFP is responsible for the reduction in infection rate observed in the U937-GFP cell line. Caution should therefore be considered when using GFP exogenous expression in this cellular model of human macrophages.

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GENETIC DIVERSITY IN LEISHMANIA DONOVANI FROM SRI LANKA: USE OF MINICIRCLE DNA FOOTPRINT ASSAY

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Leishmaniasis comprises a variety of syndromes primarily due to at least 16 species and subspecies of Leishmania. In humans, the clinical spectrum ranges from asymptomatic infections to high mortality, with three distinct forms being classically described: visceral (VL), cutaneous (CL) and mucocutaneous (MCL). Leishmaniasis is a recently established disease in Sri Lanka with over 3000 cases of CL distributed island-wide with a few visceral and mucosal cases reported during the past decade. A genetically distinct variant of the usually visceralizing L. donovani is the causative agent and has been studied here using mitochondrial minicircle footprint assay to further understand its genetic status. Extracted parasite DNA from skin lesions of 34 CL patients and bone marrow aspirates of 4 VL patients were subjected to kDNA minicircle PCR followed by its footprint assay to further understand its genetic status. Extracted parasite DNA from skin lesions of 34 CL patients and bone marrow aspirates of 4 VL patients were subjected to kDNA minicircle PCR followed by its comparative sequence analysis through dendrogram using 6 reference Leishmania species as previously reported. Sri Lankan isolates from skin lesions made a separate cluster within other known L. donovani. There
were 4 distinct subclusters seen within the Sri Lankan group with isolates that demonstrated poor response to standard drug (intra-lesional sodium stibogluconate) forming a separate subcluster. No specific clustering of clinical isolates based on their geographical origins across Sri Lanka was apparent in the dendrogram. Distinct genetic mutations with specific functional characteristics are likely to induce drug resistance. However, correlations between L. donovani minicircle based strain specific sequence heterogeneity and distinct clinical characteristics needs further investigation. Table of Contents

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APPLICATION OF MOLECULAR TECHNIQUE FOR THE DETECTION OF VISCERAL LEISHMANIASIS IN BLOOD DONORS IN ENDEMIC AREA FOR LEISHMANIASIS IN FORTALEZA-CEARÁ, BRAZIL

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Leishmaniasis is caused by protozoa of the genus Leishmania and is endemic in 98 countries, affecting the poorest mainly in developing countries. In 1991 the World Health Organization (WHO) raised the issue that there is the possibility of transmission of Leishmania by blood donors. The conditions of storage of blood products allow the survival and maintenance of infectivity of Leishmania in blood banks and to date, serologic screening is not done in blood donors in Brazil. In areas of transmission, asymptomatic carriers represent a large contingent, it is important to evaluate the possibility of blood products are a potential risk for transmission of the parasite. In this study, buffy coat of blood donors of the Center of Hematology Ceará (Hemoco), were analyzed by conventional PCR to look for circulating Leishmania and PCR positive samples were randomized to perform sequencing for confirmation of the parasite. From May to November 2011, 351 samples of buffy coat of blood donors were analyzed. All of them had a negative serology for Chagas Disease, Syphilis, HIV I and II, HTLV I and II, Hepatitis B and C. For the conventional PCR reaction, specific primers (150 and 152) for the genus Leishmania were used, described by Oliveira, 2005, referring to a sequence of the minicircle encoding the β-globin gene, generating a fragment of 252 bp described by Pizzuto, 2001. DNA of Leishmania was detected in peripheral blood from 15/351 (4.3%) donors. The sequencing of 6/15 (40%) of the positive PCR confirmed that the isolated genome is belonging to the genus Leishmania. The results indicate the presence of Leishmania in blood donors and this implies in potential risk of transmission by blood transfusion depending on the parasite load. The role of asymptomatic donors infected with Leishmania in the chain of transmission is still not well established, so the possibility of transmission of the parasite by asymptomatic carriers must be better evaluated.

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MOLECULAR CHARACTERIZATION AND DRUG SUSCEPTIBILITY OF TRYPANOSOMA CRUZI ISOLATES DERIVED FROM INFECTED HUMANS OF ARGENTINA

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The genotype of Trypanosoma cruzi was related to the transmission cycle and geographic region. Herein, we studied the phenotype and susceptibility to trypanocidal agents of 7 T. cruzi populations recently isolated from 3 chronically infected adults (AR-SE23C, BOL-FC10A and AR-FC553) and 2 children with congenital infection (AR-FC202113 and AR-FC195205) living in Argentina. Molecular analysis showed that all isolates belonged to DTU V. For drug sensitivity studies, epimastigotes were cultured in LIT media (106/ml) and treated with 0.38-150 µM of Benznidazol (BZ), Nifurtimox (NX), Pentamidine isethionate (PENT) and dihydroartemisinin (DH) during 72 h; SylvioX10/4 clone was used as reference strain. AR-SE23C, AR-FC553 and AR-FC202113 isolates were as susceptible to BZ (IC50= 3.35±0.5, 7.66±0.0 and 2.25±1.0 µM respectively) and NX (IC50=5.06 ±0.2, 5.54±0.1 and 1.21±0.2 µM, respectively) as Sylvio-X10/4 (IC50= 4.22±0.4 and 5.29±1.3 µM for BZ and and NX, respectively). In contrast, significantly higher dose of BZ (73.08±4.3 and 40.62±3.1 µM) was required to reach IC50 for BOL-FC10A and AR-FC195205 isolates, respectively, and of NX (14.11±2.3 µM) for AR-FC195205 isolate. AR-SE23C, AR-FC553, AR-FC202113 and AR-FC195205 were similarly susceptible to PENT, with ID50 ranging from 2.68±2.1 to 4.09±0.1 µM; instead, 10.27±1.7 µM of this compound were necessary to reach IC50 for BOL-FC10A. All isolates were similarly resistant to DHIC50 as compared to BZ, NX and PENT. Preliminary studies on C3H/He mice infected with AR-SE23C trypomastigotes and treated with 100mg/kg/day BZ and NX during 30 days showed that conventional treatment is effective to diminish mortality rate and parasitaemia levels. Nevertheless, histopathological analysis revealed that neither drug was effective to clear tissue parasites and ameliorate the inflammatory process generated in the acute phase. In conclusion, we here confirm the predominant circulation of T. cruzi DTU V in Argentina, which includes subpopulations with a wide range of susceptibility to trypanocidal agents in vitro. MINCYT/FOCYT-PICT 2010-2148

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UTILITY OF DIFFERENT CLINICAL SAMPLES OF LEISHMANIA (VIANNIA) BRAZILIENSIS LESIONS TO CONDUCT MICROSATELITE ANALYSIS

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Leishmania (Viannia) braziliensis (LVB) is the main cause of cutaneous and mucocutaneous Leishmaniasis in the Americas. Microsatellites are DNA sequences repeated consecutively and present in abundance in non-coding regions of the eukaryotic genome. The variation in the number of repeats creates different alleles, allowing estimation of the genetic diversity of populations and gene flow. DNA from in vitro culture has been commonly used in Leishmania microsatellite analyses because of the quality of DNA and ease of standardization. However, parasite culture is difficult to obtain in field conditions. Other clinical samples are easier to collect, transport and maintain, but their use in population diversity studies has not been evaluated. Therefore, we assessed the viability and repeatability of microsatellite analyses based on different clinical samples from the same patients. We studied 22 cutaneous Leishmaniasis cases by LVB confirmed by nested real time polymerase chain reaction (PCR) and tested 53 clinical samples including cultures (22), biopsies (13), filter paper imprints (9) and scrapings using lancets (9). Samples were collected in Lima and six other cities of Peru during one year. Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue kit. Nine previously described microsatellite markers were tested by PCR and fragments amplified were analyzed to identify alleles using capillary electrophoresis in a 3130xl Genetic Analyzer (AB). We tested if all clinical samples amplified the nine microsatellite markers equally frequently and if PCR products from biopsies, lancets and filter papers showed the same allele peaks (±2 base pairs) as PCR products from cultures. On average, cultures amplified significantly more markers than the other samples (8.4 versus 5.6 to 6.3) but all clinical samples showed the same alleles peaks as cultures for the nine markers in >90% of the cases. Seven markers had the same alleles in >95% of the cases. Culture strains of LVB amplify more microsatellite
markers compared to other clinical samples, but the alleles identified are similar across all type of samples. Considering that cultures are difficult to obtain in field conditions, the use of other clinical samples may allow a better characterization of the population structure and genetic diversity in this important parasite.

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UNDERSTANDING THE MOLECULAR EPIDEMIOLOGY OF TRYPANOSOMA CRUZI I IN NORTH AMERICA: NEW ORLEANS FIRST APPROACH

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The most current nomenclature of Trypanosoma cruzi recognizes six discrete typing units (DTUs), Tc-VI and a seventh one named Tcb. TcI is highly heterogeneous and can be divided into 5 distinct subgroups TcIa-TcIe, based on differential amplification of the miniexon intergenic region. Though T. cruzi is endemic to Latin America, it has a marked presence in the United States. Despite the presence of an adequate vector for T. cruzi in Triatoma sanguisuga and one of the United States’ few areas with an autochthonous case of human Chagas disease, there exist few studies exploring the genetic variability of Southeastern Louisiana’s T. cruzi parasite populations particularly with respect to the TcI haplotypes. In the presented study, 60 rodent hosts and 12 T. sanguisuga vectors were captured from the site of Louisiana’s local human T. cruzi infection. DNA extractions were prepared from rodent tissues and from cultures established from vector feces. T. cruzi prevalence was determined by diagnostic PCR, subsequent PCR genotyping methods allowed for detection of specific TcI sub-genotypes. Amplification of T. cruzi satellite DNA revealed 76.6% of infection among sampled rodents. Twenty two T. cruzi-positive rodents could be genotyped by differential amplification of the mini-exon and 19 (86.4%) were found to be TcI. Eight samples of feces from vector (66.6%) were trypanosomatid-positive by direct microscopic observation. Six strains were successfully isolated and genotyped as TcI. All TcI samples were further typed as haplotype TcIa. The findings presented here corroborate existing literature on North American T. cruzi genetic distribution as well as the current proposed geographical distribution of the TcI haplotype. Further studies are required to fully assess the broader applicability of this study; however, it provides an epidemiological snapshot of the sampled region, enhancing our current understanding of regional T. cruzi phylogeography.

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GENETIC EXCHANGE IN LEISHMANIA TROPICA

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Leishmaniasis is a parasitic disease caused by protozoan intracellular parasites of the genus Leishmania. Leishmania species can show different clinical presentations, ranging from non-lethal cutaneous and mucocutaneous Leishmaniasis, which typically leave disfiguring scars, to visceral leishmaniasis, which can be lethal if left untreated. While traditionally thought to propagate asexually, hybridization has been unequivocally demonstrated to occur in the sand fly stages for L. major, and evidence has also been found more recently in L. donovani. L. tropica is an emerging Old World species that is responsible for severe cutaneous disease throughout its range, from India to Northern Africa. From a sample set of 36 isolates, 25 nuclear markers and 3 kinetoplast DNA markers were amplified and sequenced for multi-locus genotype analysis (MLSA). The preliminary sequence data shows extensive heterozygosity, indicative of potential ongoing hybridization occurring in this species. From this set, 4 strains have been selected for introduction of three different drug resistance markers. Selection of double-drug resistant hybrids was performed by culturing midgut homogenates of co-infected sand flies in the presence of both drugs. Here we present genetic evidence illustrating patterns of inheritance and gene exchange in this Old World species.

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DIVERSITY OF TRYPANOSOMA CRUZI INFECTION IN PATIENTS CO-INFECTED WITH HIV AND CHAGAS DISEASE

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Chagas disease, caused by the protozoan Trypanosoma cruzi, causes an unusual and severe neurological syndrome in immunocompromised patients such as those infected with HIV. We characterized the genetic diversity of T. cruzi infection in several HIV-infected Bolivian patients with high-level parasitemia. Samples were derived from HIV-positive patients recruited into an epidemiological study of HIV/Chagas disease coinfection in Santa Cruz, Bolivia, and DNA was extracted from whole blood or guanidine-preserved samples using QiaCube extraction robot. T. cruzi infection was confirmed with RT-PCR. We used nested PCR to amplify a 327-base pair fragment of the polymorphic TcSC5D gene, which has previously been used for strain typing, and amplicon deep sequenced the region using an Ion Torrent PGM. We determined multiplicity of infection and genotyped the strains of T. cruzi using deep sequencing and conventional restriction fragment length polymorphism (RFLP) methods. Sequences were clustered to predict genotypes using a heuristic clustering algorithm. Within-host and within-population diversity indices were calculated using EstimateS. We have shown that deep sequencing of T. cruzi from clinical samples is possible. Furthermore, we have documented polyclonal infections in HIV-coinfected patients, which may have implications for the pathogenesis of the disease in this population. Further studies will examine parasite diversity in a larger sample size and compare T. cruzi strains found in blood and cerebrospinal fluid.

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ARTINM ADMINISTRATION DURING ACUTE EXPERIMENTAL INFECTION WITH TRYPANOSOMA CRUZI PROMOTES PROTECTION AGAINST THE PARASITE

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Chagas disease is caused by infection with Trypanosoma cruzi, and the profile of the host immune response is essential for the infection control. ArtinM, from Artocarpus heterophyllus, a lectin that binds to the manotriose, modulates immunity toward Th1 axes and confers resistance to intracellular pathogens. Then, we analyzed the ArtinM immunomodulatory property during acute experimental infection with T. cruzi. Male BALB/c mice (10 mice/group) were used at 10 weeks-old, and the infection was realized with T.cruzi (3000 trypomastigotes). “Colombian
strain*). The ArtinM administration (0.5µg/100µL; i.p.) was performed before infection (-5, -4 and -3 days) and post–infection (5, 10 and 15 days), and as negative control was used the vehicle (saline), followed by groups: Saline control (SC), Saline infected (SI) and Lectin infected (LI). Parasitemia was determined at 7th, 14th and 21th days post-infection. The hemogram, reticulocyte count, cytokines measurement (plasma and heart), histological analysis of heart (inflammatory infiltrate and nests of amastigotes) were performed after 23 days of infection. Inflammatory infiltrate was measured in H&E stained heart sections, through the point's grid with predetermined area that express the area (%) of inflammatory infiltrate. This method was used to determine the number of nests of T. cruzi by immunohistochemistry. IL group shows significant decrease in parasitemia at 14th and 21th days of infection. IS and IL showed increased reticulocytes, total leukocytes, neutrophils, lymphocytes and monocytes, and the hematimetrics parameters were decreased, ArtinM administration promotes a significant reduction in the inflammatory infiltrate (% of area) and nests of T. cruzi in the heart. Moreover, low levels of IL-12 p40, IFN-γ and TNF-α were found after ArtinM treatment, and IL-10 production was elevated, verified in plasma and heart. Thus, ArtinM administration demonstrates a protective effect against T. cruzi during acute experimental infection.

INFLUENCE OF LOAD PARASITIC IN INFLAMMATORY RESPONSE AND DEVELOPMENT OF INTESTINAL INJURY IN MICE INFECTED WITH TRYPANOSOMA CRUZI

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The acute phase of Chagas’ disease is reported to be multifactorial; among the forms of evolution of the most common disease have cardiac and digestive forms, this last in the esophagus and colon. Therefore, this study aims to evaluate variations of antigenic concentrations in the intestinal lesions in mice with acute Trypanosoma cruzi infection. We used 40 male mice C57BL/6WT divided into control group (A) and infected groups with 3x10^5 (B), 3x10^4 (C) and 3x10^3 (D) forms trypomastigotes of strain “Y” of T. cruzi. From a total of eight groups, four were euthanized after 7 (S) days of infection, and the remaining groups after 14 (F) days. The parasitemia was performed daily from day 3 after infection until the time of euthanasia. In the morphometric analysis, we verified the area myositis, ganglionitis and periganglionitis in relation the area of the muscle layer of the colon. Also analyzed the width and thickness of the muscular layer and of the colon mucosa. By immunohistochemistry, we detected the presence of T. cruzi nests. The quantification of the cytokines were performed by CBA. In results, there was an increase in parasitemia dependent on the concentration of inoculum between the different groups. Immunohistochemistry showed antigenic labeling in all animals of the group DF and in no animal of the BS group. The periganglionitis, ganglionitis and myositis were growing between the groups (significant differences), except to groups C and D, seven and fourteen days. The mucosal thickness showed no differences between groups. The width of the colon did not change between groups, except for DF, where there was enlargement. The thickness of the muscle layer is not changed in groups euthanized at 7 days, but after 14 days of infection in CF was hypertrophy and a reduction in DF. In the results of the cytokines, there was a noticeable change in the highest inoculum having an IL-2 after 7 and 14 days, and IL-6, IFN-g and TNF-a only 14 days, in which IL-10 did not differ significant between groups. In conclusion, the parasite load and the time of infection directly influence the number of nests of T. cruzi present in the colon, but not interfere with the mucosal thickness. There is a hypertrophy of the muscle layer in CF and decreased thickness of muscle in DF at the expense of enlargement of the colon, with alterations significant in production of cytokines proinflammatory.

LABORATORY FINDINGS OF ACUTE INFECTION WITH TRYPANOSOMA CRUZI FOR DIFFERENT CONCENTRATIONS OF THE STRAIN “Y” IN THE INOCULUM

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Hematological and urinary parameters are often used for screening and confirmation of various diseases. In Chaga’s diseases highlighted the clinical and laboratory findings are the result of a multifactorial infection with Trypanosoma cruzi. Moreover, recent studies demonstrate that variations of the antigenic concentrations may influence the course of disease, as well as the functional response of the infected organism. This way, this study aimed to report the laboratory findings in acute experimental T. cruzi infection with different inoculums through hematological and urinary parameters. Mice C57BL/6, males, aged 8 to 10 weeks, weighing 20 to 25g were infected with different parasite loads (3x10^4; 3x10^5 and 3x10^6) through strain “Y” of T. cruzi. The curve of parasitemia was performed until the 12th day after infection. Hematological parameters were evaluated using the complete blood count (automated method - ABX MICRO 60) on days 6, 9 and 12 after infection. The analysis of urinary parameters was performed through physicochemical analysis and urine sediment after 9 days of infection. The onset of parasitemia curve varied with the concentration of parasites in the inoculums on the 3rd, 4th and 5th days, for high, medium and low, respectively. The period of peak occurred for the high, medium and low inoculums on days 10, 9 and 8 respectively after infection. Any assessment of the blood count (red blood count, reticulocyte count, white cells and platelets) and urinary parameters (sediment), we observed changes in the high-inoculums when compared with the control (p <0.05). However laboratory evaluation by hemogram and urine routine are able to detect modifications, dependent on the parasitic load.

EVALUATION OF METABOLITES NITROGEN IN MICE CBL57BL/6 INFECTED WITH DIFFERENT INOCULA OF STRAIN “COLOMBIAN” OF TRYPANOSOMA CRUZI

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Chagas’ disease is a neglected tropical disease caused by Trypanosoma cruzi. Through increased catabolism, is associated also with renal disorders. This study aimed to evaluate the nitrogenous wastes from C57BL/6 mice infected with different inocula of T. cruzi. 70 mice male C57BL/6 were used at 12 weeks of age, weighing between 20 and 25g. The T. cruzi infection was performed with an inoculum of 3x10^4; 3x10^5 and 3x10^6 trypomastigotes of strain "Colombian". The animals were divided in groups: non-infected control (GC); Infected with 300 forms (GIA); Infected with 3,000 forms (GIB) and Infected with 30,000 forms (GIC), both for 22 and for 31 days. Held parasitemia every 3 days, the 24-hour urine collection in individual metabolic cages and blood collection. Urea levels were made by ultra-violet method and creatinine by colorimetric kinetic method, both in blood and in urine 24 hours a spectrophotometer. There was a decrease in urinary volume on the GIC after 22 days of infection and GIB, GIC 31 days after infection, beyond the weight decrease of the GIC group of animals on both days. For the BUN there was an increase to of levels GIC (22 days) and GIC and GIA (31 days). Levels of urinary urea reduced in the group GIB (22 days) and in GIC (31 days). The plasma creatinine showed a tendency to increase in all groups after 22 days of infection, whereas after 31 days there was a reduction in the levels for GIB and GIC. Decrease in urinary creatinine levels in groups GIB and GIC.
August 2009-September 2012 were sent to CDC Atlanta for further tests. A total of 145 samples that tested positive for influenza during CDC Atlanta, for further antigenic characterization and drug sensitivity since then laboratory sends technique started in Ethiopian National Influenza Reference Laboratory and mammals. The vaccinations offered every flu season target types A and B Influenza Virus isolates received during August 2009-September 2012 from Influenza sentinels surveillance (ISS) in Ethiopia.

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Historically, research of infectious diseases has focused on infections with single pathogens. However, infections with pathogens often occur in the context of other pre-existing viral and bacterial infections or pathological conditions. Clinically, this is of particular relevance for co-infections with pathogen Streptococcus pneumoniae and influenza virus, which both are the important cause of global morbidity and mortality in the world. However, the analysis of incidence data, representing the possible synergy between community-acquired pneumonia (CAP), which possible causative agent is S. pneumoniae, influenza and other respiratory diseases (RD) (chronic bronchitis, asthma, etc.) has never been presented before in Ukraine. The aim of the research was the model-based study of the official incidence data to identify the possible relationship between CAP, RD and influenza morbidity rates during 2007-2011 epidemic seasons in Ukraine via mathematical modeling. The official incidence data, published annually by Influenza Control Center and FG Yanovsky National Institute of Phthisiology and Pulmonology, was analyzed. As a result it was proposed conceptual synergy model of CAP, influenza RD morbidity among the population of Ukraine. The model parameters were found by the program developed in Java and based on the quasi-gradient method. The highest CAP morbidity rate in Ukraine was in 2009-2010 yrs., exceeding corresponding value for 2008 by 24.2 % and for 2011 - by 5 %. The analysis of incidence data showed significantly higher morbidity rates than average in Vinnytsia, Ivano-Frankivsk and Kyiv regions. The mathematical model implied the existence of functional relationship between the incidence of influenza and RD in their influence on the occurrence of complications such as CAP. Results of modeling showed that probably about 20% of CAP among the population of Ukraine occur as a complication after influenza (individually or against the background of bronchitis, asthma and other pathological conditions related to RD). In conclusion, it was proposed the meaningful approach to modeling that takes into account the functional relationship between RD and influenza cases that led to CAP and show strong association between these diseases and necessity of intensive preventive strategies for its control.

THE FIRST ANTIGENIC/ANTIVIRAL CHARACTERIZATION OF INFLUENZA VIRUS ISOLATES RECEIVED DURING AUGUST 2009-SEPTEMBER 2012 FROM INFLUENZA SENTINELS SURVEILLANCE (ISS) IN ETHIOPIA

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Influenza is a respiratory disease caused by RNA viruses that affects birds and mammals. The vaccinations offered every flu season target types A and B Influenza Viruses. Detection of Influenza virus using Real Time-PCR technique started in Ethiopian National Influenza Reference Laboratory during the A (H1N1) 2009 pandemic and since then laboratory sends influenza positive samples to WHO collaborating center for Influenza at CDC Atlanta, for further antigenic characterization and drug sensitivity tests. A total of 145 samples that tested positive for influenza during August 2009-September 2012 were sent to CDC Atlanta for further antigenic characterization and drug sensitivity tests. In the reference Laboratory, the Influenza positive Isolates were characterized antigenically using hemagglutination inhibition test with a panel of post ferret antisera, and also tested for functional neuraminidase inhibition assay to assess susceptibility of the viruses to the neuraminidase inhibitors oseltamivir and zanamivir drugs. The results of 136(93.8%) isolates were available till September, 2012. 48.5 % (66) of the isolates were A/ CALIFORNIA/07/2009-LIKE (H1N1) viruses, 12.5 % (17) of the isolates were B/BRISBANE/60/2008-LIKE viruses, 4.4 % (6) of the isolates were A/PERTH/16/2009-LIKE (H3N2) GP viruses, 4.4 % (6) of the isolates were B/WISCONSIN/01/2010-LIKE viruses, 1.5 % (2) of the isolates were B/FLORIDA/04/2006-LIKE viruses, 2.2 % (3) of the isolates were B/ VICTORIA/02/87,2.2 % (3) of the isolates were B/YAMAGATA/16L88 LINEAGE BY PCR, which is similar to B/Wisconsin/01/2010 virus. 21.3 % (29) of the isolates were unable to grow on the cell culture and was difficult for the characterization. 2.9 % (4) of the isolates were totally negative for Influenza Virus both by real time -PCR and cell culture techniques. All Influenza Positive Isolates were sensitive to Oseltamivir and Zanamivir on functional neuraminidase inhibition assay to assess susceptibility of the Influenza viruses to the neuraminidase inhibitors oseltamivir and zanamivir. All antigenically characterized Influenza Isolates were compatible with Influenza vaccine recommended by WHO. It also showed that diverse types of Influenza strains are circulating in Ethiopia. The inability of some positive isolates to grow in cell culture may possibly relate to sample collection, storage and sample transportation issues and need evaluations and improvements.

SIMULATION MODELLING OF SOCIAL PROTECTION INTERVENTIONS FOR TUBERCULOSIS (TB) AND CHRONIC AIRWAY DISEASES (CAD) IN MALAWI

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Catastrophic health care expenditure has emerged as one of the concerns among global health practitioners particularly in low income countries. To address this concern, social protection has been used as a framework to address healthcare related poverty and vulnerability in these countries. This has been followed by a growing number of governments in low income countries developing and adapting social protection strategies in their health plans. Similarly, there is also a rising interest in social protection among global health and health system researchers. Despite global interests and adoption of health care related social protection interventions in low income countries, the implementation, uptake, equity and effectiveness of current social protection interventions are limited by; inadequate prevailing national health budgets which are often donor dependent; policies prevalent within the wider health sector and failure of integration of social protection intervention by government agencies and the health sector in general. The research was aimed at finding ways of improving social protection interventions aimed at protecting the poor and vulnerable populations in Malawi, especially those affected by chronic airway diseases (CAD) and TB. The overall objective was to improve delivery of CAD and TB services, by successfully engaging policy makers, healthcare providers and various stakeholders, in the generation of new evidence about effective ways to strengthen the provision, uptake, equity and effectiveness of social protection mechanisms in TB and CAD treatment. Simulation modelling, econometric analysis and process evaluation studies were used to evaluate the process and impact of existing social protection intervention and generate new knowledge. Preliminary results indicate that, the involvement as partners of major stakeholders directly responsible for social protection policy and interventions will ensure policy relevance of this research and its continued impact beyond the life of the research project.
PNEUMOCOCCAL SURFACE PROTEIN A (PSPA) BASED PNEUMONIA VACCINE SHOWING ENHANCED PROTECTIVE IMMUNITY WHEN CONJUGATED TO VI POLYSACCHARIDE FROM SALMONELLA TYPHI

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Phase I data from trials using protein based pneumococcal vaccine antigens show poor immune response in humans. To address this problem protein antigen need to be presented in a way to induce a high immune response and subsequent protection in humans. We have developed a conjugate vaccine in which PspA family 1 and family 2 proteins from Streptococcus pneumoniae are conjugated to Vi capsular polysaccharide from Salmonella typhi. The conjugation technology strongly boosts protective immune responses against Streptococcus pneumoniae and Salmonella typhi.MethodWe have optimized a scalable high yielding method for fermentation and purification of Vi polysaccharide and two protein antigens of PspA Family. We have developed a method for conjugation of PspA Family 1 and 2 proteins with Vi polysaccharide and tested conjugates by ELISA for their immune response in mice. We have done preliminary challenge studies of the vaccine with pathogenic strains of Streptococcus pneumoniae to check vaccine efficacy.Results A series of Vi-PspA conjugates are prepared and tested in mice. A poor anti-Vi-PspA response was obtained when un-conjugated PspA was used as antigen but when conjugated to Vi a substantial increase in the responses were obtained. Immunized mice with selected Vi-PspA1 conjugate show 70-80% protection in the preliminary challenge studies done with Streptococcus pneumoniae. Further challenge studies with a Vi-PspA1 and Vi-PspA2 combination vaccine will be performed to enhance the protective immunity against both pathogens.Conclusion The Vi-PspA production and conjugation process is developed for the purpose of Scale up and making a cost effective vaccine targeting developing countries.

ELABORATE HEALTH RECORDS SURVEILLANCE FOR TB CASE FINDING IN AN INFANT COHORT STUDY IN PREPARATION FOR PHASE THREE TB VACCINE TRIALS IN WESTERN KENYA

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Finding every case of Tuberculosis in infant vaccine trials is important for efficacy endpoints. Health record surveillance could be a key source of TB suspects and therefore, TB Cases. We therefore, sought to evaluate the yield of health records surveillance for TB case finding in an infant cohort in preparation for vaccine trials. The study was a prospective, observational, cohort study. After enrolment, TB cases listed in TB registers were searched and matched with Health and Demographic Surveillance System data base to confirm residency and compared with the study database, if there was a match, further confirmation was done. Potential TB suspects were also generated from: Inpatient Department, TB laboratory, Patient Support Centre and X ray records. TB cases were found through both the active and passive detection systems. Interestingly the active case finding through scheduled TB screening follow-up visits generated only 8 cases (14.0%), whereas various “passive” surveillance systems located within the health system itself identified 72% of all TB cases. 12% of TB cases were diagnosed post mortem via verbal autopsy. In contrast to other infant cohort studies, incentivized individual health seeking behavior of parents likely played a significant role in increasing the effectiveness of the passive system. Elaborate Health records surveillance for TB case finding is labor and resource intensive, however, is likely to be more useful than routine TB screening to capture each endpoint in vaccine efficacy trials. Improvements are needed to reduce the proportion of TB cases detected post mortem.
isotopes of 2011 had H275 marker. A total of 28 isolates were analysed for phenotypic susceptibility assay. The mean zanamivir IC50s were 1.75nM, 2.53nM and 1.84nM for the subtypes H1N1, pH1N1 and H3N2 respectively. Most of the 2008-2009 (8) sH1N1 analysed showed highly reduced sensitivity to oseltamivir. The IC50s in the fluorescent assay ranged from 73nM-984nM. Pandemic A/H1N1 strains obtained between 2009-2011 indicated oseltamivir IC50 ranges of 1.60nM-6.32nM categorised as normal sensitivity. All the 8, influenza A/H3N2 isolates obtained between 2009-2011 were sensitive to oseltamivir with the IC50s ranging between 0.16nM and 0.94nM. The 2011, WHO range and median IC50 values for oseltamivir carboxylate were 0.4nM-10nM and 0.5nM; 0.1nM-5nM and 0.16nM and 0.94nM. The 2011, WHO range and median IC50 values for oseltamivir carboxylate were 257nM-3455nM and 458.2nM; 132nm-2179nM and 191.3nM for mutant types sH1N1, sH3N2 and pH1N1 respectively. The 2011, WHO range and median IC50 values for oseltamivir carboxylate were 257nM-3455nM and 458.2nM; 132nm-2179nM and 191.3nM for mutant types sH1N1 and pH1N1 respectively. The WHO IC50 values for zanamivir both for mutant and wildtype strains ranged between 0.2nM-3nM for all subtypes with no significant difference between the mutant and wildtype strains for each subtype. H275Y mutation increased the IC50 in the 2008-2009 sH1N1 isolates by 50-100 fold. Resistance to NAi was found to be both drug and virus subtype specific.

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CHALLENGES FACING TB CONTROL IN INDIA FOCUSING ON THE ROLE OF XPERT MTB/RIF IN THE PUBLIC AND PRIVATE SECTOR

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Globally, tuberculosis (TB) remains a major public health issue with an estimated 8.8 million new cases and 1.3 million deaths reported in 2012. In India, this health issue is compounded by the failure to diagnose cases in the national surveillance system and the high incidence of TB drug resistance. Of the estimated 3 million cases missed by national notification systems globally, 31% were in India. Drug resistance is of increasing concern with 3.6% of newly diagnosed TB cases and 20% of previously treated patients having multidrug-resistant TB (MDR-TB), defined as Mycobacterium tuberculosis resistant to isoniazid and rifampin, with or without resistance to other first-line drugs. There were an estimated 99,000 cases of MDR-TB in India in 2009, including those outside the Revised National TB Control Program (RNTCP). The Indian RNTCP introduced the Programmatic Management of Drug Resistant TB (PMDdT) to address the needs of this patient population and is rapidly scaling up its services. A key issue in the management of MDR-TB is timely and accurate diagnosis (including drug susceptibility testing). There is interest in the promise of point-of-care (POC) diagnostics for diseases of global health importance but a need for better appreciation of the POC testing process moving beyond the technology alone. One area of progress is the global scale up of Xpert MTB/RIF (Cepheid Inc.), a molecular test for TB that also enables rapid detection of rifampin resistance. However, a greater understanding of role that the large, unregulated private health care sector plays in providing TB care is essential. The Initiative for Promoting Affordable Quality TB Tests (IPAQT) endorses the use of WHO approved tests such as Xpert MTB/RIF in private labs at affordable rates. Focus group interviews were conducted with health care providers from the public and private health care sectors, the latter at sites involved with the IPAQT initiative, to ascertain current practices and perceptions and attitudes to inclusion Xpert MTB/RIF in the TB diagnostic algorithm. Perceived barriers to implementation include delays in obtaining results and cost.

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TUBERCULOSIS AND WAR IN THE U.S. MILITARY

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Studies which have demonstrated increased morbidity and mortality from tuberculosis (TB) during war have largely focused on civilian populations. TB also has a long and well-established association with the military, but the association of increased TB risk during armed conflict is less certain. The purpose of this study was to examine the U.S. military experience in times of war and armed conflict to better understand its impact on the risk of TB. This historical study estimates the risk of TB infection, disease and mortality during these conflicts, comparing those serving overseas with civilian and military populations remaining in the U.S. TB rates in the U.S. Army declined dramatically over the period from 1885 to 2012, from a high of 1,168 per 100,000 in 1917 to a low of 0.4 per 100,000 in 2012. Army rates were generally considerably lower than civilian rates, due in large part to the “healthy soldier” effect. Other than World War I, armed conflict showed very little impact on declining TB trends. Some of the focal risk groups known to have higher rates of TB included U.S. and enemy prisoners of war (POWs), the foreign born, and others found to be infected at induction into the military. Although the risk of TB in the U.S. military largely reflects that of the underlying U.S. population, the military also has unique exposures to tuberculosis during times of armed conflict. In order to protect the health of these troops and conserve military fighting strength, these unique exposures require additional surveillance and control measures.

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FEASIBILITY ASSESSMENT FOR ESTABLISHING SURVEILLANCE FOR EMERGING RESPIRATORY VIRUSES IN NON-PUBLIC HOSPITALS IN KENYA, 2013

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Influenza A H7N9 and Middle East respiratory syndrome coronavirus (MERS-CoV) are emerging respiratory viral infections with pandemic potential which can result in high fatality. As of March 2014, 395 cases and 125 deaths of influenza A H7N9 and 206 cases and 86 deaths of MERS-CoV had been reported worldwide. Kenya’s high volume of international travel poses a risk for introduction of these pathogens. Public hospitals in Kenya conduct severe acute respiratory illnesses (SARI) surveillance. Little is known about SARI surveillance in non-public hospitals where majority of foreigners and travelers are likely to seek care. We conducted an assessment to determine the capacity of non-public hospitals to conduct SARI surveillance and to identify hospitals for implementation of MERS-CoV and influenza A H7N9 surveillance. In November 2013, we interviewed managers from 44 non-public hospitals in Nairobi and Mombasa counties. Respiratory illness accounted for a range of 0-18 admissions per day. A surveillance focal person was present in 32 (73%) hospitals, of whom 10 (23%) had received training in the last 12 months. Although requested by the Ministry of Health, national reporting of SARI was done in 18 (41%) hospitals; 12 (67%) reported monthly and 6 (33%) weekly. Among 28 (64%) hospitals which recorded patients’ nationalities, foreigners accounted for a median of 10% (range: 0.5%-80%) of patients. Nasopharyngeal (NP) and oropharyngeal (OP) swab specimens were collected for clinical diagnosis in 27 (61%) and 29 (66%) hospitals respectively. In the last 12 months, 10 (23%) hospitals had trained their staff on NP/OP specimen collection, packaging, transport and reference laboratories, and 27 (63%) had training on use of personal protective equipment. Non-public hospitals are an

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important source of medical care for foreigners and the basic capacity for surveillance exists. These hospitals will be sensitized on the importance of national reporting of priority conditions, and some will be targeted for implementation of MERS-CoV and influenza A H7N9 surveillance.

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THE ACCURACY OF NON SEVERE PNEUMONIA DIAGNOSIS IN TANZANIAN CHILDREN: THE VALIDITY OF THE RESPIRATORY RATE

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Integrated Management of Childhood Illness (IMCI) guidelines recommend antibiotic treatment to all children presenting with cough or difficult breathing and an increased respiratory rate for their age. However, many pneumonia diagnoses in resource poor settings are done on the bases of sub-optimal condition; the environment may not be conducive for counting respiratory rates. We conducted a study to determine if respiratory rate was likely to be transiently raised by a number of contextual factors in a busy clinic leading to overuse of antibiotics. Respiratory rates were recorded in children aged 2 - 59 months presenting with cough or difficulty breathing to one of the two busy outpatient clinics and then repeated at 10 minute intervals over 1 hour in a quiet setting. A total of 167 children were enrolled with a mean age of 7.1 (SD±2.9) months in infant and 27.6 (SD±2.8) months in the older age group. The mean respiratory rate declined from 37.5 breaths per minute (bpm) at clinic to 35.8 bpm initial reading at quiet room and 35.0 bpm final reading (p<0.02). This resulted in 11(85%) being mis-classified with non severe pneumonia in infants and 2(13%) in older children. In a logistic model age group (infant or older child) was the only risk factor associated with over-diagnosis of non severe pneumonia. Over-diagnosis of non severe pneumonia in a busy clinic is significant and it tends to vary with age. Changing the respiratory rates cut-offs to higher threshold reduced the proportion of non-severe pneumonia mis-classification in infants. These findings have public health impact in managing these children as antibiotic over-use accelerates high levels of resistance. More studies of the accuracy and utility of respiratory rate as an indication for antibiotics are needed, especially as vaccines against bacterial pneumonia are introduced to resource poor countries.

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MICROSCOPIC OBSERVATION DRUG SUSCEPTIBILITY (MODS): A RAPID DIAGNOSIS OF PULMONARY TUBERCULOSIS IN HIV/AIDS PATIENTS IN RESOURCE-SCARCE BOLIVIA

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The need for fast, reliable diagnosis of Mycobacterium tuberculosis (MtB) infection is particularly acute in patients with HIV. This study examined the rates of tuberculosis (TB) and multidrug-resistant tuberculosis (MDRTB) in HIV patients in Bolivia. It evaluated the Microscopic Observation Drug Susceptibility rapid liquid culture (MODS) versus traditional Ziehl-Neelsen staining (ZN) and Lowenstein Jensen culture (LJ), and assessed the value of the string test and induced sputum in sputum-scarse individuals. The presence of MtB in sputum of 107 HIV-positive patients was evaluated by ZN, LJ, and MODS. MtB detection by string test was evaluated by MODS in 92 of these. The TB-HIV co-infection rate of HIV patients with respiratory symptoms by sputum sample was 44.9% (48/107). The mortality of TB+ hospitalized patients was 51.4% (18/35) and of ambulatory patients was 30.7% (4/13). The rate of MDRTB was 9%. Of 48 sputum samples positive by any diagnostic method, 63% were positive by ZN, 79% by LJ, and 96% by MODS. Median time to positive culture was 10 days by MODS versus 34 days by LJ (p<0.0001). In pts not able to produce sputum without induction, the string test had a sensitivity of 82% compared to induced sputum. Of the ten patients unable to produce a sputum sample, four were TB-positive by the string test. MODS was faster and more sensitive for MtB detection when compared to LJ, and these differences are more pronounced in smear-negative patients, who are at the greatest risk for missed diagnoses. The string test, in conjunction with MODS, is a valuable diagnostic technique for HIV positive sputum-scarse patients. Nine percent of our patients had MDRTB, which reinforces the need for rapid detection of antibiotic sensitivity testing in HIV patients in Bolivia.

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HEALTH RISK ASSESSMENT OF PESTICIDES AND POLYCHLORINATED BIPHENYLs CONTAMINATIONS IN DAIRY PRODUCTS FROM SELECTED FARMS IN GREATER ACCRA REGION, GHANA

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The residual concentrations of synthetic chemicals such as organochlorine pesticides (OCPs), pyrethroids and polychlorinated biphenyls (PCBs) in dairy products (milk, yoghurt, cheese) from selected farms in Ghana were analyzed using Gas Chromatography (GC). A total of 50 samples of dairy products (9 cheese, 25 cow milk and 16 yoghurt) were analyzed for OCPs, pyrethroids and PCBs. Of the numerous pesticides evaluated, detectable levels of OCPs (β-HCH, endrin, heptachlor, endosulfan, p,p'-DDT and methoxychlor); Pyrethroids (permethrin, allethrin, cypermethrin and deltamethrin) and PCBs (18, 28, 52, 101, 153, 138, and 180) were found in all the dairy product samples analysed. Milk samples were found to be the most contaminated with respect to the OCPs and the levels ranged between 0.0001 µg/ml and 0.0407 µg/ml. β-HCH was the highest OCP with concentration of 0.0407 µg/ml while Cyfluthrin was the highest synthetic pyrethroids recorded in yoghurt sample (0.0318 µg/ml). The highest PCB 18 (2,2,5-Trichlorobiphenyl) recorded (0.2668 µg/ml) in yoghurt samples. Data obtained from the field regarding safe use of pesticides and symptoms among farmers was very high. The estimated dose for γ-chlordane (8.5x10-5µg/ml), endrin (0.0114 µg/ml)p,p'-DDT (8.5x10-5µg/ml), DDE (8.5x10-5µg/ml), heptachlor (2.8x105µg/ml), dieldrin (6.8x10-5µg/ml) do not pose a direct hazard to human health, although present in milk samples since the values were lower than toxic threshold as well as, reference doses (γ-chlordane: 0.0005 µg/ml; endrin: 0.20 µg/ml; p,p'-DDT: 0.50 µg/ml; DDE: 0.50 µg/ml; Heptachlor: 0.0001 µg/ml; and Dieldrin: 0.005 µg/ml) and may indicate minimum risk to human. However, β-HCH (0.0375 µg/ml), endosulfan (0.0142 µg/ml), methoxychlor (0.0746 µg/ml) and PCBs (0.0498 µg/ml) levels exceeded the reference doses of (β-HCH: 0.003 µg/ml, endosulfan: 0.006 µg/ml, methoxychlor: 0.005 µg/ml and PCBs: 0.002 µg/ml) in children between the ages of 0-1 year, 1-11 years and adults indicating a great potential for systemic toxicity in all age groups especially children who are considered to be the most vulnerable population.
BACTERIOLOGICAL QUALITY OF WELL WATER USED FOR DRINKING PURPOSES IN GAROUA, NORTH CAMEROON

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Groundwater serves as a major source of drinking water in North Cameroon. Water quality is an important determinant of human health, considering in particular waterborne diseases in local communities. This study aimed at assessing the bacteriological quality and potential sources of well water contamination in Garoua, a metropolis of North Cameroon. The water quality of 23 wells was assessed through commonly used microbiological tests. Also analysed were physicochemical parameters of the water. For each well, monthly sampling was performed during 10 months; physical characteristics of sampling sites were documented and potential sources of contamination were identified. Results showed that the abundance of heterotrophic aerobic and mesophilic bacteria (HAMB) and bacterial bio-indicators in well water in Garoua all exceeded the WHO's drinking water standards. Total coliforms were present in all well water samples at high concentrations (5.0x102 to 4.8x104 CFU/100 ml of water). The water harbours relatively high concentrations of faecal coliforms (1.2x102 to 2.3x104 CFU/100 ml of water). Escherichia coli and faecal streptococci concentrations showed high spatial and seasonal variations from one well to another. The physicochemical analysis showed that, in 52.17% of wells, water was acidic with various mineralization. The principal component analysis (PCA) pointed out that seasonality had less influence on the majority of measured water parameters (pH, electrical conductivity, total dissolved solids, and salinity) than the location of the well water point. Human wastes from the traditional latrines system extensively used in this area and animal manure might have contaminated the wells.

WATER RESOURCES DEVELOPMENTS IN ETHIOPIA: POTENTIAL BENEFITS AND NEGATIVE IMPACTS ON THE ENVIRONMENT, VECTOR-BORNE DISEASES AND FOOD SECURITY

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To satisfy the growing demand for electricity, Ethiopia plans to increase its electricity production five-fold between 2010 and 2015, mainly through the construction of dams. A literature review shows that while dams can boost power and agricultural production, promote economic development and facilitate flood control, they can also lead to environmental, ecological and socioeconomic changes. Several case studies show that dams may alter the composition and density of vectors and intermediate host species, increase the incidence of malaria schistosomiasis and possibly lymphatic filariasis and lead to eutrophication of reservoirs, soil erosion and earthquakes. There is evidence that dams and commercial irrigation schemes can increase soil and water degradation, vulnerability to drought and food insecurity in riverine and lacustrine areas downstream of dams. It appears that dams in Ethiopia are also vulnerable to high soil erosion rates and earth quakes. Consequently, the current and proposed large-scale dam construction program in Ethiopia requires in-depth research to improve our understanding of the unintended negative effects of projects and to guide the location, design and implementation of appropriate preventive and remedial programs.

EXPLORING ADAPTATION MEASURES FOR INFECTIOUS DISEASES IN MACHALA, ECUADOR

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Ecuador is facing new challenges related to climate change impacts in the human health sector, especially for emerging infectious diseases, such as dengue fever. Although the public health sector is investing substantially in measures of surveillance and mosquito control, the effects of climate variability and change are not considered in the current strategies for reducing dengue virus transmission. Effective planning for adaptation measures in the public health sector would require the following: a) a better understanding of climate and environmental interactions with the vector and disease transmission in which a monitoring and surveillance climate-disease program is fundamental b) an evaluation of the effectiveness of current policies and measures in reducing the vector disease including ‘what if’ analysis in projected scenarios, and finally c) building scientific capacity in research institutions and looking for a permanent dialog with stakeholders and decision makers to discuss the adaptation measures on the climate change impacts on infectious diseases. With this perspective, a consortium of national and international institutions and local public health organizations is developing an interdisciplinary research program on dengue transmission in the city of Machala, Ecuador. This 3-year study will develop predictive models by

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linking climate factors with local neighborhood social and environmental conditions to predict when and where dengue outbreaks will occur in Machala, Ecuador. This study aims to link multi-scale climate phenomena to neighborhood-level dengue risk, fundamentally changing how we think about climate-disease dynamics. At the same time, an iterative process of monitoring and evaluation of adaptation measures linked to decision making aids and tools is proposed for developing climate change policy in the public health sector.

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OCCUPATION-RELATED SELF REPORTED HEALTH PROBLEMS AMONG SOLID WASTE HANDLERS IN A RAPIDLY URBANIZING COASTAL COMMUNITY IN GHANA

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This study applied a mixed method design to investigate exposure to waste, use of personal protective gear, and self-reported health problems among solid waste handlers in a semi-urban township of southern Ghana. A total of 280 waste handlers were studied representing all types of waste handling practices including sweepers, collectors, transporters and disposers of waste. Most waste handlers engaged in multiple of these activities (69.3%). The most commonly reported health problems were bodily pains (56.4%), headache (38.6%), fever (35.7%), feelings of physical discomfort (28.2%), and diarrhoea (11.4%). In-depth interviews with 22 waste handlers also highlighted eye problems, stomach pains and non-specific symptoms such as stress and tiredness as commonly experienced. There was a correlation between exposure of bare body parts of waste handlers and disease, with a higher likelihood to report fever among those using bare hands during waste handling (odds ratio (OR) = 1.89 (95% CI 1.37 - 2.56), p < 0.0001) compared with those who used protective gear. Interviews showed that waste handlers generally had basic knowledge about the disease preventive purpose of wearing personal protective measures. However, observations revealed that most waste handlers did not use such measures consistently mainly due to discomforts, impracticalities of wearing it in hot and humid conditions, and not being supplied with protective gear by employers or not being able to invest in it themselves. The study indicates that waste handlers experience a burden of disease which may be consequences of their occupation. Our findings stress that waste handlers in rapidly urbanizing areas need protection against occupational diseases through the wearing of affordable and suitable protective gear. Waste companies and government institutions employing a growing number of waste handlers should train waste handlers in the proper use of protective gear, educate on how to protect their health and to provide such protective gear.

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VENO-OCCCLUSIVE LIVER DISEASE IN CHILDREN AND YOUNG ADULTS: AN EMERGING PROBLEM IN DEVELOPING COUNTRIES

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Veno-occlusive liver disease (VOLD) is increasingly reported in young people from developing countries, particularly in the Arabian peninsula and in countries from the former Soviet Union. Many conditions can be associated with this multifactorial disease: parasitic diseases like CE and schistosomiasis, as well as TB, abscesses, cysts or the presence of a membranous web that obstructs the terminal portion of the inferior vena cava. Poverty, malnutrition, recurrent bacterial infections, and filariasis have been suggested to be predisposing factors for inferior vena cava occlusion in developing countries, whereas myeloproliferative neoplasms, abdominal cancer and oral contraceptives are associated with this syndrome in developed countries. However, environmental toxic causes are described in children and young adult living in rural areas: particularly, pyrrolizidine alkaloids derivatives can cause the disease by microscopically damaging the hepatic venular bed. We report a case of a 25 year old male immigrant from a rural area of Morocco admitted to our Hospital for ascites, mild cholestasis and abdominal pain in October 2013. All tests resulted negatives except quanti FERON gold test, so the patient was unsuccessfully treated for TB for 2 months. The patient clinical conditions and LFT slowly worsened and after 2 inconclusive hepatic biopsies, a third biopsy combined with ultrasound and CT scan findings allowed the diagnosis of VOLD. All commonest causes of VOLD were ruled out, therefore, a toxic cause due to pyrrolizidines alkaloid derivatives was considered. The patient is currently on the wait list for liver orthotopic transplantation due to end-stage liver failure. This finding warrants more attention to the toxic environmental agents in developing countries, as they need to be considered in the differential diagnosis of liver failure also in immigrants to Europe.

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SOCIAL AND ENVIRONMENTAL DETERMINANTS OF CHILDHOOD DIARRHEAL DISEASE IN MALAWI: A NATIONWIDE, HOUSEHOLD-LEVEL, GEOSPATIAL ANALYSIS

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Diarrhea continues to be a leading cause of death among children under-five in developing countries. Despite increased access to improved water and sanitation in some settings, many areas of sub-Saharan Africa and Asia have been left behind. The complex and multifactorial nature of associations with childhood diarrheal disease complicate understanding of the more critical determinants of risk. We analyzed data from the 2010 Malawi Demographic and Health Survey to identify risk factors for diarrhea among Malawian children. A hierarchical logistic regression model and a GIS-based raster analysis using inverse distance weighted interpolation were used to evaluate the independent effects of sanitation on childhood diarrheal prevalence, and to characterize the distribution of diarrheal disease and its determinants across Malawi. Approximately one-quarter of all households reported at least one child under-five having diarrhea in the two weeks preceding the survey. Households with an average child age between 6-12 months had six times the odds of childhood diarrhea when compared to households with an average child age less than 5 months. While 79% of households had access to an improved water source, only 12% of households were using an improved sanitation facility, with an additional 11% lacking access to any sanitation facility. Households with no sanitation facility had 45% greater odds of childhood diarrhea as compared to those with an improved and unshared sanitation facility. In multivariable analyses, the significant effect of sanitation on diarrhea prevalence remained after adjusting for child age and maternal education. This study calls renewed attention to the persistent, yet preventable, burden of diarrheal disease among children in Malawi and reveals important District- and sub-District-level variation in the distribution of determinants that could be used to inform the targeting of future interventions.
PERVERSIVE EXPOSURE TO FECAL CONTAMINATION IN LOW-INCOME NEIGHBORHOODS IN ACCRA, GHANA

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Globally, diarrhea contributes to about 800,000 fatalities in children under five each year, and is a primary cause of mortality in developing countries. Rapid urbanization in low-income countries has led to a growing sanitation crisis. A need exists for more effective WASH interventions in low-resource urban environments that can minimize the transmission of feces and reduce the rate of diarrheal illnesses. Effective interventions require evidence-based research that highlights risk behaviors and perceptions of fecal contamination risk in people's daily lives. This study examines the context of fecal contamination during daily activities among residents in low resource urban settings of Accra, Ghana. Qualitative data were collected through 16 focus group discussions to understand the daily behaviors that place people at risk of fecal contamination. Data were collected and analyzed using a grounded theory approach to develop a conceptual framework of the context of fecal contamination in low income neighborhoods of Accra. MaxQDA10 software was used for data analysis. Results show that latrine use is low in these neighborhoods leading to a range of alternative methods of fecal disposal that contribute to fecal contamination throughout neighborhoods. Feces were further spread through refuse dumping, poor refuse collection systems, recreational activities, and occupational tasks of residents. These pathways of fecal contamination underscore the pervasiveness of risk for fecal contamination throughout low-income urban neighborhoods, suggesting the need for multi-pronged interventions that target multiple pathways of feces transmission.

NEUROPSYCHOLOGICAL PROFILE OF CHILDREN IN NATIVE COMMUNITIES EXPOSED TO MERCURY CONTAMINATION IN MADRE DE DIOS, PERU

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Recent studies show that an adequate neuropsychological profile is associated to adequate levels of learning. For this reason we are interested in knowing which cognitive profiles in pediatric populations of native communities exposed to mercury contamination in Madre de Dios, which vulnerable to suffer neurocognitive deficiencies because there are located in areas of difficult access to education and present deficiencies in their basic needs so we have as objective to determinate the Neuropsychological Profile of children in native communities exposed to mercury contamination in Madre de Dios, Peru. Transversal study of a representative sample taken from the native communities Ese’eja (Palma Real, Sonene, Inferno) of Madre de Dios, 64 children were evaluated regardless of gender, ages from 3 to 7 years old. For this evaluation we used Neuropsychological Test Maturity, CUMANIN. Parametric Kormogorov-Sminnov test of a sample was applied in order to check for a normal distribution in the study variables. Frequencies established variables, measures of central tendency and dispersion in qualitative variables were used. The test of variance of a factor for Global Neuropsychological Development Profile was applied to difference between groups regarding their Neuropsychological Profile in other hand there was no variances of a factor for Global Neuropsychological Development Profile. In conclusion we found a large number of children with less than p50 during different parameters of Neuropsychological Profile in other hand there was no difference between groups regarding their Neuropsychological Profile.

ASSESSMENT OF THE QUALITATIVE IMMUNE RESPONSE INDUCED BY THE CYD TETRAVALENT DENGUE VACCINE IN HUMAN VOLUNTEERS

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Clinical efficacy observed against dengue virus (DENV) serotypes 1, 3 and 4, but not DENV2 in the Phase 2b trial of the CYD tetravalent dengue vaccine candidate (CYD23; Clinicaltrials.gov NCT00842530), contrasted with the similar levels of antibody neutralization levels observed for all 4 serotypes in a Vero cell based PRNT50 assay. To further investigate the finding, we assessed the quality of the vaccine-induced antibodies. Sera from a completed clinical trial (NCT 01134263) in volunteers who were flavivirus-seronegative at baseline, were depleted using virus-coated beads, and neutralization was assessed before and after depletion in a flow cytometry-based assay using U937-DC SIGN+ cells. These showed that in this naïve population CYD-TDV vaccination elicited mostly homotypic anti-DENV4 responses, while anti-DENV1, DENV2 and DENV3 responses included a significant heterotypic component. Antibody epitopes were then mapped in a few samples using recombinant DENVs displaying serotype-specific, E protein domain VII hinge epitopes. Homotypic anti-DENV4 responses were seen to be directed against the DIII hinge, while anti-DENV3 was not. This serotype-specific quaternary epitope was present and recognized by human monoclonal antibodies (mAbs) on the corresponding CYD-1, 2 and 3 serotypes, while cross-reactive anti-rDII-DIII or DIII mAbs recognized all 4 CYD serotypes. Potentially enhancing anti-prM responses were not dominant in vaccinees’ sera in Western Blots, and it was also observed from a FcγR+ CV1 cell-based assay (presented by Byers and others at this conference) that vaccine-induced anti-DENV2 responses were not more enhancing than against the other serotypes. In conclusion, our results suggest that despite the presence of key epitopes on the vaccine viruses, qualitative differences exist between vaccine-induced responses against serotype 4 compared to the other 3 serotypes, although it will be necessary to confirm the results obtained here in a larger number of sera. These results demonstrate nevertheless the interest of qualitatively assessing dengue vaccine immunogenicity and how it relates to protection. Studies using post-vaccination sera from clinical trials in endemic areas are ongoing, and results are expected in the coming 2 months.
INVESTIGATIONS OF THE OBSERVED EFFICACY OF THE CYD TETRAVALENT DENGUE VACCINE IN THE PHASE 2B TRIAL IN RATCHABURI, THAILAND

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The first efficacy trial (CYD23; Clinicaltrials.gov NCT00842530) of the CYD vaccine showed clinical protection against dengue virus (DENV) serotypes 1, 3, and 4, but not DENV2, while similar antibody neutralization levels (Vero cell based PRNT50 assay) were observed for all four serotypes. Post study investigations of this result included a broad array of analytical and experimental methods in four areas: host, virus, vaccine vector, and novel immunological assays. Antigenic diversity between parental vaccine virus and wild-type CYD23 isolates does not impact neutralization (PRNT50) using serum from either CYD23 vaccinees or placebo recipients, and an assessment of viremia differences between serotypes in CYD23 clinical cases was inconclusive. Pre-existing immunity impacts vaccine immunogenicity and exploratory analysis using logistic regression, suggests a relationship between probability of disease and PRNT titers. Using CV1 cells transfected with FcγRIIa, preliminary studies with post-vaccination sera from baseline-naïve subjects show no evidence of differential neutralization. Serotype-specific antibody depletion studies, and studies using recombinant DENVs displaying serotype-specific E protein domain I/II hinge epitopes show qualitative differences between serotype-specific neutralizing responses, while a Vero-cell based microneutralization assay shows certain discrimination between serotypes. Analyses using serotype-specific monoclonals show that important E protein DIII hinge region epitopes are displayed on the vaccine viruses, including CYD2. Finally, prior clinical data suggest a relationship between vaccine potency (CCID50) and neutralization, as well as in vivo competition between serotypes in different formulations, and no differences in IFNγ responses between serotypes. Additional investigations, including Phase 3 efficacy study data anticipated in the second half of 2014, will deepen our understanding of the results observed in this Phase Ib efficacy trial.

LARGE SCALE SAFETY AND IMMUNOGENICITY OF CYD DENGUE VACCINE; RESULTS FROM A RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER PHASE III EFFICACY TRIAL IN LATIN AMERICA

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In a phase III study of the efficacy of the recombinant, live, attenuated, CYD tetravalent dengue vaccine (TDV) against virologically-confirmed dengue fever in dengue-endemic areas of five Latin American countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico (N=20 875). Children and adolescents aged 9-16 were randomized 2:1 to receive 3 injections of CYD-TDV or placebo at study months 0, 6 and 12. Safety and immunogenicity were assessed as secondary objectives. Serious adverse events (SAEs) occurring at any time throughout the study in the whole study population were documented, and reactogenicity was described in a representative subset of 2000 children (300-600 per country) who were randomly selected from among those enrolled during the first months of the study. SAEs were reviewed by an independent data monitoring committee. Reactogenicity data included solicited injection site reactions, solicited systemic reactions, and unsolicited adverse events, respectively collected for 7, 14, and 28 days after each vaccination. Immunogenicity was also assessed in this subset of 2000 children using Vero cell-based PRNT50 assays against the four dengue serotypes to test serum collected at enrolment, on D28 after the 2nd and 3rd injections, and at 13 months after the 3rd vaccination. Results of these analyses are expected in September/October and will be presented here for the first time.

ClinicalTrials.gov: NCT01374516

astmh.org
EFFICACY OF THE CYD DENGUE VACCINE: RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER PHASE III TRIAL IN LATIN AMERICA

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The protective efficacy afforded by vaccination with the recombinant, live, attenuated, CYD tetravalent dengue vaccine (TDV) against virologically-confirmed dengue fever was evaluated in a phase III trial in dengue-endemic areas of five Latin American countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico. (N=20,875). Children and adolescents aged 9-16 were enrolled over a 9 month period, randomized 2:1 to receive 3 injections of CYD-TDV or placebo at study months 0, 6 and 12, and were actively followed for febrile illness (temperature ≥38°C) for ≥2 days) until the 13th month after the 3rd injection; i.e. for 25 months after the 1st injection. The primary endpoint was efficacy against cases against symptomatic, virologically-confirmed dengue occurring at least 28 days after the 3rd injection, regardless of severity or serotype. Efficacy by serotype was evaluated as a secondary objective. Active surveillance consisted of weekly contacts via phone or home visits to remind parents to consult the trial center or health care center in the event of febrile illness, considered as a suspected dengue case. An acute sample was to be collected within 5 days of fever onset, and a convalescent sample 7-14 days later. Virological confirmation was by dengue qRT-PCR or dengue N51 antigen ELISA and cases were subsequently serotyped by qRT-PCR. Together with a second phase III efficacy study in >1000 children aged 2-14 in 5 Asian countries conducted in parallel with a similar protocol, this study will provide pivotal data on the efficacy of the CYD-TDV vaccine in different populations and epidemiological settings. Results of our study are expected in September/October and will be presented here for the first time.

A SINGLE DOSE OF LIVE ATTENUATED TETRAVALENT DENGUE VACCINE TV005 IS SAFE, IMMUNOGENIC AND HIGHLY INFECTIOUS IN HUMANS

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Dengue virus remains a public health burden in many tropical and subtropical areas of the world. The lack of a vaccine or antiviral therapy and the relative unsustainability of vector control together contribute to the ongoing emergence of dengue disease. The last decade has seen an increase in vaccine development efforts, with live attenuated vaccines making significant progress. Our laboratory at the NIAID has developed live attenuated dengue vaccine candidates shown to be both safe and immunogenic in monovalent and tetravalent studies in humans. Together with a second phase III efficacy study in >10000 children aged 2-14 in 5 Asian countries conducted in parallel with a similar protocol, this study will provide pivotal data on the efficacy of the CYD-TDV vaccine in dengue-endemic areas of five Latin American countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico. (N=20,875). Children and adolescents aged 9-16 were enrolled over a 9 month period, randomized 2:1 to receive 3 injections of CYD-TDV or placebo at study months 0, 6 and 12, and were actively followed for febrile illness (temperature ≥38°C) for ≥2 days) until the 13th month after the 3rd injection; i.e. for 25 months after the 1st injection. The primary endpoint was efficacy against cases against symptomatic, virologically-confirmed dengue occurring at least 28 days after the 3rd injection, regardless of severity or serotype. Efficacy by serotype was evaluated as a secondary objective. Active surveillance consisted of weekly contacts via phone or home visits to remind parents to consult the trial center or health care center in the event of febrile illness, considered as a suspected dengue case. An acute sample was to be collected within 5 days of fever onset, and a convalescent sample 7-14 days later. Virological confirmation was by dengue qRT-PCR or dengue N51 antigen ELISA and cases were subsequently serotyped by qRT-PCR. Together with a second phase III efficacy study in >10000 children aged 2-14 in 5 Asian countries conducted in parallel with a similar protocol, this study will provide pivotal data on the efficacy of the CYD-TDV vaccine in different populations and epidemiological settings. Results of our study are expected in September/October and will be presented here for the first time.
being assessed pre-dose, post-dose 1, post-dose 2, and 1, 6, and 12 months post-dose 3. The primary endpoint for immunogenicity is based on assessment of virus neutralizing antibody responses with analysis of seroconversion and geometric mean titers. Subjects have now completed their follow-up through the primary endpoint (1 month post-dose 3) and the immunogenicity and safety data through this time point will be presented. Subjects are continuing through the long term follow-up stage of the protocol.

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SAFETY AND IMMUNOGENICITY OF TAKEDA’S LIVE ATTENUATED DENGUE VACCINE CANDIDATE IN A PHASE I STUDY CONDUCTED IN FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS

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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 VT EU (NCT01105511). Volunteers received two doses of the tetravalent dengue vaccine candidate 90 days apart and were followed for safety and immunogenicity. Serum samples were collected after each dose to measure vaccine virus replication by qRT-PCR and virus isolation and to measure neutralizing antibodies to wild-type DENV. In this phase 1 study, the vaccine was well-tolerated and no serious vaccine-associated adverse events occurred. Low levels of vaccine viral RNA were detected after prime in 46% of individuals that received the lower dose formulation and in 79% of individuals that received the higher dose formulation. Both vaccine formulations induced seroconversion rates of 67-100% to each of the 4 serotypes of DENV, and more than 90% of subjects who received two doses of the high dose formulation seroconverted to three or more DENV (trivalent response). In summary, these findings highlight the good tolerability and immunogenicity of Takeda’s live attenuated dengue vaccine in flavivirus-naïve healthy volunteers and support further development and clinical testing of the candidate dengue vaccine.

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BURDEN, RISK FACTORS AND CHARACTERIZATION OF ROTAVIRUS AMONG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA—2008-2011: THE GLOBAL ENTERIC MULTICENTER STUDY (GEMS)

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Rotavirus is the most common cause of severe diarrhea among children worldwide. Data on risk factors for morbidity and mortality are limited. We analyzed data from children <5 years old seeking health care with moderate-to-severe diarrhea (MSD) and enrolled as cases in the Global Enteric Multicenter Study (GEMS) site in Nyanza Province, Kenya. An MSD case was defined as a child with a diarrheal illness <7 days duration comprising ≥3 loose stools in 24 hrs and ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. Rotavirus VP6 antigen was detected in stools by the ProSpecT ELISA rotavirus kit. Demographic and clinical information were collected at enrollment and during a ~60-day follow-up visit. We used logistic regression to evaluate factors associated with rotavirus among children with MSD compared with non-rotavirus MSD. From January 31, 2008 to January 29, 2011, 1,476 cases were enrolled; rotavirus was detected in 217 (14.7%); among cases aged 0–6, 6-11, 12-23 and 24-59 months old, rotavirus was detected in 24.0% (59/246), 18.3% (78/427), 15.6% (64/410) and 4.1% (16/393), respectively. Compared to cases without rotavirus, cases with rotavirus infection were more likely to be: 0-11 months old (65.6% vs. 44.7%, OR= 6.08, 95% confidence interval (CI) [3.80-9.72]), be female (48.7% vs. 41.4%, OR= 1.34, 95%CI [1.06-1.70]), and present with vomiting ≥3 times/24hrs (69.9% vs. 45.1%, OR= 2.83 95%CI [2.19-3.65]), sunken eyes (97.1% vs. 92.2%, OR= 2.85 95%CI [1.45-5.59]), lethargy (18.7% vs. 11.3%, OR= 1.80 95%CI [1.31-2.47]) and restlessness (72.7% vs. 58.2%, OR= 1.92 95%CI [1.47-2.49]). Among children with MSD, rotavirus was more prevalent among infants and significantly associated with severe diarrheal illness in our study setting. Our findings support the Kenya Ministry of Health plan to introduce rotavirus vaccine.

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THE THRESHOLD OF ROTAVIRUS SPECIFIC PLASMA IGA AS A CORRELATE OF PROTECTION FROM ROTAVIRUS DIARRHEA IS AGE DEPENDENT

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Optimizing and interpreting immunogenicity data and correlates of protection may help to better identify children in whom rotavirus vaccination is failing in developing countries. Here we use the PROVIDE study to examine the ability of rotavirus specific plasma IgA to correlate with protection from future episodes of rotavirus diarrhea within the first year of life. At the icddr,b site in Mirpur, Dhaka, 700 children were enrolled at birth and followed for 24 months. Bi-weekly surveillance for diarrhea was performed and diarrheal specimens were evaluated for rotavirus by enzyme-linked immunosorbent assay (ELISA). Diarrheal disease severity was measured by a modified Vesikari-Ruuska score, with score ≥11 indicating severe diarrhea. Rotavirus plasma IgA was detected by ELISA at weeks 6, 18, and 24. Per published standards, children were considered seropositive if the plasma IgA was ≥20 U/ml. Five percent of children were seropositive at week 6 with a Geometric Mean Titer (GMT) of 1.5 (66.5 for seropositive). Irrespective of rotavirus vaccination status, at weeks 18 and 24, 26% and 37% of the children were seropositive with a GMT of 7.1 and 12.3 (114.1, 139.4 for seropositive) respectively. Seropositivity at 18 and 24 weeks of age was correlated with up to 85% and 87% protection from future episodes of rotavirus diarrhea, respectively. At six weeks, seropositivity as previously defined was not associated with protection against future episodes of diarrhea. Increasing the cut-off to 40 U/ml at six weeks increased the proportion protected to 86% however the sample size was small. Our data show that regardless of natural exposure or vaccine-induced responses, rotavirus specific plasma IgA levels at ≥20 U/ml at weeks 18 and 24 serves as a correlate of protection from rotavirus diarrhea in the first year of life. If measured at 6 weeks of age, higher IgA levels are needed to indicate protection from future episodes of rotavirus diarrhea. After unmasking, analysis will assess plasma IgA as an effective correlate of protection in rotavirus-vaccinated children.
PATHOGEN-SPECIFIC MORTALITY AMONG INFANTS AND YOUNG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA - WESTERN KENYA, 2008-2011

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Diarrhea is a leading cause of childhood morbidity and mortality in sub-Saharan Africa; one in ten child deaths in the first 5 years of life is due to diarrheal disease. We assessed pathogen-specific mortality following an episode of moderate-to-severe diarrhea (MSD) in children enrolled in the Global Enteric Multicenter Study in western Kenya. We recruited children <5 years old presenting to sentinel health facilities with MSD. At enrollment each child was assessed clinically, and provided anthropometric data and a stool sample to identify enteropathogens. Survival status was determined at 60 days. We calculated unadjusted exact odds ratios (OR) and 95% confidence intervals (CI) using simple logistic regression models. From 2008 to 2011, 1,476 children with MSD were enrolled; 52 (3.5%) died. Nineteen (37%) children died at health facilities (7 at enrollment) and 33 (63%) died at home. Case-fatality rates by age stratum were 4.5% (<12 months), 3.1% (12-23 months), and 2.3% (24-59 months). Pathogens associated with increased risk of case-fatality were Shigella dysenteriae (OR: 7.2; CI: 1.3-27.8), non-typhoidal Salmonella (OR: 2.8; CI: 1.0-6.6), typical enteropathogenic Escherichia coli (OR: 2.6; CI: 1.1-5.5), and enterotoxigenic E. coli producing heat-stable toxin (OR: 2.5; CI: 1.1-5.2). Children who died were more likely to be underweight (weight-for-age z score < -2: OR 12.6; CI: 6.4-26.8), wasted (weight-for-length/height z score < -2: OR 8.2; CI: 4.4-15.1), and stunted (length/height-for-age z score < -2: OR 4.2; CI: 2.3-7.9) at enrollment. Malnutrition and four bacterial pathogens for which no vaccines are available were associated with increased risk of mortality from MSD. In the near-term, optimizing nutritional status and water, sanitation, and hygiene interventions are urgent priorities for childhood diarrheal mortality reduction in Kenya.

PATHOGEN-SPECIFIC ETIOLOGY AND BURDEN OF COMMUNITY DIARRHEA IN THE FIRST TWO YEARS OF LIFE IN DEVELOPING COUNTRIES: RESULTS FROM THE MAL-ED MULTISITE COHORT STUDY

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Studies of diarrheal etiology in developing countries have historically focused on children presenting with severe symptoms to health centers and thus best describe pathogens associated with severe diarrhea. However, the etiologies of community diarrhea may be different. MAL-ED is a multisite birth cohort study with intensive community surveillance for diarrhea as well as collection of monthly asymptomatic stool specimens from eight sites in South America, Africa and Asia. A total of 7,068 diarrheal and 22,599 non-diarrheal control specimens from 2,073 children aged 0-24 months were comprehensively tested for a broad range of enteropathogens. In the first year of life, Campylobacter, rotavirus, ST-producing enterotoxigenic E. coli (ST-ETEC), Cryptosporidium and astrovirus were associated with the highest burdens of diarrhea in descending order of attributable fraction. In the second year of life, diarrhea attributable to Shigella was more prominent while diarrhea attributable to Campylobacter decreased. There was substantial site-to-site variation, such that 6 distinct pathogens had the highest burden of diarrhea for at least one combination of site and age. Cryptosporidium, rotavirus, ST-ETEC and Shigella were associated with more severe diarrheal episodes. This study reveals substantial heterogeneity in the pathogen-specific burden of community diarrhea including an unexpectedly high burden of disease associated with Campylobacter and astrovirus.

CHANGES IN GUT MICROBIOME COMPOSITION DURING DIARRHEA EPISODES IN NICARAGUAN CHILDREN

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The gut microbiome, the collection of bacteria in the human gastrointestinal tract, plays an important role in human health. Understanding how the gut microbiome is affected by diarrhea episodes may help explain alterations in intestinal function among children in low-income settings. This study examined the gut microbiome of Nicaraguan children during diarrhea episodes and while free of diarrhea for at least 2 months. 16S amplicon sequencing was performed to determine changes in the gut bacterial microbiome during diarrhea episodes. Sequencing data analysis was done using Qime (Caporaso, 2010). Rarefaction analysis and diversity estimates were carried out to compare the overall diversity of microbiota in diarrheal versus healthy stools. Principal Coordinate
Analysis of amplicon sequences (Hamady, 2010) was used to compare clustering by state (diarrheal vs. healthy). In all, 74 stools were provided by 27 children who were enrolled in a one-year cohort study of diarrheal etiologies. These children had a mean age of 21 months (range: 1 to 45 months), 49% were female, 63% were breastfed, and 10% had received an antibiotic during the diarrhea episode. A total of 593,509 sequences (an average of 7,707 reads per sample) were assigned to 7,880 operational taxonomic units at ≥97% similarity, clustering into 237 genera, 115 families, 54 orders, 28 classes, and 14 phyla. Diarrheal and healthy stools had statistically significant differences (p<0.05) for the phyla Firmicutes, Bacteroidetes, and Actinobacteria. Also, as compared to healthy stools, diarrheal stools had a greater relative abundance of the taxa Enterobacteriaceae (13.0% diarrheal vs. 7.9% healthy) and Streptophyta (9.4% vs. 3.3%), and a lower relative abundance of the taxa Ruminococcaceae (9.8% vs. 21.0%) and Cyanobacteria YS2 (2.1% vs. 9.7%). Phylogenetic diversity did not differ significantly between diarrheal vs. healthy stools. Principal Coordinate Analysis showed clustering by state (diarrheal vs. healthy samples) indicating an overall perturbation of the microbiota in diarrheal stools.

MARKERS OF OPV FAILURE: INFLAMMATORY AND NUTRITIONAL ENVIRONMENT CRITICAL MEDIATORS OF VACCINE RESPONSE

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The oral polio vaccine (OPV) shows reduced immunogenicity in low income countries; children often have lower antibody titers in response to the vaccine, but the biological cause is unknown. We are measuring OPV responses in infants in an urban slum of Dhaka, Bangladesh. While OPV2 had only a 1.2% failure rate, OPV1 and OPV3 failed in 6.5% and 9.9% respectively as measured by serum neutralizing antibody responses at 18 weeks of age. We hypothesized that nonpolio enterovirus infection at the time of immunization could interfere with OPV. We found that the presence of a nonpolio enterovirus at the time of the 14 week immunization was associated with failure of OPV1 and OPV3 at 18 weeks (p=.005 & .003). We further hypothesized that nonpolio enterovirus infection was interfering by inducing an innate antiviral response in the gut that prevented OPV replication. In support of this, up to a quarter of OPV failures were associated with the inability to culture OPV1 or OPV3 virus from stool samples 24 hours after the first OPV dose at 6 weeks of age (chi-square probability=.01 & .07). In addition to these analyses, blood, stool, and urine samples from the children have been tested for over 20 biomarkers of immunity, inflammation and nutritional status. We performed univariate linear regression analyses to evaluate the association of each biomarker with vaccine performance using serum antibody titers. Markers of poor intestinal health reg1B and manin1 (p=.0002 & .044) were negatively associated with vaccine performance. Serum ferritin and IL-5 (p=.028 & .029) levels, markers of systemic inflammation, were also negatively associated with vaccine performance. Serum zinc concentration and WAZ at 18 weeks of age (p=.034 & .006) positively correlated with increased antibody titers. These results indicate that competing nonpolio enterovirus infections and a chronic inflammatory response likely serve to hinder initial OPV replication and the ability to elicit an effective immune response. Further support for this hypothesis was obtained by measuring fecal excretion of vaccine virus after the final OPV dose as a measure of mucosal immunity. We found that high levels IL-5 positively trended with increased virus excretion (p = .065). We concluded that the majority of the children in the cohort exhibited high levels of inflammatory markers, resulting in an enteric environment of chronic inflammation and infection that left little room for an OPV response.

IMMUNOGENICITY IN MICE OF A CHOLERA CONJUGATE VACCINE CONTAINING VIBRIO CHOLERAE O1 INABA O-SPECIFIC POLYSACCHARIDE (OSP) AND A RECOMBINANT TETANUS TOXIN HEAVY CHAIN FRAGMENT

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Immunity against cholera is serogroup-specific and serogroup specificity is determined by the O-specific polysaccharide (OSP) of Vibrio cholerae lipopolysaccharide (LPS). Here, we describe a cholera conjugate vaccine containing Inaba OSP from a well-characterized source strain isolated in 2007 from a patient with cholera in Bangladesh. The OSP was conjugated via its core oligosaccharide to a recombinant tetanus toxin heavy chain fragment (rTThc) using squaric acid chemistry. We administered the vaccine intramuscularly to mice in the presence and absence of immunoadjuvants, a substance with mixed effects on the immune system. We immunized mice with OSP:rTThc and alum protected against lethal challenge in mice orally challenged with wild-type V. cholerae. These results suggest that a cholera conjugate vaccine containing Inaba OSP can be highly immunogenic for inducing anti-OSP responses and that such anti-OSP-based immunity can be protective even in the absence of significant vibriocidal responses. These results suggest that OSP:rTThc may warrant further development as a cholera conjugate vaccine.

SALIVARY ANTIBODIES TO WB123 PROVIDE A NON-INVASIVE TOOL FOR ASSESSMENT OF ONGOING WUCHERERIA BANCROFTI TRANSMISSION

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Significant progress has been made toward the global goal to eliminate lymphatic filariasis (LF) by 2020 though the tools for monitoring control success and certification of transmission interruption need to be refined further. WHO guidelines for transmission assessment surveys (TAS) will guide decisions about stopping mass drug administration (MDA); the tools for post MDA surveillance, however, are likely to involve antibody testing –likely based on antibodies to Wb123, a Wuchereria bancrofti (Wb)-specific antigen that is expressed early in parasite development and has been shown to be a sensitive and specific marker of exposure to Wb infective stage larvae (L3). Although serum/plasma based IgG4 anti-Wb123 testing has already proved useful in assessing prevalences in target (6-7 year olds) populations, and a rapid diagnostic test (RTD) for these same IgG4 anti-Wb123 antibodies is currently under development for use with whole blood, both require at a minimum a finger prick. To development a
non-invasive methodology for the measurement of antibodies to Wb123, a commercially available salivary collection device was used to collect saliva from a group of 321 6-7 year olds from 5 villages in Mali 3 years after cessation of 7 annual rounds of MDA with albendazole and ivermectin. At the same time, dried blood spots were collected for antibody elution and whole blood was used for measurement of circulating filarial antigen by ICT. Prevalences of serum IgG4 anti-Wb123 was performed by ELISA and compared to salivary IgG and IgG4 anti-Wb123 performed by luciferase immunoprecipitation assay systems (LIPS). While the prevalence of CFA was 4.5% (16/321) and for IgG4 serum anti-Wb123 from dried blood spots was 4.0% (13/321), the salivary antibody prevalences were higher with IgG anti-Wb123 being 8.4% (27/321) and for the more specific IgG4 anti-Wb123 being 6.5% (21/321). Our data suggest that saliva is a rich source of specific antibody to Wb123 and may provide a convenient, sensitive and non-invasive alternative for antibody surveillance following cessation of MDA.

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A COMPARISON OF TWO RAPID TESTS FOR DETECTING FILARIAL ANTIGENEMIA IN LOW PREVALENCE SETTINGS IN SRI LANKA

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Filarial antigen tests are useful for mapping the distribution of Wuchereria bancrofti infections and for detecting areas with persistent infections following mass drug administration (MDA). Prior studies have shown that the new AlereTM Filariasis Test Strip (Strip) has better analytical sensitivity than the BinaxNOW® Filariasis card test (Card), and the Strip detected 26% more positives than the Card in field studies performed in a highly endemic area in Liberia. This study compared the performance of the Strip and Card in 2 areas in Southern Sri Lanka with low level persistence of filariasis some 7 years following that country’s intensive MDA program. The study design called for testing ~400 people > 8 years of age in each site (cluster sampling of households). Card and Strip tests were performed in parallel in the field with finger prick blood samples; test results were scored at 10 and 30 min and at 12 hr. The Strip detected many more positives at 10 min than the Card (28/389 versus 13/390 in study site A and 50/462 versus 13/462 in study site B). With one exception, all blood samples positive by Card were also positive by Strip. Semi-quantitative test scores based on the intensity of “T” lines tended to be higher by Strip than by Card. Thus filarial antigenemia rates by Strip can be much higher than those by Card test in the post-MDA setting, when filarial antigen levels tend to be low, and this could affect the outcome of transmission assessment surveys. It was not uncommon for Strips to turn from negative to positive between 10 and 30 min (5.6 % of samples tested), and this rarely occurred with the Card (0.36%). However, many Cards that were negative at 30 min were positive at 12 hr (14.3%), and this was less common with Strip tests (4.0%). These results underline the importance of taking care to read filarial antigen test results at 10 minutes according to the manufacturer’s instructions. The improved sensitivity, lower cost, and longer shelf life of the Test Strip favor its use over the Card test for filariasis elimination programs.
of mass treatments. During a LF survey conducted in 26 villages in South Cameroon (1805 individuals tested), we noted that none of those 52 subjects who had a positive ICT presented W. bancrofti microfilariae (mf) in their night blood smear, whereas 91% had Loa loa mf. As blood smears were also prepared by day for all the individuals, we could analyze the association between ICT positivity and Loa microfilaria. At village level, the prevalence of ICT positivity was strongly correlated with the prevalence of diurnal Loa microfilaria (r=0.61, p<0.001). Among the ICT-positive subjects, 96% presented Loa mf by day (arithmetic mean: 36,977 mf/mL). The association between ICT positivity and Loa mf density was assessed using multivariate logistic regression adjusting on individual characteristics (age, sex, Mansonia perstans mf density). Five groups were defined: one including the amicrofilaremics (reference group) and four with increasing mf densities (divided into quartiles). Odds-ratios [95% confidence interval] associated with ICT positivity were 85 [18-294] and 315 [72-1373] in the two groups with the highest Loa microfilariae (3061-12,120 mf/mL and >12,120 mf/mL). We also compared the results of Og4C3 ELISA tests, using dried blood spots, between ICT negative subjects with no Loa mf and ICT negatives with >20,000 Loa mf/mL. Optical density values obtained with samples from the latter group were higher than those obtained in the former (mean 0.44 vs 0.25, p<0.001). Our results suggest that high Loa mf density may cause false positivity with CFA detection tests (in that case LF mapping based on ICT would have to be redone in Loa-endemic areas) or is associated with amicrofilaremic W. bancrofti infection. Antibody detection tests will be applied on our samples to resolve the issue.

ELIMINATION OF ONCHOCERCIASIS IN AFRICA: DO WE HAVE STRATEGIES AND TOOLS? UGANDA CASE ANALYSIS

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Onchocerciasis is a neglected parasitic disease that infects at least 37 million people in Africa. The first major attempt to control onchocerciasis was launched in West Africa in 1974 with vector control as the only strategy. However, the advent of ivermectin (Mectizan®) in 1987, provided opportunities for other endemic African countries to join the war against onchocerciasis. Uganda has had a long history of onchocerciasis control and elimination dating back to the 1950s. Control of onchocerciasis using annual mass treatment with ivermectin started in 1992. However, annual treatments alone were not able to interrupt transmission of onchocerciasis in some foci. The Ministry of Health adopted new strategies to address this challenge, hence the initiation of Simulium spp. vector control and semi-annual treatments. New sensitive M&E tools have been developed to delineate areas where transmission has been interrupted. Impact assessments conducted in 2004 revealed a reduction of microfilaria prevalence in most of the foci. Assessments were based on skin snip surveys; serology (Ov16), pool screening of flies using PCR and crab collections and examinations for infestation with immature stages of the vector. Based on the results in 2007, an elimination policy was adopted in some foci. Since then, interruption of onchocerciasis transmission has been achieved in a total of 7 foci, where the vectors have also been eliminated. This translates to 1,354,390 treatments halted with more than 1.2 million people living in areas where transmission has been interrupted. With a combination of strategies and available evaluation tools, Uganda has demonstrated that it is feasible to achieve onchocerciasis elimination in Africa.

FINANCIAL AND ECONOMIC COSTS FOR CONTROL, ELIMINATION AND ERADICATION OF RIVER BLINDNESS IN AFRICA

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Onchocerciasis, also known as river blindness, has been a serious public health problem in Africa; however, the transmission of the disease has been suppressed with successful mass drug administration (MDA) with ivermectin. Also, recent studies in Mali and Senegal proved the feasibility of elimination with ivermectin. These provided momentum for shifting the goal from control to elimination along with the global movement toward elimination of neglected tropical diseases. As part of an Eradication Investment Case for onchocerciasis, financial and economic costs of alternative scenarios of control, elimination, and eradication are estimated using a micro costing approach from a viewpoint of service providers (i.e. ministries of health, WHO, and NGOs). The costs are estimated up to 2045 considering regional elimination in Africa is predicted to be achievable by 2040 employing a micro simulation model for onchocerciasis transmission. Financial costs include costs for MDA and surveillance. Economic costs measure the opportunity costs for community drug
distributors (volunteers) and donated drugs. Unit cost per person living in endemic area is estimated at $1.43 with all costs included and $0.11 with only financial costs. Financial cost of staying in control mode is estimated at $52.7 million over 2013-2045. Scaling up MDA coverage and implementing regular surveillance to achieve elimination and eradication would cost $440 million - $453 million, the savings being due to decrease in the number of required treatments as MDA is stopped with elimination achieved. Economic costs are estimated to be significantly higher than financial costs, $6.3 billion for the control scenario and $3.1 billion - $3.3 billion for the scenarios of elimination and eradication over the same time horizon. The results suggest scaling up MDA coverage and implementing regular surveillance to achieve elimination and eradication, after initial resource-intensive efforts, would allow substantial financial and economic savings in the long term.

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EXPLORING THE RELATIONSHIP BETWEEN ACCESS TO WATER, SANITATION AND HYGIENE AND SOIL-TRANSMITTED HELMINTH INFECTION: A DEMONSTRATION OF TWO RECURSIVE PARTITIONING TOOLS

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Soil-transmitted helminths (STH) - a class of parasites that affect billions of people - can be mitigated using mass drug administration, though reinfection following treatment occurs within a few months. Improvements to water, sanitation and hygiene (WASH) likely provide sustained benefit, but few rigorous studies have evaluated the specific WASH components most influential in reducing infection. There is a need for alternative analytic approaches to help identify, characterize and further refine the WASH components that are most important to STH reinfection in order to improve WASH interventions for control of STH. In this paper, we introduce two recursive partitioning approaches: classification and regression trees (C&RT) and conditional inference trees (CIT). Utilizing data from a school-based randomized control trial in Kenya, we conduct an assessment of the school- and household-level WASH components and demographic indicators that contribute to reinfection of pupils 10 months following deworming treatment. We show how C&RT and CIT can be used to identify the WASH components most predictive of and associated with STH infection. In addition, we demonstrate how both tools can be used to identify complex interactions between WASH indicators and sub-populations that may be particularly susceptible to STH reinfection, both of which are difficult to identify using traditional epidemiological methods. We discuss the relative merits and weaknesses of each approach and make recommendations for their use as tools to enhance STH control programs.

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IMPACT OF WASH AND ALBENDAZOLE DISTRIBUTION ON INFECTION WITH SOIL-TRANSMITTED HELMINTHS IN TIMOR-LESTE: INITIAL RESULTS OF A CLUSTER RANDOMIZED TRIAL

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Soil-transmitted helminths (STH) infect more than two billion people worldwide, causing considerable morbidity, including malnutrition and anaemia. STH infections are most prevalent in communities where adequate water and sanitation are lacking and hygiene behaviour is poor. Deworming programmes with anthelmintic drugs are highly effective in reducing morbidity but rapid reinfection occurs if there is no change in the environment. Therefore, provision of water, sanitation and hygiene (WASH) programs is of critical importance in the sustainable control of STHs. “WASH for Worms” is a cluster randomised controlled trial assessing the impact of a community-based WASH intervention, implemented by WaterAid Australia, on infection with intestinal parasites following mass albendazole (ALB) chemotherapy in villages in Timor-Leste. In this trial, initiated in 2012, twelve intervention villages receive the WASH programme and ALB treatments every six months. Twelve control villages receive only the six-monthly ALB. All villages are followed-up for two years after the first ALB distribution. Infection prevalence and intensity is measured by a modified qPCR. The results for STH infection levels at baseline and after the first follow-up in the first 16 villages enrolled will be presented together with results of an anthelmintic efficacy trial using a single dose of ALB on STHs conducted in 8 villages. At baseline the prevalence of STHs in the first eight villages was high, with more than 90% of participants infected with at least one STH (assessed by PCR) mostly comprising Necator americanus (75.3%) followed by Ascaris lumbricoides (63.6%). At the first follow-up in 8 of the villages, it was possible to detect an additional benefit of the WASH intervention compared to deworming alone. This trial is the first reported RCT evaluating the impact of integrated WASH and deworming programmes on infection with STHs, and will provide essential evidence for scaling up integrated programmes for STH control.

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ASSOCIATION OF WATER QUALITY WITH SOIL-TRANSMITTED HELMINTHIASIS, DIARRHEA AND INFLUENZA-LIKE ILLNESS IN NUEVA SANTA ROSA, GUATEMALA – 2010

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Improved water quality is essential in reducing diarrhea. Its impacts on influenza-like illness (ILI) and the soil-transmitted helminths (STH) Ascaris and Trichuris are less well described, though some data link them to water, sanitation, and hygiene. To assess their association with water quality, we conducted a cross-sectional survey in Nueva Santa Rosa, Guatemala among persons >1 year old in randomly selected households (HH). A stool sample was tested by Fecal Parasite Concentrator and Kato-Katz method for STH. Diarrhea and ILI were identified by self-reported symptoms in the past week. We explored associations between Escherichia coli-positive drinking water (water quality) and disease outcomes using exact logistic regression models. Median unbiased estimates (MUE) are reported when maximum likelihood estimates did not exist. We interviewed 920 persons from 204 HH. Water results were available for 778 persons; 84% (650/778) lived in HH with E. coli-positive water. Among persons in HH with water quality testing, 12.4% (76/611) tested positive for E. coli-positive water. Among persons >1 year old in randomly selected households (HH). A stool sample was tested by Fecal Parasite Concentrator and Kato-Katz method for STH. Diarrhea and ILI were identified by self-reported symptoms in the past week. We explored associations between Escherichia coli-positive drinking water (water quality) and disease outcomes using exact logistic regression models. Median unbiased estimates (MUE) are reported when maximum likelihood estimates did not exist. We interviewed 920 persons from 204 HH. Water results were available for 778 persons; 84% (650/778) lived in HH with E. coli-positive water. Among persons in HH with water quality testing, 12.4% (76/611) tested positive for E. coli-positive water.

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INTEGRATING WATER TREATMENT INTO ANTENATAL CARE: IMPACT ON USE OF MATERNAL HEALTH SERVICES AND HOUSEHOLD WATER TREATMENT AMONG MOTHERS -- UGANDA, 2013

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In Uganda, high maternal and neonatal mortality rates reflect underutilization of reproductive health services; high diarrhea risk among children results from poor access to safe water. To incentivize household water treatment and reproductive health service use, water treatment kits (buckets and 30 sachets of flocculant-disinfectant powder) were provided at first antenatal visits. Refills of 30 sachets each were distributed at follow-up antenatal (ANC) visits, health facility deliveries, and postnatal check-ups. We evaluated this intervention through cross-sectional surveys of a random sample of women from participating health facilities who received reproductive health care in 2013 after project launch (intervention group) and in 2012 before project launch (pre-intervention/comparison group). We used the Chi-Square test statistic to compare groups. We surveyed 226 intervention group women and 207 comparison group women. A higher percentage of intervention than comparison group women reported treating drinking water on the day of the survey (31.7 vs 19.7%, P=0.01), and had detectable chlorine residual, an objective measure of treatment, in their stored drinking water (13.5% vs 3.4%, P<0.01). Of 226 intervention group women, 222 (98.2%) received water treatment kits. Although 96.8% of intervention group women had ≥2 ANC visits (median 4 visits, range 1-5), only 101 (45.5%) received one or more sachet refills. There was no difference in the percentage of women in intervention and comparison groups that reported ≥2 ANC visits (66.2 vs 68.5%, P=0.61) or health facility deliveries (67.8 vs 74.3, P=0.14). Intervention group women were more likely than comparison group women to treat their drinking water, but had similar low use of reproductive health services. Although water treatment kit coverage at first antenatal visit was high, inadequate distribution of, or low demand for, refill sachets may have contributed to limited program impact.

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IMPROVEMENT OF WATER, SANITATION AND HYGIENE IN TWO URBAN SLUMS IN UGANDA THROUGH COMMUNITY PROACTIVE AND SUSTAINABLE INTERVENTIONS

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In urban slum settlements in Uganda, the major risk factor for water borne diseases such as diarrhea is contaminated drinking water due to poor latrine status, low safe water coverage, and poor domestic and personal hygiene practices. To address the challenges affecting these areas, a project aimed at improving the water, sanitation and hygiene (WASH) status in 2 urban slum communities in Uganda through community proactive and sustainable interventions was implemented. The 2 slums involved were in Kampala and Mukono in the central region of the country. To establish the WASH status of the communities before project implementation, a baseline survey was carried out among 213 households. The survey involved both quantitative and qualitative methods. Several interventions with full community participation were then implemented to improve the situation. The interventions included: community sensitization on WASH, promotion of hand washing using the tippy tap technology, supporting clean-up exercises in the community, providing advisory roles in WASH, supporting health clubs in primary schools, training community members in a short course in WASH, capacity building of youth in WASH, promoting drinking safe water through household chlorination and home improvement campaigns. After the project implementation period, a final evaluation survey was carried out among 300 households. This survey involved both quantitative and qualitative data collection methods. Latrine coverage improved from 86.0% to 98.7%. Piped water usage improved from 38.0% to 86.0% with a reduction in the use of unprotected sources from 30.0% to 2.3%. Treatment of drinking water improved from 95.3% to 99.7% with more households (96.0%) boiling their water from 94.0% who did so at the baseline. Indiscriminate disposal of solid waste reduced from 18.0% to 2.0% and satisfaction with solid waste management services increased from 40.0 to 91.8%. Drainage around homes improved from 57.0% to 86.0% while presence of soak pits at households increased from 2.3% to 10.0%. Improvements in the latrine statuses, environmental hygiene and waste management practices were also registered. There were significant improvements in the WASH status of these communities after the implementation of several multi-faceted interventions. Urban slums can benefit from WASH interventions when communities are fully involved and with a focus on capacity building.

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SOME CHILDREN WITH ACCESS TO IMPROVED WATER AND SANITATION DEMONSTRATE BETTER GROWTH: FINDINGS FROM THE YOUNG LIVES COHORT STUDY IN ETHIOPIA, INDIA, PERU AND VIETNAM

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Child undernutrition is widespread in developing countries and is perceived to have large costs over the lifecycle. We used data on 8,062 children from Ethiopia, India, Peru and Vietnam at ages 1, 5, and 8 yrs from the Young Lives cohort to estimate multivariate logit regressions for associations between household and community-level access to improved water and sanitation, and stunting and underweight at these three ages. Improved drinking water was associated with stunting only in Peru, where access at age 5 yrs decreased risks of stunting at age 5 yrs (OR: 0.68; p<0.05). In Ethiopia access to improved sanitation facilities (facilities) at age 1 yr was associated with decreased odds of stunting at 1 yr, 5 yrs and 8 yrs (OR: 0.59, 0.63, 0.64; p<0.05). In India and Peru, access to improved facilities at 1 yr was significantly associated with stunting at age 5 yrs only (OR: 0.61, 0.75; p<0.05). Improved facilities access when children were 5 yrs old was associated with stunting only at age 5 in Peru (OR: 0.71; p<0.05) and age 8 in Vietnam (OR: 0.64; p<0.05). Improved facilities at age 8 was not associated with stunting at age 8 in any country. Improved drinking water at 1 yr was significantly associated with underweight at age 8 in India (OR: 0.52; p<0.05); for all other time points and countries, improved drinking water was not significantly associated with underweight. Facilities quality at age 1 yr was associated with lower odds of being underweight in Peru at 1 yr (OR: 0.59, p<0.05) and in India at age 5 yrs (OR: 0.60, p<0.05). There were no other significant associations between facilities and underweight. The combination of significant associations with quality of sanitation facilities and reduced undernutrition and the numerous insignificant associations suggest the importance of further investigating mediating factors that affect whether interventions to improve sanitation reduce concurrent and long-term child malnutrition.
cytometry. The immunization regimen resulted in robust production of CSP were also recognized to a similar extent regardless of the mouse antibody responses in all the mice strains tested against the immunogen. Analysis and ELISA. Groups of six different strains of mice were used to and antigenic integrity of the protein were defined by western blot was expressed in soluble form with high yield. The proper configuration P. yoelii. The chimeric protein a recombinant protein chimera containing immunogenic domains that protein based on the P. vivax CSP. In this study we tested the capacity of P. yoelii eradication of infections. An effective vaccine is therefore essential to the control and malaria in the past 10 years. However, these measures are not effective Africa. Existing control measures have significantly reduced the burden of Plasmodium vivax is the most widespread species of Plasmodium representing 50% of the malaria cases occurring outside sub-Saharan Africa. Existing control measures have significantly reduced the burden of malaria in the past 10 years. However, these measures are not effective against hypnozoites a dormant stage form responsible of P. vivax relapse infections. An effective vaccine is therefore essential to the control and eradication of P. vivax. A well characterized vaccine candidate is the circumsporozoite protein (CSP). Anti-CSP antibodies have the ability to inhibit the invasion of hepatocytes by P. vivax sporozoites in vitro. Cellular immune responses against CSP have also been correlated with protection. However, preclinical and clinical data of P. vivax CSP based vaccines have shown limited success in inducing cellular immune responses. Based on our reported data on the use of chimeric P. yoelii proteins to enhance cellular reactivity, we decided to design a recombinant chimeric protein based on the P. vivax CSP. In this study we tested the capacity of a recombinant protein chimera containing immunogenic domains that preserved the protein topology described for P. yoelii. The chimeric protein was expressed in soluble form with high yield. The proper configuration and antigenic integrity of the protein were defined by western blot analysis and ELISA. Groups of six different strains of mice were used to test immunogenicity. The chimeric protein was able to induce robust antibody responses in all the mice strains tested against the immunogen. Interestingly, synthetic peptides representing the allelic forms of the P vivax CSP were also recognized to a similar extent regardless of the mouse strain. Cellular immune responses were investigated by IFN-γ and IL-4 ELISPOT test as well as intracellular cytokine staining measured using flow cytometry. The immunization regimen resulted in robust production of IFN-γ, IL-2 and TNF-a by CD4+ and CD8+ T cells. The fine specificity of the cellular immune responses induced by immunization with the chimeric P. vivax CSP will be discussed.

**IMPACT OF A SCHOOL-BASED WATER, SANITATION AND HYGIENE PROGRAM ON DIARRHEA, RESPIRATORY INFECTIONS AND ABSENTEEISM: A LONGITUDINAL EVALUATION**

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School-based water, sanitation and hygiene (WASH) programs can lead to improvements in pupil health and attendance, but few rigorous studies are available. We conducted a quasi-experimental, longitudinal impact evaluation of a comprehensive school-based WASH program in Mali that provided schools with latrines, water points, drinking and handwashing stations, cleaning supplies, hygiene promotion, and training on management and governance. We randomly selected 100 primary schools participating in the WASH program and matched them to 100 control schools that were not participating in the program based on location, school population, and the presence of latrines and water points before the start of the program. Unannounced visits were conducted between February 2013 and June 2014, for a total of seven visits per school. At each visit we conducted a roll-call of all pupils in the school and asked 40 pupils to provide a one-week recall of absence, diarrhea, and symptoms of respiratory infections. We evaluated schools for adherence to the program. We will employ random effects longitudinal regression analysis with data clustered at the school level to examine the association between participation in the WASH program and roll-call absence, self-reported absence, self-reported diarrhea, and self-reported symptoms of respiratory infections. The study is powered to detect a 20% reduction in pupil absence and a 30% reduction in diarrhea. Data will be analyzed after the final round of data collection in June 2014.

**A CHIMERIC PLASMODIUM VIVAX CSP TAILORED TO ENHANCE THE CELLULAR IMMUNE RESPONSE**

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Plasmodium vivax is the most widespread species of Plasmodium representing 50% of the malaria cases occurring outside sub-Saharan Africa. Existing control measures have significantly reduced the burden of malaria in the past 10 years. However, these measures are not effective against hypnozoites a dormant stage form responsible of P. vivax relapse infections. An effective vaccine is therefore essential to the control and eradication of P. vivax. A well characterized vaccine candidate is the circumsporozoite protein (CSP). Anti-CSP antibodies have the ability to inhibit the invasion of hepatocytes by P. vivax sporozoites in vitro. Cellular immune responses against CSP have also been correlated with protection. However, preclinical and clinical data of P. vivax CSP based vaccines have shown limited success in inducing cellular immune responses. Based on our reported data on the use of chimeric P. yoelii proteins to enhance cellular reactivity, we decided to design a recombinant chimeric protein based on the P. vivax CSP. In this study we tested the capacity of a recombinant protein chimera containing immunogenic domains that preserved the protein topology described for P. yoelii. The chimeric protein was expressed in soluble form with high yield. The proper configuration and antigenic integrity of the protein were defined by western blot analysis and ELISA. Groups of six different strains of mice were used to test immunogenicity. The chimeric protein was able to induce robust antibody responses in all the mice strains tested against the immunogen. Interestingly, synthetic peptides representing the allelic forms of the P vivax CSP were also recognized to a similar extent regardless of the mouse strain. Cellular immune responses were investigated by IFN-γ and IL-4 ELISPOT test as well as intracellular cytokine staining measured using flow cytometry. The immunization regimen resulted in robust production of IFN-γ, IL-2 and TNF-a by CD4+ and CD8+ T cells. The fine specificity of the cellular immune responses induced by immunization with the chimeric P. vivax CSP will be discussed.

**INHIBITION OF PLASMODIUM VIVAX INVASION OF RED BLOOD CELLS USING ANTI-DUFFY BINDING PROTEIN ANTIBODIES**

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It has long been established that the malaria parasite, Plasmodium vivax, depends on the interaction of its Duffy Binding Protein (DBP) with the erythrocyte Duffy antigen (either FyA, FyB) for host cell invasion. Recent evidence has indicated that patients who are FyAf/Ay homoygotes are far less likely to experience severe vivax malaria and that DBP protein has a lower binding affinity for this version of the Duffy receptor. As most vaccine resources and strategies concentrate on the parasite’s use of the Duffy Binding Protein, it is important to determine if current studies of the Fy::DBP interaction should remain the sole interaction for vaccine development or if we need to explore the Fy:::DBP more closely. The focus of this study was to examine the ability of P. vivax to invade red blood cells in vitro and to determine if inhibition of the parasite’s invasion of FyAf/Ay red blood cells differs from FyBf/Bf blood cells. Reticulocyte enriched blood samples of varied Fy-positive FyBf/Bf and FyAf/Ay homozygotes were exposed to different strains of P. vivax with genetic variations of DBP (P. vivax strains Nicaragua, Indonesia, and Thailand). New invasions were measured using flow cytometry and selective staining techniques, with and without anti-DBP blocking antibodies. The results showed that the blocking ability of the anti-DBP antibodies was highly dependent upon the parasite strains used. Anti-DBP antibodies directed against the DBP Sal 1 variant showed a 97.0% inhibition of invasion into FyAf/Ay red blood cells by P. vivax Indonesia, but only a 24.8% inhibition into FyBf/Bf red blood cells by the same strain. Conversely, anti-DBP antibodies showed 1.4% inhibition and 12.7% inhibition of P. vivax Nicaragua invasion into FyAf/Ay and FyBf/Bf red blood cells, respectively. Invasion by P. vivax Thailand was not inhibited in either Fy genotype. Also, anti-DBP antibodies showed highly varied efficacy against invasion by all strains into Duffy-negative (FyFy0) red blood cells. These results demonstrate that differences in the parasite’s DBP polymorphisms may play a large role in invasion success in the absence of other complex genetic factors between donors. This work indicates that both the genotype of the P. vivax and the Fy genotype of the host are potentially important considerations in the development of vaccine candidates.

**ANTIBODIES TO A HYPOTHETICAL FALCIPARUM MALARIA ANTIGEN (PF3D7_1134300) INHIBIT ERYTHROCYTE INVASION**

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We previously screened a Plasmodium falciparum 3D7 strain blood stage cDNA phage display library using a differential approach and identified three antigens encoded by PF3D7_1135100 (MSP-7), PF3D7_1021800 (PF5EA-1) and PF3D7_1134300 that are uniquely recognized by antibodies in plasma from resistant Tanzanian children but not by those from asthmh.org
HUMANIZED HLA MICE ARE PROTECTED AGAINST CHALLENGE WITH *PLASMODIUM FALCIPARUM* SPOROZOITES (PFS2) WHEN IMMUNIZED WITH IRRADIATED PFSZ OR LIVE PFSZ UNDER CHLOROQUINE COVER BUT NOT BY IMMUNIZATION WITH HUMAN ADENOVIRUS 5-VECTOR*ED PLASMODIUM FALCIPARUM ENCODING AMA1 AND CSP

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Development of humanized mouse models that are able to act as surrogate human immune system is a highly pursued goal for testing human vaccine candidates, generating fully human monoclonal antibodies and studying the development of human immune system. Previously we have demonstrated that humanized mice expressing HLA molecules in NOD.RagKO.I2RgKO background (DRAG/DRAGA) that were infected with HLA matched human hematopoietic stem cells develop a functional human immune system and respond to vaccination. Humanized HLA mice also develop human hepatocytes, kupffer cells, liver endothelial cells and erythrocytes and sustain the vertebrate life cycle of *P. falciparum*. Herein we show that humanized mice are protected against liver stage *Plasmodium falciparum* infection upon immunization with irradiated Pfsz or live sporozoites under chloroquine cover. In contrast, humanized HLA mice were not protected by immunization with human adenovirus 5-vector*ed P falciparum* NMRC-M3V-Ad-PfCA encoding AMA1 and CSP constructs. Humanized HLA mice represent a new pre-clinical model for testing vaccines against malaria.

IDENTIFICATION OF A CORE GROUP OF *PLASMODIUM FALCIPARUM* MHC CLASS I PEPTIDES SEQUENCED FROM INFECTED LIVER CELLS

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Sterile protection from *Plasmodium falciparum* (Pf) infection can be achieved by developing potent cell mediated immunity against malaria liver stages. Liver stage immunity has been established in human clinical trials using two different but similar approaches: 1. Immunization with radiation-attenuated-sporozoites (RAS) and 2. infection-treatment-vaccination (ITV). The model for RAS immunization suggests that attenuated sporozoites enter hepatocytes, initiate development, and then die leaving behind parasite material for degradation and presentation on MHC Class I receptors. During ITV human volunteers are challenged with live sporozoites under chloroquine coverage resulting in complete hepatocyte invasion and development. Both RAS and ITV immunization suggest that liver stage immunity is mediated by Class I presentation of Pf peptides by either aborted liver stage development or during successful merozoite development. A long standing goal in the malaria vaccine field is to identify the Pf antigens that induce liver stage immunity. Towards this goal we have worked to identify Pf Class I peptides presented from *in vitro* infected human hepatocytes by direct peptide sequencing. We have identified a core group of 12 Pf proteins sequenced from three genetically

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distinct primary human hepatocyte sources infected with sporozoites. Among this core group of Pf proteins we have identified a subset that induces IFN-γ secretion from PBMCs of RAS-protected volunteers. In our future experiments we will test this core group of liver stage antigens as vaccine candidates in malaria challenge models.

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IMMUNODOMINANCE AND VACCINE DEVELOPMENT: NEW INSIGHTS FROM PROTEOME-WIDE PROFILING OF T CELL AND ANTIBODY RESPONSES TO MALARIA

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For many infectious diseases, the development of effective vaccines based on immunodominant antigens has thus far not been successful. Immunodominance is the phenomenon whereby pathogen-specific immune responses target only a small fraction of the full range of possible antigens and epitopes. It is a central feature of immunity but what distinguishes effective immune targets from less effective targets is not obvious. In experimental infections with simple organisms, immunodominance results in only a few specificities dominating the host’s response. Large pathogens such as the Plasmodium parasite represent a far greater challenge because of the complexity and scale of potential immune targets. We have generated unique datasets of proteome-wide T cell responses and antibody responses to Plasmodium using omics-scale technology platforms, including protein microarrays and epitope prediction algorithms, with specimens from humans experimentally or naturally exposed to malaria. We have integrated these datasets to develop metrics of immunological, structural and genetic parameters associated with antigen immunodominance in the context of a complex parasite. We establish that the repertoire of T cell reactive antigens is largely distinct from the repertoire of antibody reactive antigens, and that T cell target antigens are more conserved as compared with antibody targets. We further establish that the immunodominance hierarchy for antibody responses is influenced by structural or functional properties that differ from those that underlie the hierarchy for T cells. By defining the degree to which immunodominance shapes immunity against Plasmodium and identifying antigens and epitopes that represent key targets of protective immunity in humans, our studies have direct outcomes for vaccine development. These also data further our understanding of host-pathogen interactions.

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PLASMODIUM FALCI PARUM ACTIVATION OF β-CATENIN MEDIATES THE DISRUPTION OF INTERENDOTHELIAL CELL JUNCTIONS: A ROLE IN CEREBRAL MALARIA

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A high proportion of deaths by Plasmodium falciparum is caused by cerebral malaria, the most profound syndrome of severe malaria that is fatal within 24-72 hours in 20% of the cases. The disruption of the blood brain barrier (BBB), characteristic of cerebral malaria, causes diffusion of blood cells and serum into the brain tissue leading to coma and damage to the nervous system. The mechanisms underlying this process are largely unknown but the need for adjunct therapy to rescue patients from death is mandatory due the high rate of mortality. Using monolayers of human brain microvascular endothelial cells (HBMECs), we have observed that incubation with erythrocytes infected with Plasmodium falciparum promotes the disruption of interendothelial cell junctions (IEJs) between these cells. This process is mediated by the delocalization of β-Catenin from the adherent junctions to the nuclei and the activation of the Tcf/LEF pathway, which leads to the detachment of the HBMECs. Infecting HBMECs with a lentivirus carrying a dominant negative mutant tcf4 vector, we were able to inhibit the detachment of HBMECs induced by P. falciparum. Compounds that inhibit the activation of the β-Catenin pathway also inhibit the disruption of IEJs and detachment of HBMECs induced by P. falciparum and have a protective effect against experimental cerebral malaria in mice. Our findings describe an unprecedented role for β-Catenin in the maintenance of the BBB integrity in the setting of cerebral malaria and open new perspectives for the treatment of the disease.
opportunities for the early detection and prognosis of malaria as well as could provide better understanding of disease pathogenesis and host responses in malaria.

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THE ROLE OF ENDOTHELIN-1 IN THE VASCULAR PATHOBIOLOGY OF CEREBRAL MALARIA

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Cerebral malaria (CM) is a serious complication of Plasmodium falciparum infection associated with cerebral vasculopathy, high mortality, and adverse neurological sequelae. The vasoactive peptide, endothelin-1 (ET-1), has been shown to mediate blood-braint barrier (BBB) permeability, inflammation, and vascular tone, and may be important in CM pathogenesis. We previously reported that ET-1 was important in regulating cerebral blood flow, brain microvascular hemorrhage and mortality in our experimental CM model. These actions were mediated by ET-1 activation of the endothelium (ET) receptor. To test the hypothesis that ET-1 is involved in the pathological process of ECM, we investigated ET receptor mediated signaling in mice infected with P. berghei ANKA (PbA). ET receptor blockers (ETARB) significantly improved survival in ECM mice. In addition, ETRB enhanced vascular integrity during PbA infection. BBB permeability, and protein levels of angiopoietin-2 and VCAM-1 were significantly lower in ECM mice treated with ET, than in mice treated with saline. In addition, ET, RB prevented the ECM-induced decrease in angiopoietin-1 in PbA-infected mice. CM is associated with astroglisosis in both human disease and in experimental models. Our preliminary data indicate that astroglisosis is associated with abnormal protein levels of connexin 43 (Cx43), a gap junction protein critical in gliss and BBB integrity. ET, RB prevented the PbA-induced dysregulation of Cx43. We hypothesized that ET-1 mediated vascular dysfunction in ECM potentially by regulating neuroinflammation and Cx43 expression. In this report, we observed a reduction in the activation of JNK in the brains of mice with ECM. JNK, a downstream substrate of ET-1, has been demonstrated to regulate Cx43 expression and function, and is important in CM. Our data indicate that ET-1 may mediate the vascular pathology and neuroinflammation in ECM via regulation of JNK signaling and subsequent Cx43 dysregulation. The ET-1 pathway may thus be a potential therapeutic target as an adjunct in the treatment of human CM.

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PLASMODIUM FALCIPARUM IN AOTUS: A NOVEL MODEL FOR PELACENTAL MALARIA

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Pregnant women are infected with a distinct Plasmodium falciparum (Pf) phenotype that binds to chondroitin sulfate A (CSA) and grows to high density in the placenta. Placental sequestration is linked to severe pathologies in the mother and offspring, leading to tens or hundreds of thousands of deaths each year. CSA-binding parasites express VAR2CSA, a distinctly structured member of the Pf erythrocyte membrane protein 1 (PfEMP1) variant surface antigen family that is now the leading target for vaccine development. Further research progress is hindered in the absence of an animal model that recapitulates the features of human placental malaria. Previous work has shown that Pf can infect and sequester in brain, heart and spleen of non-pregnant Aotus, and we sought to establish a model of placental malaria using this parasite-monkey combination. We infected pregnant Aotus (n=2) with CSA-binding Pf-CS2 during the 3rd trimester and observed a pronounced sequestration of parasites in placental intervillous spaces, with ~30-fold higher parasite density in placental versus peripheral blood. Most placental parasites were mature blood stages, while peripheral samples were exclusively young ring stages. Aotus (n=1) infected with non-CSA-binding parasite lines (CAMP; FVO) before pregnancy developed recrudescences during subsequent pregnancy, similar to the experience of women. CS2 parasites collected from pregnant Aotus express VAR2CSA on the infected erythrocyte (IE) surface, and bind specifically to CSA. Similar to immune multigravid women, a monkey infected with Pf-CS2 parasites over successive pregnancies acquired antibodies against VAR2CSA, and purified antibodies blocked the binding of several maternal parasite isolates to CSA. In summary, Pf infections in pregnant Aotus monkeys recapitulate all the prominent features of malaria infection and immunity in pregnant women, and can be useful for basic mechanistic studies as well as preclinical studies to qualify candidate pregnancy malaria vaccines.

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SPLENIC PATHOLOGY SUPPORTS THE ACCELERATION OF SEVERE MALARIA THROUGH HIV INFECTION IN PEDIATRIC PATIENTS

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A number of cohort studies have demonstrated that HIV-1 infection correlates with severe Plasmodium falciparum malaria (higher parasitemia, more clinical malaria complications, and higher case fatality rate), and in vitro studies have shown that HIV-1 impairs phagocytosis of parasitized erythrocytes by macrophages. As macrophage-mediated clearance of parasites in the spleen plays an important role in the clearance of parasites during malaria infection, we hypothesized that histological analysis of the spleen might further elucidate the mechanisms by which HIV-malaria co-infection accelerates progression to severe malaria. As part of a post-mortem study in Blantyre, Malawi of parasiticemic comatose children, we systematically surveyed malaria parasites across organs in patients with and without HIV. The overall HIV seropositivity rate in the cohort was 21%. Using a combination of histology, immunohistochemistry and qRT-PCR, we found that HIV-positive patients had significantly higher parasite loads in brain, heart, gut, spleen and skin. The difference was most pronounced in the spleen where the difference in the mean number of parasites was highly significant (320 ± 139 vs 81 ± 20 parasites in 10 high power fields, p = 0.0018). In a focused histological analysis of the spleen, we found that HIV-positive cases had higher levels of “free” parasitized erythrocytes, i.e. those not engulfed by macrophages, while phagocytosed parasites could be readily observed in HIV-negative cases. These HIV-specific effects were significant, even across a wide range of values for peripheral parasitemia, hematocrit, and final histologic diagnosis (cerebral malaria or non-malarial coma). These data suggest that HIV infection enhances parasite sequestration in many different organ systems, and specifically in the spleen, where phagocytosis of parasites is impaired. These findings bridge in vitro mechanistic data and in vivo association study data to further elucidate the process by which HIV accelerates progression to severe malaria.
CHILDREN WITH CM MANIFEST MARKED DIFFERENCES IN GLOBAL STRESS RESPONSES WHICH ARE ASSOCIATED WITH CEREBRAL PARASITE SEQUESTRATION AND UNDERLYING PATHOPHYSIOLOGY

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Cerebral Malaria (CM), a severe complication of Plasmodium falciparum infection, is associated with a high rate of mortality and neurological sequelae. The WHO defines CM as a single clinical entity of coma and peripheral blood malaria parasitemia; however, disease heterogeneity has been suggested by the identification of brain sequestration (RET+) in some but not all patients with CM. RET+CM is associated with brain microvascular pathology, parasite sequestration, coagulopathy and heightened inflammation. To explore potential mechanistic diversity in factors resulting in CM, we carried out unsupervised clustering on whole blood transcriptomes from 98 children with CM and identified three transcriptional clusters. One cluster was significantly associated with hyperparasitemia (p<0.0001) and another cluster with lack of brain sequestered parasites (RET−) (p=0.028). To further define host features associated with parasite sequestration, we then compared transcriptional profiles between samples associated with brain sequestered parasites (RET+) to lack of brain sequestrated parasites (RET−) taking parasite load into account. Gene sets associated with RET+CM reflected heightened inflammation (cytokine activity GO.000512), changes in neutrophil (neutrophil chemotaxis GO.0030593) and platelet (alpha granules GO.0031093) biology and dysregulation of coagulation (blood coagulation GO.0007597). Elevated plasma inflammatory cytokines and neutrophil proteins were found in RET+ samples, consistent with the RNA data. Surprisingly, neutrophils isolated from RET+ patients at the time of infection displayed impaired chemotaxis towards IL-8. In contrast, the transcriptional profiles of children with RET-CM were associated with upregulation of non-inflammatory stress pathways (protein catabolism pathways: GO.0006511; DNA repair pathways: GO.0006270) and type I interferon. Our data suggests host response diversity despite similar clinical presentations of WHO defined CM. Irrespective of parasite load, we found that children with cerebral parasite sequestration demonstrate evidence of neutrophil activation and coagulopathy. Further study of how neutrophil and platelet biology is involved in sequestration and why children with RET-CM have distinct host profiles could reveal novel strategies to prevent brain sequestration in CM and improve clinical outcomes.

PHENOTYPIC VARIATION IN THE ABILITY OF PLASMODIUM KNOWLESI LAB AND FIELD STRAINS FOR THE INVASION OF HUMAN RED BLOOD CELLS

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Plasmodium knowlesi, primarily a simian parasite, is now the major cause of human malaria in Malaysian Borneo. While the majority of the cases are uncomplicated, clinical profiles similar to that of severe P. falciparum cases occur in a small subset of infected patients, with high parasitemia as the only risk factor consistently associated with disease severity. However, there has been little investigation to understand what causes such disparity in parasitemia. Recently, we showed that P. knowlesi H strain, a primate adapted lab strain, preferentially invades very young human red blood cells (RBCs). This strain however can gradually adapt to proliferate at a much higher efficiency in human blood. To expand this observation to other P. knowlesi strains, we have established in vitro culture rhesus RBCs 3 additional P. knowlesi strains. RBC age-dependent invasion assays and proliferation assays demonstrate that these lab strains exhibit different capacity in invading human RBCs. We are currently carrying adaptation experiments for these additional lines. To determine whether variations observed in the lab are also found in the field, we investigated the human RBC tropism of 32 field strains isolated from patients admitted to Kapit Hospital, Sarawak, Malaysia. The in vivo preference determination of 26 of them shows that a majority of the strains have a skew for invading younger RBCs (18/26). Indeed, 5 isolates display a very strong preference in invading reticulocytes. In vitro invasion assays performed with 9 isolates (parasite density greater than 10,000/ul) confirm that field strains differ significantly in the range of human RBCs they can invade. Together, these results suggest a significant variation in the ability of P. knowlesi strains to invade human RBCs, underlying a possible mechanism for the virulence of the parasite in human infections.

CHARACTERIZATION OF THE EXPANDED ACYL CO-A SYNTHETASE GENE FAMILY

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The plasticity of the Plasmodium falciparum genome allows this malaria parasite to adapt quickly to selective pressures encountered in the human host by the acquisition of single nucleotide polymorphisms, recombination or gene duplications. One example of such duplication events is the acyl Co-A synthetase (ACS) gene family where four conserved orthologs of ACS are predicted to perform classical ACS function while nine paralogs have expanded and diverged from the PfACS9 ortholog and their function remains unknown. ACSs activate fatty acids (FA), which can then be used for protein modification, phospholipid biosynthesis, FA elongation, and beta-oxidation. In P. falciparum the majority of FA are taken up from the host environment. Long-range haplotype analysis suggests that members of this gene family are under recent positive selection. To address the biological function of the individual members of the PfACS family, we tag individual genes with an HA tag and a protein degradation domain resulting in inducible protein knockdowns. These modifications allowed us to identify different expression patterns and distinct cellular localization of individual ACSs that could be a result of neo-functionalization. PfACS5 and PfACS1a were exported to the host cell cytosol and membrane periphery while PfACS9 remained within the parasite. Comparison of growth rates for wildtype and PfACS1a, PfACS4, PfACS5 and PfACS9 knockdown parasites in complete media did not differ and we could not detect any changes in gene expression of any other family member by quantitative RT-PCR. However, growth defects could be detected for PfACS5 knock down parasites in media containing restricted glucose and solely palmitic and oleic acid. We hypothesize that the expansion and recent positive selection of the PfACS gene family are the consequence of metabolic pressures driving parasite evolution, and understanding FA metabolism will give us insight into key metabolic pathways that might serve as potential targets for novel antimalarials.
genes encoding the β and γ subunits of the ATP synthase complex in *P. falciparum* blood stages, raising the possibility that non-enzymatic functions of the ATP synthase may be essential. To address this possibility, we aimed to design a catalytically inert but structurally intact ATP synthase mutant subunit was identified as an initial target for mutagenesis. A merodiploid line expressing a tagged mutated β subunit at an ectopic site was generated in Dd2attB and NF54attB parasites. In theory, this should reduce the overall ATP synthase catalytic activity by about 87.5%. Although the mutant subunit was correctly trafficked to the mitochondrion in both lines, the transgenic parasites grew at the same rate as the parental lines, indicating that the ectopically expressed mutant subunits did not have a dominant negative effect. Mitochondria from the NF54attB merodiploid line were isolated and initial blue native gel results indicated the mutant form of the protein being incorporated into the complex. Given that no dominant negative effect was seen with the merodiploid lines, a synthetic gene to replace the endogenous gene with a mutated version through single allelic replacement was attempted. The synthetic gene consists of two exons, the first of which is targeted with a ribozyme system. These studies will help determine the role of ATP synthase in erythrocytic and insect stages of *P. falciparum*.

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### AN ALGORITHM FOR ASCERTAINING THE VAR GENE REPERTOIRE IN PLASMODIUM FALCIPARUM FIELD SAMPLES

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*Plasmodium falciparum* erythrocyte membrane protein-1 (PFEMP1) proteins are encoded by the var gene family, present in 40 to 60 copies per genome. PFEMP1 mediates tissue-specific cytoadherence of infected erythrocytes, allowing parasites to evade clearance by the spleen and cause pathologic effects via sequestration. An infected erythrocyte usually expresses only one PFEMP1 variant on its surface at a time. PFEMP1 molecules undergo clonal antigenic variation that is central to their ability to evade the host immune response. Next generation short-read sequencing data cannot be used to accurately and comprehensively characterize sequence variation of this large, repetitive and highly variable gene family. Each var gene consists of two exons, the first of which is longer (2,500-10,500 bp) and much more variable in length than the second (1,000 - 1,500bp). We developed a novel algorithm to perform targeted assembly of var gene exons, based on a combination of Pacific BioSciences (PacBio) and Illumina data. The algorithm takes advantage of patterns of conservation at the boundaries of the single intron, and the different variation profiles of the two exons, to generate exon 1 and exon 2 sequences from a combination of k-mer walks using Illumina reads and information from PacBio-based contig assemblies. Our novel algorithm recovers a complement of exons which is similar in number and length distribution to those found in the reference 3D7 genome, suggesting that it captures most of the var exons in the genomes of field-derived samples. Using this algorithm, we have characterized the repertoire of var genes in 12 clinical samples from Mali. We are determining the constitutive expression and upstream promoters for identified var in each sample and the sequence diversity present. We will compare these results to known var repertoires. This new tool will advance efforts to understand the role of PFEMP1 variation in pathogenesis and immune evasion and may aid in the design of diversity-covering malaria vaccines.

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### COMPARATIVE ANALYSIS OF FIELD ISOLATE AND MONKEY-ADAPTED PLASMODIUM VIVAX GENOMES

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Due to the difficulty to culture *Plasmodium vivax* in vitro, significant insights into this important malaria parasite is based upon a history of successful adaptations of human infections to non-human primates. *P. vivax* strains grown in monkeys serve as a renewable source of parasites for studying relapse characteristics, mosquito species compatibilities, drug susceptibility profiles or immune response characteristics for potential vaccine development. However, little is known as to how the adaptation to a new host influences the parasite's genome and, consequently, its biology. Additionally, despite the consistent observation of complexity of infections in clinical isolates, these monkey-adapted strains are typically assumed to consist of a clonal population of a single strain. We describe here the comparative analysis of genome sequences from seven *P. vivax* parasites that have been adapted to monkey hosts with sequences obtained from six field isolate genomes. Our results reveal that the adaptation of parasites into monkey hosts is unlikely to result in any systematic modification of the genome. We also show that monkey-adapted strains are not always
homogenous and that they can still consist of a mixture of strains. Additionally, we describe the analysis of six blood samples collected during the generation of the Mauritian-I and Mauritian-II strains and show that, starting from a single complex infection, different strains became dominant in different monkeys. Overall, our study highlights some of the complications associated with studying monkey-adapted strains but also provide a solid framework for developing better, more controlled studies of this important resource for understanding vivax malaria.

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ASSSESSMENT OF POPULATION STRATIFICATION AND COMPLEXITY OF INFECTION IN CAMBODIAN PLASMODIUM VIVAX BY HIGH-THROUGHPUT SEQUENCING

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In Cambodia, malaria is one of the foremost public health problems and its control is a high priority for the government and NGOs. Among the estimated 13.6 million Cambodians, 2.5 million individuals live in forested areas where malaria transmission is highest. With the implementation of extensive control efforts, the number of reported malaria cases has globally decreased since 1997, due to a decrease in falciparum malaria. By contrast, in the same period, the number and proportion of cases attributed specifically to Plasmodium vivax has significantly increased. In order to design more efficient elimination and control strategies in this region, it is critical to first understand the dynamics and organization of the P. vivax population. Here, we described a novel sequencing assay that enables robust genotyping of 130 single nucleotide polymorphisms (SNPs) in a high-throughput and cost-efficient manner. We applied this assay to 401 P. vivax-infected patients recruited from 9 study sites throughout Cambodia between 2004 and 2013. Our analysis provides a thorough perspective on the diversity, organization and dynamics of the Cambodian P. vivax population as well as a first assessment of the factors influencing complexity of infections. Overall, our study provides a foundation to design better control strategies against vivax malaria in Cambodia and to limit the spread of antimalarial drug resistance.

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SPATIAL DISTRIBUTION OF THE MICRONEMAL PROTEIN CELTOS DURING MOSQUITO MIDGUT TRAVERSAL

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Malaria remains a major burden on the health of global populations. Transmission of the malaria parasite through the Anopheles vector has emerged as a major focus of interest with a view to developing strategies to block mosquito infection, or reinfection of the human host. To date, whilst greater detail is emerging, there are still very few molecular details of any of the stages of mosquito or host re-infection. One protein that functions in both of these stages is CelTOS (cell traversal in ookinetes and sporozoites protein) a secreted soluble micronemal antigen that plays a role in both the colonisation of the mosquito midgut by the ookinete (traversing the epithelium towards oocyst formation) and infection of the liver by the sporozoite (traversing liver cells towards hepatocyte infection). Whilst knockout of this protein has already definitively demonstrated its function in these two distinct processes we still do not know what CelTOS does or its spatial localisation during these key stages. Here we present an update on our work using a tagged line of CelTOS that we have developed along with a suit of imaging reagents and microscopy approaches to make steps towards the functional dissection of CelTOS during mosquito transmission. Given its biphasic role, further understanding of CelTOS is hoped to lay the foundations for developing this key transmission protein into a future multi-stage anti-malarial vaccine.

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GENOTYPIC DIFFERENCES IN DENV NEUTRALIZATION ARE EXPLAINED BY A SINGLE AMINO ACID MUTATION THAT MODULATES VIRUS “BREATHING”

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Flaviruses explore multiple conformations via dynamic motion of virus structural proteins. This introduces complexity to the antigenic surface, as virus “breathing” varies epitopes available for antibody (Ab) binding. A recent study explored the structural basis for genotypic differences in the neutralization potency of a DENV-1 specific mAb (E111) that binds to a poorly exposed domain III epitope on the envelope (E) protein, as reported previously. Studies with DENV strains WP and 16007 indicated that ~100-fold difference in neutralization sensitivity could not be explained by differences in the affinity of mAb E111 for each strain. Instead, we hypothesized that the ensemble of structures sampled by these two viruses was distinct. To investigate the basis for differences in the “breathing” of these two strains, we generated a panel of DENV RVP variants expressing E protein chimeras or single amino acid differences on both WP and 16007 backgrounds. By assessing E111 potency against the E protein chimeras RVPs, the difference in neutralization was mapped to three consecutive residues in domain II that differed between 16007 and WP. Further use of single amino acid mutant RVPs demonstrated that residue 204, but not neighboring residues 202 and 203, was responsible for the difference in neutralization. That residue 204 is located at a distance from the E111 epitope suggested that this amino acid dictates changes in the conformational ensembles sampled by the virus. In support of this, differences in neutralization by a panel of mAbs representing epitopes distinct from E111 were all significantly modulated by the same residue. Our results demonstrate that neutralization susceptibility can be altered in an epitope-independent manner by subtle mutations that alter the overall structural ensemble. That different conformational ensembles of flaviviruses may affect the landscape available for Ab binding has important implications for vaccine development and antibody mapping studies.

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CONSTRUCTION AND CHARACTERIZATION OF CHIMERIC DENGUE VIRUSES CONTAINING ANTIBODY EPITOPES FROM TWO SEROTYPES

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Dengue is the most prevalent mosquito-borne viral disease of humans and a major public health problem worldwide, with approximately one-half of the world’s population at risk of infection by the four dengue virus (DENV) serotypes. Dengue vaccine development has been slow to date, as achieving a balanced and robust immune response against all four serotypes has proven elusive. The reasons for imbalanced immunity elicited by candidate vaccines are not fully understood, but are thought to include variable immunogenicity and competition between the live-attenuated viruses in the vaccine formulations. However, as the precise determinants of DENV immunity are incompletely understood, improving vaccine
formulations is difficult. Here we describe the results of investigations into the role of epitopes within the DENV envelope (E) domain VII hinge region in DENV immunity. We transplanted DENV-3 EDII residues that constitute the epitope footprint of the DENV-3 specific monoclonal antibody (mAb) S17 into a DENV1 infectious clone, making the recombinant virus rDENV-1/3. We then transplanted the DENV-1 EDI residues that constitute the epitope footprint of the DENV-1 specific mAb 1F4 into a DENV-3 infectious clone, making the clone rDENV-3/1. Both rDENV-1/3 and rDENV-3/1 can be neutralized in vitro via the mAb corresponding to the transplanted residues, demonstrating that mAb epitopes can be transplanted between DENV serotypes. To test how epitope transplant affected rDENV-1/3 and rDENV-3/1 neutralization by human sera, these viruses were tested against a panel of DENV-1 and DENV-3 1° immune sera. Strikingly, both recombinant viruses are neutralized by 1° DENV-1 and 1° DENV-3 hummune sera, suggesting that the recombinant viruses display epitopes recognized by both DENV-1 and DENV-3 neutralizing Abs in polyclonal immune sera, giving them “bivalent” qualities. To test the immunogenicity of these viruses in vivo, we have subsequently challenged groups of rhesus macaques with both recombinant viruses and here present the results of the macaque neutralizing antibody response following infection.

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CHARACTERIZATION OF NEUTRALIZING ANTIBODY RESPONSES FOLLOWING NATURAL SECONDARY DENGUE VIRUS INFECTIONS

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Dengue Virus (DENV) is an arthropod-borne flavivirus and the causative agent of dengue fever and dengue hemorrhagic fever. The goal of this study is to characterize the human neutralizing antibody response that develops after secondary DENV exposure. As there are four serotypes of DENV, people can be infected multiple times, each time with a new serotype. Based on recent studies, the dengue field has learned a lot about the properties of neutralizing antibodies generated in people exposed to dengue for the first time (primary infections) but little is known concerning the response generated during a repeat infection with a new serotype (secondary infection). Following primary infections, people develop lifelong protective immunity against the serotype of infection. Primary infections stimulate serotype-cross reactive and serotype-specific (to the serotype of infection) antibodies. However, only a small fraction of serotype-specific antibodies that bind to quaternary structure epitopes at the hinge region between domains I and II of the viral envelope protein are responsible for DENV neutralization. In the present study we investigated the properties of neutralizing antibodies produced during a secondary infection. Following a secondary infection, people develop neutralizing/protective antibodies to multiple serotypes including serotypes that have not infected the individual. An antibody depletion technique using beads coated with purified virus was used to measure levels of serotype specific and cross reactive antibodies and their relative contribution to neutralization. We observed two types of responses: in some sera, dengue virus neutralization was dominated by cross reactive antibodies, whereas in other sera both type-specific and cross-reactive antibodies contributed to neutralization. Thus, unlike primary sera, secondary sera contain antibodies that cross neutralize and presumably cross-protect from DENVs. Ongoing studies with recombinant DENVs and human monoclonal antibodies indicate these secondary infection antibodies recognize novel conserved epitopes that are distinct from the E protein domain VII hinge epitopes targeted after primary exposure.

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TRANSPANTATION OF A COMPLEX QUATERNARY SEROTYPE-SPECIFIC NEUTRALIZING ANTIBODY EPITOPE BETWEEN DENGUE 3 AND 4 REVEALS DETERMINANTS OF POLYCLONAL NEUTRALIZATION RESPONSES

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Dengue virus (DENV) is the most significant human arboviral disease worldwide with upwards of 300 million infections annually; however the determinants of human immune responses to DENV infection remain largely unknown. Thus we set out to develop tools with which to characterize antibody (Ab) responses to DENV infection in humans. Using reverse genetics we developed infectious clones (IC) for all 4 DENV serotypes which allow us to study Ab-virus interactions. Characterization of a panel of monoclonal Abs (mAb) identified a strongly type-specific neutralizing Ab of DENV3. Using a structure-guided approach a 12Å region of the envelope (E) protein domain VII (EDI/II) hinge region encompassing mutations that lead to escape of neutralization was identified and transplanted from DENV4 into DENV3 (rDENV3/4) to assess the contribution of this epitope to the polyclonal immune response in humans. Interestingly, this rDENV3/4 gained full sensitivity to neutralization by human DENV4 immune sera while becoming resistant to DENV3 sera, indicating that this EDII hinge region contains major determinants of type-specific neutralization responses. When the reciprocal transplant was made into DENV4, mAb binding was not retained and there was not a significant shift in neutralization profiles, indicating that the adjacent residues in the recipient DENV serotype play a role in epitope presentation on the virion surface. The addition of 5 amino acid residues from DENV3 into DENV4 was able to restore mAb binding and neutralization, however polyclonal serum neutralization remained largely unchanged. Finally, we moved a complex quaternary epitope encompassing residues spanning multiple E dimers into DENV4 (rDENV4/3). This rDENV4/3 was viable and grew to high infectious titers and exhibited sensitivity to DENV3 immune sera, while neutralization responses to DENV4 remained largely unchanged. These results provide insights into the determinants of type specific neutralization responses that could guide future development of rationally designed DENV vaccine platforms.

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GENERATION OF RECOMBINANT DENGUE VIRUSES ENCODING VARIANT ENVELOPE GENES FROM INTRASEROTYPIC GENOGROUPS REVEALS BREATH OF TYPE-SPECIFIC NEUTRALIZATION RESPONSES

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It is estimated that over 300 million individuals are infected with dengue virus (DENV) each year, yet a detailed understanding of the humoral response to infection is poorly understood. Recent clinical trials of tetravalent DENV vaccine platforms have shown that eliciting a balanced and robust immune response against all 4 DENV serotypes has proven difficult. Within each DENV serotype exist multiple genogroups; however the breadth of serotype-specific immune responses across these genogroups is not well characterized. Using reverse genetics, we have developed infectious clone (IC) systems for all 4 DENV serotypes allowing for the generation of recombinant DENV (rDENV) for use as novel reagents for studying determinants of DENV-specific immune responses.
LONG-TERM MAINTENANCE OF DENGUE VIRUS-SPECIFIC CELL MEDIATED IMMUNE RESPONSES

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All 4 serotypes of dengue virus (DENV) can cause dengue fever (DF) and severe dengue (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS), mediated by cross-reactive T cells. Infection with any of the 4 DENV viruses is thought to confer life-long immunity to re-infection with that same serotype only, and heterotypic secondary infection is associated with development of severe disease. Our understanding of how memory T-cells may contribute to anti-DENV immunity is incomplete, and the duration of DENV-specific T-cell-mediated immune responses is not known. We enrolled volunteers in Hawaii who recalled experiencing a dengue-like illness in 1943, and following an outbreak of DENV-1 in 2001, the first known autochthonous transmission since 1943. Dengue is not endemic in Hawaii and in the absence of repeated exposures we were able to assess the duration, memory phenotype, and effector function of DENV-specific T cell-mediated immune responses in volunteers who experienced a single infection 3, 9, or 60+ years previously. DENV-specific proliferation was observed in all previously infected subjects, but not in any Control (seronegative) subject. Both CD4+ and CD8+ T-cells proliferated in response to stimulation with DENV-1 however there were marked differences between the two subsets with respect to longevity of responses. CD4+ responses were maintained at similar levels for the 3yr, 9yr and 60+yr groups, whereas CD8+ T-cell memory declined with time, with substantially diminished responses apparent at 9yrs; in one subject this decline was already apparent 6 yrs after infection. B*3501/ NS3500 tetramer analysis in age-matched 3yr and 60+yr subjects showed that the frequency of polyfunctional IFN-γ+TNF-α + DENV-specific CD8+ T-cells declined significantly between 3yr and 6yrs after infection, reaching values similar to those seen at 60+ yrs, and that IFN-γ production declined while TNF-α was maintained. We demonstrate the long-term persistence of dengue-specific T-cell immunity and show that the CD4+ and CD8+ memory pools are regulated independently.

INTERACTION OF A DENGUE-SPECIFIC CD8+ T CELL NS1 EPEITOE WITH KIR3DL1 ON NK CELLS REVEALS AN UNDERAPPRECIATED ROLE FOR NK CELLS IN IMPACTING DENGUE DISEASE SEVERITY

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Killer immunoglobulin-like receptors (KIRs) interact with HLA class I ligands and play a central role in the regulation and activation of natural killer (NK) cells. During our investigation of CD8 T cell responses to a highly conserved HLA-B57 restricted DENV epitope, we observed substantial binding of a tetramer (B57-NS1 19-34 TET) to an NK enriched population. Since HLA-B57 is a known binding partner to KIR3DL1, we hypothesized that the B57-NS1 19-34 TET bound KIR3DL1 on NK cells. Staining of KIR3DL1 transfectant cell line confirmed that B57-NS1 19-34 TET bound KIR3DL1. Consistent with the function of an inhibitory KIR, incubation of PBMC with HLA B57 expressing NS1 19-34 pulsed target cells suppressed the degranulation of only KIR3DL1+ NK cells. Furthermore, staining of PBMC from our clinical cohort revealed marked activation of NK enriched cells only in HLA B57+ patients who developed severe dengue disease DHF. These observations reveal a previously unappreciated role for a dengue T cell epitope in modulating NK cell function and have important implications for the pathogenesis of severe dengue disease.
1:3 by date of birth (within 14 days), proximity (control within 3 km of a case), and gender. Cox regression analysis was used to calculate hazard ratio (HR). In multivariable analysis, we controlled for distance from the clinic. In total, 1618 cases were matched to 3541 controls. Half of cases and controls were female and no difference in socio-economic status was detected (p=0.12). Mean distance to the clinic was 1.9 km for cases and 1.8 km for controls (p<0.01). In all, 229 deaths occurred, 31 among cases (1.9%) and 198 among controls (5.6%). Cases contributed 2719 person years (py) and controls 5335 py of observation. Mortality rates were 0.01 and 0.04 deaths/py among cases and controls, respectively; the adjusted HR was 0.30 (95% CI: 0.19, 0.47) corresponding to a 70% (95%CI: 53, 81) reduction in mortality. Children enrolled in the clinical trial at KEMRU/CDC experienced a marked reduction in all-cause mortality. These data suggest that considerable reduction in child mortality could be achieved by reducing barriers to health care and providing quality care according to national guidelines.

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REACTIVE VACCINATION IN THE PRESENCE OF DISEASE HOTSPOTS

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Reactive vaccination has recently been adopted as an outbreak response tool for cholera and other infectious diseases. A global revolving stockpile of cholera vaccine was recently established for emergency use, but there is only enough vaccine globally - within and outside the stockpile - to vaccinate approximately one million people. When responding to outbreaks, public health officials must quickly decide who and where to distribute limited vaccine. Transmission hotspots have been discussed as one potential mechanism to efficiently allocate vaccine, however the effectiveness of this approach is likely to be context dependent. We compared strategies for allocating vaccine across multiple areas with heterogeneous transmission efficiency. We constructed meta-population models of a cholera-like disease and compared simulated epidemics where: vaccine is targeted at areas of high or low transmission efficiency, where vaccine is distributed equitably across the population, and where no vaccine is used. We find that connectivity between populations, transmission efficiency, vaccination timing, and the amount of vaccine available all shape the performance of different allocation strategies. In highly connected settings, like cities, when vaccinating proactively or early in the epidemic, targeting limited vaccine at transmission hotspots (i.e. areas with high transmission efficiency) is often optimal. However, once vaccination is delayed, targeting the hotspot is rarely optimal, and strategies that either spread vaccine between areas or those that focus on non-hotspots will avert more cases. Although transmission hotspots may seem like an intuitive target for outbreak control, we show that in many situations the hotspot epidemic may proceed so fast that hotspot-targeted vaccination will prevent relatively few cases, and vaccination shared across areas where transmission can be sustained is often the best approach. Our results suggest a general rule of thumb for vaccination in cities: reactive distribution of vaccine across areas that can independently maintain transmission is generally preferred to targeting any particular transmission hotspot, and hotspots should only be targeted when they are thought to be necessary drivers of transmission. These results provide new insights on how to efficiently vaccinate in response to an epidemic when vaccine is limited.

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ASSESSMENT OF REASONS FOR POLIO VACCINE REFUSALS IN NORTHERN NIGERIA - OCTOBER, 2012

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Oral polio vaccine (OPV) refusals during supplemental immunization activities (SIAs) contribute to the spread of polio virus in Nigeria. In September 2012, the Expert Review Committee (ERC) on Polio Eradication and Routine Immunization in Nigeria recommended that social research be conducted to better understand the reasons for polio vaccination refusals among caregivers in Northern Nigeria. Following the OPV campaign in October, 2012, we conducted a cross-sectional study using semi-structured questionnaires to assess polio risk perception, reasons for refusals and perception of OPV campaigns. We interviewed caregivers who refused OPV for their children in two purposively selected high refusal districts (one rural and one urban) in five north-western Nigeria states. Median age of the 148 study participants was 39.5 years, 82.4% were male, all were Muslims and 28% had primary level education or higher. Polio risk perception was low (77%). 89% of participants believed that the polio vaccine was neither necessary nor helpful, and possibly harmful. Most participants (75%) had an unfavourable or indifferent view of the polio campaigns. Religious belief was reported as an important driver of the participants’ understanding of health and disease (70%). Most (85%) study participants indicated they were more concerned about other health issues such as malaria. Caregivers refuse OPV largely due to poor polio risk perception, unmet felt-needs and religious beliefs. Partners have therefore adopted strategies including; communication schemes aimed at increasing awareness of polio as a health threat; educating communities about the safety of the vaccine; engagement of religious leaders, polio survivor groups and polio/pro-OPV Community Development Volunteers, comprehensive health camps and provision of basic amenities like water boreholes to address identified issues. The adoption and implementation of the recommendations of this study contributed to the 59% reduction in the incidence of polio in Nigeria between week 52 of 2012 and week 52 of 2013.

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CHARACTERISTICS ASSOCIATED WITH THE REGULAR CONSUMPTION OF MILK OR SOLIDS IN BREASTFEEDING INFANTS IN MAL-ED, AN EIGHT SITE COHORT STUDY

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The WHO recommends exclusive breastfeeding for the first 6 months of life in order to maximize nutrient intake, increase immune function, and decrease exposure to pathogens. We expect that mothers choose to stop exclusive breastfeeding or introduce milks or solids based on multiple infant (size, illness, behavior), maternal (maternal age, education, number of live births) and environmental (food security, resources) factors. Using data from an 8-site birth cohort study, we explored the relationship between child, maternal and environmental characteristics and the timing
of regular consumption of milks or solids during the first six months of life. Overall, 1,967 children were included in this analysis, approximately 200 per study site. The median age of first regular consumption of milk or solids ranged from 41 days (Pakistan site) to 176 days (Bangladesh site). We used Cox proportional hazards models with time (in days) to regular consumption of milk or solid as the outcome, including both fixed (sex, maternal age, parity, etc.) and time varying (weight, height, illness, etc.) exposures. Higher weight-for-length (WLZ) and length-for-age (LAZ) were associated with later introduction of milk or solids. Food insecurity, as well as illness (diarrhea, cough in the past 30 days), were also associated with later consumption of milks and solids. Factors related to breastfeeding initiation (colostrum, prelacteal feeding), maternal education, and parity were not strongly associated with timing of regular consumption of milks and solids. We included interactions between time and WLZ, LAZ, and cough and found that the protective effect of higher values of WLZ, LAZ, and cough decreased over time. These preliminary results indicate that the diet of the breastfed child may be altered according to the child’s size or health status. These data will improve our understanding of the site-specific and overall timing of the regular consumption of milk and solids as it relates to child and maternal factors, and will allow us to make recommendations regarding how to incorporate breastfeeding in future analyses in order to avoid the potential for reverse causality. In addition, we can explore the factors associated with early regular consumption of milk and solids in these populations in order to understand and appropriately evaluate their impact on growth and overall health.

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**HEMOGLOBIN CONCENTRATION DURING PREGNANCY AND INFANT COGNITIVE AND MOTOR DEVELOPMENT: A PROSPECTIVE COHORT STUDY**

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There have been several studies on the consequences of anemia during pregnancy but to date, very little is known about the impact of prenatal anemia on infant neurocognitive function. The objective of this study was to assess the impact of anemia during pregnancy on the cognitive and motor functions of one-year-old children in Benin. Our prospective cohort study included one-year-old children born to women enrolled at their first antenatal care (ANC) visit, before 29 weeks of pregnancy, within the MiPPAD trial comparing sulfadoxine-pyrimethamine and mefloquine.

Hemoglobin (Hb) concentrations of pregnant women were determined from venous blood samples collected at first and second ANC visits of at least, one-month interval and at delivery. Women were prescribed oral iron, folic acid and anthelminths as part of the ANC package in Benin. A total of 635 children (76.7% of eligible children) were assessed for cognitive and motor functions, using the Mullen Scales of Early Learning (MSEL), at twelve months of age by trained research nurses. Prevalence of anemia decreased from 67.8% at first ANC visit to 40.1% at delivery. Children of mothers who were anemic at second ANC visit had better gross motor function (an estimated mean increase of 2.4 points, 95% Confidence Interval, CI, 0.1 to 4.7). We observed a significant negative quadratic relationship between infant gross motor function and Hb concentration at first ANC visit for women of gestational age greater 22 weeks (p=0.025). Thus, infant gross motor scores increased with increasing maternal Hb concentration until 88 g/L where it plateaued and began to decline at 110 g/L. We found no significant association between Early Learning Composite scores and maternal Hb concentration. There appears to be an optimal range Hb concentration (88-110 g/L) between 22 and 32 weeks of gestation that may be beneficial to infant gross motor function at age one year. These may reflect physiological hemodilution, which peaks between 22 and 32 weeks of gestation. Further studies are required to corroborate our findings.
CLINICAL ASSESSMENT OF CHILDREN WITH FEBRILE ILLNESS AT HEALTH FACILITIES IN THREE DISTRICTS IN SOUTHERN ZAMBIA

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Given the decline in malaria prevalence in many areas in sub-Saharan Africa, fever in children can no longer be treated presumptively as malaria. The purpose of this evaluation was to examine health system capacity and clinicians’ ability to accurately diagnose and treat pediatric febrile disease in three districts in Zambia with varying malaria endemicity. We conducted health facility surveys, health worker (HW) interviews, and observed patient-provider interactions for children under the age of 5 who presented with history of fever in the previous 48 hours. Quality of the clinical evaluation was assessed using the Zambian Ministry of Health IMCI guidelines. We observed 161 patient-provider interactions and interviewed 53 HWs at 24 health facilities with regard to their assessment of danger signs. Inability to feed was the most commonly assessed (53%) danger sign, followed by persistent vomiting (47%), convulsions (31%) and lethargy (29%). All four danger signs were assessed in just 8% (13/161) of children. None of the HWs conducted all, and less than 15% performed half of, the physical examination and history taking items as prescribed by IMCI guidelines. All facilities had capacity to test for malaria with either RDT or microscopy, but only 57% of children presenting with fever were tested. Clinical diagnosis of pneumonia was also incomplete. Less than one third of children with cough or difficulty breathing had their respiratory rate counted. Adherence to the malaria treatment protocol was high, as all patients who were RDT positive received ACT, while only 1 of 85 RDT-negative received ACT. However, none of the pneumonia-diagnosed children received the recommended treatment (amoxicillin), and 48% of those with fast breathing (WHO-defined, non-severe pneumonia) did not receive appropriate treatment. Identification of danger signs, diagnosis and treatment of febrile illness were not conducted in accordance with IMCI guidelines. There is an urgent need for interventions to improve management of pediatric febrile illness at the health facility level in Zambia.

THE TRANSCRIPTOMIC ANALYSIS OF EARLY ADULT ECHINOCOCCUS GRANULOSUS

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Despite the substantial efforts have been made to control echinococcosis there is a clear need for new tools in its prevention. Dogs are pivotal in Echinococcus granulosus transmission and we contend that interruption of the parasite life cycle in the definitive host stage provides a very practical and cost-effective vaccination strategy. Specific or differential genes expressed in the scolice of adult worms are likely the vaccine targets for dogs against E. granulosus. To identify the genes, adult E. granulosus worms aged two weeks were collected from experimentally infected dogs. The worms were fixed and stored in ethanol. Each of the worms was cut into two parts, head and neck region. mRNA were extracted from the two parts respectively and RNA-seq technique was used to precisely quantify transcript levels in the two parts. A real time PCR was used to confirm the gene expression in the tissues. We identified 953 genes specifically or differentially expressed in the scolice of E. granulosus. Most of the up-regulated genes are novel, indicating these genes may compose a network in regulating specific function of worm head, and they may play an important role in settlement of E. granulosus in dog intestinal surface. Known genes include calcium-transport, dynein light chain, early growth response protein 2-like isoform 4, myosin heavy chain and transmembrane protein 144. Interestingly, neuropeptide f was highly expressed in the head of E. granulosus. The peptide acts as a neurotransmitter in the central nervous system(CNS), indicating the peptides may involve in the early CNS of the parasite. In addition, lamininaminotransferase was also up-regulated in the head, which may involve in nervous development in the early stage of adult worm. As scolices are an important worm part for attachment, the specific and differential genes are likely the targets for drug and vaccine development against adult E. granulosus.

A HIGH SENSITIVITY DOT-BLOT FOR THE DIAGNOSIS OF HEPATIC CYSTIC ECHINOCOCOSIS USING WHOLE BLOOD SAMPLE

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The diagnosis of hepatic cystic echinococcosis (CE) is based on ultrasonography (US) and confirmed by serology. However, a high proportion of inactive cysts, as well as early CE1 cysts, remain seronegative with conventional serodiagnostic tests, which poses diagnostic problems where no pathognomonic signs of CE are present on US examination. We present preliminary results of the performance of a whole blood dot-blot with human hydatid cyst fluid (HCF) especially in patients with hepatic CE who are seronegative with routine diagnostic test. HCF was collected from a CE patient after percutaneous aspiration of an active CE1 liver cyst. Dot-blot membranes were prepared with 50ng of total proteins, and whole blood samples were diluted 1:2000. We tested a total of 34 whole blood samples, 24 of which from CE patients with active, transitional and inactive cysts and 10 from volunteers and patients with non-parasitic cysts (control group). All CE patients with different cyst stages recognized the proteins present in the HCF, while all samples in the control group did not
recognize any proteins. These preliminary results are encouraging and we plan to evaluate our test on a higher number of CE patients and patients with other helminthic infections. This diagnostic test could be useful for the development of a rapid finger prick test for the serodiagnosis of CE in remote endemic areas. Further identification of the different antigens recognized will support the development of diagnostic tools that could improve the sensitivity of CE diagnosis.

LONG-TERM FOLLOW-UP OF PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS IN GERMANY

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From 1992 to 2011, 312 patients with alveolar echinococcosis (AE) were diagnosed and treated at the specialized outpatient clinic of the Ulm University. Demographic and clinical data were assessed and updated from the patients’ first visits. At that time, 74.7% of the patients were alive, 12.8% had died, and 12.5% were lost to follow-up. Patients were treated either by surgery with subsequent benzimidazole (BZM) prophylaxis for at least 2 years (n=133), or continuous BZM treatment in case of inoperability (n=157). At first diagnosis, 17 patients had inactive lesions. Imaging and treatment schemes changed during the 20 years of observation. AE was diagnosed more often by chance in patients from 2000 onwards (48.0%) than before 2000 (28.7%). Since 2000, the disease was detected more frequently with lesions at PNM stages I and II (27.0% vs. 15.8%) according to WHO classification; as a consequence, radical resections were feasible in more patients (57.7% vs. 20.0%). Surgical resections were less frequent since 2000 (38.2% vs. 50.5%). Since 1993, PET-CT-scans with 18FDG were used routinely to visualize larval activity at time of diagnosis. For follow-up, the rationale for performing a PET-CT every other year was to monitor the effect of continuous BZM treatment, and to detect relapses after surgery. At the end of follow-up, medical treatment had been interrupted for 25.3% of the patients. Of 56 patients with R0 resection, 42 had stopped medical treatment. At their last visit, the disease status of 73.1% of the patients was judged as stable, in 5.1% as progressive while under medical treatment. AE was considered as being cured in 15.7% of the patients. The 5- and 10-years’ survival rates in this cohort were 96.8% and 90.5%. Data analysis of two decades’ experience in the management of AE showed that best care can be provided to the patients when they present at an early stage of the disease. As the disease is rare, expertise is best acquired in a single specialized institution; a benefit for the patients results from strict adherence to the WHO treatment recommendations.

NEW CT-CLASSIFICATION OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

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Computed tomography, mostly combined with PET, provides one of the most important diagnostic tools in suspected alveolar echinococcosis. Aim of the study was to establish a new CT-classification based on a large patient collective with confirmed hepatic alveolar echinococcosis. In 224 patients, CT-morphology of liver lesions due to an alveolar echinococcosis was retrospectively examined. The findings were grouped into the new classification scheme. Within the classification a lesion was dedicated to a “primary morphology” as well as to a “pattern of calcification”. The primary morphology distinguishes following types: I. Diffuse infiltrating (with / without cystoid portion), II. Primarily circumscribed tumor-like (with / without cystoid portion and with / without offshoot at the edge), IIIa. Primarily cystoid, intermediate (with / without more solid portions at the edge), IIIb. Primarily cystoid widespread (with / without more solid portions at the edge), IV. Small-cystoid / metastatic* and V. Mainly calcified. Except for the “primary morphology” type V, following patterns of calcification were attributed additionally: without calcifications; with feathery calcifications; with focal (p.r.n. central - just possible with*) calcifications; with diffuse calcifications; with calcifications primarily at the edge. The various classification patterns are demonstrated by image examples. The proposed CT-morphological classification shall facilitate the interpretation of lesions due to a hepatic alveolar echinococcosis. This could help to interpret different clinical courses better and shall assist in the context of scientific studies to improve the comparability of CT findings.

COMPARING GROUP MEANS AND INDIVIDUAL RESPONSES TO TREATMENT OF SOIL-TRANSMITTED NEMATODES WITH BENZIMIDAZOLE DRUGS: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Monitoring anthelmintic treatment efficacy is important to detect suboptimal response where drug pressure is high. The recommended method is egg reduction rate (ERR): percentage reduction in the mean number of eggs per gram (EPG) in excreta post- vs pre-treatment calculated using arithmetic rather than geometric mean (ERRam, ERRgm). We compared these group mean outcomes to individual patient outcomes: centile distribution of individual ERR (ERRic) from which we derived: proportion cured (cure rate, CR), or with reduced EPG (rEPG) or no change/ increased EPG (nEPG); and median ERR (mERR). We analysed trials which treated Ascaris lumbricoides (AL), Trichuris trichiura (TT) or hookworms (HW) with benzimidazoles (1832 subjects): four trials of albendazole (ABZ: n=613; AL: n=121, TT: n=297, HW: n=195); two of mebendazole (MBZ: n=1219, AL: n=174, TT: n=513, HW: n=532). Both drugs were very effective on AL: CR were 90.9% with ABZ and 94.2% with MBZ. Treating TT with ABZ gave CR=15.4%, rEPG=48.8%, nEPG=35.7% and mERR=39%; and MBZ gave CR=22.4%, rEPG=58.1%, nEPG=19.4%, mERR=76%. Treating HW with ABZ gave CR=69.2%, rEPG=23.1%, nEPG=7.7, mERR=100%; MBZ gave CR=14.1%, rEPG=51.7%, nEPG=34.1%, and mERR=56%. Efficacy estimates expressed as ERRgm were systematically higher the ERRam for both drugs. As opposed to group mean estimates ERRam/ERRic within one analysis, describes better the distribution of individual responses (centile distribution) and calculates proportions cured, with partial or no response, and median ERR; it should be tested on larger datasets to pinpoint changes in patterns of response and poor responders.
WATER AND SANITATION TARGET DIFFERENT SOIL TRANSMITTED HELMINTHES ACCORDING TO THEIR ROUTE OF INFECTION

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Soil transmitted helminthes (STH) are a group of parasites of public health relevance due to its burden of disease and prevalence. The global strategy of the World Health Organization for the control of the morbidity related to STH includes preventive chemotherapy but also highlights the need for developmental improvements in the living conditions of affected populations. As part of a project for the development of strategies for the control of STH in northwestern Argentina, a survey was performed in randomly selected families of Wichí aboriginal communities of Tartagal with known high endemic prevalence of STH; socio-demographic information originating in the primary care routine sanitary data forms was incorporated into the database. The surveys were designed to include a representative randomized sample that included whole family groups. STH were surveyed through examination of single fresh stool specimens with 5 methods and an ELISA assay for Strongyloides stercoralis based on NIE antigen. In a community of 2914 individuals, 229 stool samples and 255 serum samples were evaluated and revealed an overall prevalence of STH of 54% with species specific prevalences of 49% for hookworms, 41% for S. stercoralis, 1% for Ascaris lumbricoides and 0.5% for Trichuris trichiura. The sanitary conditions of this communities revealed improved drinking water availability in 98% of the individuals which contrasts with a mere 2.3% with improved sanitation (unimproved pit latrines 94%, open defecation 1.4%, no data 1.9%). These results highlight the distinctly contrasting prevalence between STH with life cycles that infect through skin penetrator of filarial larvae (hookworms and S. stercoralis) versus those that infect through the oral ingestion of embryonated eggs (A. lumbricoides and T. trichiura) according to environmental conditions. In the communities described in this report the lack of adequate sanitation favored skin penetrators but the availability of improved water blocked those transmitted trough oral ingestion. Life cycles determine risk factors and the interplay of these elements should guide control measures.

DETECTION AND CHARACTERIZATION OF AN IMMUNODOMINANT ANTIGEN PRESENT ON THE SURFACE OF ASCARIS L3 LARVAE

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The human roundworm Ascaris lumbricoides is estimated to infect over 800 million people and is a significant public health problem. The closely related pig parasite A. suum plays an important role in veterinary medicine and represents a suitable model for A. lumbricoides. A continued exposure to Ascaris induces immunity at the level of the gut in pigs, protecting the host against the migrating larvae. The objective of this project was to identify and characterize parasite antigens targeted by this immune response that may be crucial for parasite invasion and survival. Pigs were immunized by trickle infection (100 A. suum eggs 5 times per week) for 30 weeks, challenged with 1,000 eggs at week 32 and euthanized two weeks after challenge. At necropsy, there was a 100% reduction in L3s recovered from the intestine and a 97.2% reduction in white spots on the liver in comparison with challenged non-immune controls. Antibodies purified from the intestinal mucus of immune pigs were subsequently used to probe L3 larval extracts resulting in a strong specific recognition of a 12kDa antigen (As12). This antigen is present on the surface of infective L3 larvae and is shed actively. As12 appears to be a glycolipid that contains phosphorylcholine, and it cannot be visualized by protein staining. Furthermore, As12 is highly resistant to different enzymatic and chemical treatments. This molecule could be of significant importance to the survival of the parasite during the initial stages of infection. Further studies are needed to investigate its molecular nature and its role at the parasite-host interface.

AVIAN TROPISM-ASSOCIATED PATHOGENESIS OF WEST NILE VIRUS; CHARACTERIZING THE ROLE OF AVIAN LEUKOCYTE INFECTION

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West Nile virus (WNV) is an arbovirus that was first reported in North America in 1999 and, by 2003, had spread nearly 3,000 miles from New York to southern California. The spread of WNV across the U.S. has been shown to be dependent on long-range movements, though sporadic sampling and an ascertainment bias from selecting isolates not associated with disease in humans may have affected these analyses. In addition, methods of viral overwintering and viral genetics associated with viral spread are not well understood. Herein, we report sequences for more than 125 WNV isolates collected from 15 counties of California. These isolates were made from mosquito pools collected as part of routine surveillance by the California Vectorborne Disease Surveillance System from 2003 - 2012. This unique dataset allows inference of fine-scale spatial and temporal movements of WNV. Using the Bayesian MCMC approach implemented in BEAST, we performed phylogeographic analyses and demonstrate that 3 independent introductions of WNV (2 WN02 strains and 1 SW03 strain) occurred in California between 2002 and 2003 via the Coachella and Sacramento Valleys. The two genotypes of WNV have remained in co-circulation in California from 2003 to 2012, though SW03 viruses were primarily restricted to southern California. An association between WNV genotype and WNV neuroinvasive disease (WNNID) in humans has not been consistent, as both WNV genotypes have circulated in counties with high and low WNNID incidence. Multiple examples of short-range movements of WNV across a few hundred miles, as well as geographic constraint of WNV strains within a single region for up to 8 years, suggest viral transmission has been driven by resident, rather than migratory, birds. In addition, while most mosquito pools containing infectious virus were collected in summer months, 2 viruses from mosquitoes collected during winter months were phylogenetically consistent with continued viral transmission as an overwintering mechanism for WNV. These data show that dense sampling across space and time can help to explain basic biological and ecological properties of WNV transmission.
comparison with WNV tropism in mammals, we hypothesized that early WNV replication in AMCRs is driven specifically by replication in circulating monocytes. Therefore, peripheral blood mononuclear cells (PBMC) were isolated from AMCRs and infected with WNV in an ex vivo culture system. The degree of replication attained by various WNV strains and mutants in this ex vivo system recapitulated their relative viremia and pathogenicity in vivo in AMCRs. Flow cytometric analysis suggested that the cells infected in ex vivo PBMC culture were predominantly monocytes. A WNV virus engineered to express target sequences for miR223, a miRNA found specifically in myeloid lineage cells, was generated to assess the specific role of infectivity of these cells for modulating in vivo avian virulence phenotypes. This cell-restricted recombinant virus failed to replicate in AMCR ex vivo PBMC cultures, demonstrating the myeloid lineage as the source of viral replication in the leukocyte preparations. Furthermore, the myeloid-restricted virus exhibited peak viremias in AMCRs 200-fold lower than the parental WNV strain or a control virus expressing a mosquito miRNA target sequence. Additionally, mortality of the miR223 target sequence WNV was 50% with an average survival time of approximately ten days, while the wild type virus yielded 100% mortality with birds succumbing within approximately five days. Thus, several lines of evidence point to the importance of leukocytes, including monocytes, in WNV infection of AMCRs. The ex vivo PBMC culture system may be a useful model for pathologic assessment of WNV strains, and can be used to further elucidate the mechanism of action of viral mutations that affect WNV host competence in avians.

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ROLE OF TNF-α RECEPTOR 2 IN WEST NILE VIRUS INFECTION
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West Nile virus (WNV) is the most common cause of arboviral encephalitis in the USA. Several lines of evidence suggest that production and release of TNF-α is one of the early cellular events in WNV infection. While the role of TNF-α receptor 1 (TNF-R1) in WNV infection is controversial, there are no studies that have evaluated the role of receptor 2 (TNF-R2) in WNV-associated neuropathogenesis. We hypothesized that TNF-α acting through TNF-R2 promotes survival of WNV-infected neurons and immune cells by counteracting WNV-induced apoptosis and enhancing regulatory T cells (Tregs) functions. Therefore, TNF-R2 knock out (KO) mice infected with WNV will have more severe disease and higher mortality than the wild type (WT) mice. The objective of this study was to examine the role of TNF-R2 signaling in protecting WNV-infected mice. Age- and gender-matched 8-12 weeks old, KO and WT (C57Bl/6) mice were inoculated with 100 or 10 plaque forming units (pfu) of WNV NY-99 strain and monitored daily for 3 weeks. Mice survival was analyzed using Log-rank (Mantel-Cox) tests and viremia was quantitated by quantitative reverse transcriptase real-time PCR (qRT-PCR). When infected with 100 pfu of WNV, 97% of KO mice succumbed to death whereas 31% WNV-infected WT mice survived (N=29 in each group, Chi-square 14.38, df 1, p= 0.0001). While WT mice had lower viremia (7±3x10^3 vs. 4±7x10^4 pfu equ/mL) and slightly longer median survival time (11 days vs. 10 days), these differences were not significant. Interestingly, when infected with 10 pfu of WNV, there was no difference in the survival between WT and KO mice suggesting the role of TNF-R2 while is critical in heavy infection, it may not have any role in low dose WNV-infection. Therefore, because of limited distribution of TNF-R2 in immune, hematopoietic and neuronal cells that are primary targets of WNV, specifically augmenting the TNF-R2 could be a better and safer therapeutic strategy in heavy WNV infection.

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RESTRICTION OF 2’-O METHYLCATION DEFICIENT WEST NILE VIRUS IN INSECTS
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Interferon (IFN) induced proteins with tetraecopeptide repeats (IFITs) were recently identified as mammalian sensor molecules that recognize non-self viral RNA lacking 2’-O methylation at their 5’ end and selectively block translation. Similar to mammals, the host mRNA of insects is methylated at both the N-7 and 2’-O positions yet insects lack any known IFN response pathway or apparent IFIT gene orthologs. Here, we show that a mutant West Nile virus (WNV) lacking 2’-O methyltransferase activity (WNV-NS5-E218A) is attenuated in mosquito cells and intact mosquitoes. Wild type WNV (WNV-WT) grows to approximately 2 logs higher titer than WNV-NS5-E218A in both C6/36 and AAG2 mosquito cells and WNV-WT had an increased infection rate and higher titer of infection in Culex tarsalis mosquitoes at day 3 and 5 post infection in comparison to WNV-NS5-E218A. These results suggest that WNV-NS5-E218A is restricted by a novel effector pathway that recognizes the loss of 2’-O methylation, analogous to our observations in mammals; alternatively, insects use a 2’-O methylation for efficient translation and thus, restrict non-self RNA lacking this modification. To identify the genes and pathways responsible for restricting WNV-NS5-E218A, a genome-wide RNAi screen that targets 12,870 Drosophila genes was performed. We identified 140 genes within the primary screen that increased infection of WNV-NS5-E218A when silenced. Of these 140 genes we are currently pursing four genes with mammalian homologs that increase WNV-NS5-E218A infection to a greater extent than WNV-WT. We will discuss how these genes preferentially restrict WNV lacking 2’-O methylation in insect cells.

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SUBSTITUTION OF THE PREMEMBRANE AND ENVELOPE PROTEIN GENES OF MODOC VIRUS WITH THE HOMOLOGOUS SEQUENCES OF WEST NILE VIRUS GENERATES A CHIMERIC VIRUS THAT REPLICATES IN VERTEBRATE BUT NOT MOSQUITO CELLS
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Most known flaviviruses, including West Nile virus (WNV), are maintained in natural transmission cycles between hematophagous arthropods and vertebrate hosts. Other flaviviruses such as Modoc virus (MODV) and Culex flavivirus (CxFV) have host ranges restricted to vertebrates and insects, respectively. The genetic elements that condition the differential host ranges and transmission cycles of these viruses have not been identified. In this study, fusion-PCR was used to replace the capsid (C), premembrane (prM) and envelope (E) genes and the prM-E genes of a full-length MODV infectious cDNA clone with the corresponding regions of WNV and CxFV. Fusion products were directly transfected into baby hamster kidney-derived cells that stably express T7 RNA polymerase. At 4 days post-transfection, aliquots of each supernatant were inoculated onto vertebrate (BHK-21 and Vero) and mosquito (C6/36) cells which were then assayed for evidence of viral infection by RT-PCR, Western blot and plaque assay. Chimeric virus was recovered in cells transfected with the fusion product containing the prM-E genes of WNV. The virus could infect vertebrate but not mosquito cells. The in vitro replication kinetics and yields of the chimeric virus were similar to MODV but the chimeric virus produced larger plaques. Chimeric virus was not recovered in cells transfected with any of the other fusion
products. In conclusion, our data indicate that genetic elements outside of the prM-E gene region of MODV condition its vertebrate-specific phenotype.

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THE YELLOW FEVER ICEBERG: THE PROBABILITIES OF MILD, SEVERE AND FATAL DISEASE FOR PEOPLE INFECTED WITH YELLOW FEVER VIRUS

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Yellow fever virus, like other pathogens, only causes disease in a proportion of individuals it infects. Severe illness and death represent the tip of the iceberg relative to asymptomatic and mild infections, which is more critical for determining the overall disease burden and prevalence of infection. We compiled data on asymptomatic infections, mild disease, severe disease, and fatalities from eleven outbreaks affecting hundreds to thousands of people in Africa and South America between 1969 and 2011. We defined severe disease as fever with jaundice or hemorrhagic symptoms and mild disease as fever or other reported illness not meeting the severe disease criteria. Using a Bayesian model, we estimated the probabilities and 95% credible intervals (CI) for asymptomatic infection, mild disease, and severe disease in infected individuals. The average probability of being asymptomatic was 0.65 (95% CI: 0.55-0.74). The probability of infection resulting in mild disease was 0.33 (95% CI: 0.13-0.52) and severe disease was 0.12 (95% CI: 0.05-0.26). We also estimated the case fatality rate for individuals experiencing severe disease; the probability of fatality for severe cases was 0.47 (95% CI: 0.31-0.62). For unknown outbreaks, the uncertainty and variability between studies indicates that for every severe case of yellow fever, there may be 1 to 70 additional infections, representing asymptomatic or mild infections. The large range in all of these estimates reflects the scarcity of data, intrinsic variability, and variation between outbreaks, possibly due to study design or differences in environmental, host, vector, or virus characteristics. Nonetheless, the results are generally inline with previous estimates, which are derived only from individual studies. As it is generally only the most severe cases that are recognized and reported, these estimates will help improve the understanding of the burden of disease and the estimation of the potential risk of spread during YF outbreaks.

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FLAVIVIRUS EXPOSURE IN NORTHEASTERN KENYA

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Flaviviruses are transmitted throughout the world, though their burden is still largely underestimated in Africa. The objective of this study was to assess the seroprevalence of flavivirus exposure in northeastern Kenya and to link previous exposure to risk factors and reported historical symptoms associated with prior febrile illness. Questionnaires on demographics, reported symptoms, and mosquito exposures were administered to 891 participants among 6 northeastern Kenyan villages in February and March of 2011. Visual acuity tests and physical exams were also performed. Sera were tested via standardized ELISA protocols for anti-dengue virus IgG. Risk factors associated with seropositivity were determined using Fisher’s exact test and logistic regression models. Forty-three (4.9%; 95% CI 3.6-6.5%) participants were flavivirus seropositive (aged 3-85 years). Prevalence differed significantly among villages (p<0.001) with higher risk in Sabenale (19%), Golabele (10%) and Tumtish (9%), and lower risk in Gedilun (4%), Korahindi (2%) and Matarba (1%). Children (<16 years old) were less likely to be seropositive than adults (p<0.001). Women tended to be less at risk than men (p=0.057). Seropositivity was associated with poor measured visual acuity (p=0.026) and the following reported lifetime historical symptoms: red eyes (p=0.005), poor vision (p=0.038), eye pain (p=0.005), photophobia (p<0.001), backache (p=0.001), malaise (p=0.001), and sleepiness (p=0.045). Seropositivity was also associated with home flooding (p=0.0215). Flavivirus exposure is rare in northeastern Kenya, but more common among adults than children. Differences in seroprevalence between residents of nearby villages with similar ecology may be due to varied mosquito control practices or economic factors. Flavivirus exposure is associated with many historical visual symptoms and poor measured visual acuity. Despite low prevalence and no reported outbreaks, ongoing interepidemic transmission is demonstrated by documentation of seropositive children.

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MALARIA IMPORTATION AND ELIMINATION IN SWAZILAND

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Achieving country-wide elimination of malaria requires the cessation of local transmission caused by imported malaria. Swaziland, a sub-Saharan country that has worked towards malaria elimination for over a decade, has brought local transmission down to extremely low levels. As Swaziland makes further progress towards elimination, the methods and metrics typically used to assess the risk of local transmission initiated from imported malaria become inadequate. Additionally, independent of method or metric, accurate accounting of both local and imported cases becomes ever more critical as the relative weight carried by each individual case increases. To both assess transmission dynamics in a low-transmission setting such as Swaziland, as well as evaluate surveillance efforts, we developed algorithms to estimate malaria transmission chains. Using comprehensive case data from 2010 through 2013, these estimated chains - based on identifying likely causal links between successive cases through the use of spatio-temporal kernels - provided insight on the frequency and length of chains of local transmission. By identifying secondary cases that appear to have no likely primary case we catalogue likely gaps in surveillance. Our analysis suggests than most locally acquired cases were caused by a few imported cases and that most imported cases resulted in no extended local transmission. Further, we highlight regions in space where local transmission chains appear to be longest. Our results suggest these regions would benefit from more systematic control and surveillance efforts to identify and curtail extended local transmission in the future.

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MAPPING SEASONAL INTERACTIONS BETWEEN POPULATION MOVEMENTS AND MALARIA TRANSMISSION FOR STRATEGIC MALARIA ELIMINATION PLANNING

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In most countries that are planning for malaria elimination a strong seasonality in malaria transmission can be observed. This often drives the timing of intervention and surveillance efforts, and the seasonal patterns can potentially be exploited to optimally target resources..
for achieving elimination. Factors that have received lesser attention in designing elimination strategies are population movements, their seasonal patterns and their demographic composition. As a country or area transitions towards malaria elimination, imported cases make up an increasingly larger proportion of those seen, and the importance of accounting for population movements rises. Throughout the world, the volumes and major routes of population movements tend to follow seasonal patterns, with certain times of year showing significantly greater amounts of movement than others, and this varying by demographic groupings. These movements can impact substantially on the dispersal of parasites in a region, depending on how the timings and routes of seasonal movements interact with the seasonality of malaria transmission. Here, we demonstrate how the simple combination of national malaria surveillance system data, case investigation information and population mobility metrics derived from mobile phone records can inform on these seasonal interactions, using Namibia as an example. Using anonymized cellphone call detail records to determine the mobility patterns of nearly 2 million residents, we show that the timing, duration of stay and magnitude of movements vary substantially across time and space, with significant movement peaks in December and January aligning with peak malaria transmission. Further, case investigation data from more than 100 malaria patients in the region with highest transmission (Zambezi) enables validation of these mobility patterns and provides valuable additional demographic and epidemiological insights into risk groups and contact patterns. The approaches presented can be updated rapidly and used to identify which regions would benefit from coordinating efforts at certain times of year and how spatially progressive elimination plans can be designed to account for the interacting seasonality in transmission and mobility.

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A MONITORING AND EVALUATION TOOL FOR REACTIVE CASE DETECTION: PILOT EXPERIENCE IN ACEH PROVINCE, INDONESIA

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Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many low transmission settings worldwide as a critical part of malaria elimination programs. ACD strategies include determining the origin of infection, case investigation, and responding to locally acquired cases of malaria, known as reactive case detection (RACD). Effectively implementing RACD requires substantial programmatic and human resources. Aceh Province has a goal to eliminate malaria by 2015. Between June and September 2013, a pilot was conducted to evaluate RACD activities and identify best practices to inform RACD efforts in Aceh Province. Using a standardized monitoring and evaluation (M&E) tool, a sample of 5 districts including 34 health facilities were evaluated to measure RACD indicators, in addition to staff interviews regarding RACD procedures. To measure case investigation and RACD rates and timeliness, quantitative data was extracted from the district health offices and health facility registers and measured against defined indicators. Questionnaires were administered to 59 health facility staff involved in conducting RACD. From January to December 2012, a total of 289 cases (range 0 to 153) were reported at health facilities with a completeness of 92% when compared with the district health office records. Mean case investigation rates were 78% (range from 62 to 100). Screening by microscopy was conducted on a total of 931 community individuals (16.3 individuals per index case) with 3 new infections identified. Mean timeliness of RACD conducted within 7 days was 82% (range from 43 to 100). These findings indicate the need for improved patient data collection upon presentation at the health facility, and for staff to visit index cases after normal work hours to prevent loss due to follow up during case investigations. Rollout of the M&E tool to additional districts in Aceh Province and the monitoring of currently sampled districts will improve timeliness and reporting completeness of facilities and optimize RACD program effectiveness.

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SUSTAINABLE MALARIA ELIMINATION ON ANEITYUM ISLAND, VANUATU, 1991 - 2014

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Weekly mass drug administration (MDA) of chloroquine, pyrimethamine/ sulfadoxine, and primaquine was carried out on the entire population of 718 inhabitants of Aneityum island for 9 weeks in 1991 before the onset of the rainy season. Simultaneously insecticide-treated bednets (ITNs) were distributed to the entire population. Microscopy showed the immediate disappearance of Plasmodium falciparum, whereas P. vivax disappeared from 1996 onwards until new cases were reported in January 2002. In July 2002 P. vivax infections were detected by microscopy in 22 of 759 individuals: 20/298 born after 1991, 2/126 born between 1991-82, and 0/335 born before 1982. Subsequent PCR increased the total to 77 (36, 21, and 20 in respective age groups). The age distribution was similar to those before elimination and on other islands. In November a similar age pattern was found but with fewer (39) infections. In December 2002, the 2nd MDA of weekly chloroquine for four weeks and daily primaquine for 14 days was carried out as a containment measure on the population born after 1982, in concert with re-strengthening of the community-based provision of ITNs. Population-wide mass blood surveys detected by PCR no cases in December 2002 (n=436, only those born after 1982), immediately after the MDA, but 26 in 2003 (730), 20 in 2004 (732), 34 in 2005 (836), and 15 in 2007 (719). No positive cases were detected in 2010 (950) and 2013 (1093). The age distribution of 2003-2007 positive cases was different from those before elimination and on other islands: i.e. a substantially lower prevalence was observed in the population born after 1982 (0.8%) than those between 2002-1992 (3.7%), between 1991-1982 (5.3%), and before 1982 (2.1%), suggesting that these submicroscopic infections mainly reflected relapses from liver stages. Sero-epidemiological monitoring suggested that the persistence of antibodies against P. vivax may partially explain the lower parasite prevalence in the oldest age group. On Aneityum, indigenous P. falciparum transmission has never re-established after the 1st MDA in 1991, despite surveillance by community microscopists that showed continuous parasite importation from other islands. A high degree of community engagement to prevent resurgence, in addition to high ITN coverage (1.05 net/person) and usage (95%, 2014), sustains malaria freedom on this island.

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GENETIC CORRELATES OF DECLINING TRANSMISSION: PLASMODIUM VIVAX IN SRI LANKA

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We examined whether the recent dramatic decline in malaria transmission in Sri Lanka led to a major bottleneck in the local P. vivax population, with a substantial decrease in the effective population size. To this end, we
typed 14 highly polymorphic microsatellite markers in 185 P. vivax patient isolates collected from 13 districts in Sri Lanka over a period of five years (2003-2007). Overall, we found a high degree of polymorphism, with 184 unique haplotypes (12-46 alleles per locus) and average genetic diversity (expected heterozygosity) of 0.8744. A marginal decline in Allelic diversity (15.4 to 10.9) as well as heterozygosity (0.87 to 0.82) was observed from the 2003-4 to 2006-7 period. Almost 69% (n=127) isolates had multiple-clone infections with no major changes observed over time. Significant spatial and temporal differentiation (F_st=0.04 - 0.25; p<0.0009) between populations was observed. The effective population size was relatively high but showed a decline from 2003-4 to 2006-7 periods (estimated as 45,661 to 22,896 or 10,513 to 7,057, depending on the underlying model used). We used three approaches - namely, mode-shift in allele frequency distribution, detection of heterozygote excess and the $M$-ratio statistics - to test for evidence of a recent population bottleneck but only the low $M$-ratio statistics (ranging between 0.15-0.33, mean 0.26) were suggestive of such a bottleneck. The persistence of high genetic diversity and high proportion of multiple-clone infections, with little change in effective population size, despite the collapse in demographic population size of P. vivax in Sri Lanka indicates the importance of maintaining stringent control and surveillance measures to prevent resurgence. It also appears that more samples (over decades) might be required to detect the genetic signatures of shrinking malaria transmission.

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**EVALUATION OF TOPICAL REPELLENTS AS ADDITIONAL VECTOR CONTROL MEASURES TO CONTROL RESIDUAL TRANSMISSION IN MALARIA PRE-ELIMINATION AREAS**

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In Southeast Asia a substantial decrease of malaria has been achieved during the last decade and elimination is now becoming a realistic goal. However residual transmission due to outdoor and/or early biting vectors is not tackled by wide coverage of insecticide treated nets (ITNs). This may compromise the elimination efforts. For this purpose we set up a study to evaluate the public health value of mass use of topical repellent in addition to ITNs. A randomized community based design has been adopted covering a population of 40,000 inhabitants in the province of Ratanakiri in Cambodia. The 98 clusters were randomly divided in two arms after a pre-trial survey: one intervention arm (ITN and repellent) and one control arm (only ITNs). Comparing to a randomized household trial, present design has the advantage to avoid the risk of the repellent aversion effect and the exchange of products between the households. The principal indicators of effectiveness is the prevalence of parasite carriers measured by PCR techniques using a mobile molecular lab in the field, and the measurement of malaria antibodies. While parasite-prevalence provides a snapshot of the exposure to malaria at a certain moment, serological indicators provide a picture of the “force of malaria infection” over a prolonged period. Moreover passive case detection provides a measurement of malaria disease incidence in both arms. We expect a community protection against residual transmission when a high adherence in the use of repellents is achieved. To address this working hypothesis mass effect of repellents on the vector population entomological surveys are carried out in both arms. The effectiveness of the intervention is dependent on the efficacy of the repellents against vector bites and the effective use of repellents by the population and both are addressed in the study design. First results will be presented. The outcome of this study will be crucial in the development of new strategies to control not only the indoor transmission during sleeping time (ITN) but also the increasing proportion of residual transmission which occurs mainly outdoors, and before and after sleeping time.

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**A CLUSTER-RANDOMIZED TRIAL OF TARGETTED CONTROL TO ELIMINATE MALARIA IN CENTRAL SENEGAL: STUDY DESIGN AND ACCEPTABILITY OF THE INTERVENTIONS**

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The purpose of this trial is to evaluate the extent to which a targeted malaria control strategy combining vector control with indoor residual spraying (IRS) and chemotherapy, delivered by district health staff to villages reporting clinical cases, can reduce the transmission of malaria, in a region where scaling up of control measures has been effective in reducing the incidence of malaria. The trial will also determine whether, as part of this strategy, chemotherapy should be delivered to all members of targeted communities (MDA, Mass Drug Administration) or only those who have been tested and are known to be infected (MSAT, Mass Screening and Treatment). Methods: 45 health posts, each serves about 10,000 people, were randomized in 40 clusters; 15 to receive IRS and MSAT, 15 to receive IRS and MDA, and 10 to serve as controls. In intervention clusters, villages with evidence of transmission (hotspots) were identified on the basis of confirmed malaria cases reported the previous year. Interventions are delivered over two years, the primary outcomes are the incidence of malaria and the prevalence of parasitaemia just after the main peak period of transmission in the second year. To assess effects on transmission, incidence in non-targetted areas in each cluster will be compared. In 30 clusters, all households in hotspot villages were targeted to receive IRS with Actellic 300CS in July, followed in 15 of the clusters by MDA with dihydroartemisinin-piperaquine (DHA-PQ) administered to all persons in the household in September and again in October, and in the other 15 clusters, instead of MDA, all persons in the household were screened using a malaria RDT and those who test positive treated with DHA-PQ. In all trial clusters, LLIN coverage was topped-up by providing persons diagnosed with malaria with a LLIN. Results and conclusion: Preliminary results after the first year of intervention will be presented, including description of the logistics of MDA and MSAT delivery, the coverage of the interventions, the sensitivity of RDTs for detecting infections, and the acceptability of both interventions by the community.

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**ZOONOTIC RISKS OF NON-TUBERCULOUS MYCOBACTERIA BETWEEN HUMANS AND SMALL MAMMALS (POTENTIAL TRANSMISSION OF BURULI ULCER) IN COTE D’IVOIRE AND GHANA**

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The prevalence of Buruli ulcer, caused by the non-tuberculous Mycobacterium ulcerans (MUs), ranges from 1-150/100,000 infected individuals in Ghana and Côte d’Ivoire, making it the second most important mycobacterial disease. The mode of transmission is unknown and compounding this is the fact that mycolactone, the major virulence factor, has been found in other environmental mycobacteria species (MPMs) which cause disease in some animals. Although there are no
reports of human disease caused by these novel species, they share similar ecological niches in the endemic aquatic environments. Our work is based on the One Health concept and suggests that overlapping environmental habitats of the pathogen, animals and humans directly influence transmission. Using (i) active case surveillance of human disease burden; (ii) molecular characterization of NTMs; (iii) and zoonotic risk analysis, we are conducting a study in 6 communities of Côte d’Ivoire and 4 communities in Ghana to understand transmission. Comparative analysis of our socio-anthropological data suggests that individuals in Ghana have a higher level of Buruli ulcer awareness than in Côte d’Ivoire. In both countries, association with dirty water is a risk factor and affected people are generally poor. Molecular analysis of our environmental samples indicate a high presence of mycobacterial DNA including MPMs. Almost all of our suspected human cases also type positive for MU DNA. Using Variable Nucleotide Tandem Repeat (VNTR) typing, we have been able to profile and identify one common genotype that is present in both humans and the environment within the same community in both countries. Interestingly we have identified mycobacterial DNA with MPM profiles in lesions and swellings from trapped small mammals within our study sites. Most of the animals were rats, with the majority from homes close to water bodies in both countries. We intend to develop a model of infection dynamics, and identify key risk factors that will ultimately improve control and surveillance strategies for National Control Program.

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LINKED HUMAN AND LIVESTOCK STUDY ON SEROPREVALENCE AND RISK FACTORS FOR BRUCELLOSIS IN KENYA, 2012

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Brucellosis is an endemic zoonotic disease in Kenya with dual burden with significant economic losses among livestock and illness and disability in humans. Brucellosis studies in Kenya have previously focused on either animals or humans. We conducted a cross-sectional survey that examined humans and livestock (cattle, sheep, bovine, camels) in the same households. We assessed the seroprevalence and risk factors for human brucellosis, animal brucellosis and their association. The study was conducted in three counties with different livestock production systems; Random households were selected with a two-stage cluster sampling method by sub-location and then household. Three persons were enrolled and livestock species proportional to the size of the herd were sampled per household. A questionnaire was administered to household heads and the persons sampled. Human sera were tested for Brucella IgG antibodies using competitive ELISA and animal sera were tested using an indirect ELISA. Risk factors were assessed using multivariate logistic regression. A total of 1,099 households, 2,811 persons and 11,039 livestock were enrolled. Overall, 14% (95% CI 12-16) of households had at least one Brucella seropositive person and 15% (95% CI 12-18) of herds had at least one seropositive animal. Among humans sampled, 6.7% (95% CI 5.6 - 7.8) were seropositive. Risk factors for human seropositivity included taking unboiled milk (aOR=3.9, 95% CI 2.0-7.6), exposure to goats [herding, milking, feeding] (aOR=2.5, 95% CI 1.6-4.0) and handling hides (aOR=3.9, 95% CI 2.5-6.2). Animal seropositivity was associated with intermingling with wildlife (aOR=4.3, 95% CI 2.3-8.1) and keeping goats (aOR=2.7, 95% CI 1.4-5.2). The odds of human seropositivity given a seropositive animal in the same household was 5.3 (95% CI 3.2 -8.8). This linked survey shows that human and animal brucellosis seropositivity is associated with factors that increase exposure to seropositive animals. The survey contributes to understanding the burden of brucellosis in Kenya and targeting of health education programs.
surveillance activities under the Ministry of Health and gain insight into transmission dynamics associated with MPX disease. The second study, a population-based serological survey, collected health behavior information from healthy participants in the same geographic region. Both studies collected information on demographic, health, and animal exposure data as well as tissue samples from all active cases and blood samples from healthy participants. We subsequently preformed a matched case-control analysis using these two similarly collected datasets to identify potential risk factors for human MPX in the DRC. Samples were collected from 595 suspected MPX patients identified through active surveillance and from 2,345 healthy persons enrolled in the population-based study. Suspected cases of human MPX were investigated based on the presence of monkeypox-like lesions and confirmed with real time Polymerase Chain Reaction (PCR) using vesicle fluid or scabs; controls were chosen based on the absence of both skin lesions and specific MPX antibodies in serum by laboratory diagnosis. 390 MPX-positive cases were included in the analysis, stratified by the presumed source of infection (animal (n=252) or human (n=138) source), and were matched to a total of 653 MPX-negative controls based on sex and age categories. Initial findings from univariate and multivariate analysis suggest individuals with evidence of smallpox vaccination are at a reduced risk for MPX infection (adjusted OR (aOR): 0.12, 95% C.I: 0.03, 0.56) while exposures to animals including Gambian rats (aOR: 2.58, 95% C.I: 1.63, 4.07), large terrestrial rodents (aOR: 1.80, 95% C.I: 1.24, 2.75), and non-human primates (aOR: 2.66, 95% C.I: 1.44, 4.90), may be associated with an increased risk of MPX infection, particularly in unvaccinated populations.

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**SOUTH AMERICAN EASTERN EQUINE ENCEPHALITIS: POSSIBLE ENZOOTIC HOSTS, HUMAN EPIDEMIOLOGY AND ASSOCIATIONS WITH LAND USE**

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South American eastern equine encephalitis virus (SA EEEV, recently reclassified into the species *Madaranga virus*) had not been associated with significant human disease, unlike its North American counterpart, until a 2010 Panama outbreak. SA EEEV is an alphavirus (family *Togaviridae*) transmitted by Culex mosquitoes (subgenus *Melanoncolum*). Since transmission and epidemiology of SA EEEV is not well understood, we conducted a serosurvey of small mammals and birds, coupled with a human serosurvey. We hypothesized that SA EEEV seroprevalence would be higher in agricultural areas compared to forested areas, mediated by habitat preferences of generalist species likely to serve as enzootic hosts. From January to December 2012, rodents and marsupials were trapped in 42 locations (village, cultivated fields, pasture, shrub, forest; 12,005 trap nights, n=556 rodents; n=20 marsupials). Mist nets were also placed at night for bat trapping (n=52), and bird samples obtained during a prior field effort were tested (n=162). During this period, 776 people from 3 population centers were surveyed. Sera were tested for SA EEEV and the closely related and endemic Venezuelan enzootic equine encephalitis virus (VEEV), by IgG ELISA and plaque reduction neutralization tests (PRNT), and mammal spleens were tested for virus by RT-PCR. We found that the short-tailed cane mouse (*Zygodontomys brevicauda*), a generalist species, had the highest overall EEEV seroprevalence (8.3%), and was the most numerous rodent species trapped (n=229). The abundance of this species was highly associated with cultivated fields and pasture. The abundance of EEEV-positive animals was greater for sites with high rodent diversity (using Shannon-Wiener and Simpson’s indices). The overall human SA EEEV seroprevalence was 4.8%, with no age trend, suggesting that SA EEEV exposure may be recent, consistent with a lack of seroprevalence in past Panamanian surveys. The sites with highest abundance of EEE positive animals did not coincide, however, with highest seroprevalence in humans. Since human VEE seropositivity in these sites was as high as 78%, we hypothesize that this geographical discrepancy may be due to cross-protective immunity. Future studies to further clarify the impact of land use change on EEEV transmission are necessary to guide public health policy in Central and South American countries.

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**CANINE RABIES VACCINATION TO PREVENT HUMAN RABIES IN RURAL TANZANIA**

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The annual mortality rate of human rabies in rural Africa is 3.6 deaths per 100,000 individuals. Rabies can be prevented by prompt post-exposure prophylaxis, but this is costly and often inaccessible in rural Africa. As 99% of human exposures occur through rabid dogs, canine vaccination also prevents transmission of rabies to humans. We evaluate the cost-effectiveness of rabies control through annual canine vaccination campaigns in two districts of rural Tanzania, Ngorongoro and Serengeti, using a dynamic model of transmission in both dogs and wildlife and incorporating empirical uncertainty in the biological parameters to make probability-based evaluations of cost-effectiveness. We find that annual canine vaccination campaigns are very cost-effective in both districts compared with no canine vaccination. In Serengeti, annual campaigns up to 70% coverage are cost-saving. Across a wide-range of parameter assumptions and levels of societal willingness-to-pay for life-years, vaccination campaigns are always cost-effective and life-saving, and therefore preferred.
to approximately 2% nucleotide diversity), but all were restricted to hosts of a single species. Apicomplexan parasites of the genus *Hepatocystis*, related to *Plasmodium*, were detected using microscopy and PCR. “Deep sequencing” of the parasite cytochrome *b* gene showed one *Hepatocystis* lineage to be transmitted frequently between red colobus monkeys (*Procolobus rufomitratus*) and olive baboons (*Papio anubis*), whereas the remaining five lineages infected hosts of a single species. Analyses of fecal samples revealed helminth parasites of the genera *Oesophagostomum* (nodule worms) and *Trichuris* (whipworms), which were further characterized by PCR and sequencing of the internal transcribed spacer region of the ribosomal DNA complex. Both parasite genera contained multiple cryptic lineages, some representing putative novel taxa, and several infecting primates of multiple species, including humans. Collectively, these results suggest that pathogens with highly variable and mutable genomes (e.g. RNA viruses) are not necessarily more likely to cross species barriers than more genomically complex pathogens (e.g. protozoa and helminths), in certain ecological settings. Efforts to prevent zoonotic transmission in such settings should focus on common transmission pathways inferred from empirical studies of a range of pathogen taxa, rather than on specific classes of pathogens assumed to have high cross-species transmission potential.

**SAFETY AND IMMUNOGENICITY OF RTS,S/AS01 MALARIA VACCINE CANDIDATE IN HIV INFECTED INFANTS AND CHILDREN: A PHASE III RANDOMIZED, DOUBLE-BLIND, CONTROLLED TRIAL**

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Malaria and HIV remain important global health problems. The RTS,S/AS01 malaria vaccine candidate, which showed protection against malaria with a favorable safety and immunogenicity profile during trials in sub-Saharan Africa, had previously not been evaluated specifically in HIV-infected participants. We measured safety and immunogenicity in children with WHO stage 1 or 2 HIV disease at two centers in Kenya (NCT01148459). Children aged 6 weeks to 17 months were randomized 1:1 to receive 3 doses of RTS,S/AS01 or rabies vaccine, administered monthly. The primary objective was occurrence of serious adverse events (SAEs) from first vaccination until 14 months post-dose 1. A secondary objective, incident clinical malaria, was captured by passive case detection. Baseline characteristics were similar between study arms. Out of 200 children enrolled, 177 (89%) completed follow-up. The frequency of SAEs was 41.4% (95% CI: 31.6-51.8) among RTS,S/AS01 recipients and 36.6% (95% CI: 27.3-46.8) among rabies vaccine recipients. At least one SAE was reported within 30 days of vaccination in 20.2% (95% CI: 12.8-29.5) of RTS,S/AS01 recipients and 11.9% (95% CI: 6.3-19.8) of rabies vaccine recipients. At least one episode of pneumonia occurred in 13/99 (13.1%) RTS,S/AS01 recipients compared to 5/101 (5.0%) rabies vaccine recipients within 30 days post-vaccination. Fatal SAEs occurred in 5.1% (95% CI: 1.7-11.4) of RTS,S/AS01 recipients and 4.0% (95% CI: 1.1-9.8) of rabies vaccine recipients. One related SAE was reported: a febrile convulsion in an RTS,S/AS01 recipient. Solicited adverse event frequency 7 days post-vaccination was: injection site pain 18.1% and 6%, fever 41.7% and 18.8%, irritability 25.3% and 10.7%, and loss of appetite 17.7% and 8.7% in the RTS,S/AS01 and rabies vaccine group respectively. No evidence of differential HIV disease progression (CD4+ HIV viral load and WHO HIV Clinical Staging) was seen between study arms. In the RTS,S arm, anti-CS antibody geometric mean titer (EU/mL) was 0.3 (95% CI: 0.3-0.4), 329.2 (95% CI: 260.6-415.8) and 18.4 (95% CI: 13.3-25.5) at baseline, one and 12 months post-dose 3. Vaccine efficacy against first episode of clinical malaria was 31% (95% CI: 19-60). RTS,S/AS01 showed no serious safety concerns and was immunogenic in HIV-infected children. When considered with efficacy data, HIV-infected children need not be excluded from potential future vaccination programs with RTS,S/AS01.

**MATHEMATICAL MODELING OF THE SITE-SPECIFIC IMPACT OF THE RTS,S VACCINE UNDER MULTIPLE ROLL-OUT SCENARIOS**

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Vaccines against malaria are one promising avenue for reducing the global burden of the disease beyond what has been achieved with current interventions. The pre-erythrocytic vaccine RTS,S is the candidate furthest along the development pipeline, and as such there is great interest in the potential impact of a large-scale roll-out. Here, we addressed this question by using the previously developed EMOD model of malaria to simulate the outcome of such a campaign. The central model is a mathematical description of the vector life cycle coupled to within-host parasite and immune dynamics. To study RTS,S, we incorporated a representation of the vaccine, characterized by an initial efficacy against infection and a half-life of protection. An iterative stochastic approach was used to optimize the modeled vaccine properties by sampling parameter space and evaluating a likelihood function that assesses the fit between the simulations and clinical trial data. Having obtained a calibrated model of RTS,S, we subsequently explored a variety of different roll-out scenarios to test the effects of policy choices (e.g., age of first administration, booster inclusion) and deployment setting characteristics (e.g., demographics, seasonality). Additionally, sensitivity analyses were performed on other axes, such as coverage level and treatment-seeking behavior, quantities that are at once difficult to measure and highly relevant to the success of a campaign. Simulated clinical and severe incidences were incorporated into a cost analysis in order to establish the incremental impact of the vaccine over other interventions, such as insecticide-treated nets (ITNs). We concluded that the cost effectiveness of population-wide infant vaccination is greatest in regions of moderate endemicity, though the specifics depend on a number of factors, including pre-existing ITN coverage and local anopheline feeding preferences.
Alhydrogel® transmission blocking vaccine has been well tolerated and functional activity will be available in October 2014. Overall, Pfs25-EPA/Alhydrogel® results following the fourth vaccination in the Mali study and associated vaccination in the high dose groups. Anti-Pfs25 antibody response was the same between the two studies following the second and third responses at levels ≥ vaccination. The percentage of responders with anti-Pfs25 antibody each subsequent dose of vaccine given but diminished quickly following vaccination. The antibody response at each vaccine dose increased with dose, five volunteers (low dose of 16 µg Pfs25-EPA/Alhydrogel®) received 2 doses (Days 0, 56) and 100 volunteers (high dose of 47 µg Pfs25-EPA/Alhydrogel®) have thus far received 3 doses (Days 0, 56, 112) with a scheduled fourth vaccination on Day 480. The US Phase 1 study was an open label dose escalating study in which 47 volunteers (safety dose of 8 µg Pfs25-EPA/Alhydrogel®) received 2 doses (Days 0, 28, 56, 84, 140; total dosage: 13.5x105 PfSPZ) by the end of July 2014. For decades it has been known that humans can be protected against malaria by repeated immunization with radiation-attenuated Plasmodium falciparum sporozoites (SPZ). Traditionally, those PfSPZ have been administered by exposing vaccinees to the bites of >1,000 PfSPZ-infected, irradiated mosquitoes. Recently, a process for manufacturing aseptic, purified, radiation attenuated cryopreserved PfSPZ has been developed. When administered by IV injection, this product, Sanaria® PfSPZ Vaccine, induced sterile protection against controlled human malaria infection in 668 malaria naïve adults who received the highest dosage. To begin the process of assessing the vaccine in Africa, we are conducting a double blind, randomized, controlled Phase 1 clinical trial to assess PfSPZ Vaccine’s safety, immunogenicity, and protective efficacy against naturally occurring malaria infection in healthy, malaria exposed, 18-35 years old Malians. Among the 105 volunteers vaccinated by direct venous inoculation (DVI), 12 volunteers (pilot safety group) have received 2 doses of PfSPZ Vaccine (Day 0: 1.35x10⁵ and Day 14: 2.7x10⁵ PfSPZ) and 93 volunteers will have received 5 doses of 2.7x10⁵ PfSPZ or normal saline placebo (Day 0, 28, 56, 84, 140; total dosage: 12.5x10⁵ PfSPZ) by the end of July 2014. Nine subjects in the pilot group will join the larger group to receive an additional 3 vaccinations (total dosage: 12.5x10⁵ PfSPZ). The incidence and severity of local and systemic adverse events occurring within 7 days after dose are being recorded. During the malaria transmission season all volunteers will be examined every 14 days and as clinically indicated for blood-stage parasitemia by microscopy. All volunteers have been immunized twice. Immunizations have been well tolerated with no local reactogenicity, minimal mild to moderate systemic reactogenicity, no serious adverse events, and no patent parasitemia. These early results show PfSPZ Vaccine administered via DVI is safe and well tolerated in healthy malaria-exposed, African adults. Results of all immunizations will be presented.
ASSESSING EFFICACY OF THE PFSPZ VACCINE BY CONTROLLED HUMAN MALARIA INFECTION IN TANZANIA

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The rich potential for African scientists to spearhead phase 1 studies that have a controlled protection component has not been tapped because controlled human malaria infections (CHMI) have been available only at centers in the USA and Europe with access to infected mosquitoes. Sanaria has developed aseptic, purified, cryopreserved infectious Plasmodium falciparum (Pf) sporozoites (SPZ) (PfSPZ Challenge) manufactured in compliance with cGMPs, suitable for parenteral injection and shippable to any location. PFSPZ Challenge has been tested in the Netherlands, US, UK, Tanzania, Kenya, Germany and Spain using different dosages and inoculation routes. 3,200 PFSPZ administered by direct venous inoculation (DVI) reproduces the 100% infection rate and 11-11.5 day pre-patent period of 5 mosquito bite CHMI. In the present, first of its kind study, we will use PFSPZ Challenge to test the efficacy of Sanaria’s radiation attenuated, non-replicating PFSPZ Vaccine against CHMI administered by DVI. PFSPZ Vaccine showed 100% efficacy in a recent study by the Vaccine Research Center (VRC), NIAID, NIH in the study group receiving the highest dose tested (5 doses of 1.35x10^5 PFSPZ by DVI). The Bagamoyo Clinical Trial Unit, Ifakara Health Institute, is now comparing the same regimen shown to be 100% protective at VRC with a regimen that delivers twice the dose (2.7x10^5 PFSPZ) at each of the 5 time points, in minimally malaria-exposed Tanzanian adults. Both groups will be assessed for protection against CHMI using PFSPZ Challenge administered by DVI at 3 and 24 weeks after the 5th dose (40 immunized and 8 control subjects). An additional 6 volunteers will receive the higher dose and undergo CHMI only at 24 weeks. We will present safety, tolerability, and preliminary immunogenicity data for this unique study that is opening an exciting pathway for malaria vaccine and drug research and development in Africa and the world.

POTENT CELLULAR AND HUMORAL IMMUNOGENICITY OF CHAD63 MVA ME-TRAP IN AFRICAN INFANTS AND CHILDREN

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Vaccination is one of the most cost-effective health care interventions available and an effective malaria vaccine against Plasmodium falciparum could save more than half a million lives each year. We developed a prime-boost immunisation approach employing the viral vectors ChAd63 and MVA, both encoding the pre-erythrocytic malaria antigen TRAP. Previous studies with ChAd63 MVA ME-TRAP have shown excellent immunogenicity and significant efficacy in adults with protection correlated with frequency of mono-functional CD8+ T cells secreting IFNγ. We report here T cell phenotypes and antibody titres measured in 138 children vaccinated during three Phase I dose-finding and age-escalation studies to assess safety and immunogenicity of the ChAd63 MVA ME-TRAP vaccine in malaria-exposed children in The Gambia and Burkina Faso. Age groups at first immunisation were 2-6 years, 5-12 months and 10 weeks in The Gambia and 5-17 months in Burkina Faso. IFNγ ELISPOT responses to TRAP were significantly lower in 2-6 year old and 5-12 month old children in The Gambia and 5-17 month old children in Burkina Faso than in malaria-naïve UK adults receiving the same vaccines. Immunogenicity in 10 week-old infants in The Gambia was high and comparable to that in adults. Flow cytometry revealed IFNγ secretion from both CD4+ and CD8+ T cells with IL-2 and TNFα also detected from CD8+ T cells. Anti-TRAP IgG responses varied by age group and dose, with significantly higher titres detected after boosting in vaccinees primed with a higher dose of ChAd63 ME-TRAP. Titres were also significantly higher in 5-12 month and 10 week old children than 2-6 year-old children in The Gambia that received the same dose. IgG titres in 5-17 month-old children in Burkina Faso were comparable to those in 5-12 month-old children in The Gambia. IgG responses were predominantly composed of IgG1 and IgG3 isotypes and we also detected IgA and IgM responses. We demonstrate excellent cellular and humoral immunogenicity of a pre-erythrocytic malaria vaccine in key target populations for vaccine deployment.

SAFETY AND IMMUNOGENICITY OF THE BLOOD-STAGE PLASMODIUM VIVAX VACCINE CHAD63-MVA PVDDB

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There has been comparatively little research into vaccines for Plasmodium vivax in the past, with only two antigens previously reaching Phase I
clinical trials. More recently, the importance of a vaccine for *P. vivax* has been recognised and included in the 2013 update to the Malaria Vaccine Technology Roadmap. Here we report on the first blood-stage *P. vivax* vaccine Phase Ia clinical trial. This has been carried out in Oxford using recombinant simian adenovirus ChAd63 and the poxvirus MVA encoding the *P. vivax* antigen PvDBP (Duffy-binding protein region II) in a heterologous prime-boost regimen. The *P. vivax* parasite requires an interaction between the Duffy-binding protein ligand and its host receptor, the Duffy antigen receptor for chemokines (DARC), in order to invade reticulocytes, making PvDBP a promising antigen for vaccine development. Twenty-three healthy volunteers have been vaccinated and followed up in this Phase Ia dose escalation study of ChAd63-MVA PvDBP. The ChAd63 PvDBP priming vaccine was initially given alone to 4 volunteers at a dose of 5 x 10^8 vp before being increased to 5 x 10^10 vp following a planned safety review. MVA PvDBP has been given to 15 volunteers 8 weeks after ChAd63 PvDBP prime at doses of 1 x 10^8 pfu – 2 x 10^9 pfu. The primary objective of the study was safety, and the vaccines have been well tolerated with no serious adverse events. Follow-up of volunteers will be complete at the end of June 2014. The secondary objective was humoral and cellular immunogenicity which has been assessed using assays including PvDBP IFN-γ 1 cell ELISPOT, B cell ELISPOT, PvDBP IgG antibody ELISA and functional antibody analysis. The vaccine regimen is immunogenic, with increases in the cellular and humoral responses seen after MVA PvDBP boost (compared with ChAd63 PvDBP alone). This is the first vaccine against PvDBP to be assessed in humans, and has shown a favourable safety profile and promising levels of immunogenicity which support onward clinical development towards proof-of-concept efficacy testing.

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ELQ-300 PRODRUGS FOR ENHANCED DELIVERY AND EFFICACY AGAINST MALARIA

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ELQ-300 is a preclinical candidate of the Medicines for Malaria Venture that targets the liver and blood stages of malaria, as well as the forms that are crucial to transmission of falciparum malaria: gametocytes, zygotes, and ookinetes. In mouse models of the disease, a single oral dose of 0.03 mg/kg prevented sporozoite infections while 4 daily doses of 1 mg/kg achieved complete cures of patent infections. A significant obstacle to the clinical development of ELQ-300 relates to its physical-chemical properties. Its relatively poor water solubility and high crystallinity limits absorption to the degree that only low bloodstream concentrations can be achieved by oral dosing. While these low bloodstream concentrations are sufficient for therapy the levels are too low to establish an acceptable safety margin required by regulatory agencies for clinical development. One way to address the challenging physical-chemical properties of ELQ-300 is through the development of prodrugs. In this presentation we will profile a series of ELQ-300 prodrugs, focusing primarily on the bioreversible nature of ELQ-337. At the equivalent dose of 3 mg/kg the delivery of ELQ-300 from ELQ-337 is enhanced by 4-5-fold, reaching a Cmax of 6.9 micromolar by 4 hrs after oral administration. The superior in vivo efficacy of this compound will be discussed. Apart from highlighting the outstanding in vivo efficacy of ELQ-337, this data demonstrates that the prodrug strategy represents a viable approach to overcome the physical-chemical limitations of ELQ-300 to deliver the active drug to the bloodstream at high enough concentrations sufficient for safety and toxicology studies as well as achieving single dose cures.

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UNDERSTANDING THE MECHANISM FOR EFFICACY AND TOXICITY OF 8-AMINOQUINOLINE ANTIMALARIALS: IN VITRO AND IN VIVO STUDIES WITH HYDROXYLATED METABOLITES OF PRIMAQUINE

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Hydroxylated metabolites are presumed to be responsible for toxicity and/or efficacy of primaquine and other 8-aminoquinoline antimalarials. However, no definitive data are available on pharmacokinetics, pharmacodynamics and in vivo plasma/tissue profiles of the hydroxylated metabolites. Recent in vitro studies with primary human hepatocytes and application of stable isotope (13C) labeled PQ provided evidences for generation of hydroxylated metabolites presumably through CYP mediated pathways. Previous studies by us have shown that 5-hydroxyprimaquine (5-HPQ) and 6-Methoxy 8-N-Hydroxy aminoquinoline (MHQ) generated robust methemoglobin formation and oxidative stress in normal and G6PD deficient human erythrocytes. Both 5-HPQ and MHQ produced selective depletion of GSH in G6PD deficient erythrocytes. The studies were extended to include 2-, 3-, 4- and 8-N-hydroxyprimaquine (8-NHPQ). Both 2-HPQ and 4-HPQ are relatively stable analogs. 2-HPQ and 4-HPQ did not generate significant methemoglobin and only marginal ROS in normal and G6PD deficient RBCs with & without microsomes. Neither metabolite showed activity in vivo in the blood stage Plasmodium berghei mouse malaria model (i.v. route of administration). Until recently, no definite evidences have been available regarding generation of 3-HPQ and/or 8-NHPQ in vivo & in vitro. 3-HPQ can be further metabolized with pooled human liver microsomes to generate more toxic/reactive species. 8-NHPQ is a reactive metabolite, which generates significant methemoglobin and oxidative stress. Among 2-, 3-, 4- HPQ and 8-NHPQ, none affected GSH levels in normal or G6PD-deficient human erythrocytes. Formation of 2- and 3-HPQ metabolites was confirmed in vitro on incubation of PQ with recombinant human CYP2D6; another HPQ, perhaps 4-HPQ is also formed. However, 2-HPQ, 3-HPQ and 4-HPQ metabolites appear not to be responsible for toxicity/efficacy of PQ.

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A PHASE I/IB STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY AND PHARMACOKINETIC PROFILE OF DSM265 IN HEALTHY SUBJECTS AND THEN ITS ANTIMALARIAL ACTIVITY IN INDUCED BLOOD STAGE PLASMODIUM FALCIPARUM INFECTION

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DSM265 is a new antimalarial that targets the malaria parasite’s pyrimidine biosynthetic enzyme dihydrotocatechol dehydrogenase (DHOHD). A Phase I/Ib study was designed to collect safety, PK and efficacy data. It was undertaken in two parts: the first comprised a single ascending dose (SAD) design to assess safety, pharmacokinetics, and the maximum tolerated dose. Embedded within this first part was an assessment of food effect. The second part comprised exploration of the antimalarial activity of...
DSM265 in a single-dose cohort using the induced blood-stage malaria (IBSM) system at a dose guided by data from preclinical efficacy prediction and emerging PK data. To date, 5 cohorts comprising 49 volunteers have participated in the SAD up to 400mg, study of food effect, or the IBSM phase. To date, no significant drug-related clinical or laboratory toxicity have been observed. Fitting of a population PK model to the data indicates mono-exponential disposition, with a blood clearance of 0.45 L/hr (95%CI: 0.41-0.50), and an elimination t1/2 of 91 hrs. DSM265 shows dose proportionality, both in terms of Cmax and AUCinf. No food effect was observed. A dose of 150 mg was selected for the IBSM phase by combining the MPC predicted from preclinical data (0.5-1 µg/mL) with the human PK, aiming to observe parasite clearance kinetics, including recrudescence. When tested in the IBSM system in 7 healthy volunteers, the drug showed encouraging antimalarial activity with 99.9% parasite clearance by 130 hrs, with recrudescent infection observed in 5/7 subjects between 10 and 21 days after drug administration. No mutations in the DHODH gene were observed in recrudescent parasites. Fitting of a population PK/PD model to the parasitemia data led to an estimate of the MPC of 954 ng/mL (95%CI: 678-1267). Population estimates of PRR and parasitemia t1/2 were 2.2 (95%CI: 2.0-2.7) and 6.6 hrs (95%CI: 5.4 - 7.2) respectively. These results demonstrate that it is possible to obtain well characterised efficacy data early in the clinical development of an anti-malarial. The combination of early safety, PK and efficacy data with the PK/PD model show that future clinical trials of DSM265 will benefit by being smaller and focused towards confirming the observed efficacy. The observed duration of action of DSM265 suggests its promise as a component of a new combination single-dose treatment for malaria.

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TARGETING DRUG RESISTANCE: HARNESING EVOLUTIONARY FITNESS IN PLASMODIUM FALCIPARUM FOR DRUG DISCOVERY

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Management of drug resistance with combination therapies is key to prolonging the effectiveness of new antimalarials. Typical combination therapies pair drugs that are effective as monotherapies and employ differing binding sites. Pairing drugs that target independent sites presents new modes of action and can provide an alternative to selection. Here we explore new kinds of antimalarial combination therapies where the changes giving rise to resistance become the target for the second drug. This approach incorporates built-in protection for the partner drug from development of resistance. In the wild-type organism the partner drug has little or no effect and the organism is not subject to selective pressure to develop resistance. When resistant mutants to the first drug occur then, and only then, does the second drug act, greatly diminishing the population subject to selection. In the event that mutations occur conveying resistance to the second drug, those mutations will drive the organism back towards sensitivity to the first drug. We have taken a systematic approach in the study of resistant organisms and their unique susceptibilities. We screened the Malaria Box against a panel of mutant parasites with well-defined resistance mechanisms to identify compounds with differential activity between mutant and wild-type parasites. We show that a significant percentage of the Malaria Box targets one of the pathways tested. In particular, we find a number of compounds with negative cross-resistance, representing promising leads in pursuing inhibitor pairs as described above. Importantly, multiple chemotypes inhibit each target, indicating there are overlapping modes of action in the parasite despite broad chemotypic diversity in the compound set. This suggests the parasite has a limited number of chokepoints that can be exploited in drug development making it all the more critical to develop a means of protecting the long-term efficacy of compounds in the pipeline with combination strategies as discussed.

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NON-INFERIORITY CLINICAL TRIAL COMPARING FIXED-DOSE COMBINATION AND CHLOROQUINE FOR PLASMODIUM VIVAX UNCOMPLICATED INFECTION IN THE BRAZILIAN AMAZON

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In Latin America, Plasmodium vivax is responsible for around 80% of malaria episodes. As Chloroquine (CQ) efficacy decreases, alternative therapies such as artemisinin-based combinations recommended by WHO must be evaluated. This phase III, noninferiority randomized trial, compared the antischizontocidal efficacy and safety of a 3 days supervised treatment of the fixed-dose artemunate-amodiaquine Winthrop® (ASAQ) versus CQ for the treatment of uncomplicated Pvixiv infection in children above 6 months-old and adults. Primary endpoint was the efficacy rate at day 28. Patients were followed-up until day 42. 380 patients were included in Manaus, Brazil. Baseline characteristics and dropout rates were similar between arms. In Per-Protocol analysis, adequate clinical and parasitological response (ACPR) at day 28 was achieved in 100% and 92.4% of patients in the ASAQ and CQ arm (93.7% versus 89.5% in the Intention-To-Treat analysis). Non-inferiority was achieved in both populations and subsequent bilateral tests concluded in superiority of ASAQ (p=0.001% and < 0.001% in ITT and PP analysis respectively). Clearance of parasite from D1 to D3 was observed in significantly more patients (p<0.001) and significantly more patients were afebrile at D1 (p=0.001) in the ASAQ group. A significant reduction in the number of gametocyte carriers was also observed in the ASAQ group versus CQ group during follow-up. A significant difference in the proportion of recurrence during the 42 days of follow-up was also observed (26.5% versus 3.9% in the CQ versus ASAQ groups, p<0.001). 79% of recurrences in the CQ arm, and all in the ASAQ, occurred after D28. The occurrence of emergent adverse events (AEs) was similar in both arms (CQ=115; ASAQ=110), with no serious AE in the CQ and 5 (related to 3 patients) in the ASAQ arms. ASAQ is a safe and efficacious alternative to treatment of uncomplicated P. vivax infection. 42-days-follow-up enables better assessment of CQ efficacy. Microsatellite genotyping correction and CQ levels results will be presented.

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QTC PROLONGATION AND DRUG SAFETY OF AN INCREASED DOSE OF DIHYDROARTEMISININ-PIPERAQUINE IN YOUNG CHILDREN 5-24 KG IN MALAWI

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Dihydroartemisinin-piperaquine (DHA-PPQ) is recommended for the treatment of uncomplicated falciparum malaria and is being explored as mass drug administration to reduce transmission. Recent pharmacokinetic (PK) studies and a pooled meta-analysis of individual patient efficacy data by the Worldwide Antimalarial Resistance Network have raised concerns...
about potential under-dosing of young children with current weight-based dose recommendations and advocated higher dosing regimens in this vulnerable population. In contrast, the European Medicines Agency has called for more data to substantiate the cardiac safety of DHA-PPQ and its effect on QTc intervals. We conducted an open-label dose optimization study aimed at describing the population PK profile of PPQ and the tolerability of a higher dose regimen of DHA-PPQ in children of 5–24 kg with uncomplicated falciparum malaria [PACTR201303000506302]. In the first step of the study, 100 children received a dosing regimen based on supervised treatment with doses of 1.7–3.8 mg/kg DHA and 13.6–30.0 mg/kg PPQ given once daily over three days, using whole or half-tablets (20/160 mg and 40/320 mg DHA/PPQ tablets); children were followed-up for 63 days. In the second step 100 children received a more pragmatic regimen using whole tablets with wider ranging doses of 2.0–4.0 mg/kg DHA and 16.2–32.0 mg/kg PPQ. QTc was measured 4–6 h after the last DHA-PPQ dose and compared to baseline and Day 28 using digital 12-lead electrocardiograms and the vertical caliper on median overlapped template technique. We will present findings from the first step. Findings from the second step will be presented, dependent on study progress. Optimising the weight-based dose regimen for DHA-PPQ in the main high-risk group of young children ahead of its roll-out into control programmes in sub-Saharan Africa will help inform the translation of dosing recommendations into programmatically feasible, user-friendly, safe weight- and age-based dosing regimens.

**SCHEDULED SCREENING VERSUS PREVENTIVE TREATMENT FOR THE CONTROL OF MALARIA IN PREGNANCY IN MALAWI: A RANDOMIZED CONTROLLED TRIAL**

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The decline in effectiveness of intermittent preventive treatment in pregnancy with SP (IPTp-SP) due to high levels of sulphadoxine-pyrimethamine (SP) resistance by *Plasmodium falciparum* malaria parasites necessitates urgent alternative approaches for malaria control in pregnancy. The objectives of this study is to determine whether scheduled intermittent screening with a malaria rapid diagnostic test (mRDT) and treatment of mRDT-positive women with Dihydroartemisinin-Piperazine (ISTp-DP) from the second trimesters is more efficacious than IPTp-SP in reducing adverse birth outcomes and malaria infection at term among HIV-sero-negative women protected by insecticide-treated bed nets in a malaria endemic setting. This two arm, open label, multicentre, randomized controlled superiority trial was designed to assess a 25% or greater reduction in placental malaria in multigravidae.

**ECOLOGICAL GENOMICS AND PLASTICITY OF GENETIC REGULATION FOR SALTWATER TOLERANCE**

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Evolution of osmoregulatory systems is a key factor in the transition of species between fresh- and saltwater habitats, including anopheline mosquitoes known for their role as disease vectors. Here we use RNA-Seq to investigate gene expression differences between an obligate freshwater (*Anopheles coluzzii*) and euryhaline malaria vector (*An. merus*). After rearing in freshwater (FW), both young and old larval instars of each species were briefly (6 h) exposed to either saltwater (SW) or FW conditions to test the impact of water salinity on mRNA levels. We aimed to describe the transcriptomic response of anophelines to water salinity, and how the response differs between *An. merus* and *An. coluzzii*. We also tested how the transcriptomic response to water salinity differs with age, particularly for the tolerant species *An. merus*. Our results are congruent with the ability of gene induction to mediate SW tolerance, with the intolerant *An. coluzzii* exhibiting little difference in gene expression (<2% of the transcriptome), in contrast to greater plasticity by *An. merus*. In the latter, >16% of the 11,025 genes assayed responded to saltwater exposure, with similar levels of up- and down-regulation. The impact of age at exposure was less dramatic than species identity, with 567 genes significantly differentially expressed in response to water type between young and old *An. merus*. Besides effector genes with putative roles in ion transport (e.g., Na+/K+-ATPase), we also report differential expression in response to water salinity by genes involved in general stress responses such as heat shock proteins, and potential cross-talk between the immune response and osmoregulation. Additionally, we report on a network of 115 co-expressed genes associated with SW tolerance. Finally, we complement our investigation of gene expression with QTL mapping from a backcross of *An. merus* and *An. coluzzii*. We report regions of sequence divergence associated with SW tolerance, and discuss the presence of differentially expressed genes within these regions.

**A COMPARATIVE APPROACH TO IDENTIFY PATHWAYS REGULATING FEMALE REPRODUCTIVE BIOLOGY IN ANOPHENE MALARIA VECTORS**

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Female *Anopheles gambiae* mosquitoes exhibit major behavioral and physiological changes in the first 24 hours after mating, including egg laying and refractoriness to further mating. These post-mating responses are in part mediated by transfer of seminal secretions contained in the mating plug, a complex of proteins, lipids and hormones, formed in the male, and deposited in the female atrium (uterus) during mating. However, not all Anophelines produce and transfer a plug, with this structure absent in the Nyssorhynchus mosquito series (*Anophelles albinanus*). This raises the question of how female post-mating changes are induced in absence of a plug? To address this question, we compared the molecular processes underpinning the mating responses of *An. gambiae* and *An. albinanus* females via RNAseq analysis of the atrium. A significantly larger response was observed in *An. gambiae* after mating (2235 genes, p<0.05) than in *An. albinanus* (212). Comparison of seminal secretions from the two species indicates that this >10-fold increase in *An. gambiae* response may be due to the transfer of potent transcriptional regulators. Enrichment analysis (DAVID) identified pathways of epithelial transport and molecule trafficking regulated in both species, suggesting the exchange of material across the atrium epithelium. Among the genes exclusively modulated in *An. gambiae*, were proteins associated with mating plug processing and energy production, suggestive of high-energy demands in mated females,
while in An. albimanus enriched processes included chitin metabolism, which may indicate structural changes to the uterus after mating, as well as carboxylesterase activity suggestive of xenobiotic metabolism. This comparison provides important insight into the different pathways shaping female post-mating behavior in An. gambiae and An. albimanus. Ultimately, a better understanding of Anopheles reproductive biology will aid vector control efforts aimed at reducing mosquito fertility, and may highlight conserved reproductive pathways that could be targeted in all malaria vectors worldwide.

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PFS47 ROLE IN THE ADAPTATION OF PLASMODIUM FALCIPARUM TO NEW WORLD ANOPHELES

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The Pfs47 gene, a member of the 6-cys protein family, is required by Plasmodium falciparum to evade the Anopheles gambiae immune system. This led us to propose that Pfs47 may be important for the malaria parasite adaptation to different Anopheles mosquitoes around the world. Pfs47 is a polymorphic gene with signatures of diversifying selection and a strong geographic genetic structure. The highest genetic diversity of Pfs47 was detected in Africa, and the lowest diversity was detected in the New World with one haplotype. Phylogenetic analysis of Pfs47 haplotype sequences present a geographic structure with most African haplotypes forming a clade separate from most of the Asian alleles. The haplotype found in the New World appears to be more closely related to the African clade. Infection of the New World malaria vector A. albimanus with P. falciparum was found to be highly dependent on the geographic origin of the parasite strain. The A. albimanus mosquito immune system was found to be responsible for the local infectivity found in African P. falciparum NF54 infections. Allele replacement showed that a Pfs47 haplotype from the New World can rescue the NF54 parasite infection of A. albimanus. This provides evidence that the mosquito immune system can be an important barrier for adaptation of P. falciparum to new vectors and that selection of particular Pfs47 haplotypes may be required to overcome this barrier.

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DISCOVERY OF NOVEL LONG NON-CODING RNAS AND PROTEIN-CODING GENES IN ANOPHELES GAMBIAE USING DEEP RNA SEQUENCING

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Recently, an expanding class of long non-coding RNAs (lncRNAs) that function in epigenetic regulation, the regulation of gene expression transcriptionally and post-transcriptionally, and the regulation of genomic stability have been described in mammals, Drosophila melanogaster and Caenorhabditis elegans. We have analyzed the transcriptome of the malaria vector Anopheles gambiae, based on deep-read RNA sequencing technology that generated over 500,000,000 reads from first and third instar larvae and adult females and males. Our de novo transcriptome assembly, and comparisons with gene models defined in VectorBase annotation Release 3.7, revealed over 1,100 novel IncRNAs and more than 200 previously unannotated putative protein-coding genes. The IncRNAs exhibit differential expression across life stages of the mosquito and display an increased rate of divergence over evolutionary time across the genus Anopheles when compared to the newly discovered protein-coding genes and previously annotated protein-coding genes. This initial description of IncRNAs in An. gambiae offers the first (large-scale) insights into non-coding RNAs in this mosquito and defines another potential set of targets for the development of vector-based interventions that may curb the malaria burden in disease-endemic countries.

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THE EXPRESSION OF THE PIRNA PATHWAY GENES, PIWI, AUBERGINE AND ARGONAUT 3, DURING HEAT AND COLD STRESS RESPONSE IN THE MALARIA MOSQUITO, ANOPHELES STEPHENSI

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Stress-induced mobilization of transposons is well-documented in many organisms. An RNA-interference pathway, designated the piRNA pathway, is responsible for repressing transposon mobilization in germ-line tissues of the fruit fly, Drosophila melanogaster. The genes encoding the components of the piRNA pathway, Piwi, Aubergine (Aub) and Argonaute 3 (Argo3), were identified and characterized in the malaria vector mosquito, Anopheles stephensi. Preliminary experiments show that they are induced in embryos following heat or cold stress. Current experiments in adult female mosquitoes are designed to assay the effects of heat and cold stress on the expression levels of the mosquito orthologs of the heat-shock protein genes, hsp70 and hsp90, as well as Piwi, Aub and Ago3 and endogenous transposase genes. Additionally, mosquitoes mutant for Piwi, Aub and Ago3 are being generated and will be tested for a phenotype affecting the heat-shock response. The results of this work are expected to inform the development of transposon-based gene-drive systems for introgressing beneficial traits into vector mosquitoes.

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THE ROLE OF INSECT INNATE IMMUNITY IN CONTROLLING RICKETTSIA TYPHI INFECTION

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Rickettsia typhi, the causative agent of murine typhus, is an obligate intracytosolic bacterium that is transmitted by the flea. Upon infection, R. typhi systemically infects fleas. However, infection level is kept manageable so that flea fitness is unaffected, presumably by the flea innate immune system. Two main arthropod innate immune signaling pathways, the Imd and Toll pathways, respond to Gram-negative bacteria and Gram-positive bacteria/fungi, respectively. Upon activation by the Imd or Toll pathway, Rel/NF-κB transcription factors Relish and Dorsal/DIF increase transcription of antimicrobial peptides. The immune pathways that control R. typhi infection in the flea have yet to be elucidated. We hypothesize that the Imd pathway is critical for controlling Gram-negative R. typhi burden within the flea. Dissecting the flea immune response to R. typhi has been hampered by the lack of a flea genome. Therefore, R. typhi infection was modeled in Drosophila S2R+ cells. R. typhi infected and replicated within S2R+ cells. To identify which innate immune signaling pathway(s) is critical for controlling R. typhi burden, S2R+ cells were treated with dsRNA to knockdown either the Imd or Toll pathway. Knockdown of negative regulators caspase and cactus, of the Imd and Toll pathways, decreased R. typhi burden suggesting both the Imd and Toll pathways control R. typhi burden. Knockdown of the NF-κB factor Relish but not Dorsal increased R. typhi burden, further confirming the role of the Imd pathway in control of R. typhi burden. More extensive screens will provide further evidence of which pathway(s) is controlling R. typhi burden. Additionally, a flea dorsalis-like sequence has been identified and we are working to identify a flea relish-like sequence in hopes of parlaying the knowledge garnered from the Drosophila model into the flea. Identification of immune pathways in the flea and understanding the flea innate immune response to R. typhi could potentially lead to new treatment modalities that will decrease transmission and burden in the flea.

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**SIRNA NANOPARTICLE-MEDIATED TARGETING OF DOUBLESEX, A REGULATOR OF SEX-SPECIFIC DEVELOPMENT IN Aedes aegypti**

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Sexually dimorphic behaviors, including blood feeding and other sex-specific behaviors linked to reproduction, contribute to the global spread of mosquito-borne illnesses. Exploration of the developmental genetic basis for sexual dimorphism in mosquitoes has been hampered by a lack of methods to pursue functional developmental genetic studies. Although male and female spice variants of Doublesex (Dsx), a terminal transcription factor in the sex-determination pathway, have been detected in the dengue vector Aedes aegypti, the sex-specific expression patterns of these transcripts have not been detailed in developing tissues. Furthermore, although sex-specific Dsx splice forms are believed to regulate sex-specific development in mosquitoes, this hypothesis has not been functionally tested. We have implemented a powerful methodological toolkit, including molecular markers for developing neural tissue subtypes and siRNA nanoparticle-mediated gene targeting, to characterize Dsx function during A. aegypti development. These studies uncovered sex-specific Dsx expression patterns in developing tissues, including the pupal brain mushroom body, antennal lobe, and optic lobe. These results provide insights into previously unappreciated events in intracellular invasion by T. cruzi and suggest that the transgenic over-expression of appropriate endogenous proteins may significantly improve the protective capacity of viral vector based or live attenuated vaccines for T. cruzi and perhaps other pathogens.

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**UNTARGETED METABOLOMICS TO STUDY MULTIDRUG RESISTANCE IN LEISHMANIA DONOVANI**

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Chemotherapy is the most important tool for the control of visceral leishmaniasis, but its efficacy is jeopardized by the growing resistance and treatment failure against first-line drugs. Antimonials have become obsolete in the Indian subcontinent because of resistance, the efficacy of miltefosine is decreasing, and isolated cases of amphotericin B failure are of concern for both the scientific and health community. To further delay the emergence of resistance, the WHO recommended combinations of anti-leishmanial drugs. Until today, the mode of action and drug resistance mechanisms of the four available drugs (antimonials, miltefosine, amphotericin B and paromomycin) are poorly understood.

In this study, we performed untargeted LC-MS metabolomics to identify differentiating metabolites in Leishmania donovani promastigotes with induced resistance to single drugs and drug combinations. The most significant metabolic changes were found in the lines resistant (R) towards SbIII, amphotericin B (AmB) and miltefosine, and the combinations SbIII + AmB and SbIII + paromomycin. A clear additive or synergistic effect of the single resistant lines in a combination-resistant line was not found, but significant overlap between differential metabolites in the single R lines and their combination-resistant line was observed. The detected metabolic changes upon drug exposure in wild-type and resistant lines were experimentally validated and showed that resistant parasites have (i) an increased capacity for protection against oxidative stress, and (ii) an altered fluidity of the plasma membrane. Our results elucidate the mechanisms underlying the ability of Leishmania to develop resistance to combinations of anti-leishmanial drugs: single and multidrug resistant parasite cell lines show distinct metabolic adaptations, but these all converge on the same defensive mechanisms.

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**THE SACRIFICED FLAGELLUM OF Trypanosoma cruzi PROVIDES VERY EARLY TARGETS OF PROTECTIVE CD8+ T CELLS**

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The CD8+ T cells crucial to the control of infection with Trypanosoma cruzi, the agent of human Chagas disease, are predominantly directed at members of large gene families, including trans-sialidase (ts)-like proteins that are diverse and variable within and among T. cruzi isolates. We hypothesized that CD8+ T cell responses directed against sub-dominant, invariant proteins may induce more potent cross-strain protection from T. cruzi infection and thus sought to identify such targets. Investigation of the early events in intracellular invasion by T. cruzi revealed that trypanastigotes sacrifice their flagella via an asymmetric division during amastigogenesis and thus release flagellar proteins into the host cell cytoplasm. Peptides derived from flagellar proteins appear to be among the earliest T. cruzi proteins to enter the class I MHC processing and presentation pathway. Overexpression of one of these proteins, the abundant paraflagellar rod protein (PAR) 4, by transgenic T. cruzi enhances the potency of the PAR4-specific CD8+ T cell response, resulting in significantly improved control of a challenge infection. This enhanced protection despite the relatively low abundance of PAR4-specific CD8+ T cells was associated with the ability of PAR4-specific CD8+ T cells to detect host cell infection by T. cruzi significantly earlier than the immunodominant ts-specific T cells. These results provide insights into previously unappreciated events in intracellular invasion by T. cruzi and suggest that the transgenic over-expression of appropriate endogenous proteins may significantly improve the protective capacity of viral vector based or live attenuated vaccines for T. cruzi and perhaps other pathogens.

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**DISSECTING THE PROMASTIGOTE TO AMASTIGOTE DIFFERENTIATION IN Leishmania amazonensis**

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Leishmania is a genus of protozoan parasites transmitted by the phlebotomine sand fly that causes an array of disease ranging from cutaneous to visceral leishmaniasis resulting in significant morbidity and mortality. These parasites have a dimorphic life cycle, existing as a promastigote in the sand fly vector and an intracellular amastigote in the human host. While it is known that temperature and pH changes induce this change, the molecular basis of this differentiation is still unknown.

To uncover the molecular mechanisms behind this transition, we used Hsp90 inhibitors, which are also capable of inducing the promastigote to amastigote change. In addition to the expected transition we observed a dose-dependent morphological change in response to the inhibitor, a situation not seen when changing the temperature or pH. We hypothesize that the morphological variability induced by the Hsp90 inhibitor is
associated with intermediate states in the promastigote to amastigote transition not seen under normal circumstances. We have employed different techniques to characterize these forms such as morphological analysis by microscopy and FACS, and gene expression analysis by qRT-PCR. We have also generated reporter plasmids expressing fluorescent proteins under the control of promastigote and amastigote specific promoters to follow the transition between the two forms in response to the typical heat and pH as well as in the presence of the Hsp90 inhibitor. Finally, proteomic analysis of promastigotes, amastigotes and intermediate forms has helped us proposed effector proteins responsible for the parasite transition. Ultimately, these signaling molecules will represent potential drug targets against leishmaniasis, a neglected disease that badly needs new therapeutic options.

**A NOVEL FIELD-APPLICABLE MOLECULAR TEST FOR VISCERAL LEISHMANIASIS**

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In the Americas there are several thousand reported annual cases of full blown visceral leishmaniasis (VL) and mortality ranges between 7 and 10%. Furthermore, underreporting of VL is underestimating the actual disease burden. American VL is a zoonotic disease produced by Leishmania infantum and transmitted between dogs and humans by urbanized sand flies. Dogs are the principal reservoir hosts and, therefore, are critical targets for controlling urban transmission. The rK39 serological test is widely used to identify and remove infected dogs from the transmission cycle, but it has low sensitivity (<33%) to detect subclinically infected dogs. The lack of sensitivity of serological tests is considered as the main reason for the inefficacy of control programs. We have developed a sensitive and specific molecular test to detect L. infantum using a Recombinase Polymerase Amplification method coupled with Lateral flow reading (RPA-LF). This innovative isothermal amplification test is as sensitive as real time PCR (gold standard) but it does not require sophisticated equipment, is fast, and the result is read with the naked eye. This makes it ideal for point of care diagnosis and field epidemiology studies. In the lab RAP-LF detected <2 parasites in blood samples. Furthermore, evaluation of dog blood samples from an endemic area showed that RPA-LF detected more subclinically infected dogs than rK39 (51.9% vs. 14.3% positivity, respectively; p< 0.01). The RPA-LF fills the need for an effective diagnostic tool that will play a critical role in control interventions to reduce urban transmission of visceral leishmaniasis to humans.

**USE OF CLINICAL PREDICTORS AND MOLECULAR DIAGNOSIS TO IDENTIFY THE SPECIES RESPONSIBLE FOR SNAKEBITE IN RURAL NEPAL**

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Snakebite is an important medical emergency in rural Nepal. Correct identification of the biting species is crucial for clinicians to choose appropriate treatment and anticipate complications. This is particularly important for neurotoxic envenomation which, depending on the responsible species, may not respond to antivenoms. Adequate species identification tools are lacking. This study used a combination of morphological and molecular approaches (PCR-aided DNA sequencing from swabs of bite sites) to determine the relative contribution of venomous and non-venomous species to the snakebite burden in southern Nepal. The study also investigated the performance of baseline patient history and clinical characteristics to distinguish between cobra (Naja spp.) and krait (Bungarus spp.) bites. Out of 749 patients admitted to one of the 3 study centres with a history of snakebite, the biting species could be identified in 194 (25.9%). Out of these, 87 had been bitten by a venomous species, most commonly Naja naja (n=42) and Bungarus caeruleus (n=42). When both morphological identification and PCR/sequencing results were available, a 100% agreement was noted. Among patients bitten by venomous snakes, 71 (81.6%) presented with signs of envenomation including neurotoxic signs in 55 (77.4%). Being bitten at night (OR= ∞), while sleeping (OR= 56 [95% CI= 9.9-318.2]), indoors (OR= 9.4 [95% CI= 1.9-46.9]), and presenting with abdominal pain (OR= 23.4 [95% CI=3.8-142.5]) was associated with krait bite, and local signs of envenomation (e.g., edema) with bites by cobras and pit vipers. This study is the first to report the use of forensic genetics methods for snake species identification in a prospective clinical study, and to identify epidemiological and clinical features associated with krait and cobra envenomation in Nepal, thereby providing decisive guidance to improve patient care.
MULTIPLEX PCR DETECTION OF ENTEROPATHOGENS CAUSING TRAVELERS’ DIARRHEA FROM SPIKED STOOL SMEARS ON WHATMAN FTA CARDS USING MULTIPLEX PCR

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Molecular diagnostics offer significant advantages over conventional testing for enteropathogen detection in travelers’ diarrhea. There is limited data on the performance of PCR assays using stool samples and smeared stool cards. We sought to determine the limits of detection (LoD) for diarrheal pathogens using a multiplex PCR assay on spiked stool samples, and spiked stool smeared onto Whatman FTA® Elute cards. The multiplex PCR panel used a combination of Luminex xTAG analyte-specific reagents (ASRs) and in-house primers to detect: enterotoxigenic Escherichia coli (ETEC), enteropathogenic E coli (EPEC), enterohemorrhagic E coli (EHEC), Shigella spp., Salmonella enteritidis, S typhimurium, Campylobacter spp., norovirus [GI, GII], Giardia lambia and Cryptosporidium spp. LoD analysis was carried out by performing serial 1:10 dilutions, with the 4th and 5th dilution performed in triplicate. We evaluated the impact of prolonged storage of stool cards (1 and 3 months) on the LoD. The mean fluorescence intensity value for detection was ≥300. LoDs in spiked stool ranged from 10^2 to 10^4 cfu/g for bacterial pathogens, 10^2 to 10^3 pfu/g for Norovirus GI and GII respectively and 10^2 cyst/g for Giardia. Cryptosporidium could not be reliably detected in the stool or stool card. The LoD for ETEC, EHEC and S enteritidis were similar in the stool card and spiked stool, while the LoD for EAE, S typhimurium, Shigella, Campylobacter and norovirus GI and GII were 1 log higher in smeared stool cards compared to stool. Giardia had a lower LoD in the stool card (1 cyst/g). Significant variability (2-4 logs) in the LoDs was observed with prolonged storage of stool cards. LoDs for S enteritidis and ETEC increased from 10^1 cfu/g at 1 month to 10^3 cfu/g at 3 months, while EHEC decreased from 10^3 cfu/g to 10^2 cfu/g during the same time-points. Campylobacter increased from 10^1 cfu/g at baseline to 10^2 cfu/g at 1 month and could not be detected at 3 months. Further evaluation of the multiplex PCR assay using spiked specimens and diarrheal samples is needed before deployment in field studies.

REAL-TIME QUANTITATIVE PCR SURVEILLANCE OF GASTROINTESTINAL PARASITES IN A SYMPTOMATIC RURAL ARGENTINIAN POPULATION: INITIAL RESULTS OF THE LATIN AMERICAN MULTICENTER PARASITE STUDY (LAMPS)

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There are over 2 billion people infected with gastrointestinal (GI) parasites. Diagnosis of GI parasites relies on stool microscopy that has low diagnostic sensitivity and specificity. To better understand the parasitic etiology of abdominal symptoms, we implemented our rapid, high throughput, multi-parallel quantitative real-time PCR (qPCR) for the 8 common GI parasites including the helminths, Ascaris lumbricoides (Al), Ancylostoma duodenale (Ad), Necator americanus (Na), Strongyloides stercoralis (Ss), Trichuris trichiura (Tt) and protozoa, Cryptosporidium parvum (Cp), Entamoeba histolytica (Eh) and Giardia lambia (G1). This assay was used to analyze stool samples collected from 99 patients seen in a rural Argentinian clinic. For Al, qPCR identified (56.6%) positives whereas McMaster’s microscopy technique identified (47.5%) with 91.3% sensitivity and 90.5% negative predictive value (NPV). For hookworm, there was 37.4% detected by qPCR compared to 22.2% by microscopy (p < 0.05), with a 95.5% sensitivity and 98.4% NPV. Hookworm ova are indistinguishable by microscopy, but qPCR is species specific. While Na was the predominant hookworm detected, Ad DNA was detected in higher concentrations (0.61 versus 0.0001). This has important implications, since Ad is more aggressive in causing anemia. The difference between qPCR and microscopy was dramatically seen for G1 (63.6% versus 8.1%) with 55 additional positives for G1(p = 0.001). For Ss, qPCR identified (21.2%) positives whereas microscopy identified (3.0%)(p < 0.05) with 100% sensitivity and negative predictive value. qPCR was also able to detect polyparasitism by a factor of 7 compared to microscopy (p < 0.05). We have deployed a quantitative molecular based system that has improved diagnostic accuracy than stool microscopy. This is the first time this assay has been used in Argentina and has shown the prevalence of GI parasite infections in symptomatic patients. The results will help refine treatment options on a public health scale and lead to better health outcomes in endemic settings.

A HOSPITAL-BASED SURVEY ON HUMAN BRUCELLOSIS ITS ASSOCIATED FACTORS IN KANO METROPOLIS, KANO STATE- NIGERIA 2011

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The global burden of human brucellosis remains enormous; it causes more than 500,000 infections per year. It is one of the most widespread zoonotic diseases globally. Brucellosis is a multi-systemic, acute to chronic, disease characterized by fever, headache, joint pains, musculo-skeletal pains, sweating, malaise and body wasting. It is a severe debilitating disease that requires prolonged treatment, if untreated can result in permanent disability and loss of productivity. Human brucellosis presents a great variety of clinical manifestations making it difficult to diagnose clinically. In some endemic areas every case of human fever of unknown origin is assumed to be due to brucellosis. Therefore, the diagnosis must be confirmed by laboratory tests. A hospital-based survey on human brucellosis was undertaken to determine its prevalence, associated factors and the extent of missed diagnosis. A descriptive study and laboratory analysis were conducted. A suspected case was defined as any patient ≥ 15 years with fever (>37.5°C) plus any two of the clinical signs suggestive of human brucellosis. Socio-demographic features, clinical information and assessment of potential risk factors were obtained through questionnaires.

Their sera were screened with Rose Bengal plate test (RBPT) and positive cases were subjected to Enzyme Linked Immunosorbent Assay (ELISA) to confirm and detect evolution of the infection. A total of 250 suspected cases were enrolled and 50(20%) were confirmed positive. There were 131 males and 119 females. Mean age was 24years ± 16. Sensitivity and specificity were 90% and 85%. Of the 50 cases, 31(62%) were males; clinical signs consistent were recurrent fever (p 0.04), cough/sneeze (p 0.02) and osteoartthritis (p 0.00). Risk factors for human brucellosis were consumption of fresh milk (p 0.02) and local yoghurt kindirmo (p 0.00), keeping goats (p 0.00), assisting animal parturition (p 0.01), processing and eating raw meat (p 0.03 and p 0.00). Thirty six (72%) of the 50 cases were positive by IgG thus chronic infection. Diagnosis of human brucellosis is missed in hospitals in Kano. Risk factors identified were processing and consumption of unprocessed milk and meat; keeping goats and assisting parturition. Authorities should educate the populace on the risks of this disease and its prevention; physicians should raise the index for diagnosis of human brucellosis in patients that present with signs suggestive of it.
UTILITY OF PROVISIONAL DIAGNOSES OF DENGUE FROM A SENTINEL-ENHANCED SURVEILLANCE SYSTEM IN PONCE, PUERTO RICO

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Dengue and a number of other acute febrile illnesses (AFI) present with similar signs and symptoms. The unavailability of sensitive and specific rapid diagnostic tests poses a significant challenge to early and accurate diagnosis of dengue because early identification is important for management of patients to reduce mortality. We used data from the first year of a Sentinel Enhanced Dengue Surveillance System (SEDDS) in Ponce, Puerto Rico between May 7, 2012 and May 6, 2013 to evaluate the accuracy of clinicians’ diagnoses of patients presenting with an AF (N = 2,027) in a dengue-endemic area. A classification system was developed, and diagnoses were grouped into syndromes defined as dengue, influenza, viral infection, gastrointestinal, respiratory, genitourinary, and other. Laboratory diagnostic testing was performed for 23 pathogens associated with AFI, including the four dengue viruses (DENV-1-4). Of 1,152 cases in which an infecting pathogen was identified, 579 (50.3%) were DENV-positive, 301 (26.1%) were positive for influenza, and 272 (23.6%) were positive for another pathogen. Among the DENV-positive cases, 243 (42%) received a provisional clinical diagnosis of dengue, while 183 (32%) and 60 (10%) were diagnosed as viral infection and respiratory illness, respectively. At initial clinical presentation, the DENV-positive cases with a provisional diagnosis of dengue had a higher proportion with rash (53% vs. 0.38%; p = 0.001) and diarrhea (44% vs. 32%; p = 0.005), more pronounced leukopenia (3,100 vs. 4,600; p < 0.001), thrombocytopenia (93,203 vs. 155,937; p < 0.001), and elevated aspartate transaminase (175 vs. 112; p = 0.001). Multiple logistic regression modeling and stratification analysis by age group will be performed to determine the clinical and laboratory factors most associated with a clinical and laboratory diagnosis of dengue. This will both inform physicians in their diagnostic approach to dengue and facilitate the use of provisional diagnoses as an early detection tool for increases in dengue in Puerto Rico.

CARDIAC INVOLVEMENT IN PEDIATRIC DENGUE; A SERIAL ECHOCARDIOGRAPHIC STUDY

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Dengue is the most prevalent vector-borne viral infection of humans. There have been case reports of severe cardiac involvement including myocarditis in dengue, although the true incidence is not well defined. To better characterize cardiac involvement we performed daily echocardiographic studies in children with suspected dengue from 2010 to 2012 at a tertiary care center in Bangkok, Thailand. Plasma levels of cardiac troponin-T were measured daily. We analyzed 180 confirmed dengue cases classified as dengue fever (DF) and dengue hemorrhagic fever (DHF) according to the 1997 WHO case definitions. There were 119 cases of DF, and 12, 27, 21, and 1 cases of DHF grade 1, 2, 3, and 4, respectively. On the day of presentation (approximately 4.5 days of illness), DHF cases had significantly lower stroke volume, left ventricular end-diastolic volume, ejection fraction, and cardiac index, and higher systemic vascular resistance compared to cases with DF (p < 0.001 to < 0.05). Flow and tissue Doppler studies demonstrated a decreased early diastolic component of flow at the mitral valves as well as decreased tissue plane excursion of the mitral valves in DHF cases. These early differences were attributed to findings from DHF cases with ultrasound-documented plasma leakage at this time point. Serial studies showed that differences in hemodynamic and cardiac functional indices between DF and DHF were most pronounced around the time of defervescence. There were no clinically evident cases of cardiac failure in this study. An abnormal ejection fraction (EF <55%) was observed in 6%, 9%, 18%, and 32% of cases of DF, DHF grade 1, DHF grade 2, and DHF grades 3/4, respectively (p < 0.001). However, the abnormal EF was transient and usually coincided with the development of plasma leakage. No cases with abnormally elevated troponin-T levels were identified. We conclude that cardiac involvement is uncommon in pediatric dengue and is not a major contributing factor for dengue shock syndrome. Subtle changes in cardiac function are common but appear to be transient and may reflect plasma leakage and volume status.

CHAGAS DISEASE TRANSMISSION AND CARDIAC MANIFESTATIONS AMONG TEXAS BLOOD DONORS

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Although well established as an important cause of morbidity and mortality in Latin America, Chagas disease is increasing in recognition as a potential cause of heart disease in the United States. Screening of blood donors in the greater Houston area for Chagas disease (Trypanosoma cruzi infection) began in 2007. The transmission mechanisms and potential cardiovascular manifestations of these T. cruzi positive individuals have not been previously studied. An one-time assessment of Houston area blood donors that screened positive from 2008-2012 for T. cruzi infection (n=30) included 1) blood draw for confirmatory T. cruzi diagnostic testing, 2) a questionnaire to evaluate source of infection, cardiac symptoms and health co-morbidities, 3) high-sensitivity troponin T biomarker evaluation, 4) an electrocardiogram, and 5) an echocardiogram if electrocardiogram was abnormal. We found 57% (17/30) of blood donors had two or more positive tests confirming infection. Of those with confirmed infection, 41% (7/17) had an echocardiographic abnormality consistent with Chagas cardiomyopathy. In addition, 36% (6/17) were suspected to be locally acquired cases. High-sensitivity troponin serum levels were increased in a linear manner with cardiac severity. Cardiologists should consider the changing transmission dynamics associated with Chagas disease in the southern United States and should consider Chagas disease in patients who may have clinically-compatible electrocardiogram or cardiomyopathy, even if the patient has no history of residing in a Chagas-endemic country.

EXAMINATION OF 2006-2013 MALARIA INCIDENCES IN RELATION TO THE SCALING OF PREVENTIVE CONTROL INTERVENTIONS IN MULEBA DISTRICT IN NORTHWEST TANZANIA

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Following outbreak of malaria in Muleba district in 2006, intensive malaria control interventions, including Indoor Residual Spraying (IRS), and distribution of LLINs were introduced. IRS was introduced as an outbreak preemptive measure in selected areas of the district in 2007 and 2008, with gradual scale-up of one round of IRS per year covering the entire
district until 2011. About 80,000 LLINs were distributed to under-fives in 2009 and 170,000 nets distributed in early 2011 to the remaining population. However, due to universal attainment of LLINs in 2011, as well as limited resources, IRS was scaled down in 2012-2013, with the last round targeting only 25% (17,000 house structures) of the district. After 2011, no significant efforts have also been made to keep-up LLIN coverage. We compared health facility based malaria incidence per 1000 population of respective village catchment areas from Jan 2006-May 2013, a period in which a total of 352,488 out-patient malaria cases were recorded. District incidence in 2006 (pre-control period) was 118 per 1000, with the rate declining to its lowest level of 37 per 1000 in 2010. This peak decrease of 67% in malaria incidence at 2010 compared to 2006 was most likely due to intensification of effective multiple control interventions over that period. However from 2011 onward, scale down and/or low maintenance of control interventions, coupled with reported stock-outs of ACTs, and low net use (68%-THMIS 2011-12) likely contributed to a rise in malaria incidence; by 2013, increasing to 181 per 1000 (adjusted for 12 month period). The 2013 rate was 3500 higher than the pre control era of 2006. Muleba’s case suggests that scaling-down malaria control efforts has resulted in loss of initial gains in controlling malaria. This could have serious implications with possible rebound of malaria to pre-intervention levels as well as frequent malaria outbreaks. Once the control of malaria has reached to manageable levels, it is important to advocate for effective monitoring and response so as to sustain the fight against malaria.

FOCAL SCREENING AND TREATMENT (FSAT) CAMPAIGN IN FOCI OF MALARIA TRANSMISSION: IMPACT ON MALARIA PREVALENCE AND COMPLEXITY

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Mass screening and treatment campaigns have had limited success in curbing malaria transmission, most likely due to the prevalence of subpatent infections missed using the field based diagnostic tools. It has been shown that subpatent malaria infections are more likely to occur in households where patent infections are identified. Therefore it is possible that a more focal approach to treatment campaigns using patent infections as a marker for the presence of a subpatent reservoir may be more effective at reducing the infectious reservoir in humans. To test this strategy, a focal screening and treatment (FSAT) intervention in foci of malaria transmission (prevalence 18.6%, 95% CI. 10.8-26.5%) was conducted in the western Kenyan highlands as part of a larger cluster randomized trial. All consenting individuals under 15 years old or febrile adults residing within the foci were tested for malaria with a rapid diagnostic test (RDT) and if found positive all individuals residing in the compound received a curative dose of artemisinin combination therapy. Blood spots on filter paper were collected from all household members (N=2083), regardless of RDT result. To assess the impact of FSAT, parasite prevalence and complexity using nested polymerase chain reaction and merozoite surface protein-2 genotyping was determined. The impact of the FSAT approach on parasite prevalence and allelic diversity was assessed with two follow-up cross-sectional surveys at 2-month intervals post intervention. Of the compounds within the intervention foci, 168 of 406 (41.4%) households sampled received treatment. Twenty-seven households declined or had no individuals present during time of sampling. Initial results indicate that, at baseline, PCR prevalence in compounds with a patent infection and therefore targeted for treatment was 34.0% compared to 11.1% in those that had no patent infections (p<0.0001) with the FSAT approach successfully identifying 78.3% of parasite carriers. This strategy could provide a useful and operationally attractive alternative to detecting subpatent infections in foci of infection.

SPATIAL PATTERNS OF MALARIA TRANSMISSION OVER ONE YEAR IN A CLINIC CATCHMENT AREA OF CHONGWE DISTRICT, ZAMBIA

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As malaria transmission declines, it may be more cost-effective and efficient to focus control efforts on high-risk areas. Describing spatial patterns of malaria transmission may identify opportunities to develop and improve current interventions. Individuals testing positive for malaria at a single clinic in Chongwe District, Zambia, were approached for enrollment in the study. These index cases were administered a questionnaire and a blood sample was taken for a rapid diagnostic test (RDT) and microscopy. Upon enrollment their homes were visited, all household contacts were enrolled, administered a questionnaire, and gave a blood sample for RDT and microscopy. GPS coordinates were recorded at each household. If an individual from a previously enrolled household sought care, these were considered re-infection households. Spatial patterns of malaria in this clinics catchment area were analyzed using SaTScan for cluster detection and programs from R statistical software to describe patterns of spatial clustering. From June 2012 to June 2013 a total of 472 index cases and 1,901 household contacts (43% RDT positive) were enrolled. Two statistically significant space-time clusters of index case households were identified controlling for age and gender; one in December 2012 and one in January 2013 (the peak transmission season). No clustering of RDT positive household contacts was identified; factors associated with being an RDT positive household contact included distance to the clinic, socioeconomic status, and bednet ownership (p<0.05 for all comparisons). In conclusion, transmission in this area appears to be high with a seasonal peak in incidence during the rainy season. Two space-time clusters of index case households were detected during the peak transmission season in close proximity to the clinic. No clustering of malaria comparing household contacts was detected likely because of high transmission in the entire catchment area.

CESSATION OF COTRIMOXAZOLE PROPHYLAXIS IN HIV EXPOSED CHILDREN DOES NOT INCREASE THE INCIDENCE OF MALARIA AND OTHER MORBIDITIES

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HIV-exposed infants born to mothers with HIV should receive cotrimoxazole prophylaxis (CPT) until HIV infection can be excluded and the child is no longer exposed through breastfeeding. As daily CPT provides prophylaxis against malaria, it could modulate the development of malaria-specific immunity and increase the incidence of malaria after it is stopped (a rebound effect). To determine if this is the case, we investigated the incidence of malaria and other morbidities during and after CPT in the first two years of life in HIV exposed children in southern Malawi. A cohort of 500 HIV exposed children on CPT for 12 months from the age of 6 weeks to one year and 500 non-HIV exposed children not on CPT were followed until their second birthday. HIV exposed children were recruited at the Prevention of Mother to Child Transmission (PMTCT)
HIV clinic while location and age-matched non-HIV exposed children were recruited from the same population. The incidence of malaria, all-cause morbidity, admissions and mortality was compared during the first and second year of life in the two study groups using multivariate, negative binomial regression analyses. In year-1, the incidence of uncomplicated malaria in HIV exposed children was 65% lower compared to the non-HIV exposed group (IRR = 0.35, 95% CI 0.25, 0.49, p<0.001). In year-2 the incidence of malaria in the HIV exposed group was similar to that in non-HIV exposed group (IRR 0.94, 95% CI: 0.53, 1.68, p=0.839) among the first 315 children that completed the follow-up period. The same pattern was observed for all-cause morbidity and hospital admissions. CPT was associated with marked reductions in the incidence of uncomplicated malaria, all-cause morbidity and hospital admissions during the period in which it was given. The follow-up in year-2 is on-going but preliminary results suggest that the incidence of malaria does not increase after cessation of CPT at around 14 months of age.

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STANDARDIZED MULTIDISCIPLINARY METHOD FOR THE EVALUATION OF THE EFFECTIVENESS OF MALARIA CONTROL INTERVENTIONS

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In a global context of international funding plateauing, control programs are looking for innovative options to better perform with a constant budget. In absence of groundbreaking new control tools, the only option is to make the best use of the control measures that are already available. In order to guide policy making, we propose to carry out a broad and comprehensive evaluation of interventions deployed in a given setting, including an evaluation of their effectiveness and the identification of the key determinants affecting their effectiveness. We wrote and selected 20 Standard Operating Procedures (SOP) through a multi-country multidisciplinary workshop, including experts from Belgium, Benin, Cameroon, Côte d’Ivoire, France, Madagascar and Niger. These SOP cover the following fields: analysis of health systems (1), anthropology (1), biological diagnosis (4), drugs resistance (2), entomology (3), epidemiology (4), health economics (2), and immunology (3). All 20 SOP are combined in a single toolbox that is being implemented in Benin and Madagascar in 2014. Preliminary results will be presented. For each malaria control intervention the following indicators are evaluated: coverage, protective effectiveness against infection, protective effectiveness against morbidity, cost-effectiveness, socio-anthropological determinants of effectiveness, entomological determinants of effectiveness (vector behavior, insecticide resistance) if applicable, and in vivo and in vitro measure of antimalarial drug resistance. Results also include an analysis of health systems and management of malaria control in general. The toolbox-named PALEVALUT- will be further reviewed before and after implementation in Cameroon, Côte d’Ivoire and Niger in 2015. The whole tool will be soon available with free access on the internet.

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CHANGING HEALTH CONDITIONS AND THE DECLINE OF ALL-CAUSE UNDER-FIVE MORTALITY IN RWANDA 2000-2010: A DECOMPOSITION ANALYSIS

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All-cause child mortality (ACCM) has declined rapidly over the past decade in Rwanda, from 196 deaths per 1,000 live births during 1996-2000 to 76 deaths per 1,000 live births during 2006-2010. Rapid development and improvements in many socioeconomic and health indicators between 2000 and 2010 are likely to have played a role in determining these declines. For example, the percentage of households with improved toilets increased from 9% to 72%, the percentage of households with mosquito nets rose from 8% to 94%, and the percent of children whose mothers used ITNs increased from 5% to 77%. In this analysis, decomposition models were used to describe the decline in ACCM between two Demographic and Health Surveys (DHS) (Rwanda DHS 2000 and Rwanda DHS 2010). The relative importance of changes in the distribution of key socioeconomic and health-related variables (coverage) and changes in the magnitude of association between these variables and ACCM (coefficients) across these surveys was examined. The model explains a decline of 83 deaths per 1,000 live births from 2000 to 2010, or 69% of the reduction observed from the surveys. Results show that the combined effect of all of the changes in intervention coverage explained almost all (99%) of the modeled reduction in ACCM. Holding other factors constant, the observed increase in household bednet ownership could have explained as much as 45% of the modeled decline in ACCM between 2000 and 2010, and 31% of the observed decline in ACCM from survey data. The increasing percentage of children whose mothers used ITN between 2000 and 2010 could have explained an additional 4.2% of the modeled reduction in ACCM, presumably through reductions in neonatal mortality. Improvements in coverage of antenatal care and increasing birth intervals could have explained an additional 13.4% and 3.1% of total modeled ACCM decline, respectively. Changes in the distribution of household wealth and of multiple births between surveys would have led to small but significant increases in ACCM, holding other variables constant. These results clearly show the important role of malaria control interventions in the reduction of child mortality in Rwanda.

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VARIATIONS IN MALARIA EPIDEMIOLOGY IN RELATION TO VECTOR CONTROL COVERAGE IN NINE DISTRICTS OF UGANDA

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Malaria has been a major public health problem in Uganda. The disease is highly endemic in 95% of the country where 90% of the population live. A scale up of malaria control interventions in the past decade coupled with environmental, social and economic factors appears to have contributed to a changing pattern of the epidemiology of the disease in the country. This study was part of a larger study on the distribution of insecticide resistance and resistance management. The aim of this component of the study was to understand the impact of varying coverage of vector control interventions on the observed epidemiological pattern of malaria
in different groups of districts. Three groups of nine districts were selected among all highly endemic districts that were in existence since 2001, using criteria based on coverage with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS): 1) Districts that had undergone several rounds of IRS (Apac, Gulu, Pader), 2) Districts where LLINs had been distributed but no IRS had taken place (Kayunga, Kiboga, Mbale), and 3) Districts that had not received IRS or LLINs as part of large campaigns at the time of selection (Bugiri, Mayuge, Soroti). Forty-five cluster were sampled in the nine districts in which a cross-sectional survey was conducted during September-October 2012 transmission season. A total of 528 interviews were conducted in the 45 clusters, an average of 12 households per cluster. Blood samples were gathered from all household members and mosquitoes were collected using the pyrethrum spray catch method. Anopheles gambiae s.l. and A. funestus s.l. were the main vectors collected. Prevalence of Plasmodium falciparum and density of malaria vectors varied between groups of districts with different levels of vector control coverage. The highest prevalence was observed in the unsprayed districts that had not received IRS or LLINs as part of large campaigns distributed but no IRS had taken place (Kayunga, Kiboga, Mbale), and 2) Districts where LLINs had been distributed but no IRS had taken place (Kayunga, Kiboga, Mbale), and 3) Districts that had not received IRS or LLINs as part of large campaigns at the time of selection (Bugiri, Mayuge, Soroti). Forty-five cluster were sampled in the nine districts in which a cross-sectional survey was conducted during September-October 2012 transmission season. A total of 528 interviews were conducted in the 45 clusters, an average of 12 households per cluster. Blood samples were gathered from all household members and mosquitoes were collected using the pyrethrum spray catch method. Anopheles gambiae s.l. and A. funestus s.l. were the main vectors collected. Prevalence of Plasmodium falciparum and density of malaria vectors varied between groups of districts with different levels of vector control coverage. The highest prevalence was observed in the unsprayed group with historically low coverage with LLINs. Pyretroid resistance was widespread in nearly all districts. The role of vector control interventions is discussed in the light of observed variations in malaria epidemiology.

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PERCEPTIONS OF COMMUNITY-LED TOTAL SANITATION ON SANITATION BEHAVIORS IN RURAL ZAMBIA: A QUALITATIVE STUDY

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Despite emerging initiatives in the sanitation sector, inadequate sanitation remains a leading global contributor to morbidity and mortality in children. The government of Zambia, UNICEF, and partners are engaged in the rollout of an ambitious hygiene and sanitation program aimed at reaching 3 million new users of improved sanitation hand washing practices. One of the key approaches used to reach this objective is Community Led Total Sanitation (CLTS), a grassroots, subsidy-free strategy with the goal of changing sanitation practices to create open defecation free (ODF) villages. During CLTS, community members participate in activities that lead them to realize and declare that they are “eating each other’s stool”. This qualitative study explores community members’ and stakeholders’ sanitation behaviors, knowledge and perceptions during early CLTS implementation in Zambia. We conducted 67 key informant interviews and 24 focus group discussions with community members and other WaSH stakeholders in 6 districts in Zambia who were selected using purposive sampling based on recommendations from project staff. The study was conducted in July and December of 2013, 12 to 18 months after initiation of CLTS implementation. According to key informants, triggering activities elicited a strong emotional response involving shame, disgust and peer pressure which persuades individuals to build and use latrines. Pride and dignity were also reported as important influential factors, as individuals and communities become empowered. Traditional leaders and community Sanitation Action Groups have strong hierarchical influence that is also persuasive in changing behaviors. Respondents frequently mentioned that children help to influence their families to improve sanitation behaviors. Overall, participants reported an increase in latrine construction and usage after triggering; however, poor (e.g. rocky or sandy) soil conditions and taboos prohibiting certain family members from sharing the same toilet act as barriers in many areas. CLTS results in powerful individual and community emotional responses that serve to encourage construction and use of latrines and adoption of improved hygiene practices. This formative research suggests that CLTS has potential to be an effective approach for improving sanitation beliefs and behaviors in Zambia, which in turn may result in improved child health.

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FACTORS ASSOCIATED WITH PUPIL LATRINE USE IN KENYAN PRIMARY SCHOOLS

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Little empirical data exist on how the type, design, and maintenance of school latrines affect pupil latrine use. The purpose of this study was to characterize how school sanitation conditions are associated with latrine use patterns in 60 primary schools in Nyanza Province, Kenya. We conducted a longitudinal assessment, using structured observations to measure both latrine conditions and latrine use during the 30 minute morning break. We modeled the association between pupil to latrine ratio and latrine use (pupil used a latrine vs. not), using multivariable logistic regression. Lower pupil to latrine ratios were associated with increased latrine use, with the odds of use more than doubling when comparing schools with the lowest pupil to latrine ratios (<15:1) to schools with the highest ratios (>75:1; odds ratio=2.18, 95% CI: 1.69-2.80). We also modeled the association between different latrine characteristics and the number of uses at specific facilities, using multivariable negative binomial regression. Pupils preferred to use newer latrines over older latrines (incidence rate ratio (IRR)=1.13, 95% CI: 1.01-1.26), and also preferred ventilate improved pit latrines (IRR=1.15, 95% CI: 1.00-1.31) and urinals (IRR=1.96, 95% CI: 1.57-2.44) over prefabricated plastic latrines (IRR=0.68, 95% CI: 0.54-0.85) and traditional pit latrines (referent). An increased number of latrines in a block (a group of conjoined latrines) was associated with increased use at that block, although the increase in use was not proportional to the block’s added capacity (IRR for two doors compared to one=1.13, 95% CI: 0.96-1.33; IRR for four doors=1.71, 95% CI: 1.39-2.10; IRR for six or more doors=2.26, 95% CI: 1.73-2.93). We found some evidence suggesting latrine dirtiness was a deterrent to pupil latrine use, although the 95% CI included one (IRR=0.91, 95% CI: 0.82-1.02). Our study provides insights into factors affecting latrine use, potentially leading to a better allocation of resources for school sanitation, with the end goal to improve pupil’s health and educational outcomes.

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THE IMPACT OF HOUSEHOLD IMPROVED SANITATION ON HUMAN FECAL EXPOSURE IN RURAL INDIA: APPLICATION OF MICROBIAL SOURCE TRACKING TECHNIQUES

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In India, where 626 million mostly rural people practice open defecation and 535,000 children under age five die each year from diarrhea, improved sanitation is clearly necessary to reduce fecal exposure. As part of a large cluster randomized controlled sanitation trial in Odisha, India, to measure the impact of household latrines on diarrhea diseases, human fecal contamination and exposure in intervention and control communities was measured and compared using microbial (fecal) source tracking (MST) methods based on host specific genetic markers of the gut organism Bacteroidales. MST is an emerging approach to discriminate and quantify human and other animal sources of fecal contamination. The goals of this study were (1) to measure levels of total, human and animal fecal contamination in the public domain (improved and unimproved water sources) and the domestic household domain (household stored drinking water, mother and child hand rinses) of fecal-oral disease transmission, and (2) to compare observed levels of total and human

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fecal contamination in intervention and control villages for a better understanding of pathways by which latrines reduce exposure in rural India. In intervention (n = 30) and control (n = 30) villages, 20-L samples were collected from improved (public deep and private shallow tube wells for drinking, n = 209) and unimproved (community ponds for bathing and hygiene activities, n = 94) water sources along with samples from 5 to 6 households per village, comprising 300-mL of household stored drinking water (n = 348), and hand rinses of mothers (n = 349) and children (n = 346), during the monsoon seasons of 2012 and 2013, after the intervention had ended. After concentration by filtration, total-, human-, and ruminant-associated markers were measured using quantitative PCR assays validated in India. Our results show that despite relatively high detection rates of total Bacteroidales in the public domain (55 to 100%), human-associated markers were rarely detected (1 to 5%). In contrast, detection rates of human-associated markers were significantly higher in the domestic domain (18 to 21%) while total Bacteroidales detection rates were lower (53 to 70%). We present and discuss results of in-depth analyses of observed levels of human fecal exposure, including effects of household latrines on the different pathways tested in the public and domestic domains of disease transmission in study communities.

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IMPACT OF A COMMUNITY-LED TOTAL SANITATION INTERVENTION ON CHILD HEALTH IN RURAL MALI: EVIDENCE FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Globally 2.5 billion people lack access to an improved sanitation facility; only 15% of rural households use improved sanitation in Mali (JMP 2014). Community-led total sanitation (CLTS) uses participatory approaches to mobilize communities to build their own toilets, facilitate sustained behavior change, and eliminate open defecation. Although CLTS has been implemented in over 50 countries, there is a lack of rigorous and objective data on its impacts on sanitation behaviors and child health. This cluster-randomized trial evaluated a CLTS program implemented by the government of Mali with support from UNICEF among 121 villages from the Koulkoro district of Mali. Household survey data (N=4299) collected 1.5 years post intervention delivery revealed that CLTS almost doubled access to private latrines (65% vs. 35%), as well as reduced open defecation rates by over 70% among adults and by 50% among children (p<0.001). CLTS households were half as likely to have human feces observed in courtyards (p<0.001). Latrines in CLTS communities were 3 times more likely to have soap and have a cover over the pit, as well as 20% less likely to have flies visible inside (p<0.001). Among children under five, CLTS did not reduce the case definition of diarrhea (relative risk [RR] 0.96, 95% CI 0.80-1.16), although risk of loose stool as measured by an image chart was reduced by 24% for those children not exclusively breastfeeding (RR 0.76, 95% CI 0.59-0.98). When accounting for baseline height, children under five in CLTS villages were taller (+0.16 height-for-age Z-score, 95% CI 0.0-0.32) and less likely to be stunted (RR 0.87, 95% CI 0.75-1.0). Improvements in child weight (+0.09 weight-for-age Z-score, 95% CI -0.04 to 0.21) and a reduction in the proportion of children underweight (RR 0.86, 95% CI 0.71-1.04) were observed but were not statistically significant. This study provides evidence that a pure behavioral intervention with no monetary subsidies substantially increased access to sanitation facilities in rural Mali. CLTS may have improved child growth through pathways other than preventing diarrhea, such as lessening the subclinical condition of environmental enteropathy through potential reduced exposure to environmental fecal contamination.

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THE EFFECTIVENESS OF A RURAL SANITATION INTERVENTION ON HEALTH AND ORISSA, INDIA: A CLUSTER-RANDOMIZED, CONTROLLED TRIAL

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We undertook a cluster-randomized, controlled trial to assess the effectiveness of a rural sanitation intervention under the Government of India's Total Sanitation Campaign (TSC) to prevent diarrhoea, child malnutrition and soil transmitted helminth infection. 100 rural villages in Orissa, India were selected for participation in the study. We enrolled households with a child <4 years or a pregnant woman at the time of enrolment. Following a baseline survey, 50 villages were randomized to the intervention arm and undertook latrine promotion and construction in accordance with the TSC; control villages received no intervention. Following the implementation period, we collected and assayed stool samples for soil-transmitted helminths (STH) and provided deworming tablets to assess the rate and intensity of reinfection; we also measured the height of children <2 to assess HAZ. Thereafter, we visited study participants 7 times over 18 months, collecting self-reported diarrhoea prevalence for children <5 (primary outcome) and all members of the household as well as weights for children <5 to assess WAZ. At endpoint stools were again assessed for STH eggs and heights measured for children <2. In sub-samples of households, we also assessed faecal contamination of household water supplies, hands of child caretakers and sentinel toys given to children and monitored the density of synanthropic flies that can serve as mechanical vectors of faeces. We assessed latrine coverage and use throughout the study villages with spot checks at mid-line and endline of the surveillance period. The intervention increased mean village-level latrine coverage from 9% to 63% in intervention villages compared to an increase of 8% to 12% in control villages. 63% of households with any latrine reported using them. Health surveillance data was collected from 1437 households with children under 5 in the intervention arm (1919 <5s, 10014 individuals overall), and 1465 (1916 <5s, 10269) in the control arm. The intervention had no effect on diarrhoeal disease among children <5 (period prevalence ratio 0.97, 95%CI: 0.83-1.12) or all ages (1.02, 95%CI: 0.88-1.18). Neither did it impact HAZ for children under 2, WAZ for children <5, or the prevalence or egg count of STHs. There was no evidence that the intervention impacted contamination of household drinking water, hands or sentinel toys, or impacted density of flies in food preparation areas.

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CURRENT STRATEGIES AND CHALLENGES TO IMPLEMENTING HANDWASHING HARDWARE IN HUMANITARIAN EMERGENCIES

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Diarrhoea and respiratory infections are prevalent in humanitarian emergencies but can be prevented by handwashing with soap. The diarrhea and respiratory infections are prevalent in humanitarian emergencies but can be prevented by handwashing with soap. The diarrhea and respiratory infections are prevalent in humanitarian emergencies but can be prevented by handwashing with soap.

astmh.org
staff of United Nations agencies, non-governmental and government organizations. They reported that communal handwashing stations are common in the acute phase of an emergency but maintenance of soap and water are problematic due to lack of ownership. Organizations aim to distribute soap according to SPHERE standards but SPHERE does not recommend quantities needed for handwashing. Consistency and frequency of distribution of water dispensers and soap is highly variable in the post-acute phase due to funding constraints, prioritization by response agencies, and local market availability. There is a tradeoff between using local materials which are lower in cost and readily available and improved materials that are typically costly but more desirable. Sanitizer was not deemed a viable option for community-wide use due to acceptability, cost and sustainability. Evaluations of hardware uptake and acceptability are rare despite perceived utility of such data. Respondents indicated a strong interest in identifying hardware that is most acceptable among what is already available to emergency-affected populations. Assessing the type of soap most attractive to populations and how hardware choices affect handwashing behavior could provide useful guidance to improve handwashing promotion during emergencies.

**HANDWASHING BEHAVIOR CHANGE STRATEGIES IN HUMANITARIAN EMERGENCY SITUATIONS: THE PERSPECTIVES OF EXPERTS**

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Handwashing can prevent diarrhea and acute respiratory infections, but is practiced infrequently in long-standing refugee camps. Little information is available on handwashing behavior change strategies targeting displaced persons during emergencies. We conducted key informant interviews between February and April 2013 with professionals supporting water, sanitation and hygiene (WASH) services for emergency-affected populations to understand behavior change strategies and to identify research gaps. We used purposive and snowball sampling to identify experts involved in handwashing promotion. Twelve respondents representing United Nations agencies, non-governmental organizations, and a government agency were interviewed. Respondents reported that technical and infrastructure aspects of WASH are not well coordinated with behavioral approaches; behavior change strategies are less of a priority. Typically, implementing organizations set no specific goals related to improving handwashing practices. Respondents described a dearth of behavior change expertise at the global, regional and local levels. Information on pre-existing knowledge and practices related to hand hygiene is generally not collected prior to implementation of handwashing promotion. Messages focusing on health benefits are given over prolonged timeframes, with little effort to understand motivators to handwashing or assess effectiveness and modify messages. A relatively unskilled workforce is expected to deliver often complex, participatory methods to improve behavior. Effectiveness of behavior change strategies is rarely assessed. Our findings underscore the need to strengthen behavior change expertise at all levels. The current reliance on anecdotal evidence hampers promotion of appropriate handwashing behavior. Lack of understanding of pre-existing behaviors and motivators for handwashing restricts adaptations to the local context and likely undermines behavior change efforts. Establishing specific behavior change objectives and developing contextually specific approaches could improve the effectiveness of handwashing promotion in emergency settings.

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**DETERMINANTS OF MORTALITY AMONG HUMAN IMMUNODEFICIENCY VIRUS AND TUBERCULOSIS (HIV/TB) CO-INFECTED PATIENTS IN AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA**

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Management of HIV and tuberculosis co-infection remains a major challenge for clinicians and public health authorities. We conducted a prospective cohort study to determine risk factors of death among HIV/TB co-infected patients in Aminu Kano Teaching Hospital, Kano, Nigeria. We recruited 160 consenting, newly diagnosed HIV/TB co-infected patients 18 years or older between June and December 2012. We diagnosed TB by clinical features and any of positive sputum acid fast bacilli (AFB), chest radiographic features of tuberculosis or biopsy proven TB adenitis. We excluded patients with previous exposure to anti-TB or anti-retroviral drugs. We administered structured questionnaires to collect socio-demographic, clinical and laboratory information. Patients started on highly active antiretroviral therapy (HAART) within 8 weeks of starting anti-TB were considered as commencing early treatment; delayed commencement defined as starting HAART after 8 weeks of starting anti-TB. Patients who reported ever missing a dose of either or both of anti-TB and HAART drugs were considered as having sub-optimal adherence. All patients were followed up for 6 months. We conducted bivariate and multivariate analyses to determine independent risk factors of death during the study. A total of 71 (44.4%) patients were females. The median (IQR) age was 33 (28 – 401) years). The mean (range) hospital stay for admitted patients was 20 (5 – 37) days. The mean CD4 counts for patients who commenced early and those who delayed treatment were 144 cells/mm3 and 112 cells/mm3 respectively. On bivariate analysis, sputum AFB positivity HR (P-value): 3.1 (0.01); Hepatitis C co-infection HR (P-value): 9.8 (0.03) and sub-optimal adherence HR (P-value): 4.3 (0.001) to increase risk of dying among HIV/TB co-infected patients. In contrast, early commencement of HAART HR (P-value): 0.2 (<0.001) was found to decrease risk of dying. On multivariate analysis, risk of dying was reduced by early commencement of HAART HR (P-value): 0.2 (0.005), while hepatitis C co-infection HR (P-value): 12.3 (0.03) and sub-optimal adherence HR (P-value): 2.8 (0.04) remained independent risk factors of death. Early commencement of HAART among HIV/TB co-infected patients improves survival. Clinicians should adhere to universally accepted guidelines on timing of commencement of HAART. Sub-optimal adherence should be addressed by strengthening adherence units in HIV programs.

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**RECOVERY OF CD4+ T CELL COUNTS IN HIV-ASSOCIATED TUBERCULOSIS ACCORDING TO AGE, NUTRITIONAL STATUS AND ANTIRETROVIRAL THERAPY**

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We quantified associations between age and the monthly change in absolute CD4+ T cell count among HIV-infected tuberculosis (TB) patients during six months of TB therapy with or without concurrent antiretroviral therapy (ART). We determined whether this association is modified by sex and baseline nutritional status. The parent study for this secondary analysis was a randomized clinical trial of concurrent versus delayed ART during TB treatment. The study population included 208 non-pregnant, HIV-seropositive TB patients who had CD4+ T cell counts >350 cells/µL and were naive to anti-retroviral therapy. Age at enrollment was
defined in years within categories as ≤24, 25-29, 30-34, 35-39 and ≥40. Nutritional status was classified as normal (BMI > 18.5 kg/m²) or low (BMI ≤18.5 kg/m²). Multivariate random effects linear regression models were used to estimate mean differences in absolute CD4-cell count in relation to concurrent ART status and baseline age. Concurrent ART during TB therapy was associated with a monthly rise of 15.7 CD4+ T cells/µL (p<0.0001). There was no overall difference in CD4+ T cell response by age during TB therapy (P=0.6550). However, among patients who received ART with TB treatment, greater gains in CD4+ T cell counts were seen among younger patients (age*time*ART, p-value=0.0443), whereas the same effect was not seen among patients who delayed ART. This inverse association between age at ART initiation and CD4+ T cell increase during concurrent ART and TB therapy was strongest in females (p-value: age*time=0.0059) and in patients with BMI ≥18.5kg/m² at enrolment (p-value: age*time=0.0061). Our findings suggest that older HIV-seropositive patients on ART might experience a slower rate of immune restoration, especially if female or BMI ≥18.5kg/m² at initiation. Older HIV-positive patients may benefit from closer monitoring of immune status and nutritional support during ART.

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FOOD INSECURITY, DIET DIVERSITY AND BMI OF HIV INFECTED INDIVIDUALS ON ANTIRETROVIRAL THERAPY IN RURAL HAITI

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Food insecurity and malnutrition are both important risk factors for poor outcomes in the treatment of people living with HIV. Food supplementation is increasingly offered in conjunction with HIV programs. Current programs focus on those with acute or chronic malnutrition. The optimal target population is unknown. We evaluated 490 HIV-infected individuals receiving antiretroviral therapy (ART) in rural Haiti. Baseline data including body mass index (BMI), household food security (using the Household Hunger Scale), socioeconomic status (SES), medication adherence and dietary diversity were collected via structured interview. We analyzed factors associated with low BMI and severe food insecurity using logistic regression. Moderate to severe food insecurity was present in 89% of individuals. Amongst severely food insecure subjects, 49% had a normal or above normal BMI. After adjusting for age, sex, BMI and presence of a garden in the home, severe food insecurity was associated with relative poverty [odds ratio (OR) 2.37, 95% confidence interval (CI) 1.58 - 3.54, p<0.001], illiteracy (OR 1.79, 95% CI 1.19 - 2.71, p< 0.01), and having no source of income generation (OR 1.63, 95% CI 1.04 - 2.56, p< 0.05). Individuals with severe food insecurity had a less diverse diet, with less frequent consumption of proteins. Food insecurity was highly prevalent in patients with HIV infection receiving ART. Normal or high BMI did not rule out severe food insecurity. Current guidelines regarding the use food support to supplement HIV treatment that are based on BMI will miss a significant proportion of patients who may benefit from this intervention.

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HIV AND CHAGAS DISEASE: AN EVALUATION OF THE CLINICAL PRESENTATION AND THE USE OF REAL-TIME QUANTITATIVE PCR TO MEASURE LEVELS OF TRYpanosoma CRUzi PARASITEMIA IN HIV PATIENTS

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With the persistence of Chagas disease in Latin America and with increased migration from rural to urban settings- where rates of HIV/AIDS are increasing- there has been an increase in the number of persons co-infected with HIV and Chagas disease in recent years. This study evaluated the clinical presentation and laboratory results of patients co-infected with HIV and Chagas in Cochabamba, Bolivia, with a focus on the levels of Trypanosoma cruzi parasitemia measured by real-time quantitative PCR (qRT-PCR) of blood clot. Clinical evaluation, electrocardiogram, chest radiograph, CD4 count, viral load, serology for T. cruzi, direct microscopy of blood, and real-time PCR of blood clot was performed on each patient. Some patients also have a sample for qRT-PCR from whole blood with EDTA. 38 of the 133 HIV patients evaluated were co-infected with Chagas disease (28.6%). Four of the 38 (10.3%) were positive by direct microscopy, thus meeting the criteria for reactivation. Two of the patients with reactivation had very high levels of parasitemia (>1000 parasites/ml) by qRT-PCR of clot and by whole blood, however two patients with reactivation had no parasitemia detected by qRT-PCR of clot and only low levels by whole blood (<40 parasites/ml). Our results demonstrated that high levels of parasitemia are associated with high HIV viral loads and low CD4 counts; however, quantifiable levels of parasitemia did not show a strong correlation with symptoms of Chagas disease or reactivation. Although the levels of T. cruzi parasitemia detected by qRT-PCR do not show direct correlation with reactivation of Chagas disease or with clinical symptomatology in all patients, the level of parasitemia in HIV patients may be an indication of those at risk for progression of disease. Further studies are needed to determine the significance of parasitemia detected by PCR, and whether asymptomatic patients with detectable T. cruzi parasitemia should be treated with antiprotozoal agents.

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RISK OF ANTIRETROVIRAL TREATMENT FAILURE AMONG CLINICALLY STABLE ADULTS IN BLANTYRE, MALAWI

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Millions of people living with HIV infection are receiving antiretroviral therapy (ART) in sub-Saharan Africa without access to routine viral load monitoring due to its expense and associated logistical challenges. If those likely to have detectable HIV viral load could be identified by readily identifiable risk factors, such patients could be prioritized for targeted viral load monitoring. Targeted monitoring in patients who are clinically well will allow for interventions before they develop severe illness or advanced ART resistance and will likely be a more affordable and feasible strategy than universal testing. As part of an on-going clinical trial at Ndirande...
Health Centre in urban Blantyre, we conducted viral load and CD4 count assessments for 945 healthy adult outpatients who had been on ART for a minimum of six months. Ninety (10%) had HIV viral load >1000 copies/mL and 64 (7%) >5000 copies/mL. Among patients with a detectable viral load of greater than 40 copies/mL, the geometric mean was 3157 copies/mL (95% CI 2083 - 4786 copies/mL). The median CD4 cell count was 454 cells/mm³ (95% CI 433 - 475), 147 (16%) had a CD4 cell count <250 cells/mm³. We will use logistic regression to investigate factors associated with elevated viral load. The covariates in the modelling will include age, sex, BMI, CD4 cell count, reason for ART initiation, current ART regimen, duration of ART and self-reported adherence. We will also explore the association between infections including TB and malaria and viral load. Our goal is to identify patient characteristics that are associated with increased risk of virological failure on ART that can be identified prior to overt ART clinical failure. Targeted viral load testing among high risk individuals may be a cost-effective strategy to improve patient survival and limit the spread of ART resistance in resource-limited settings.

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TRYPANOSOMA CRUZI PARASITEMIA IN IMMUNOSUPRESSED PATIENTS WITH HIV INFECTION OR ORGAN TRANSPLANT RECIPIENTS

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Chagas disease is now an active and emergent disease in urban centers of endemic and non endemic areas and affects around 7 million inhabitants in Latin America and a contingent of infected immigrants in other countries. Considering the high morbimortality of chronic Chagas disease reactivation associated with immunosupression, we analyzed the levels of parasitemia in immunosupressed patients under solid and stem cells transplantation (N=12) and in patients with HIV/Trypanosoma cruzi co-infection (N=43 patients). Blood samples from organ transplants recipients were analyzed between the 4-6 months post transplant. A control group of 91 patients with Chagas disease was also included. Inclusion criteria for Chagas disease and HIV infection were the presence of anti-Trypanosoma cruzi antibodies by ELISA or immunofluorescence and presence of antibodies for HIV antigens by ELISA confirmed by immunoblot, respectively. For diagnosis of Chagas disease reactivation, the parasite was identified by direct microscopy on peripheral blood or other biological fluid. Quantitative PCR was performed with genomic sequencies as previously described (Freitas et al, 2011). Higher parasitemia was observed in the co-infected group in comparison to the control group with Chagas disease, and is related to the presence of reactivation of Chagas disease in seven HIV infected patients in this group. Increased parasitemia was detected in two of the seven kidney transplant patients: one patient with reactivation of Chagas disease, and one patient without reactivation but increased number of parasite DNA copies patient (higher than 200x the pre transplant levels). Increased parasitemia is also observed in co-infected HIV/T. cruzi patients without reactivation in association with high parasitemia showed in xenodiagnosis test (higher than 20% of nymphs positive in each exam). Monitoring parasitemia by quantitative PCR should be considered as an useful tool for the management of chronic Chagas diseases in patients under immunosupression.

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DEVELOPMENT AND EVALUATION OF MULTIMEDIA INFORMED CONSENT TOOL FOR A LOW LITERACY AFRICAN RESEARCH POPULATION

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International guidelines recommend the use of appropriate informed consent procedures in low literacy research settings because written information is not known to guarantee comprehension of study information. This study developed and evaluated a multimedia informed consent tool for people with low literacy in an area where a malaria treatment trial was being planned in The Gambia. Methods: We developed the informed consent document of the malaria treatment trial into a multimedia tool integrating video, animations and audio narrations in three major Gambian languages. Acceptability and ease of use of the multimedia tool were assessed using quantitative and qualitative
Livestock and Agriculture, Phnom Penh, Cambodia, 4Ministry of Health, recent Cochrane publication on antiviral effectiveness (10th April 2014) we most urgent public health investment for Cambodia. Note: In light of the to this are the considerable uncertainty inherent in these estimates and ventilators are cost effective pandemic preparedness options. The caveats marginal returns from increased stockpile size. However there is US$20 million a mixed investment is preferable, reflective diminishing and US$4 million stockpiling is most cost effective, investment level of point estimates for most parameter inputs. We also present a cost-investment strategy. The analysis was repeated at three investment levels stockpiling and ii) additional mechanical ventilators and iii) a 1:1 mixed (CUA) on three pandemic preparedness investment options i) antiviral pandemic simulation model including age structure, health system capacity preparedness investments focus almost exclusively on high relative wealthy well-resourced countries. Yet evaluations of cost effective pandemic preparedness investments focus almost exclusively on high income countries. We undertake an economic evaluation of two pandemic preparedness investment options in Cambodia. We developed a dynamic pandemic simulation model including age structure, health system capacity and cost of illness. The model was used to perform cost utility analysis (CUA) on three pandemic preparedness investment options i) antiviral stockpiling and ii) additional mechanical ventilators and iii) a 1:1 mixed investment strategy. The analysis was repeated at three investment levels US$250k, US$4 million and US$20 million. Due to the inherent uncertainty in pandemic modelling we use sampling distributions in place of single point estimates for most parameter inputs. We also present a cost-consequence analysis (CCA) to place model results within the context of non-quantified costs and consequences. At investment levels of US$250k and US$4 million stockpiling is most cost effective, investment level of US$20 million a mixed investment is preferable, reflective diminishing marginal returns from increased stockpile size. However there is substantially more uncertainty in incremental cost effectiveness ratio (ICER) estimates for antiviral stockpiling. Also, the CCA highlights that investing in ventilators would have considerable utility between pandemics. It is likely that both antiviral stockpiling and investment in mechanical ventilators are cost effective pandemic preparedness options. The caveats to this are the considerable uncertainty inherent in these estimates and that depending on the payer, they may or may not be affordable or the most urgent public health investment for Cambodia. Note: In light of the recent Cochrane publication on antiviral effectiveness (10th April 2014) we will be updating our analysis within the coming weeks.

PANDEMIC INFLUENZA PREPAREDNESS IN CAMBODIA: AN ECONOMIC EPIDEMIOLOGICAL DECISION ANALYSIS

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Pandemic burden is predicted to be high in low income countries, relative wealthy well-resourced countries. Yet evaluations of cost effective pandemic preparedness investments focus almost exclusively on high income countries. We undertake an economic evaluation of two pandemic preparedness investment options in Cambodia. We developed a dynamic pandemic simulation model including age structure, health system capacity and cost of illness. The model was used to perform cost utility analysis (CUA) on three pandemic preparedness investment options i) antiviral stockpiling and ii) additional mechanical ventilators and iii) a 1:1 mixed investment strategy. The analysis was repeated at three investment levels US$250k, US$4 million and US$20 million. Due to the inherent uncertainty in pandemic modelling we use sampling distributions in place of single point estimates for most parameter inputs. We also present a cost-consequence analysis (CCA) to place model results within the context of non-quantified costs and consequences. At investment levels of US$250k and US$4 million stockpiling is most cost effective, investment level of US$20 million a mixed investment is preferable, reflective diminishing marginal returns from increased stockpile size. However there is substantially more uncertainty in incremental cost effectiveness ratio (ICER) estimates for antiviral stockpiling. Also, the CCA highlights that investing in ventilators would have considerable utility between pandemics. It is likely that both antiviral stockpiling and investment in mechanical ventilators are cost effective pandemic preparedness options. The caveats to this are the considerable uncertainty inherent in these estimates and that depending on the payer, they may or may not be affordable or the most urgent public health investment for Cambodia. Note: In light of the recent Cochrane publication on antiviral effectiveness (10th April 2014) we will be updating our analysis within the coming weeks.

RELATIONSHIP OF ANGIogenic AND INFLAMMATory BIOMARKERS AT MID-PREGNANCY TO SMALL-FOR-GESTATIONAL AGE OUTCOMES IN A PROSPective COHORT OF TANZANIAN WOMEN

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Intrauterine growth restriction (IUGR) is a major public health problem that affects an estimated 27% of pregnancies in low and middle-income countries. Inadequate fetal growth is associated with increased risk of neonatal morbidity and mortality as well as developmental delay and cardiometabolic disorders in adulthood. We examined the relationship between a panel of angiogenic and inflammatory biomarkers measured in mid-pregnancy and small-for-gestational age (SGA) outcomes in Tanzanian women. Plasma samples were collected from a prospective cohort of 432 primigravid women at enrolment (12-27 weeks gestation). Levels of 18 biomarkers (Ang-1, Ang-2, Ang-L3, VEGF, sFlt-1, sTNFR2, PGF, MIP-1β, MCP-1, Leptin, IL-1β, IL-18 BP, sCAM-1, FAC-D, sEndoglin, CRP, CHI3L1, C5a) were analyzed by ELISA. Infants falling below the 10th percentile of birth weight for gestational age relative to the applied grow standards were considered SGA. Multivariate binomial regression models were used to determine the relative risk of SGA associated with levels of each biomarker. Receiver operating curves obtained from multivariate logistic regression models were used to assess the discriminatory capability of selected biomarkers. A total of 60 participants (13.9%) gave birth to SGA infants. Compared to those in the first quartile, the risk of SGA was reduced among those in the fourth quartiles of VEGF-A (adjusted risk ratio (RR) 0.38, 95% Confidence Interval (CI), 0.19-0.74), PGF (adjusted RR 0.28, 95% CI, 0.12-0.61), sFlt-1 (adjusted RR 0.48, 95% CI, 0.23-1.01), MCP-1 (adjusted RR 0.48, 95% CI, 0.25-0.92), and Leptin (adjusted RR 0.46, 95% CI, 0.22-0.96). Our findings provide evidence of altered angiogenic and inflammatory mediators, at mid-pregnancy, in women who went on to deliver small for gestational age infants. Studies are currently under way to validate these findings in both internal and external cohorts.

MULTINATIONAL DISEASE SURVEILLANCE PROGRAMS FOR CROSS-BORDER EPIDEMIOLOGIC INFORMATION EXCHANGE

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Cross-border disease surveillance and response efforts in regions sharing borders depend on effective international collaboration. The Border Infectious Disease Surveillance (BIDS) program facilitates epidemiologic information exchange between the United States and Mexico. To understand the global context for BIDS, we conducted a survey of multinational disease surveillance programs (MNDSPs) operating worldwide. Our survey assessed program organization, goals, operations, decision-making processes, and evaluation. From January 2013 to March 2014, we identified MNDSP representatives through internet search and querying domestic and international colleagues. We contacted 12 MNDSPs and obtained responses from ten programs spanning all continents except Antarctica. In general, responding programs aim to enhance epidemiologic surveillance capabilities, strengthen cooperation for infectious disease monitoring and prevention, and increase consistency among countries. Reportable conditions are jointly selected by participating countries based on common importance. Most programs have specific algorithms for disease surveillance and laboratory testing. Eight (80%) of the ten programs have a central database that obtains information through...
manual data entry or electronic linkage. E-mail is the primary mode of communication. Eighty percent of the programs have multinational emergency notification contact lists. All ten programs meet at least annually in person or via video or teleconference. Fewer than half (40%) of the programs share specimens or laboratory testing reagents among countries. Local and national public health laboratories are the primary infrastructure for diagnostic testing. Seven of the ten programs have dedicated funding allocated to MNDS operation. Few programs have implemented a routine quality assurance program. Despite variation in health priorities, geography, and socioeconomic context, our survey identified key operational commonalities among MNDSs and provides an important perspective on global disease surveillance efforts.

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KNOWLEDGE, ATTITUDES AND BEHAVIOR TOWARD PARASITE INFECTIONS AMONG HIGH SCHOOL STUDENTS IN SOLOMON ISLANDS

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Pacific region is an epidemic area of different parasite infections. The countries have provided examinations and medical supports to their citizens. However, few statistic data and information on attitudes and behavior toward parasite infections are available. The survey was trying to investigate the knowledge, attitudes and behavior toward parasite infections among high school students in Solomon Islands. The survey was conducted in August, 2012. Total of 679 participants, aged from 12 to 18 years old, were randomly selected from St. Nicholas and St. Joseph high schools. Approximately 95% of participants indicated that they had clean water for food preparation or cooking, however nearly 72% showed they didn’t have drinking water boiled. 67% and 13% of the students said the main source of their drinking water was from rain and bottled water, respectively. 90% of the students had toilets at home, and 82% indicated they had the habit of hand washing. However, up to 53% of participants didn’t wear shoes, while 40% with excessively long fingernails. Nearly 28% of them had been diagnosed for parasite infections. 40% claimed they had never learned about parasite prevention. A screening program executed by Taiwan Health Center in Solomon Islands showed the prevalence of parasite infection among local students was up to 34%. The understanding of parasite prevention among high school students in Solomon Islands is still deficient, and it is not taught as part of the curriculums in schools. Appropriate medical and educational resources need to be prompted to make significant changes in parasite prevention.

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A LOW-COST ICT TOOL KIT FOR IMPROVED DENGUE SURVEILLANCE, LABORATORY MANAGEMENT AND CLINICAL DECISION SUPPORT IN NICARAGUA

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Dengue is a mosquito-borne viral disease of major medical and public health importance worldwide. We have developed and tested three low-cost informatics tools in Nicaragua to help improve laboratory, surveillance and clinical management practices to reduce dengue-related morbidity and mortality. As part of the Dengue FIRST initiative (Fighting Infections through Research, Science and Technology), these tools were developed collaboratively between the NGO the Sustainable Sciences Institute and the Ministry of Health of Nicaragua. Input from stakeholders at the national, state and local levels was incorporated at multiple phases. “DENGUE-ALERT” is an innovative automated early warning system for outbreak detection and response that provides a customizable “dashboard” with information from traditional epidemiological disease reports and entomological, climatic and crowd-sourced data. It targets a wide range of end-users including public health and community-based actors and is designed to enable earlier detection and reporting of virus circulation or outbreak indicators to increase the efficiency of response and resource use. “DENGUE-SPECIALIST” is a web-based mobile application designed to improve the efficiency and accuracy of clinical data access and analysis in hospitalized dengue cases. End-users are clinicians and nurses in public sector hospitals. Simulations of SPECIALIST were tested with data from multiple years of a clinical study in a Nicaraguan public hospital. “DENGUE-LAB” is a web-based platform that supports a national-level information management system for integrating laboratory results and reporting to streamline information flow around the numerous tests used for dengue diagnosis. LAB is designed to improve quality control measures, simplify and automate some of the complexities of dengue diagnosis, and impact both the quality and the reliability of diagnostic results. Both quantitative and qualitative indicators were used in a mixed method evaluation of the pilot of these tools to assess their ability to support earlier and more accurate disease response and outbreak prevention. Following this design and testing phase, the goal is to extend the use of these tools to Mexico and to other Central American countries that are interested and able to adapt and implement “customized” versions of ALERT, SPECIALIST and LAB as appropriate in their country contexts.

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DENGUE TORPEDO: A NOVEL APP TO MOTIVATE COMMUNITY-BASED DENGUE VECTOR CONTROL

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Dengue is a mosquito-borne viral disease that continues to expand geographically in part due to failed efforts in vector control. Residents of communities affected by dengue are potentially the best agents to control vector breeding sites, since the Aedes mosquitoes that transmit dengue virus breed in clean water in and around people’s homes. We present the design and piloting of Dengue Torpedo (DT), an interactive cellphone and web platform that combines mobile technology and game concepts to motivate community residents to report and eliminate mosquito breeding containers. DT (a) crowdsources the identification and mapping of breeding sites (problem of information); (b) motivates residents to act (problem of execution); (c) develops a collaborative model of software production that involves local user-residents in researching, testing, and designing the application; (d) promotes civic engagement and active citizenship; (e) involves residents in public health education; and (f) engages youth in the creative development of communication technologies. Using a community-based collaborative model of software design and development, we created alpha and beta versions of DT in Rio de Janeiro, Brazil. The mobile and web interface is interactive and allows residents to create their own profiles and exchange information about dengue in their neighborhoods. DT also has an educational component that relates to other relevant issues in the community as well as specific information regarding dengue. Players earn badges and points that can be exchanged for community or personal prizes donated by local sponsors and small businesses. DT has 5 participatory features in dengue vector control that make it pioneering: it is interactive, connects mobile and web technologies, uses gameplay to motivate residents, institutes a community-based collaborative model of app development, and helps local institutions sponsor related educational activities. In parallel, we are developing DT for the Mexican health sector, incorporating contextually appropriate information and designing the interface with input from community members and researchers in Morelos, Mexico. DT can improve
public participation in combating dengue, generate new correlations and visualizations of scientific data of potentially great scale and low cost, reduce mosquito infestation, and eventually decrease rates of dengue infection and disease.

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OPENHDS: USE OF TABLET COMPUTERS IN HEALTH AND DEMOGRAPHIC SURVEILLANCE

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Health and Demographic surveillance systems (HDSS) can provide essential information in areas where routine vital registration is absent or incomplete. Also play an essential role in health intervention studies in such areas. Setting up and running an HDSS poses an operational challenge, and a reliable and efficient platform for data collection and management is a key prerequisite. OpenHDS is an HDSS data system that provides data entry, quality control, and reporting to support demographic and health surveillance. It consists of two components: web and mobile. OpenHDS mobile is integrated with the Open Data Kit (ODK) a software platform for data collection using mobile devices running the Android operating system, and allows direct data entry using tablet computers. This offers a number of advantages over traditional paper-based systems: it reduces the workload of the data management team, no IDs need to be typed in (removing one of the biggest causes of errors on data collection in HDSS systems); it allows for near real-time quality control, and it can provide guidance for the project logistics. The web interface allow viewing of the data collected and correction of errors/perform eventual amendments. Here we present an overview of the openHDS/ODK software platform, and report on the experience of using this platform to set up a HDSS in support of a trial of odour baited traps as a malaria intervention study on Rusinga Island, Kenya, (Solarmal) Project. We also present the experience of migrating the data systems of established HDSS sites from an older system (HRS) to openHDS (Ifakara and Rufiji, Tanzania). We show how OpenHDS addresses specific operational problems and the use of the complete OpenHDS/ODK system for data collections offers a number of advantages over paper-based systems both with respect to data quality and cost savings.

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AVAILABILITY OF SIGNAL FUNCTIONS AND QUALITY OF EMERGENCY OBSTETRICS CARE FOR POPULATION IN RURAL BANGLADESH

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The maternal mortality ratio in Bangladesh is high at 220 deaths per 100,000 live births. Emergency obstetrical care (EmOC) interventions in health facilities are effective at reducing maternal mortality. Facility deliveries in Bangladesh have grown from 16% in 2000-2006 to 29% in 2007-2012. This study assessed the quality of obstetrical services in health facilities serving rural areas of Bangladesh in order to identify opportunities to improve EmOC. We randomly sampled 50 rural villages in 46 of 64 districts of Bangladesh and interviewed the administrator of 8-9 hospitals nearest to each sampled village. We categorized the quality of EmOC at each hospital according to its capacity to provide 6 basic EmOC signal functions (administrations of antibiotics, oxytocin, and anticonvulsant; assisted vaginal delivery; manual removal of placenta and removal of retained products after delivery), staffing, and availability of phone and ambulance for referring patients. EmOC quality categories included high (6 signal functions; ≥3 staff on call [24-hour coverage], an ambulance and a phone), moderate (≥4 signal functions; ≥2 staff [or no 24-hour coverage], a phone), low (≥2 signal functions; ≥1 staff, a phone), and sub-standard (no minimum criteria). Administrators of 432 hospitals were interviewed. Administration of antibiotics was available in 99% of the hospitals, whereas anticonvulsant administration was only available in 65%. The quality of EmOC was high in 31%, moderate in 55%, low in 4%, and sub-standard in 9% of the hospitals; 32% did not have 24-hour coverage of skilled birth attendance. Approximately one-third of health facilities providing obstetric care in rural Bangladesh lack anticonvulsants needed to manage eclampsia, an important cause of maternal mortality. Similarly, the lack of 24-hour availability of skilled birth attendants increases the risk of peripartum complications. Given the high rate of maternal mortality, there is a pressing need to improve provision of EmOC in health facilities in rural Bangladesh.

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OCCURRENCE OF SEVERE DENGUE FEVER IN AN ENDEMIC CITY OF BRAZIL: AN ECOLOGICAL STUDY

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Background: Brazil accounts for more than 70% of dengue cases notified on the American continent. In this context, Rio de Janeiro became one of the most endemic cities in the country during the last decades, presenting a long history of co-circulation of all four dengue serotypes with a recent trend for the clinical and epidemiological profile of the disease to change. Methods: This ecological study aimed to analyze the relationship between incidence of severe dengue during the 2008 epidemic in Rio de Janeiro City and socioeconomic, previous circulation of other dengue serotype and health service availability indicators. The data was incorporated into a negative binomial regression model. Results: Districts with more cases of dengue in the 2001 epidemic and where higher percentages of the residents who declare their skin color/race as black showed higher incidence rates of severe dengue in the 2008 epidemic. Meanwhile, districts with lower incidences of severe dengue in 2008 were those with more Family Health Strategy (FHS) clinics. Conclusion: The findings suggest existing health inequities possibly due to greater socioeconomic vulnerability among black population. Additionally, the protective effect of FHS clinics may be due to facilitated access to other levels of health care or even by reducing vulnerability to transmission afforded by local practices in health promotion. These aspects reinforce the importance of better understanding of social determinants in order to identify key-points for developing and implementing interventions for dengue control.

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CLIMATE-DRIVEN DENGUE EPIDEMIC EARLY WARNINGS FOR BRAZIL

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In this study, we address the potential to predict dengue fever epidemics across Brazil, during the peak dengue season (austral summer period). We produce probabilistic dengue forecasts for the 553 microregions of Brazil, from 2000 to 2014, using a novel spatio-temporal modelling framework. The dengue forecasts are driven by multi-model ensemble seasonal climate forecasts and the observed epidemiological situation in Brazil at the forecast issue date. The multi-model ensemble comprises several quasi-operations forecast systems, among them two systems
from the European EUROSIP initiative and six systems from the North American Multi-Model Ensemble (NMME) project. This precursory information allows dengue warnings to be released three months ahead. We evaluate the past performance of the dengue early warning system by verifying probabilistic predictions against out-of-sample observed data. Timely dengue early warnings provide the opportunity for the ministry of health and local authorities to implement appropriate, city-specific mitigation and control actions. This model framework could be applied to predict outbreaks of other climate-sensitive diseases in other parts of the world. This is especially pertinent as climate change is likely to make diseases, such as dengue and malaria, more widespread. The successful implementation of seasonal climate forecasts in disease early warning systems depends on close collaboration between public health specialists, climate scientists and mathematical modellers. The overall objective of this study is to bring awareness to scientists, policy makers and international health surveillance teams of the data and tools required in order to make timely predictions. We hope that this early warning framework will help to improve prevention strategies for vector-borne diseases and establish a landmark towards the use of climate data to benefit society.

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COMMUNITY HEALTH WORKER TIME USE: METHOD EVALUATION AND TIME USE FINDINGS IN MALAWI

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Understanding community health worker (CHW) workload is critical for maintaining program quality in the face of competing tasks. Time motion studies are considered the gold standard in time use research, but are prohibitively time and resource intensive. Four alternative measurement methods (3 interview methods and time diary) were piloted to assess time use among CHWs as a subcomponent of a cost effectiveness evaluation of the Rapid Scale-up of MNCH in Malawi in 2013. Based on pilot results, 2 interview methods (week-long vs. year-long reference period) were deployed to measure time use among 248 CHWs (126 implementing community cases management (CCM)) in 6 districts. Reported total hours and hours spent on CCM activities in an average week were higher and more variable using the year-long vs. week-long interview method. Correlation between methods in estimated work hours/week (0.12) and CCM hours/week (0.27) was very low. Based on implausible variability in time use estimated by the year-long method, final results were reported using the week-long method only. On average a CHW reported working 42.29 (95% CI: 38.88, 45.70) hours/week and those who implemented CCM spent 18.08 (95% CI: 16.24, 19.92) hours/week on CCM. CCM (Adjusted coeff: 10.32; 95% CI: 5.09, 15.56) and each additional intervention implemented (Adjusted coeff: 1.86; 95% CI: 0.81, 2.90) increased total number of hours worked per week. CHWs who implemented CCM spent significantly less time on nutrition (Coeff: -2.24; 95% CI: -3.12, -1.37), HIV (Coeff: -3.49; 95% CI: -6.56, -0.42), and community based maternal and newborn health interventions (Coeff: -1.31; 95% CI: -2.33, -0.30), compared with CHWs implementing those services but not CCM. Findings highlight the importance of an accurate and efficient means of assessing CHW time use to determine feasibility and impact of introducing additional tasks. Time use interviews offer promise for collecting time use data when a large number of CHWs are targeted, although further refinement and validation against a gold standard time assessment is needed.

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BREASTFEEDING PRACTICES AND CHILDHOOD DIARRHEA MANAGEMENT AMONG WOMEN IN RURAL COMMUNITIES IN MOZAMBIQUE

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Early childhood nutritional practices, including exclusive breastfeeding for the first 6 months of life and adequate hydration of children with diarrhea, are critical for minimizing morbidity and mortality from infectious causes in the developing world. Gorongosa National Park in Mozambique is surrounded by rural communities with significant geographic and economic barriers to health resources. These communities are located in two groups: those located in the mountain region and those in the plains region, with the mountain communities being especially isolated. In 2011, a comprehensive household survey was conducted to study health behaviors in these regions as part of an ongoing Ecohealth Program. 1625 women of reproductive age were surveyed by trained interviewers in the local language: 1074 women from 4 mountain communities and 551 women from 2 plains communities. 192 women with children age 6 months or younger answered questions about breastfeeding, and 1035 women with children age 5 years or younger answered questions about previous nutrition and diarrhea education, and diarrhea management. Results showed that women in the plains communities were more likely to have received education about early childhood nutrition vs. women in the mountain communities (85% vs. 28%, p<.0001), but in both populations, there was no association between previous nutrition education and exclusive breastfeeding (plains: RR .85, CI .31-2.28; mountain: RR 1.01, CI 0.81-1.26). In the mountain communities, 46% of women withhold water completely during a child’s episode of diarrhea; however, those who received previous education were significantly more likely to hydrate their child though the episode (RR 3.43, CI 2.19-5.38). No such association was found in the plains communities (RR 1.10, CI 0.92-1.32), where only 7% of women will withhold hydration. These results can be used to tailor a planned intervention involving a new nutrition educational program. The data suggest that previous programs did not effectively teach best breastfeeding practices, and that there is an opportunity to increase the number of women exclusively breastfeeding, especially in the plains communities where women are more likely to use breast milk alternatives. The data also suggest that prior education about rehydration in diarrhea has been successful in the mountain communities, and any future nutrition programs should model educational materials accordingly.

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ACCEPTABILITY AND EFFECTIVE USE OF A RAPID HOME DIAGNOSTIC TEST FOR MULTIPLE INFECTIOUS DISEASES: FORMATIVE RESEARCH IN PERU AND CAMBODIA

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In 2012, the U.S. Defense Threat Reduction Agency Joint Science and Technology Office initiated a program to develop novel point-of-need
diagnostics for surveillance of emerging infectious diseases including dengue, malaria, plague, and melioidosis. Prior to distribution of devices to community members in Iquitos, Peru, and Phnom Penh, Cambodia, research was conducted to assess acceptability of use, determine conditions in which use would occur, examine incentives to use the device on someone with fever >5 years of age in their home, and assess device use competency. In February 2014, 9 focus group discussions (FGD) with community members and 5 FGD with health professionals were conducted in Iquitos, and 9 FGD with community members and 9 in-depth interviews with health professionals in Phnom Penh. In both places, participants agreed to use the device themselves (involving finger prick) or could identify someone to do so. The main incentive identified in Iquitos was the ability for tentative results to be used for care facilitation (as devices would not provide usable results for individuals), specifically reduced wait times to be seen or obtain a diagnosis. In Phnom Penh, the main incentives were monetary compensation (~US$2.50/test) or results from a simultaneous rapid test; also, free medicine for the sick was acceptable in lieu of usable results. To assess device use competency in Iquitos, participants were asked to read instructions and apply the device to research team members. Most steps were done correctly; the most difficult was proper recording of test results. In Phnom Penh, participants were asked to describe each step after reviewing the instructions; however, they struggled with comprehension. Health professionals’ main concerns were their community’s ability to accurately use the test, complicated instructions, and safety. Motivation and ability to use home diagnostic devices depended on local attitudes that varied between the two disease endemic sites, illustrating the value of formative research before deployment of novel technologies.

THE PERCEPTIONS OF PHYSIOTHERAPISTS AND PATIENTS ABOUT INTEGRATION OF HEALTH PROMOTION IN PHYSIOTHERAPY PRACTICE

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This study was undertaken to determine the status of integration of health promotion in physiotherapy practice from both physiotherapists and patients’ perspectives. A qualitative research method was followed. A rich purposive sample of physiotherapists patients from different health care settings provided information for data collection during semi-structure interviews which lasted from 45 to 60 minutes. Data were collected over a period of six months. All interviews were audio taped followed by verbatim transcription. The Nvivo version 10 program was used. The following sequence was followed in data analysis: Familiarisation with data, code book construction, thematic analysis, synthesis and identification of similarities, identification of overarching themes and formulation of concept. A total of 35 physiotherapists, 69% females and 31% males, as well as 21 patients, 55% females and 45% males, participated in the interviews. All physiotherapists had a work experience of more than 5 years whilst patients’ age ranged between 24 years to 60 years. Physiotherapists’ workload comprised of in- and out-patients whilst patients attended physiotherapy sessions at hospital and Primary Health Care (PHC) settings. Concepts formulated for both groups were knowledge, attitude and practice. Patients do have an understanding of what health promotion means and expect health promotion service during their health. The physiotherapists are unable to differentiate between health education and health promotion though they have a positive attitude towards health promotion in practice. They rarely integrate health promotion in their daily practice. In conclusion, the need to integrate health promotion in practice exists. Hospital and PHC based physiotherapy differ. There are gaps between the patients’ needs and physiotherapy practice requiring policy and guidelines to drive health promotion in physiotherapy. Focus should be on continuous professional development to improve knowledge. Education and training curriculum should be reviewed.

TREATMENT OF POSTPARTUM HEMORRHAGE WITH MISOPROSTOL BY GUATEMALAN TRADITIONAL MIDLWIVES

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Postpartum hemorrhage (PPH) due to uterine atony is a leading cause of maternal mortality in Guatemala, especially in rural areas where homebirth is common. In a pilot project, 36 Guatemalan traditional midwives received training on the recognition and treatment of PPH using 800 µg buccal misoprostol. Training was designed to be linguistically and culturally appropriate. Pre- and post-evaluation showed participants’ knowledge of PPH signs, prevention and treatment using misoprostol significantly increased following training. Ongoing data collection, to be completed in June 2014, demonstrates that midwives are capable of recognizing PPH and using misoprostol for its treatment. We plan to expand our work to reach more midwives, further addressing the shortage of data on misoprostol for PPH treatment in homebirth, particularly in Latin America.

MAPPING THE ENVIRONMENTAL AND SOCIOECONOMIC COVERAGE OF THE 2012 INDEPTH INTERNATIONAL HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM NETWORK

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The International Network for the Demographic Evaluation of Populations and their Health (INDEPTH) was initiated in 1998 and has produced reliable longitudinal data about the lives of people in low- and middle-income countries (LMICs) and the impact on those lives of development policies and programs through a global network of health and demographic surveillance system (HDSS) sites. Since stable and reliable health and demographic data are scarce across many LMICs, here we examine the environmental and socioeconomic similarities between existing HDSS field sites and the rest of the LMICs in Africa and Asia, so as to provide evidence in terms of levels of confidence in extrapolating the findings from HDSS field sites to other regions. A ‘signature’, consisting of 15 environmental and socioeconomic variables, was constructed for each HDSS field site. The field sites were then hierarchically grouped by the similarity of their signatures to quantify the variability in terms of environmental and socioeconomic conditions between sites, and these similarities were mapped. The current INDEPTH HDSS field site network covering Africa and Asia spans a wide range of climatic and environmental conditions. The similarity maps produced provide valuable information in determining the confidence with which relationships derived from present HDSS field sites can be extended to other areas, and to highlight areas where the location of new HDSS field sites would improve significantly the environmental and socioeconomic coverage of the network. The results also indicate suites of field sites that form cohesive groups and from which data can be logically summarized.
EVALUATION OF POINT-OF-CARE AND POINT-OF-NEED MULTIPLEX DIAGNOSTIC DEVICES FOR EASE OF USE AND FOR TRANSMITTING RESULTS THROUGH A SECURE ENCRYPTED REMOTE NETWORK

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The United States Department of Defense’s (DoD) Defense Threat Reduction Agency recently initiated a program designed to drive development of novel point-of-need (PON) diagnostic devices within a biosurveillance network for capturing emerging infectious diseases in austere locations. DoD biomedical laboratories are evaluating two devices in South America (Peru), Australia, Southeast Asia (Thailand, Cambodia), and Africa (Sierra Leone) to mimic real-world settings similar to those encountered by deployed military personnel. These multiplex devices are designed to detect pathogens that cause dengue fever, malaria, plague, and melioidosis: a clinic/medic-based point-of-care (POC) molecular diagnostic device for use in health care clinics and hospitals with health-care providers as the end-user, and a patient-administered “buddy-test” PON device to be used in community households with the lay person as the end-user. For POC testing, 11 Investigational Use Only (IUA)-labeled systems and 1000-2000 test pouches will be distributed to each site. Nearly 1400 POC subjects will be enrolled in Peru with an expected dengue disease rate of 20-25%, and 2500 subjects will be enrolled in SE Asia with an expected melioidosis disease rate of 10%. For PON testing, 7000 to 9000 Research Use Only (RUA)-labeled devices will be distributed across all locations to detect dengue (Peru), malaria, plague, and melioidosis (all other locations). De-identified and blinded results from these PON and POC tests will be compared to gold standard testing for study of device sensitivity and specificity (aiming for 85% for Role 1; 75% for Role 0), then wirelessly communicated through a secure encrypted remote network (SERN) using an FIO reader (success considered at 90% performance towards elimination of measles). Further studies are needed to effectively plan risk mitigation activities and to evaluate risk assessments can be used for advocacy to communicate risk to policymakers, mobilize resources, and strategically guide immunization response accordingly to the level of risk. Ongoing evaluation of indicators is needed to effectively plan risk mitigation activities and to evaluate performance towards elimination of measles.

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U.S. DEPARTMENT OF DEFENSE GLOBAL FEBRILE AND VECTOR-BORNE ILLNESS SURVEILLANCE PROGRAM

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Originally established by Presidential Directive in 1997, the Department of Defense (DoD) Global Emerging Infections Surveillance and Response System (GEIS) was expanded in 2006 and subsequently incorporated as a Division of the Armed Forces Health Surveillance Center (AFHSC) in 2008. AFHSC-GEIS divides its surveillance approach into five infectious disease categories: respiratory, gastrointestinal, febrile and vector-borne, antimicrobial resistant, and sexually transmitted. The goal of the GEIS febrile and vector-borne illness (FVBI) surveillance program is to integrate febrile illness, arthropod-vector, and pathogen discovery surveillance systems that contribute to Force Health Protection and global public health through: 1) characterizing the geographic distribution, transmission, and risk of FVBI pathogens and related illnesses; 2) outbreak detection and response; 3) generation of actionable surveillance data supporting informed patient care and FVBI disease risk assessments; 4) promoting FVBI-related research, training, and capacity-building initiatives. During fiscal year 2014, the FVBI program funded 36 competitive proposals supporting surveillance efforts in 32 countries. Recent program accomplishments include extensive global surveillance for drug-resistant malaria with initiation of a multi-site clinical trial evaluating Plasmodium falciparum artemisinin resistance in Thailand, Kenya, and Peru. Flea, chigger, and animal reservoir collections data (>90,000 data points) were also incorporated into the GEIS-funded VectorMap program, a publicly available repository for global arthropod vector collection data (www.vectormap.org). AFHSC-GEIS coordinates a worldwide surveillance network comprised of military and civilian laboratory partners who conduct FVBI surveillance in US military as well as foreign military and civilian populations. GEIS-funded initiatives yield enhanced recognition of the risks and threats from FVBI supporting the public health needs of military and associated populations in a growing number of partner nations.

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PROGRAMMATIC ASSESSMENT TOOL FOR RISKS OF MEASLES OUTBREAKS IN THE PHILIPPINES

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Measles is a highly contagious viral disease and remains an important cause of death and disability among children globally. In 2012, the World Health Organization (WHO) Regional Committee for the Western Pacific Region (WPR) reaffirmed its commitment to eliminate measles and urged member states to interrupt endemic measles virus transmission as rapidly as possible. Despite the Philippines’ continued commitment to this goal and a nationwide supplemental immunization activity conducted with measles and rubella vaccine targeting children aged 9 months to <8 years in 2011, a total of 1499 cases of measles were reported in 2012. Identifying areas at risk of outbreaks can provide an effective strategy in targeting immunization efforts and preventing future outbreaks. Based on a polo risk assessment tool developed in WPRO and applied in the Philippines, an assessment tool was developed to assess risk of measles outbreaks. Overall risk of an outbreak was assessed as a function of indicator scores that fall into four main categories of risk components (with corresponding weights): population immunity (50%), surveillance quality (24%), program delivery performance (6%), and threat probability assessment (20%). Cut-off criteria for each indicator score were created to assign an overall risk category score (RCS). The RCS was categorized into very high, high, medium, or low risk using available data at the second subnational administrative level. Preliminary results of the risk assessment compared with reported measles cases in 2013 showed clusters of laboratory-confirmed measles cases in areas identified as high and very high risk. Risk assessments can be used for advocacy to communicate risk to policymakers, mobilize resources, and strategically guide immunization response accordingly to the level of risk. Ongoing evaluation of indicators is needed to effectively plan risk mitigation activities and to evaluate performance towards elimination of measles. Further studies are needed to pilot test the risk assessment tool in other countries and regions.

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RISK FACTORS AND PREDICTORS OF PRETERM BIRTH IN DAR ES SALAAM, TANZANIA

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Preterm birth is associated with early life mortality and morbidity. In Tanzania, 1900000 babies are born each year of which 11.45% are preterm (less than 37 weeks gestation). Complications of preterm birth account for 40% of neonatal mortality and survivors are at high risk for long-term neurodevelopmental and behavioral sequelae. We aim to identify the incidence, risk factors, and predictors for preterm birth in Dar es Salaam, Tanzania. We prospectively followed 8428 mother-newborn pairs enrolled from 2001-2004. Maternal and socioeconomic characteristics, obstetric history, and medical illnesses were collected during pregnancy. Birth outcomes included small for gestational age (SGA, weight below the 10th percentile for gestational age) and preterm birth (less than 37 weeks gestation). We conducted bivariate and multivariate analyses using log-binomial regression models to examine the effect of predictor variables on outcomes. Of the 8428 pregnant women, 8003 (95.0%) gave birth to live newborns and were eligible for analysis. The incidence of preterm birth was 16.7%. Risk of preterm birth was associated with older maternal age greater than 34 years of age (RR=1.14 [unit=1 year]; 95%CI 1.07-1.20), weight in the lowest quintile (RR=1.24; 95% CI 1.11-1.39), less than one year of education (RR=1.67; 95%CI 1.44-1.93), and lower mid-upper arm circumference (RR=1.06 [unit=1cm]; 95%CI 1.04-1.07). In Dar es Salaam, preterm birth is associated with maternal age, wealth, and education, which may be surrogates to unmeasurable factors affecting fetal development. Mid-upper arm circumference, a measure of maternal nutrition, is one modifiable factor associated with preterm birth.

PREDICTORS OF EARLY MODERATE TO SEVERE STUNTING IN BOLIVIAN INFANTS (0-6 MONTHS)

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Childhood malnutrition, particularly stunting (low length-for-age), can have long-term adverse effects like impaired cognitive function, increased risk of chronic disease, decreased economic potential, and increased risk of maternal mortality. Bolivia, a lower-middle-income country, has one of the highest prevalence of infant malnutrition in the Americas with an estimated 9.5% of six month old infants being moderately to severely stunted. The purpose of this study was to identify predictors of moderate-to-severe stunting (length-for-age Z score <-2) among urban and peri-urban Bolivian children in early infancy (0-6 months of age). Convenience sampling at outpatient well-child visits was used to recruit 185 mother-infant pairs in El Alto, Bolivia, from June to October 2011. Researchers collected anthropometric data from mothers (height, weight) and infants (length, weight, head circumference) at two visits (4 to 6 weeks apart) as well as baseline socio-demographic, clinical, and perinatal characteristics. Multivariable logistic regression was used to identify predictors of being stunted at both visits. The prevalence of being stunted at both visits was 15.7%. Multivariable logistic regression showed that breastfeeding (OR:0.29 95%CI[0.1-0.81]), preterm birth (OR: 10.25 95% CI[3.26-32.23]), small-for-gestational age (OR:6.67 95% CI[2.21-20.18]), and inter-birth spacing of less than 24 months (OR:7.21 95% CI[2.08-24.94]) were significantly associated with stunting in this study population. Although this study was limited in its small sample size (leading to large standard deviations), its results were consistent with prior literature identifying preterm birth and small-for-gestational age (SGA) as predictors of childhood stunting. These results emphasize the need for targeted interventions to foster optimal in-utero growth and prevent small-for-gestational age and preterm births, which would lead to lower rates of stunting in Bolivia.

THE PUBLIC HEALTH IMPACT AND COST-EFFECTIVENESS OF DIAGNOSTIC AND PROGNOSTIC TOOLS FOR CASE MANAGEMENT OF NON-MALARIAL FEBRILE ILLNESS IN CHILDREN UNDER FIVE

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As malaria transmission declines globally, there is increasing recognition of the importance of correct identification and treatment of non-malarial febrile illness (NMFI). Studies have shown that in many regions, a decrease in antimarial therapy has been accompanied by a simultaneous increase in antibiotic use. In the absence of appropriate diagnostic and prognostic tools, currently recommended guidelines for integrated management of childhood illness (IMCI) can potentially lead to overuse of antimarials and antibiotics as well as under-referral of patients with early signs of severe illness requiring hospital treatment. We developed a Markov chain model to assess a range of diagnostic and prognostic tools for NMFI implemented at different levels of the healthcare system in terms of their impact on mortality and cost. The analysis was undertaken in a range of developing country healthcare infrastructure scenarios, including a comparison of factors such as high vs. low health facility access and public vs. private vs. community health systems. The model is parameterised using data on healthcare access, care-seeking behaviour, and epidemiological and clinical characteristics of febrile illness across a range of countries. Sensitivity analysis is conducted using data on the sensitivity and specificity of diagnostic and prognostic tools for identifying NMFI etiology and symptoms of early severe illness. The methods outlined here can be used to optimise case management strategies across a portfolio of potential diagnostic and prognostic tools considered for development and implementation.

STRENGTHENING MALARIA AND ANTENATAL CARE PROGRAMS WITH A SYSTEM COMBINING AVAILABLE TECHNOLOGIES

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RDTs are considered a viable alternative to deliver accurate results at POC. However, there are still obstacles to the widespread implementation of this strategy, such as lack of proper quality assurance of RDT-based programmes at POC and reporting constraints, especially in remote areas of low-income countries where the strategy is mostly needed. In collaboration between IHI and NIMR, an implementation research study was conducted in the Geita District to test the feasibility and usability of a system designed to overcome the issues described above. In brief, the system called Fionet is designed to assist peripheral HCWs in the processing and interpretation of RDTs, and collect and transmit clinical data to a cloud information service using local cell phone networks. Information captured is useful for evaluation of quality of RDT by district authorities. HCWs were trained in the use of mobile devices which contained electronic survey forms easily completed through a touch-
screen interface and at the same time guide RDT processing and perform automated interpretation of the results. All information collected and a high-resolution image of the RDT was transmitted to a central database located in a cloud information service. Data were safely stored and organized according to predetermined reports and was accessed via a website. Two patient populations were included in the current study: 1) general population (children and adults, male and female) in whom a malaria test was required; and 2) pregnant women attending ANC clinics in whom screening for both malaria and syphilis was performed to prevent mother to child transmission and adverse outcome of pregnancies. HCWs at all government facilities participating in the pilot were able to operate the system and collected over 5,000 patients in the course of 8 weeks. Health program managers were able to login to the portal and review cases uploaded, aggregated and organized in predefined reports, enabling to make recommendations about program management including monitoring of RDT processing in the field. Scale up of a system such as Fionet to at least a full-district level is warranted after the encouraging results of the present study, to fully demonstrate health system strengthening opportunities in delivery of care, monitoring and evaluation and improvement in system efficiencies.

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TRAINING AND CAPACITY BUILDING INITIATIVES IN SUPPORT OF GLOBAL HEALTH SECURITY, 2012-2014

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The Global Health Security Agenda, launched on 13 February 2014, called upon US government agencies to work together towards a vision of “a world safe and secure from global health threats posed by infectious diseases” by engaging with partner nations to strengthen their capacity for preventing avoidable epidemics, detecting infectious disease threats early, and responding rapidly and effectively. The Global Emerging Infections Surveillance and Response System Division of the Armed Forces Health Surveillance Center (AFHSC-GEIS) supports programs that strengthen global networks for real-time biosurveillance capabilities, train an effective biosurveillance workforce, and strengthen laboratory systems. In fiscal years 2012-14, AFHSC-GEIS supported a global health capacity building portfolio that included 67 projects in 34 countries. Specifically, AFHSC-GEIS partners executed health system improvements in six main areas: 1) electronic disease and biosurveillance systems (29 projects in 15 countries); 2) workforce development in WHO-approved epidemiology and outbreak response methods (23 projects in 20 countries); 3) tropical medicine training for host-country civilian and military personnel (11 projects in 10 countries); 4) training in, and capacity for, entomological surveillance and control methods (10 projects in 7 countries); 5) accredited laboratory practices and quality assurance efforts (34 projects in 21 countries); and, 6) health care facility and laboratory diagnostic capacity development (18 projects in 16 countries). The results of qualitative analysis of quarterly and annual reports from AFHSC-GEIS partners indicate that, while challenging, there has certainly been a progressive evolution of health security capacities within the network.

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DETERMINANTS OF DENGUE MORTALITY BEYOND BIOLOGICAL FACTORS: A SCOPING REVIEW

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Dengue is a viral disease which clinical spectrum varies from unapparent to severe forms and fatal outcomes. Though dengue death is avoidable in 99%, every year around 20000 deaths are estimated to occur in more than 100 countries. Beyond biological factors, social determinants of health (SDH) are related to fatal outcomes. A scoping review and content analysis using QDA Miner was conducted to document the role of SDH in dengue mortality. Inclusion criteria were any document with information of dengue fatal cases in humans, written in English, Spanish, Portuguese or French, peer reviewed or grey literature. Using a set of key words related to dengue mortality, PubMed LILACS /COCHRANE/ScienceDirect/WHOUS and Google Scholar, were the electronic databases used. From a total of 971 documents retrieved, 78 articles met the criteria and were reviewed. The documents were published in the Americas region (50%), Asia (38%), Europe (9%) and Africa (3%). The main article’s source of information was hospital records (56%) followed by a mix of surveillance data and hospital records (33%). Ninety-three percent included any information about the SDH. Information about individual dimension was found in 88.5% of reviewed articles, where age, education and type of infection/immunological status were considered determinants for dengue deaths. Sixteen articles (20.5%) did mention about health systems and described determinants were access, opportunity, quality of attention and health staff knowledge. Three articles (3.8%) reported socioeconomic and political context were poverty and social behavior were the determinants described. Gender and opportunity of attention were considered as dengue determinants dependent on social/personal health seeking behavior. Ethnicity was considered as biological determinant that also depends on cultural and socioeconomic context. These findings reveal the need for more studies about the role of SDH in dengue mortality, in order to design and implement interventions beyond biological factors in areas such education and the health systems (more data will be available at the presentation).

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LABORATORY QUALITY MANAGEMENT SYSTEM: EXPERIENCE WITH IMPLEMENTATION IN LATIN AMERICA AND THE CARIBBEAN

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Laboratories are essential for public health activities to include disease surveillance and control and in the treatment of diseases. The Quality Management System (QMS) in clinical laboratories emphasizes efficient use of resources, processes and personnel to reduce errors and ensure reliable and safe results. The aim of the joint CDC DHAPNAMRU-6 collaborative effort sponsored by DHP and PEPFAR is to strengthen QMS in clinical laboratories throughout Latin America and the Caribbean (LAC) to improve and measure compliance with quality laboratory services and prepare regional laboratories towards accreditation. Since 2012, 18 clinical laboratories in Belize, El Salvador, Guatemala, Honduras, Nicaragua, Dominican Republic, Colombia and Peru were enrolled in an improvement program. It began with a 5-week training workshop in Lima, Peru to include 2-weeks of training in Strengthening Laboratory
Management Towards Accreditation (SLMTA), followed by distance mentoring and technical assistant visits. We conducted baseline laboratory assessments using the Stepwise Laboratory Improvement Process towards Accreditation Checklist (SLIPTA WHO/AFRO), developed by WHO/AFRO for evaluating and monitoring the improvement progress of laboratories aiming to achieve the ISO 151889 standards. We report 12 laboratories which have now completed 12 months of the program with before and after evaluations. Among the 12 laboratories assessed, average baseline score was 118 (78-144), representing 44% compliance with the SLIPTA WHO/AFRO checklist. The average score after 12 months was 14% higher at 151 (120-180) representing 58% compliance. Among the 12 quality management systems evaluated, the three areas with greatest improvement included, customer management and customer service (28%), purchasing and inventory process control (16%), and Evaluation of internal and external quality control (15%). Information management (9%) and facilities and safety (4%) showed the least improvement. In conclusion, implementation of the QMS improved multiple areas of laboratory service and processes in diverse settings across LAC. Results of the initial post-testing evaluation will guide future training and resources for further improvement and help lead to the permanent adoption of effective laboratory practices.

CHARACTERIZATION OF ANOPHELES GAMBIAE HEME OXYGENASE

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Heme is a double-edged sword, being vital for oxygen transport yet highly toxic at high concentrations. Heme oxygenase (HO) plays a key role in detoxification through heme catabolism. Present in many organisms it is particularly well characterised in humans. Surprisingly, however, HO is poorly characterised in hematophagous insect vectors such as mosquitoes, yet such organisms deal with enormous influxes of dietary heme in their bloodmeals. Thus one might expect the enzyme to play a key role in survival. Here we are investigating the role of HO in mosquitoes and tsetse flies, which transmit malaria and sleeping sickness respectively. HOs in both organisms were identified via genome database searches, amplified, cloned, and expressed in E. coli. Spectroscopic assays have been done to confirm the catalytic attributes of vector HO in vitro, as well as Western blotting, qRT-PCR and immunolocalisation studies to investigate tissue-specific and temporal variation in HO expression. HPLC analysis is being conducted to identify the products of the HO reaction pathway. Finally, inhibition of HO activity has been carried out in vivo to determine the physiological role of HO. Here, a competitive HO inhibitor (zinc protoporphyrin) was fed to Anopheles gambiae populations. This resulted in a significant reduction in oviposition, suggesting a key role for HO in egg production. It is hoped that the results of these studies will identify new targets for the development of novel vector control agents.

THE CIRCADIAN CLOCK AND LIGHT/DARK CYCLE INFLUENCE RNA EXPRESSION IN THE Aedes aegypti MOSQUITO

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The biological rhythms of mosquitoes regulate and/or modify many behaviors and physiological processes underlying disease transmission, survivability, and fitness. The circadian clock regulation of RNA expression influences physiological and behavioral output on a daily basis. The Aedes aegypti mosquito is the major vector of Yellow fever and dengue virus, and exhibits 24 hr rhythms in biting, flight, and oviposition. To better understand the patterns of specific gene expression controlled by the daily light/dark (LD) cycle or the circadian clock in Ae. aegypti, we undertook a microarray analysis (>90% transcriptome) of adult female mosquito heads collected every 4 hr over 2 days maintained under LD or constant dark (DD) conditions. Data were subjected to cosine wave analysis (JTK_CYCLE) to determine for ~24 hr rhythmicity. We generated the Aedes aegypti Circadian Database, available at http://www.nd.edu/~bioclock/, in which these gene expression profiles and accompanying statistical analysis are searchable. This database provides the capability to examine the potential rhythmicity of the expression profile for each gene, as influenced by the LD cycle or the endogenous circadian clock. We identified 4 classes of genes present within the transcriptome: rhythmic only under LD cycle conditions, rhythmic only under DD conditions, rhythmic under both conditions, and non-rhythmic. The sets of genes active in vision, metabolism, and olfaction were investigated further, and those associated with vision were in good agreement with an earlier study on An. gambiae mosquitoes (Rund et al., 2011 PNAS 108:e421-30). Similarly, Ae. aegypti genes involved in metabolism and detoxification were under circadian control or non-rhythmic, as predicted by studies of An. gambiae. Our Ae. aegypti studies demonstrate similarities and differences in regulation of transcripts in different mosquito species, and suggest temporal modulation of processes such as vision and metabolism. These gene expression patterns likely identify both shared traits and the basis of species-specific behaviors.

DISSECTING THE FINE-SCALE MATING INTERACTIONS IN THE DENGUE VECTOR MOSQUITO Aedes aegypti

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Understanding mosquito mating biology is an important step towards identifying novel targets for mosquito control. We have begun dissecting the fine-scale mating biology in male Aedes aegypti. Seminal fluid proteins are produced in the reproductive tract tissues of male insects, primarily in the accessory glands, and are transferred... along with sperm... to females during mating. Seminal fluid proteins induce numerous physiological and behavioral changes in mated females, each of which might be targeted for novel control strategies. Our work on top candidates for genetic manipulation and the biology of male accessory gland function will be presented. Finally, and to further comprehend mosquito mating, we analyzed transcriptional changes within the lower female reproductive tract in response to male seminal fluid protein receipt. Our results suggest a wealth of new targets for control of these important disease vectors.

IMPACT OF INCREASED INSULIN SIGNALING IN THE FAT BODY OF ANOPHELES STEPHENSI AND Aedes aegypti MOSQUITOES ON LIFESPAN AND REPRODUCTION

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Lifespan is a key factor in determining the transmission efficiency of mosquito borne diseases. Finding a novel mechanism affecting mosquito lifespan could be a valuable tool to control mosquito borne disease transmission. The insulin/insulin-growth factor signaling (IIS) pathway may provide a novel endogenous solution to vector control. In mosquitoes, the IIS plays an important role in the regulation of many physiological processes, including longevity and reproduction. Here we aimed to increase insulin signaling in the fat body of An. stephensi and Ae. aegypti mosquitoes by creating a transgenic line expressing an active form of Akt, a key component of the IIS, specifically in the fat body tissue. We observed the effects on longevity and reproduction in a heterozygous line. However, contrary to the expected results, we observed an increase in the life span of heterozygous females positive for the transgene. We also observed no significant difference in the reproductive potential of heterozygous positive versus heterozygous negative females, an effect that was opposite of the
anticipated result. Ongoing work on this transgenic mosquito may yield unique insights into how the insulin signaling cascade regulates lifespan in mosquitoes and other eukaryotes.

**CROSSTALK OF IMD AND TOR SIGNALING PATHWAYS IN THE IMPORTANT DENGUE VECTOR Aedes aegypti**

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Mosquitoes transmit several devastating infectious disease such as malaria, dengue fever, yellow fever, Japanese encephalitis and filariasis. Due to the lack of effective vaccine and the increasing drug and insecticide resistance, alternative approaches for these mosquito-borne diseases are urgently required. It has been demonstrated that the Toll and the Immune Deficiency (IMD) signaling pathways play crucial roles in the production of antimicrobial peptides in Drosophila. Recently, the JNK pathway was shown to be activated by transforming growth factor-β-activated kinase 1 (TAK1), the component of IMD pathway, and to play an important role in tissue modeling in Drosophila. On the other hand, Matrix metalloproteinase 1 (MMP1) was demonstrated to be essential for the embryonic development in the Drosophila. Our previous study showed that Aedes aegypti TAK1 (AaTAK1) is responsible for the production of Cecropin A. We also showed the novel role of Aedes aegypti Matrix metalloproteinase 1 (AaMMP1) in the regulation of vitellogenesis. Our results revealed that silence of AaTAK1 by RNA interference approach resulted in the inhibition of AaMMP1 in the translatable level. In this study, we showed that the transcriptional pattern of AaTAK1 is highly expressed from 12 to 72 hours after a blood meal and particularly in the ovary and midgut. By RNAi-mediated silencing of AaTAK1, we showed that the egg production was reduced in the absence of AaTAK1. Interestingly, the expression of Vitellogenin (Vg) was inhibited in the absence of AaTAK1 or with the application of JNK inhibitor (SP600125) in the *in vitro* fat body culture system. In addition, by RNAi mediated silencing of AaJNK, the egg production was also reduced. Furthermore, we showed that silencing of AaTAK1 or AaJNK inhibit the phosphorylation of S6K, a key component of TOR pathway, and also inhibit the expression of Vitellogenin (Vg) and AaMMP1 in the fat body. Taken together, our data suggest a novel function of AaTAK1 and AaJNK in the regulation of vitellogenesis and AaMMP1 through TOR signaling pathway.

**GENETIC BASIS OF PYRETHROID RESISTANCE IN A POPULATION OF ANOPOHELES ARABIENSIS, THE PRIMARY MALARIA VECTOR IN LOWER MOSHI, NORTHERN EASTERN TANZANIA**

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Pyrethroid resistance has been slower to emerge in Anopheles arabiensis than in An. gambiae s.s and An. funestus and, consequently, studies are only just beginning to unravel the genes involved. Permethrin resistance in An. arabiensis in Lower Moshi, Tanzania has been linked to elevated levels of both P450 monoxygenases and β-estesases. We have conducted a gene expression study to identify specific genes linked with metabolic resistance in the Lower Moshi An. arabiensis population. Microarray experiments employing an An. gambiae whole genome expression chip were performed on An. arabiensis, using interwoven loop designs. Permethrin-exposed survivors were compared to three separate unexposed mosquitoes from the same or a nearby population. A subsection n of detoxification genes were chosen for subsequent quantitative real-time PCR (qRT-PCR). Microarray analysis revealed significant over expression of 87 probes and under expression of 85 probes (in pairwise comparisons between permethrin survivors and unexposed sympatric and allopatric samples from Dar es Salaam (controls). For qRT-PCR we targeted over expressed ABC transporter genes (ABC ‘2060’), a glutathione-S-transferase, P450s and esterases. Design of efficient, specific primers was successful for ABC ‘2060’ and two P450s (CYP6P3, CYP6M2). The primers for CYP4G16 used were previously used in a microarray study of An. arabiensis from Zanzibar islands. Over expression of CYP4G16 and ABC ‘2060’ was detected though with contrasting patterns in pairwise comparisons between survivors and controls. CYP4G16 was only up regulated in survivors, whereas ABC ‘2060’ was similar in survivors and controls but over expressed in Lower Moshi samples compared to the Dar es Salaam samples. Increased transcription of CYP4G16 and ABC ‘2060’ are linked directly and indirectly respectively, with permethrin resistance in Lower Moshi An. arabiensis. Our study provides replication of CYP4G16 as a candidate gene for pyrethroids resistance in An. arabiensis though its role may not be in detoxification and this requires further investigation.

**WNT SIGNALING IS ESSENTIAL FOR THE REGULATION OF TOR SIGNALING-MEDIATED EGG PRODUCTION IN THE MOSQUITO Aedes aegypti**

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Mosquito-borne diseases are the most devastating agents for human being, due to its high diversity of transmissible pathogens like protozoan and viruses. Despite the efforts from government agencies that have contributed the eradication of the mosquito-borne diseases for several decades, the goal has not been achieved yet. Therefore, many research institutes turn their attentions toward the mosquito life cycle and immune system to halt the disease transmission. Previous studies have already been demonstrated that Target of Rapamycin (TOR) pathway plays an important role in mosquito vitellogenesis, whereas WNT pathway participates in the embryonic development and cell polarity in Drosophila. However, the interactions between these pathways are poorly understood. In this study, we propose a hypothesis that factors of TOR and WNT signaling pathway play synergistically in the mosquito vitellogenesis. We attempt to characterize Wnt signaling components in the mosquito, *Aedes aegypti*. Our results showed that silencing of *Aedes aegypti* Frizzled2 (AaFz2), a transmembrane receptor of Wnt signaling pathway, resulted in the decrease of fecundity in *Ae. aegypti*. We showed that AaFz2 is highly expressed in the mosquito fat body at 6 hours post blood meal in terms of transcriptional and translational level, suggesting the amino acid stimulated feature of AaFz2. Interestingly, the phosphorylation of S6K and the expression of Vg were inhibited in the absence of AaFz2. These findings determine a direct link between Wnt and TOR signaling in the regulation of mosquito reproduction.

**FUNCTIONAL ANALYSIS OF THIOESTER-CONTAINING PROTEIN COMPLEX IN DENGUE VIRUS INFECTION**

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Dengue fever is one of the most devastating arthropod-borne diseases. The WHO reported some 2.5 billion people are now at risk from dengue and estimates that there may be 50 million cases of dengue infection worldwide every year. Up to now, no effective dengue vaccine or drug has been developed. Therefore, intensive study for potential host factors in the mosquito vector and construction of transgenic mosquito together with gene drive technique to replace the vector populations became an alternative strategy to combat dengue virus. Here, we made use of
the well-established reverse genetic approach by silencing potential host factors in the mosquitoes. Our results showed that two immune responsive genes, Thioester-containing protein 1 (TEP1) and Leucine-Rich Immune Gene 1 (LRIM1), were highly expressed in response to dengue virus infection in the mosquito midgut. Silenced of TEP1 or LRIM1 resulted in the over-expression of DENV E-protein in the mosquito midguts. Immunofluorescent assay revealed that silenced of TEP1 or LRIM1 resulted in the over-expression of DENV E-protein in the mosquito midguts. We also demonstrated that the translation level of TEP1 is highly taken in the mosquito midgut after an infectious blood meal. Taken together, our results suggest TEP1 or LRIM1 are important candidates for the establishment of transgenic mosquito against dengue virus. We are currently in the construction of gain-of-function TEP1 transgenic mosquito line with the blood meal inducible carboxypeptidase promoter. Our results will provide new insights into the understanding of dengue-vector interaction and new strategy for dengue eradication program. Data revealed by this proposal will be crucial for future studies on vector competence and vector control in the field.

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**MOSQUITOCIDAL PROPERTIES OF IGG TARGETING THE GLUTAMATE-GATED CHLORIDE CHANNEL IN THREE MOSQUITO DISEASE VECTORS (DIPTERA: CULICIDAE)**

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Mosquito-borne diseases account for an estimated 1,434,000 deaths annually and 60,056,000 disability adjusted life years. Historically, the most successful strategies to control disease transmission have been by targeting mosquito vectors through the use of chemical insecticides. The glutamate-gated chloride channel (GluCl) is a conserved and highly sensitive target of the drug ivermectin. As an alternative to using chemical insecticides to kill mosquitoes, we tested the effects of purified immunoglobulin G (IgG) targeting the extracellular domain of GluCl from *Anopheles gambiae* (AgGluCl) on the survivorship of three mosquito disease vectors that vary in their sensitivity to ivermectin: *Anopheles gambiae* s.s. > *Culex tarsalis* > *Aedes aegypti*. AgGluCl IgG mixed in a single blood meal significantly reduced the survivorship of *A. gambiae* (LC50 = 2.82 mg/mL [2.68-2.96]), as did serially blood feeds containing 1/10th of the LC50 concentration. However, AgGluCl IgG did not affect the survivorship of *A. aegypti* or *C. tarsalis*. Transcriptional analyses showed AgGluCl mRNA was present in the *An. gambiae* adult female head and thorax, but not the abdomen and immunohistochemical staining showed AgGluCl expression only in the antenna, Johnston's Organ, supraesophageal ganglion and thoracic ganglia. Interestingly, injection of AgGluCl IgG into the hemocoel equally reduced the survivorship of *An. gambiae*, *A. aegypti* and *C. tarsalis*, suggesting that AgGluCl IgG sensitivity of blood fed *A. gambiae* is due to permissive antibody diffusion across the midgut. To analyze AgGluCl IgG’s mode of action, we fed *An. gambiae* blood meals containing both AgGluCl IgG and ivermectin (a GluCl agonist). AgGluCl IgG attenuated the mosquitoicidal effects of ivermectin, suggesting that AgGluCl IgG acts as a GluCl antagonist. These data characterize mosquito GluCl as an important insecticide and immunological target. Further, the science of developing mosquitocidal vaccines, and lend insight into the unique properties of the *Anopheles* midgut relative to other mosquitoes.

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**POST-INSEMINATION TRANSCRIPTIONAL RESPONSE IN THE LOWER REPRODUCTIVE TRACT OF AEDES AEGYPTI FEMALEs**

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Insemination induces substantial changes in female *Aedes aegypti* mosquitoes. Physiologically, inseminated females accelerate blood digestion and oogenesis. Behaviorally, they increase oviposition, alter host-seeking behavior, and become refractory to subsequent insemination.

While it is known that these changes are induced by male seminal fluid proteins, what happens in the female to produce these changes is largely unknown on the molecular level. In order to better understand these changes, their associated pathways, and the specific genes involved, we used RNA-seq to conduct a differential expression transcriptome analysis of the lower reproductive tract (LRT, i.e. the bursa copulatrix, spermathecae, common oviduct, and lateral oviducts) of female *Ae. aegypti*. Comparisons were made between virgin females and those at 0, 6, and 24 hours post insemination. Our results provide a framework for further investigation of the postcopulatory physiology and behavior of this important disease vector.

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**INVESTIGATION ON ENTOMOPATHOGENIC FUNGAL FAUNA OF MOSQUITOES IN BURKINA FASO: TRANSGENIC BIOCONTROL PERSPECTIVES**

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Unlike bacteria and viruses, fungi infect mosquitoes through direct contact with the cuticle and so lend themselves to strategies currently used for delivery of chemical insecticides, and in addition may provide environmentally preferred alternatives. Much attention has focused on the ascomycetes *Metarhizium* spp. and *Beauveria* spp. However it should be noted that not much is known about fungal pathogens of adult malaria mosquitoes in the field. In that respect this study aims to investigate entomopathogenic fungi which naturally infect mosquitoes in Africa and their potential use as genetically engineered biocontrol agents. The field collection of mosquitoes was carried out in Burkina Faso. Mosquitoes were collected alive and maintained in insectary conditions with access to sugar water till death. Every morning, dead mosquitoes were collected and isolated individually in a petri dish for fungal growth. Fungal isolation and culture was conducted in a sterile environment. Two different growth media were used. Potato Dextrose Agar, a common medium for isolating fungi, and Potato dextrose agar + CTAB + Chloramphenicol, selective medium. The analysis is ongoing to unravel an exhaustive list of entomopathogenic fungi. The natural virulence of these fungi toward malaria vectors will be subsequently tested. Final results will be available in coming months, before October 2014.

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**DETECTION OF POINT-MUTATIONS IN THE KDR GENE OF THE DENGUE VECTOR Aedes albopictus**

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The Asian tiger mosquito, *Aedes albopictus*, is the most invasive mosquito in the world and a main vector of dengue, chikungunya and other viruses. Though *Ae. albopictus* originated from east Asia, in recent 30 years, it has migrated to all of the continents except Antarctica. Treatment of public areas with high population density of *Ae. albopictus* with adulticides, mainly pyrethroids, is a recommended protective measure in response to Dengue epidemics and routine treatments with insecticides is performed alongside source reduction through environmental modification. Intensive pyrethroid application poses selection pressure on mosquito populations for increased resistance. Physiological resistance to pyrethroids has been documented already in several *Ae. albopictus* populations, mainly from the native home range in Asia, abradng the sustainability of control programs based on these insecticides. Field collections of *Ae. albopictus* were conducted in six sites throughout the Guangdong province in South China and subjected to standard WHO tube assays with 0.045% deltamethrin based on discrimination dose determined by the *Ae. albopictus* reference susceptible strain. Between...
375 and 658 females were tested per site. Mortality ranged between 28.3% and 84.9%, indicating wide-spread phenotypic resistance in south China. Apart from standard WHO tube assay, rapid and reliable detection of resistance is recognized as an important element of resistance management. Historically, the most used markers for pyrethroid resistance are mutations in the para-gated sodium channel gene (kdr) gene. Here we report the current polymorphism of the kdr gene in 10 world-wide populations across the world. A total of seven non-synonymous mutations were identified. Although the frequency of non-synonymous mutations was generally low, one population from southern China showed 41.9% frequency, suggesting that kdr point mutations are widely spread among Ae. albopictus population in various part in the world.

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THE ANOPHELES GAMBIAE MOSQUITO MIDGUT TRANSCRIPTOME BY RNA-SEQ

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The mosquito midgut is the first organ to interact with malaria parasites. This tissue can mount effective antiplasmodial responses that limit parasite survival and disease transmission. RNA-Seq by Illumina sequencing of the midgut transcriptome was used to identify new genes and transcripts, contributing to refinement of Anopheles gambiae genome annotation. We sequenced ~223 million reads from An. gambiae midgut cDNA libraries generated from susceptible (G3) and refractory (L35) mosquito strains after Plasmodium berghei or P. falciparum infections. In total, 22,889 unique midgut transcript models were generated from both An. gambiae strain sequences combined, and 76% are potentially novel. Of these novel transcripts, 49.5% aligned with annotated genes and appear to be isoforms or pre-mRNAs of reference transcripts, while 50.5% mapped to regions between annotated genes and represent novel intergenic transcripts (NITs). Predicted transcripts were validated for midgut expression using qRT-PCR and microarray analysis, and novel isoforms were confirmed by sequencing. Coding potential analysis revealed that 43% of total midgut transcripts appear to be long non-coding RNA (lncRNA), and functional annotation of NITs showed that 68% had no homology to current databases from other species. Reads were also analyzed using de novo assembly and predicted transcripts compared with genome mapping-based models. Finally, variant analysis of G3 and L35 midgut transcripts detected 160,742 variants with respect to the An. gambiae PEST genome, and 74% were new variants. This in-depth sequencing and assembly of the An. gambiae midgut transcriptome doubled the number of known transcripts and tripled the number of variants known in this mosquito species. It also revealed existence of a large number of lncRNA and opens new possibilities for investigating the biological function of many newly discovered transcripts.
LIFE SHORTENING EFFECT OF OLYSET® DUO, A LONG-LASTING INSECTICIDAL NET INCORPORATING A MIXTURE OF PYRETHRROID AND PYRIPROXYFEN, AGAINST PYRETHRROID-RESISTANT MOSQUITO

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The increase in pyrethroid resistance in mosquitoes across Sub Saharan Africa has become a serious threat for malaria vector control, and threatens to reverse the recent gains achieved by the widespread use of bed nets. New bed net products are clearly needed to meet these gains. It has previously been shown that contact of adult mosquitoes with pyriproxyfen treated materials can dramatically reduce egg laying and shorten the life expectancy of exposed females. Pyriproxyfen treated bed nets could therefore be used as a resistance management tool against to help prevent the selection of pyrethroid resistance. These findings led to the development of Olyset® Duo which is a long-lasting insecticidal mosquito net incorporating a mixture of 2% w/w permethrin and 1% w/w pyriproxyfen on all sides of the net. In this study we evaluate the life shortening effect of this net against susceptible and pyrethroid-resistant malaria vectors in the laboratory. Olyset Duo and PPF LN, a pyriproxyfen alone net, were washed 3 times according to WHO guidelines and exposed to a susceptible (Kisumu) and a kdr resistant strain (VK7) of Anopheles gambiae in the WHO tunnel test. Contact with the PPF LN significantly reduced the survival rate of blood-fed females of both strains, while contact with Olyset Duo killed the susceptible strain and reduced the longevity of surviving blood-fed females of the resistant strain, when compared with the effect of exposure to untreated netting. These results indicate that Olyset Duo could reduce vectorial capacity of malaria vectors through the life shortening effect of pyriproxyfen and thus disrupt the malaria transmission cycle (since a reduction in longevity of as little as 30% can reduce vectorial capacity by approximately 300 times). The cumulative impacts of mortality of mosquitoes from exposure to permethrin, and the suppression of progeny combined with the observed life shortening effects on females that survive permethrin exposure are anticipated to have significant impacts on malaria transmission under field conditions.

ANOPHELES GAMBIAE SENSU LATO POPULATION SUSCEPTIBILITY TO THE COMMONLY PUBLIC USED INSECTICIDES FOR MALARIA CONTROL IN MALI

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Current vector control strategies are mainly based on the use of chemical insecticides for insecticide-treated nets (ITNs) and indoor residual spraying (IRS) of houses. Mosquito resistance to at least one of the insecticides used for malaria control has been observed in many countries including Mali. Consequently, monitoring insecticide resistance is a key element of the implementation of insecticide-based vector control interventions. The aim of this study was to update data on vectors resistance to insecticides in National Malaria Control Program’s sentinel sites in Mali. World Health Organization standard bioassay method was used to assess resistance in 3-5 days old F0 or F1 adult female An. gambiae s.l. Insecticides were Lambda-cyhalothrin 0.05%, DDT 4%, Permethrin 0.75%, Deltamethrin 0.05%, Bendiocarb 0.1% and Fenitrothion 1.0%. Results: Suspected to confirmed phenotypic resistance of Anopheles gambiae s.l. population were observed for all tested pyrethroids and DDT in all sites except in Yanfolila where it was susceptible to lambda-cyhalothrin (98.0%) [IC 95% 98.0-98.9] and to DDT (100%). An. gambiae s.l. was susceptible to the bendiocarb in Gao, Bougouni, Djenné, Yanfolila and Tombouctou, while suspected resistance was observed in Kati, Niono, Bandiagara and Kita. Except in Niono, a rice cultivation area (92% [IC 95% 88.3-94.8] mortality), An. gambiae s.l. population was fully susceptible to the Fenitrothion in all sentinel sites. These results showed resistance of Anopheles gambiae s.l. population to pyrethroids in the majority of the sentinel sites. Therefore, we suggest to the NMCP to alternate the pyrethroid with the Fenitrothion as management strategy to the current resistance.

EVALUATING THE EVIDENCE FOR EFFECTIVENESS OF VECTOR CONTROL OF DENGUE OUTBREAKS BY SYSTEMATIC REVIEW AND META-ANALYSIS

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Dengue is the most widespread mosquito-borne arboviral disease worldwide with an estimated 400 million cases occurring annually and almost half of the world’s population at risk. At present, preventing and responding to dengue outbreaks both rely on vector control to suppress vector populations or reduce dengue virus transmission, most commonly by eliminating or larviciding mosquito breeding sites and space-spraying with insecticide, respectively. Other approaches are also available and used widely, but with none considered to be sufficiently proven or reliable enough for recommendation, perceptions or beliefs regarding the effectiveness of each approach vary widely among decision-makers in the public health community, often without foundation. Addressing this within the aims of the European Union supported IDAMS research consortium (International Research Consortium on Dengue Risk Assessment, Management and Surveillance; www.idams.eu), we undertook a systematic review and meta-analysis of the evidence available from published studies, to determine if specific vector control tools could impact on vector abundance and/or dengue incidence. A total of 945 studies were found during systematic searches of peer-reviewed databases and grey literature; 35 of these satisfied all inclusion and exclusion criteria and were subject to the Quality Assessment Tool for Quantitative Studies (QATQS). The PRISMA guidelines were followed to ensure rigorous application of review principles. Control approaches included insecticide fogging/space-spraying, insecticide-treated materials, vector trap devices, house screening, mosquito coils and other repellents, clean-up or environmental management and biological control agents. Initial analyses indicate that house screening and community-based programmes can potentially have a beneficial impact, while evidence suggests that repellents and traps do not. Complete results of all final analyses will be presented and the implications of the findings for dengue control in the face of today’s realities will be fully considered.

INVESTIGATING THE MECHANISMS OF DDT AND DIELDRIN RESISTANCE IN FIELD POPULATION OF ANOPHELES FUNESTUS IN SENEGAL

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Insecticide resistance in Anopheles funestus, one of the main malaria vectors, is threatening malaria control in Africa. Elucidation of the mechanisms of resistance is crucial to the design of suitable resistance management strategies. Therefore, we have investigated the mechanisms responsible for DDT and dieldrin resistance in An. funestus population
from Senegal. Insecticide susceptibility assays were carried out using 2-5 day old F1 adults generated from indoor-collected, blood-fed female of An. funestus from Gankette, in Northern Senegal. WHO bioassays indicated that An. funestus is resistant to lambda-cyhalothrin 0.05% (74.64% mortality / n = 222), DDT 4% (83.36% mortality / n = 158) and deltamethrin 0.05% (88.53% mortality / n = 114). Suspected resistance was observed to permethrin 0.75% (91.19% mortality / n = 139), bendiocarb 0.1% (94.13% mortality / n = 157) and dieldrin 4% (96.41% mortality / n = 306). However this population is fully susceptible to malathion 5% (100% mortality / n = 50) and fenitrothion 1% (100% mortality / n = 55). Genome-wide transcription analysis using microarray and quantitative RT-PCR revealed that the cytochrome P450 CYP6M7 was the detoxification gene most commonly over-expressed in DDT resistant mosquitoes and field unexposed to insecticide compared to a laboratory susceptible strain. In addition, several others genes with diverse functions including glutathione S-transferases were also overexpressed. Using the pyro-sequencing method, The A296S Rdl(R) target site mutation was detected in all dieldrin resistant mosquitoes but at a low frequency (14%) in the field sample. Our study has revealed a strong association between the dieldrin resistance phenotype and the presence of the Rdl mutation. TaqMan genotyping revealed that the L119F mutation in the GSTe2 gene conferring DDT resistance in Benin is completely absent in Senegal. This indicates a shift of DDT resistance mechanisms in West Africa An. funestus. These results could help to guide the implementation of suitable control interventions against this vector in Senegal.

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EXTENSIVE ALTERNATIVE SPICING IN THE VOLTAGE GATED SODIUM CHANNEL OF ANOPELEHES STEPHENSI

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Anopheles stephensi, a sub-tropical species distributed throughout the Middle-East and South Asia is one of the major malaria vectors in India mainly in urban areas. Resistance to DDT in this vector has been reported in India since 1965, and development of incipient resistance against pyrethroids has recently also been reported. One of the mechanisms of resistance in insects is reduced sensitivity of the insects to these two insecticides due to alteration in the target site, the voltage gated sodium channel (VGSC). A single amino acid substitution in the VGSC (kdR mutation) is known to significantly alter the susceptibility of vector to insecticides by altering channel kinetics. The role of alternative splicing which reorganizes a primary transcript to generate multiple transcripts, in insecticide resistance is not yet known in insects although such events are known to significantly alter the channel kinetics. To understand the extent of alternative splicing in An. stephensi, and the possible role in generating altered sensitivity to DDT and pyrethroids, the whole coding region of VGSC gene of DDT- and pyrethroid-resistant mosquitoes was amplified in two overlapping fragments, cloned and sequenced. Analysis of sequences revealed extensive alternative splicing events. These include several different exon-skipping events, four alternative acceptor sites, two mutually exclusive exons and one intron-retention. In addition to these alternative splicing events, modification at the 5’ un-translated region has also been recorded where quadruplet repeats of 30 nucleotides are seen. Preliminary data in our study showed two splicing events to be pronounced in resistant clones. The role of such splicing events is being examined to determine correlation with DDT and pyrethroid resistance.

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INSECTICIDE RESISTANCE IN ANOPELEHES FUNESTUS IN SOUTHEASTERN AFRICA AND ITS IMPACT ON MALARIA CONTROL PROGRAM FAILURE

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Malaria parasites are transmitted to humans by anopheline mosquitoes. The parasite undergoes an obligatory sexual stage within the mosquito midgut that takes up to 14 days to complete. This presents a window of opportunity for us to control the mosquito populations before they have sufficient time to become infective. Unfortunately, both mosquitoes and parasites have been around a lot longer than humans and so far have managed to find ways of getting around all the insecticides and drugs that we throw at them. In Africa today there are approximately 140 recognised species of Anopheles mosquitoes. Only 4 of these are really good vectors of malaria parasites and of these 4, Anopheles funestus is the most important vector in the south-eastern African region. It is highly adapted to humans and human habitations and should, therefore, be easy to control using current technology. Unfortunately, it has developed high levels of resistance to the pyrethroid insecticides that are used for both treating bed nets and spraying on the inside walls of houses. Recent entomological surveys in Zimbabwe, Zambia, Malawi and Mozambique show an almost uniform profile of resistance in An. funestus with unpublished data from Zimbabwe indicating a definite impact on programme failure. There is an urgent need for resistance management strategies to be implemented in these countries.

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UNDERPINNING SUSTAINABLE VECTOR CONTROL THROUGH INFORMED INSECTICIDE RESISTANCE MANAGEMENT

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There has been a rapid scale-up of vector control for malaria in the last ten years. Both of the primary strategies, long-lasting insecticidal nets and indoor residual spraying, rely on the use of a limited number of insecticides. Insecticide resistance has rapidly increased in prevalence and has come to the forefront as an issue that needs to be addressed in order to maintain the sustainability of control efforts in the drive to elimination. Zambia’s programme reported high levels of resistance to the insecticides it used in 2010. As a result, it increased its investment in resistance monitoring to support informed resistance management decisions. A national survey on insecticide resistance in Zambian malaria vectors, covering 26 districts in all 10 provinces, was performed using WHO bioassays to detect resistant phenotypes. Molecular techniques were used to detect target-site mutations and microarray to detect metabolic resistance mechanisms. Anopheles gambiae s.s. was resistant to pyrethroids, DDT and carbamates, with potential organophosphate resistance in one population. The resistant phenotypes were conferred by both target-site and metabolic mechanisms. Anopheles funestus s.s. was largely resistant to pyrethroids and carbamates, with potential resistance to DDT in two locations. The resistant phenotype was conferred by elevated levels of cytochrome p450s. Currently, the Zambia National Malaria Control Centre is using these results to inform their vector control strategy. The methods employed here can serve as a template to all malaria-endemic countries striving to create a sustainable insecticide resistance management plan.
INCREASED EXPRESSION OF METABOLIC RESISTANCE CANDIDATE MUTATIONS IN THE MALARIA VECTOR ANOPHELES GAMBIAE SENSU STRICTO IN DAR ES SALAAM, TANZANIA

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One of the major challenges facing malaria control is the development of insecticide resistance in vector mosquitoes. Anopheles gambiae, which is a major vector of the malaria parasite Plasmodium falciparum in Africa, has over the years developed resistance to dieldrin, 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane (DDT), and pyrethroids. The objective of this study was to determine the mechanisms contributing to DDT resistance in Anopheles gambiae s.s. in Dar es Salaam, Tanzania. Female mosquitoes of standard age, reared from larvae sampled across a variety of natural breeding sites, were used in the study. Members of the An. gambiae complex were PCR-identified and screened for target-site mutations (Vgsc-1014S and Vgsc-1014F). A DDT-resistant population of An. gambiae s.s. and controls (sympatric and allopatric controls) were screened for GSTε2 and P450 genes-expression profiles using real-time quantitative polymerase chain reaction (qPCR) tests. A significantly higher allelic frequency of the Vgsc-L1014S mutation was found in DDT-resistant An. gambiae s.s. than in the control mosquitoes (p<0.001). The cytochrome P450 genes Cyp6m2, Cyp6p3, Cyp6Z3 and Cyp6Z1 were significantly over-expressed in DDT-resistant An. gambiae s.s. compared with the control populations. We report increased expression of multiple DDT-associated resistance mechanisms in the primary African malaria vector, An. gambiae s.s. in Dar es Salaam. The presence of multiple resistance mechanisms in An. gambiae that are common to both DDT and pyrethroids may have confounding effect in resistance-management strategies. However, the geographical extent of the insecticide resistance mechanisms observed in this study needs to be investigated further.

A NOVEL LONG-LASTING INSECTICIDAL NET BASED ON A MIXTURE OF CHLORFENAPYR AND ALPHACYPERMETHRIN CONTROLS MULTI-RESISTANT ANOPHELES MOSQUITOES UNDER FIELD CONDITIONS AFTER MULTIPLE WASHING: EXPERIMENTAL HUT RESULTS FROM EAST AFRICA AND IMPLICATIONS FOR WHOPES TESTING GUIDELINES

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The emergence and spread of pyrethroid-resistant Anopheles across Africa is a grave threat to malaria control. There is an urgent need for non-pyrethroid, wash-resistant LLINs to be developed. Chlorfenapyr is a pyroile insecticide with a distinct mode of action which disrupts cellular respiration. Interceptor 2 is a “coating” LLIN containing a mixture of chlorfenapyr (CPF) and alphaphycpermethrin-α. This study assessed wash-resistance in experimental hut field trials and explored the properties of CPF in laboratory tests based on current WHOPES guidelines and behavioural tests. Hut trials in Tanzania with the mixture LLIN killed 63% and 66% of An. arabiensis when unwashed and 10 times washed respectively, compared with 46% for the pyrethroid LLIN (Interceptor 1-α only) washed 10 times. HPLC showed that after 10 washes Interceptor 2 had 75% CPF and 95% α still remaining. This is the first report of a non-pyrethroid insecticide on a LLIN performing successfully after multiple washes. By contrast the standard WHO bioassays failed to predict the performance of chlorfenapyr and therefore need urgent revision otherwise suitable insecticides may be overlooked. The three minute bioassay of CPF nets produced <15% mortality while LLIN require >80% mortality to pass the LLIN test. In laboratory testing there was a strong positive correlation between temperature and mortality above and below standard test temperature. Bioassay tests conducted during the night produced consistently higher levels of mortality than the same tests in the day time. It appears that conversion of CPF to the active metabolite and its disruption of respiratory pathways is enhanced at night when the host seeking mosquito is more metabolically active during the active phase of its circadian rhythm. Testing according to WHOPES guidelines are not suitable for certain types of non-neurotoxic insecticide which, though highly effective in field trials, would be overlooked at the screening stage of evaluation through conventional bioassay.
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Insecticide resistance is threatening the reduction in malaria burden achieved by the massive scale up of vector control measures. One of the most effective vector control tool is the long lasting insecticidal nets (LLNs). LLNs rely on pyrethroid insecticides to provide a repellent barrier between humans and mosquitoes and kill mosquitoes before they can transmit malaria. Pyrethroid resistance is now occurring in most countries and can undermine the effectiveness of the LN. The development of new tools combining chemical with different mode of action is a necessity to control resistant vector and sustain the gain achieved in controlling malaria. Olyset Duo is a new LN combining a permethrin and pyriproxyfen as an insect growth regulator. Pyriproxyfen can sterilize adult mosquitoes and incorporated on a net reduce the vector density. The efficacy and wash resistance of Olyset Duo is currently evaluated in Muleba, Tanzania. Standard WHO cone, cylinder and tunnel test has been performed in the laboratory to evaluate mortality, blood feeding inhibition, reduction in fecundity and fertility of adult female anophelines exposed to Olyset Duo. Experimental hut trial has been also carried out to evaluate the impact of the mixture net on the wild free flying An. arabiensis compared to standard LNs. Anopheles exposed to unwashed Olyset Duo in cone, cylinder and tunnel show higher mortality and lower blood feeding rates than to the standard Olyset Net (permethrin alone). The surviving Anopheles exposed to Olyset Duo were all sterilized. This should have impact on the population size of the next generation. Initial results of this novel LN's mixture show great potential for the control of resistant malaria vectors in Sub Saharan Africa. The full outcomes of the study will be presented.

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Insecticide treated nets (ITN) and indoor residual spraying (IRS) are two of the primary methods of malaria prevention in Africa. In order for these methods to be effective it is essential that adequate concentrations of insecticide are present on nets and walls surfaces to kill mosquitoes. There is no easy assay to quantify insecticide levels without expensive laboratory equipment and procedures. To address this, LSHTM has developed a simple field-applicable kit for monitoring pyrethroid residues on insecticide-treated nets- the Pyrethroid Quantification Kit (PQK). This kit can be adapted to other types of treated surfaces. During the initial trials the PQK kit was calibrated against a variety of sprayed surfaces and with different concentrations of lambda-cyhalothrin before being taken into the field. Mosquito cone bioassay was conducted to show whether the surface concentrations of insecticide detected by the PQK were sufficient to kill a susceptible strain of mosquitoes. Houses in six villages were visited 3 months after IRS had been conducted in Muleba, Tanzania. The samples were analysed in the field using a handheld spectrophotometer. In each house, five areas of the wall were examined to give an indication of insecticide distribution and within-wall variation. Results showed that the actual spraying results differed from expectation. Preliminary results showed that only 28\% of houses had all rooms sprayed, leaving 72\% of houses partially sprayed, and insecticide concentration varied dramatically across sprayed walls. The PQK is an easy to use quality assurance tool for monitoring of pyrethroid application rates and improving the quality of IRS campaigns.

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The Anopheles Ace-1 gene encodes the neurotransmitter acetylcholinesterase, the target of organophosphate and carbamate insecticides, which are of growing importance for vector control. A single base mutation (G119S), which causes a major conformational change in the protein, gives resistance to both insecticide classes in mosquitoes. However, the nature, evolution and phenotypic consequences of the more recently identified copy number variation (CNV) of Ace-1 are far less well understood in An. gambiae. We show that CNV is present throughout West Africa and appears to be ubiquitously associated with resistant (119S), but is much less commonly associated with wild-type susceptible (119G) alleles. In Culex piapiensis Ace-1 CNV is well known and involves duplication which pairs a 119G and a 119S allele on the same chromosome to create a permanent heterozygote. However, in An. gambiae many Ace-1 allele copies can be found in 119S homozygotes and sequence analysis suggests that resistant alleles may have originated, or at least spread, following gene duplication. Duplicated alleles are expressed though not necessarily in direct proportion to copy number, and Ace-1 overexpression is linked to high-level carbamate resistance. Diagnostic assays and neutral markers show that Ace-1 119S duplicated resistant alleles are strongly selected and increasing in frequency. Given strong predictive links with carbamate and organophosphate resistance, molecular monitoring should be a key component of programmes using these insecticide classes for control of An. gambiae.

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Long-Lasting Insecticidal Nets (LLINs) are the mainstay of malaria control, particularly in sub-Saharan Africa. However, despite many National Malaria Control Programs (NMCPs) adopting partial or universal LLIN coverage, there is still only limited knowledge of the effective life of nets after user conditions - commonly known as LLIN durability. Our ABCDR study retrospectively investigates four aspects determining LLIN durability - attrition, bioefficacy, chemical content and physical degradation - of national campaign LLINs in eight districts in Tanzania covering a range of epidemiological and ecological settings. Nets were collected from 3,420 households, and a questionnaire was conducted to determine net ownership and net use. The number, size and location of holes and bioefficacy against anopheline mosquitoes were measured in a sub-sample.
of identified campaign nets. A total of 6,832 nets were collected, though not all of them were LLINs. Preliminary results suggest that net ownership, coverage and use are highly variable between districts (household with at least 1 net per sleeping space ranged from 29 - 66%) and by equity (those in lower socio-economic quintiles used nets less frequently). 27% of nets were reported not to have been used the night before the survey, mainly because there were no mosquitoes, the primary user did not sleep at home that night and net age. The results of the bioefficacy and physical degradation analysis are still outstanding, but will be presented and correlated with net use characteristics. Increasing the data on LLIN durability after user conditions to understand the median lifespan of nets for programmatic and cost-effectiveness decisions is becoming a priority for the World Health Organization and NMCPs. In addition to informing the Tanzanian NMCP about the nets distributed during national campaigns between 2009 and 2011, we are also extending the ABCDR study prospectively to compare the durability of three LLIN products over at least three years.

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DIRECT EVIDENCE OF CHEMICAL CONTAMINATION OF ANOPHELES GAMBIAE S.L. BREEDING SITES UNDERLYING THE SELECTION OF PYRETHROID RESISTANCE IN COTTON GROWING AREAS REVEALED BY GPC: POTENTIAL IMPACT ON THE EFFICACY OF VECTOR CONTROL TOOLS IN BURKINA FASO

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Since the detection of the first case of Anopheles gambiae resistance to pyrethroid recorded in Ivory Coast in 1993, several studies had reported the role of agriculture in the selection and the spread of pyrethroid resistance in natural populations of An. gambiae s.l. Unfortunately no direct evidence was reported enhancing the presence of such chemicals in anopheline breeding sites. It was what we addressed in the current study performed in Dano, a cotton growing area located in the South West of Burkina Faso by monitoring the insecticide content both in water and sediments sampled from randomly selected breeding sites using GC analysis from August to October 2013. The resistance status of local populations of An. gambiae s.l. was estimated using standard WHO tube assays. Early in August some herbicides as Diuron were detected from the soil residue in concentrations ranged from 22.63 to 105.5 mg/Kg of soil without any insecticide in the water. In October two pyrethroids namely lambicyclothrin and deltamethrin were found in the breeding water at concentrations ranging from 0.0147 µg/l to 1.49 µg/l together with other chemicals occurring in very low concentration from the soil residue (benoxyprenyl, dioxacar, chlorozone). A reduced mortality rate was observed both with deltamethrin 0.05% and bendiocarb 0.1% reaching 52.04% and 66.67% respectively. High kdr allele frequencies reaching 0.95 and 0.4 respectively for 1014F and 1014S alleles and 0.12 for the ace-1 allele accompanied this strength resistance phenotype. Data on the efficacy of long lasting insecticide treated bednets (LLINs) in use in the region obtained by WHO cone test, showed mortality rates ranged from 10% to 83% depending to the type of LLIN. The significance and the impact of such resistance on the efficacy of malaria vector control strategy in short and long terms were discussed.

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ANALYSIS OF THE DNA SEQUENCE OF ACE.1 GENE IN ANOPHELES GAMBIAE S.S: DUPLICATION AND RECOMBINATION HISTORY

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Gene duplication is a source of molecular innovation throughout evolution. However, even with massive amounts of genome sequence data, correlating gene duplication with other events in natural history can be difficult. This is especially true in its most interesting cases, where rapid and multiple duplications are likely to reflect adaptation to rapidly changing environments and life styles. This may be so for the Anopheles ace-1 gene which encodes for the neurotransmitter acetylcholinesterase, the target of organophosphate and carbamate insecticides. Anopheles gambiae-resistant is known to possess only one mutation on the acetylcholinesterase (AChE) gene (ace) that is involved in insecticide (carbamates and organophosphate) target site insensitivity. To better understand a preferred model for the natural history of ace.1 duplication in the main malaria vector, we proceeded to the cloning and sequence analysis of DNA fragment including the G119S mutation from four West Africa countries. Here we show that phylogenetic trees produced from the nucleotide sequences of 34 individual ace.1 gene consisted of 4 main clusters, with ace.1 copies of different specimens grouping together in three out of the four clusters as expected if there was multiple duplication events. Furthermore, Phylogenetic analysis displayed an individual mosquito bearing more than two different resistant haplotypes with unexpectedly high copy number (at least six) of susceptible haplotype in the same individual. Our data indicate that this high copy number in both susceptible and resistant haplotype have evolved through gene duplication following by recombination event.

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EVALUATING THE EFFICACY OF ORGANOPHOSPHATE-COMBINED PAINT AGAINST PYRETHROID-RESISTANT ANOPHELES GAMBIAE S.L. IN VALLÉE DU KOU, BURKINA FASO

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Malaria control is currently treated by the rapid spread of insecticide and drug resistance among mosquito and parasite respectively, and operational difficulties on the field. It is necessary to find alternative and innovative tools for more effective malaria control. In the current study we evaluated the efficacy of organophosphate-combined paint containing insect growth regulator in indoor application on the walls in Vallée du Kou where Anoph eles gambiae is resistant to pyrethroids. The main entomological parameters such as mortality rates and paint insecticidal residual efficacy were compared in a village scale between treated and untreated huts. The residual insecticidal efficacy of the paint tested both with the susceptible An. gambiae “Kisumu” and wild local populations of An. gambiae s.l. showed high mortality rates ranged 98 to 100% even six months after the treatment. The indoors catches of wild An. gambiae performed during four consecutive days per month from July to December 2013 revealed that all mosquitoes entered in the paint-treated houses were died reaching 100% mortality rates throughout the six months collection. The frequency of kdr-L1014F (98%) mutation which was higher in this area did not differ between treatments. That of the Ace-1R remained low less than 5% and did not differ whatever the treatment. These results are very promising in terms of new perspectives to control resistant malaria vectors. Contrary to the classic indoor sprays, this tool is very simple and does not require
any special equipment or qualified personnel. But a large-scale assay is required to address its impacts on malaria transmission and the perception and acceptability of targeted human communities.

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INSECTICIDE RESISTANCE PROFILES IN Aedes aegypti STRAINS FROM THE CARIBBEAN REGION OF COSTA RICA

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Dengue is the most important vector-borne disease in Costa Rica. Although control of the vector, Aedes aegypti, includes repeated use of pyrethrin and temephos, insecticide resistance is not monitored continuously. In this study, resistance profiles to deltamethrin, cypermethrin, and temephos were evaluated in strains of Ae. aegypti from the cities of Guacimo and Limon in the Caribbean Region. Bioassays were carried out by exposing groups of 20 larvae for 24 hours to each of the insecticides at a series of concentrations that would generate from 2% to 100% mortality. Tests were performed by quintuplicate using larvae from the second to fourth generations, and a 50% lethal concentration (LC50) was estimated. A 50% resistance ratio (RR50) was calculated using the LC50 of the Rockefeller strain as a susceptible control. When resistance occurred, the enzymatic mechanism was evaluated by exposing the larvae to the synergists piperonyl butoxide (PB) and 5, 5, S, S, tributylphosphorotrithioate (DEF) and repeating the assays. No resistance to temephos or deltamethrin was detected in Ae. aegypti strains from Guacimo and Limon. Emerging resistance to cypermethrin was detected in both Guacimo (LC50 = 0.00845 mg/L, range = 0.00664 to 0.01038 mg/L; RR50 = 6.07) and Limon (LC50 = 0.01016 mg/L, range = 0.00876 to 0.01177 mg/L; RR50 = 7.30). The analysis of cypermethrin with PB resulted in synergism ratios of 19.2 and 8.7 for Guacimo and Limon strains, respectively. Synergism ratios for DEF were 0.9 for the Guacimo strain and 1.8 for the Limon strain. Results show that Ae. aegypti in both areas studied are in the process of developing resistance to cypermethrin, which is associated at least in part with the activity of cytochrome P450 monoxygenase. Therefore, local authorities should begin replacement of cypermethrin to ensure effectiveness of vector control and limit the development of resistance.

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USE OF NEXT GENERATION SEQUENCING (NGS) TO IDENTIFY NOVEL SNPS ASSOCIATED WITH PYRETHROID RESISTANCE IN Aedes aegypti

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The identification of single nucleotide polymorphisms (SNPs) associated with insecticide resistance has been performed mainly through genomic DNA and cDNA sequencing. Several SNPs conferring insecticide resistance have been identified in target site genes and in less extent at some detoxification genes. Recent Next Generation Sequencing (NGS) technology allows screening the whole genome to identify SNPs associated with insecticide resistance. In this study we identified SNPs associated with pyrethroid survival in an Aedes aegypti field population collected in Viva Caucel, Yucatan, Mexico, which already has high frequency for pyrethroid associated mutations in para. Four genomic DNA libraries were built following the recommended Illumina HiSeq protocols. Two replicate libraries contained DNA from mosquitoes that had survived one hour exposure to 25 μg permethrin (active ingredient per bottle). The remaining two libraries contained DNA from mosquitoes that died from the same exposure. Sequences were obtained from an Illumina HiSeq2000/2500 sequencer. We constructed a reference library including the total transcript sequences released by Vector Base. We aligned the paired read data to our locally build reference library using the GSNAP software. SNPs with coverage <15 or >1000 were excluded. SNPs association with resistance was assessed using LOD scores. SNPs in 38 genes were associated with permethrin resistance; none of these belonged to the expected target site or putative detoxification genes. Instead, we identified genes involved with GTP and ATP binding, calcium ion binding, zinc ion binding, glutamate ion channel transport and enzymes with specific activity. These candidate genes could become markers that will allow tracking pyrethroid resistance in Ae. aegypti.

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INSECTICIDE SUSCEPTIBILITY, CHARACTERIZATION OF BREEDING SITES AND COMMUNITY PERCEPTIONS ON MALARIA VECTOR CONTROL INTERVENTIONS ON KNUST CAMPUS

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Control of malaria vectors with insecticides remains an essential component to fight and eliminate malaria. There have been reports of insecticide resistance to all four classes of insecticides. Spread of resistance is said to be due to the excessive use of insecticides in Public health and in Agriculture. Insecticide-treated nets (ITN) and Indoor residual spraying (IRS) have proven to be the two most powerful and most broadly applied vector control interventions over the years. Despite the reports of resistance to pyrethroids, a lot of ITNs are still being mass distributed for free in most communities in Ghana. This study seeks to find out the level of resistance in Anopheles mosquitoes, to insecticides on the University campus which has an urban ambience, to determine other chemical vector control methods used by inhabitants and their perception on ITN use. Due to the extensive agricultural practices on the campus, the breeding sites of the Anopheles mosquitoes were characterized using physico-chemical parameters to find out the impact of spraying on the larvae. For the susceptibility tests, 2-5 days old non blood fed female Anopheles mosquitoes were tested against 0.05% deltamethrin, 4% DDT, 0.1% fenitrothion and 1% bendiocarb using standard WHO tube assay. A well-structured questionnaire was administered to residents to determine their knowledge, attitudes and practices to malaria. A total of 3,766 mosquitoes were identified as An. gambiae s.l (98.9%) and An. funestus (1.2%). Resistance was recorded to all classes of insecticides with mortality rates of 15-54% deltamethrin, 10-50% bendiocarb, 7.5-38.75% DDT and 5-42.5% fenitrothion. Overall knockdown rates was 60-21% for deltamethrin, 36.25-11.25% for fenitrothion, 26.25-12.5% DDT and 55-10% for bendiocarb across all breeding sites. Susceptibility status of mosquitoes indicate most are resistance. There was no association between susceptibility status and physical parameters of breeding sites. Inhabitants use ITN’s, aerosol sprays, mosquito coils and repellents, impregnated curtains and screens on windows amongst other traditional methods; knowledge on use of insecticides in Public health and in Agriculture. Insecticide-treated nets (ITN) and Indoor residual spraying (IRS) in twelve African countries, namely Angola, Benin, Burkina Faso, Ethiopia, Ghana, Liberia, Madagascar, Mali, Mozambique, Nigeria.
Rwanda and Zimbabwe with support from the President’s Malaria Initiative in 2012/2013. The project regularly collects data on susceptibility of the malaria vectors to the four classes of insecticides approved by WHO for IRS with the aim to guide selection of appropriate insecticides for IRS programs. WHO tube tests were used for susceptibility tests, and most of the tests were carried out against Anopheles gambiae s.l. (144 and 188 tests in 2012, and 2013 respectively), and a total of 14 tests were also carried out against the An. funestus in Mozambique and Zimbabwe. Data were entered into the database and collated to inform the decision making processes. An. gambiae s.l. was found to be fully susceptible (98-100% mortality after 1 hour exposure and 24 hrs. holding period) to permethrin and 70% to the pyrethroids deltamethrin, cypermethrin, and cypermethrin. An. funestus was resistant to pyrethroids in Zimbabwe and in some sites of Mozambique. Resistance to malathion has been observed in Ethiopia. Variable levels of susceptibility were reported for the carbamate bendiocarb against An. gambiae s.l. Resistance to pyrethroid insecticides is widespread for An. gambiae s.l. in most countries. However, in Angola, Mozambique, Madagascar and Zimbabwe, An. gambiae s.l. is susceptible to deltamethrin for most sites. A high level of resistance to DDT (0-26% mortality after 1 hour exposure and 24 hrs. holding period) has been reported in most countries, except Zimbabwe, Mozambique, and in some areas of Madagascar. An. funestus was resistant to pyrethroids in Zimbabwe and in some sites of Mozambique. It was, however, susceptible to organochlorine and organophosphate insecticides in the southern parts of Africa. The results are discussed in the context of malaria vector control interventions, geographical locations and epidemiology of malaria in the countries.

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A SEASONAL COMPARISON OF HIGH AND LOW EPIDEMIOLOGICAL CLUSTERS OF DENGUE TRANSMISSION IN SURINAME

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The primary vector of the dengue virus, Aedes aegypti, is well established in the increasingly urbanized capital of Paramaribo, Suriname. Due to the complex interaction of the environment and the mosquito, dengue transmission risk differ within the geographic area of concern. This study characterizes dengue-related entomological and environmental factors affecting disease transmission. Previously, high and low epidemiological rate clusters of dengue were identified in Paramaribo. Within the clusters, houses were randomly selected for inspection before and after the short rainy season. An environmental survey was conducted and entomological indices were calculated for each cluster. Presence of larva and pupae were recorded and samples of pupae were collected during the surveys. All inspected locations were geospatially coded. In total, 536 houses were surveyed in Paramaribo: 242 during the pre-rainy season and 294 post-rainy season. The findings show significant difference between pupae-positive houses and pupae-positive containers (house & container index) pre- (37.60% & 16.53%) and post- (24.83% & 7.73%) rainy season, p<0.001 and p=0.033. The Breteau indices pre- and post-rainy season were 0.68 v. 0.27. These pupae indices were higher in the low cluster pre-rainy season but higher in the high cluster post-rainy season. The dispersion index remained unchanged between the seasons (3.99 and 3.70 for pre- and post-rainy season, respectively) but decreased from 5.10 to 3.81 in the high cluster and increased from 2.95 to 3.16 in the low epidemiological cluster between the rainy seasons. The variation in the dispersion index indicates that factors within clusters and seasonality affect the proportions in which pupae are present among different categories of containers. This is the first attempt to demonstrate how entomological and environmental data influence the rate of dengue transmission in Suriname.
DO SEASONAL TEMPERATURES MATTER IN THE LARVAL HABITAT? EFFECTS ON ADULT SIZE, LONGEVITY, AND R\(^*\) IN THE MOSQUITO Aedes triseriatus, VECTOR OF LA CROSSE VIRUS

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Mathematical models of mosquito transmitted diseases suggest that female longevity is an important factor for determining disease risk. As ectothermic organisms, temperature regulates mosquito growth and development. It is well understood that bellow a critical maximum, hotter temperatures decrease development time and adult size. Adult size is correlated with fecundity, but it is less well understood how size is related to longevity and how temperatures experienced in the larval habitat influence adult longevity. Additionally, most experimental studies that have tested effects of temperature use constant temperatures and ignore daily or seasonal temperature fluctuations that mosquitoes encounter under natural conditions. I simulated fluctuating daily temperature and photoperiods consistent with those experienced in St. Louis, MO in June and August to test the hypotheses that 1) Aedes triseriatus mosquito larvae developing under different seasonal conditions will differ in size and adult longevity 2) that fluctuating temperatures yield adults that differ in size and adult longevity from those produced in stable conditions. There was no effect of size on female longevity. Despite early differences in survival probability and different ages at senescence, there was no statistically significant difference between August and June treatments. The August treatment was significantly different from the constant temperature control, which experienced reduced longevity. Simulated August, June, and constant treatments also differed in larval survivorship, median day to eclosion, size, and \(r^*\) (a cohort performance index). The June and constant treatments only differed in female size. These results suggest seasonal temperature fluctuations in the larval habitat do not affect adult longevity in Ae. triseriatus, but do affect other measures of performance typically reported in studies that investigate down-stream effects of the larval habitat.

TRANSGENIC ANOPHELES STEPHENSI FITNESS AND SUSCEPTIBILITY TO VARIOUS INFECTIONS

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Various mosquitoes from the genus Anopheles spread the causative agents of human malaria. Traditional malaria control efforts have been unable to eliminate the disease, mainly because of insecticide and drug resistance arising among both the mosquitoes and the Plasmodium ssp. parasites that cause the disease. Therefore, new tools to combat the disease are needed, and one potential new technology to limit malaria transmission is the use of transgenic mosquitoes refractory to malaria infection. Multiple laboratories have created multiple mosquito lines that do not transmit the malaria parasite, but have not yet reached the stage of releasing such mosquitoes, partly due to concerns about their fitness relative to wild-type conspecifics and their ability to resist a broad range of malaria parasites and other human pathogens. We studied various aspects of mosquito fitness in five separate transgenic lines representing different transgenes, insertion points and promoters in order to determine the fitness costs that may arise due to transgenesis. Of the five lines tested, four have shown no fitness cost and the fifth has some fitness reduction due to position effects. We also challenged our mosquitoes with different \(P. falciparum\) strains. Our results indicate that the mosquitoes are highly resistant to infection by multiple \(P. falciparum\) strains and do not suffer a large fitness cost as a result of transgenesis, thereby illustrating their potential utility as part of a larger malaria control program and warranting further testing in large cage or field trials. Ongoing studies are focused on testing transgenic mosquito resistance to the \(P. vivax\) parasite, \(O\)'nyong'nyong virus and the filarial worm Brugia malayi.

AEDES AEGYPTI POPULATION STRUCTURE IS DRIVEN BY BOAT TRAFFIC IN THE PERUVIAN AMAZON

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In the Americas, as in much of the rest of the world, the dengue vector Aedes aegypti is predominantly found in urban areas. Its presence in rural areas is more limited, and the factors favoring its potential geographic expansion to rural and smaller urbanized settings are poorly understood. In the Peruvian Amazon, this vector has been expanding its range into rural communities over the last 5-10 years. Understanding Ae. aegypti dispersal patterns is important for anticipating the future range expansion of dengue and other viruses transmitted by this mosquito. To examine if human transportation networks play a significant role in the population expansion of this mosquito, we analyzed Ae. aegypti population structure using a panel of 10 microsatellite markers. Adult and immature Ae. aegypti (>20 individuals per site) were collected from the city of Iquitos (pop: 380,000) and several neighboring communities that are connected by river transport, including Nauta (pop: 14,000), Indiana-Mazan (pop: 7,000), Barrio Florida (pop: 750), Tamshiaco (pop: 4,500), and Aucayo (pop: 800). We detected significant departures from Hardy-Weinberg Equilibrium in all study sites due to lower than expected number of heterozygotes. FST statistics show significant differentiation for the majority of study site pairs. Population structure among Ae. aegypti in different towns is not correlated with the geographic distance between towns, suggesting that human transportation networks may be responsible. Furthermore, Ae. aegypti gene flow among sub-populations is greatest between locations with heavy boat traffic such as Iquitos-Nauta (which also has heavy road traffic) and Iquitos-Indiana-Mazan, and lowest between locations with little or no boat (or road) traffic such as Barrio Florida-Iquitos interior. Bayesian clustering analysis using Structure program suggested definite admixture, with 5-6 genetic clusters. Our results provide strong evidence for the hypothesis, especially via boats, is responsible for Ae. aegypti spread in the Peruvian Amazon.

ADVANCEMENTS AND CHALLENGES IN USING NEAR INFRARED SPECTROSCOPY (NIRS) TO DETERMINE THE AGE OF FEMALE Aedes aegypti Mosquitoes WITH VARYING LARVAL AND ADULT DIETS

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Interventions targeting adult mosquitoes are often used to combat the transmission of vector-borne diseases, including dengue. In the absence of a vaccine, we rely on control measures targeting the primary dengue vector, Aedes aegypti, to prevent transmission. Due to the 7 day extrinsic incubation period of the virus in the mosquito, older mosquitoes (>7 days) are of most concern in the dengue transmission cycle. Identifying the age of female mosquitoes is therefore a crucial step in determining if vector control interventions are altering the age structure of the mosquito population, thereby reducing transmission potential. We have developed
a model using near infrared spectroscopy (NIRS) to determine the age of adult female Ae. aegypti. This technique quantitatively measures organic compounds, and has previously been used successfully to age-grade other mosquito species. To determine if the larval and/or adult diet of female Ae. aegypti affects the ability of NIRS models to predict mosquito age, 2 groups of mosquito larvae were raised in identical settings and fed either fish food or infant cereal. Emerged adult females were separated and fed either blood or sugar, resulting in four experimental groups. These adult females were then killed 1, 4, 7, 10, 13 or 16 days post-emergence. The head and thorax of each mosquito were scanned using a LabSpec 5000. The spectral scans from each group were analyzed independently and collectively, to determine if the diet of the mosquito affected the spectrum. Multiple models were developed using GRAMS PLPlus/Q, with different spectral ranges. The best model included all experimental groups, and was able to positively predict the age group (< or ≥7 days) of 88.8% of mosquitoes. Models using a single experimental group to predict the others were less robust. These results indicate both larval and adult diet affect the predictive ability of models developed using the spectral scans of female Ae. aegypti. This potentially complicates the application of NIRS age grading to field populations, as larval and adult nutrition can vary greatly in the field.

**SPECIES DIVERSITY, SEASONAL, AND SPATIAL DISTRIBUTION OF MOSQUITOES (DIPTERA: CULICIDAE) CAPTURED IN AOTUS MONKEY-BAITED TRAPS IN A FORESTED SITE NEAR IQUITOS, PERU**

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This study was conducted to determine the relative abundance, diversity, seasonal, and vertical distributions of potential mosquito vectors in the Amazon Basin, Peru. A total of 66,097 mosquitoes (50 mosquito species from 12 genera) were collected from May 2001 through March 2002 at a forested site near Iquitos, Peru. Mosquitoes were collected using Aotus nancymae Hershkovitz monkey-baited CDC light traps set for 12-h day and night periods at varying heights (e.g. ground and canopy) in the forest. Of the 12 genera, three accounted for 75% of all mosquitoes collected: Culex (33%), Aedes (23%) and Psorophora (18%). The most prevalent species collected were Aedes serratus (Theobald), Culex pedrooi Sirivanakarn & Belkin, Psorophora albignu (Peryassu), and a combination of Mansonia indubitans Dyar & Shannon and Mansonia titillans (Walker), which accounted for 56% of all mosquitoes captured. In general, mosquitoes were collected more often at night and on the ground. Exceptions include Coquillettidia venezuelensis (Theobald), which were collected in relatively even numbers at both day and night and most Mansonia and some species of Anopheles, which were collected more often in the canopy. Total mosquito populations had two peaks, June-July (M. indubitans/titillans, Cq. venezuelensis) and December-January (Ps. albigenu, Cx. pedrooi, Ae. serratus). Observations of the eight most collected mosquitoes indicated that behavioral shifts were not observed between collection months. These data provide a better understanding of the species diversity, population density and seasonal distribution of potential mosquito vectors within the Amazon Basin region and allow for the development of appropriate vector and disease prevention strategies.

**HUMAN IGG ANTIBODY RESPONSE AGAINST RECOMBINANT Aedes aegypti SALIVARY PROTEINS MODIFIED DURING DENV2 INFECTION**

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Arthropod saliva has been shown to modulate the success of infection success for the microorganisms they transmit. Our group has found that DENV infection of Ae. aegypti salivary gland induces changes in the expectorated salivary content. We have produced by recombinant technology three of the proteins we found to be reduced in saliva. The immunogenicity against these proteins was evaluated in subjects from a DENV endemic area in Colombia by ELISA. We found a significant increase in the IgG antibody levels after exposure to mosquito bites. We also found a differential antibody response among healthy, febrile DENV (-) and DENV (+) subjects. We hypothesize that the decrease of these proteins in saliva may be advantageous to the virus. Differential IgG response between DENV (+) and negative subjects suggests 1) dengue positive subjects are significantly at higher exposure risk to mosquito bites 2) the immune response against these proteins may play an important role in disease progression.

**ALTERNATIVE MOSQUITO SAMPLING TO MONITOR LYMPHATIC FILARIOsis TRANSMISSION IN PAPUA NEW GUINEA**

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Entomological markers of lymphatic filariasis (LF) transmission provide key information regarding progress toward LF elimination and subsequent monitoring. However, “gold standard” approaches of human landing catches (HLC) can be inefficient and expensive as transmission approaches zero. In addition, HLC can be complicated by and/or violate cultural and ethical standards. Recently barrier screens have been used with success in the Pacific region to estimate human blood index and quantify malaria transmission in anopheline mosquitoes. Since Wuchereria bancrofti (Wb) is exclusively transmitted by anopheline vectors in Papua New Guinea, this study compares HLC, light traps, and barrier screens with regard to mosquito species captured, anopheline infectivity (as detected by microscopy for stage 3 Wb larvae), and presence of Wb DNA. 2mx40m barrier screens were sampled every 15 minutes by trained collectors in communities with simultaneous HLC collections (plus light traps in one sampling week). Collections occurred between 6pm and 6am in four communities for one week in November 2013, March 2014, and July 2014. Mosquitoes were separated according to hour captured, capture method, and the side of the screen collected (village side vs. bush side). Anopheline capture density and composition will be compared for each mosquito sampling method. Xenomonitoring for LF elimination will be quantified by both microscopy of anopheline mosquitoes and PCR for Wb DNA in all blooded mosquitoes. Human blood index and presence of Wb DNA will be compared across the study communities. Negative binomial models will be fit to compare agreement between mosquito capture
methods for mosquito species densities and presence of L3. The results are relevant to the selection of the most efficient mosquito collection techniques for xenomonitoring LF during elimination programs in PNG.

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**IGG ANTIBODY SUBCLASSES AGAINST VECTOR SALIVARY PROTEINS AS A MEASURE TO RISK OF Aedes aegypti BITE EXPOSURE AFTER IMPLEMENTATION OF ATTRACTIVE LETHAL OVITRAPS**

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Aedes salivary proteins induce specific immune responses in the vertebrate host that can be used a tool to measure vector host-contact. Specifically, an increase in IgG antibody subclasses (IgG1 predominantly associated with the anti-microbial response and IgG4 representing chronic exposure to an antigen) has been associated with chronic exposure to mosquito bites and the risk for arbovirus infection. We evaluated the levels of antibodies against Aedes aegypti salivary gland extract in subjects from dengue endemic areas in Iquitos-Peru, before and after the implementation of Attractive Lethal OviTraps (ALOTs). Serum samples were collected from the household occupants before (Time 0) and after one year of the treatment (Time 12) in the ALOT treated and the untreated control areas. PRNT70 tests for DENV were performed on all sera for determination of viral exposure. Our results showed that IgG4 antibody concentrations were significantly higher than IgG1 concentrations for both groups at both times. When comparing the antibody response between times, we found that the concentration of both IgG1 and IgG4 decreased in subjects from the treated area over 12 months. No significant differences were observed in the control area during that time. The relationship between the exposure to DENV and IgG4/IgG1 will be discussed. Our results suggest that IgG4 levels are indicative of cumulative exposure to mosquito bites and it represents a useful tool for monitoring vector control interventions.

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**INFECTION EVALUATION ON FIELD-CAPTURED MOSQUITOES FROM THREE COLOMBIAN CITIES WITH DIFFERENT ENDEMICITY PATTERNS OF DENGUE**

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Dengue fever is endemic in Colombia, where the four virus serotypes circulate in highly variable epidemiological scenarios. This heterogeneity is illustrated in the following three cities: Riohacha, with the highest values of Breteau index (BI) and lowest number of dengue cases; Villavicencio, which has the highest number of dengue cases and the second highest BI values; and Bello, which has the lowest BI values, but exceeds Riohacha in number of dengue cases. To explain these differences, adult mosquitoes were captured in four neighborhoods in each city over 3 to 5 sampling periods across 2012 and 2013. Virus presence and serotype were detected through examination of pooled mosquitoes from each sampled house. We found that mosquitoes from Bello have the highest infection rate, followed by Villavicencio and finally Riohacha. In samples from Riohacha and Villavicencio, we detected the serotypes DENV-1, 3 and 4, while in Bello we detected serotypes DENV-2, 3 and 4, and the highest proportion of pooled mosquitoes with more than one serotype. 13.3% of pools presented infection with more than one serotype, including two pools of just one single mosquito, which is the first reported case of a mosquito co-infected with two dengue serotypes in Colombia. DENV-4 serotype is the most prevalent in pools from the three cities (75.5%), followed by DENV-3 (28.8%), DENV-1 (6.6%) and DENV-2 (2.2%). Our results differ from those of dengue serotype presence in humans from the whole country reported by the Colombian National Institute of Health, where DENV-1 is the most prevalent and DENV-4 the least. This suggests that human populations are more refractory to DENV-4 infection, the most abundant serotype, while being more susceptible to DENV-1 and DENV-2 infection. These patterns highlight the importance of including evaluation of mosquito infection when developing dengue control strategies.

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**VALIDATION OF A PREDICTIVE MODEL: ENVIRONMENTAL INFLUENCES ON THE PREVALENCE OF WEST NILE VIRUS IN CULEX MOSQUITOES, LONG ISLAND, NEW YORK**

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To better understand the dynamics of West Nile Virus (WNV) transmission, we revisit a model describing the spatial-temporal distribution of positive Culex mosquito pools collected in Suffolk County, Long Island, New York. The original statistical model (m09) used meteorological and hydrological conditions to simulate WNV infection in Culex mosquitoes during 2001-2009. Here, we use Culex mosquito WNV infection data collected during 2010-2012 to validate m09 predictions for these years and to explore whether inclusion of additional environmental variables improves model performance. The m09 predictions for 2010-2012 are well correlated with observed WNV activity during the same time period (0.65). In evaluating the full 2001-2012 record (m12), we explored smoothing the meteorological and hydrological data temporally over 3-month seasons, increasing the spatial resolution of the analysis to the locations of the mosquito traps rather than the aggregated scale of the meteorological and hydrological data, and incorporating other socioeconomic and environmental variables (median household income, population density, a metric of the built environment, and household occupancy status); however, the results remained consistent, with wetter winter conditions, wetter and warmer spring conditions, and drier summer conditions favoring increased prevalence of WNV among Culex mosquitoes. In general the socioeconomic variables did not further constrain the models, suggesting that the distribution of WNV activity in Culex is most intimately linked to climate. Temporal cross validation of the m12 model confirmed consistency of the associations between environmental variables and WNV activity. The different spatial and temporal considerations of these analyses indicate robust associations between meteorological and hydrological conditions and WNV activity. Validation of this model indicates that as meteorological observations and hydrology model estimates of land surface wetness are collected, areas at heightened risk for WNV activity can be predicted.

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**OCHLEROTATUS GENICULATUS, A NATIVE EUROPEAN MOSQUITO WITH A HIGH POTENTIAL FOR TRANSMISSION OF CHIKUNGUNYA VIRUS**

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Ochlerotatus (Finlaya) geniculatus (Olivier), a tree-hole mosquito that bites humans aggressively, is present throughout Europe, North Africa and Asia Minor. In Albania it shares a common habitat with Aedes (Stegomyia) albopictus and our ovitrap collections have confirmed its abundance to at least 1350m. In the laboratory, both species were highly susceptible
OPTIMIZATION OF FEEDING ASSAYS TO EVALUATE MALARIA TRANSMISSION-BLOCKING VACCINES IN MALI

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Transmission-blocking vaccines (TBV) are critical tools for malaria elimination. However, the assays used to measure TBV functional activity, including direct membrane feeding assays (DMFA) and direct skin feeds (DSF) involve multiple biological systems with inherent variability. The objective of this research study is to establish optimal conditions for our assays used in clinical trials in Mali. Since January 2014, field experiments have been carried out to address different aspects of the feeding assays (DSF/DMFA). Experiments have been conducted to confirm optimal feeding parameters for DMFA including membrane selection (parafilm versus baudruche), mosquito age, and mosquito starvation duration. The impact of anatomical location (arm, calf, ankle) on subsequent mosquito infectivity by DSF is being assessed. Finally, we want to assess the effect of the time of day the DSF is performed has on mosquito infectivity by DSF and DMFA. The evaluated endpoints for the exploration of these variables includes: mosquito feeding rates, mosquito survival rates, and mosquito infectivity rates. Optimal observed parameters will be applied to improve the feeding assays conducted for transmission-blocking activity (TBA) measures. Data from pilot experimental hut (EH) assays, procedure by which wild, blood-fed mosquitoes are collected from the room where the participant slept alone overnight, will be presented. First, molecular tools will be deployed to pair fed mosquitoes with the individual volunteer participating in the EH assay. If fed mosquitoes captured are consistently matched to the EH volunteer, the EH assay will be expanded to evaluate TBV impact on naturally occurring transmission.

DESIGN AND ASSESSMENT OF A MOBILE DATABASE MANAGEMENT SYSTEM FOR ARTHROPOD-BORNE DISEASE SURVEILLANCE IN BELIZE

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Successful implementation of information and communication technologies for development (ICT4D) hinge on dynamic collaboration among all key stakeholders - particularly intended users. In dengue endemic areas, where vector control practices have shown diminishing returns, the gap between surveillance practices and ministerial response represents a unique opportunity for mobile health technologies to fill a need. The incorporation of real-time data collection and archiving, open-source QGIS mapping capabilities, and regression tools into a single platform aims to support ministry officials in streamlined action for both preventive and reactionary measures. This study presents a three-tiered, workshop-based approach to the adoption of a novel mobile application and database management system by the Northern Region of the Ministry of Health (MOH) in Belize. In general, the first phase consists of training MOH field technicians on theory and technical use of the mobile application; the second phase simulates field conditions in an outdoor setting and extends to risk map generation; while Phase III monitors real surveillance conditions in village settings. Shortcomings of the mobile system identified by MOH officials and field technicians at each phase guides reprogramming, ensuring a user-oriented product. Data will be available by mid-July 2014.

MOSQUITO VECTOR MICROBIOME DIVERSITY ACROSS HABITATS IN CENTRAL THAILAND ENDEMIC FOR DENGUE AND OTHER ARTHROPOD-BORNE DISEASES

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Anthropogenic environmental change is among the most frequently identified factor linked to emergence of infectious diseases. The proposed mechanism by which anthropogenic environmental changes affect infectious disease spread is through the perturbation of the ecological communities involved in transmission, resulting in changing distribution and relative abundance of the key organisms. The objective of this study is to characterize the diversity of mosquitoes, relative abundance of vectors, and their associated microbial communities along a forest-agro-urban habitat gradient in Thailand to determine how habitat changes affect multi-level communities. Various adult mosquito traps were set-up along a habitat transect to capture components of mosquito communities in each characterized habitat. Over 62,000 female mosquitoes were identified morphologically and selected vector species, Aedes aegypti, Ae. albopictus, and Culex quinquefasciatus, were pooled by species and habitat type. Segments of 16s and 18s rRNA genes were sequenced from the pools using 454 pyrosequencing technology to assess genetic and taxon-based diversity of the non-eukaryote and eukaryote microbiota. Female mosquito abundance was highest in rice fields and lowest in forests. Based on extrapolated species richness estimators (Chao1 and ACE), forest and fragmented forest habitats had the most diverse mosquito communities, followed by the rural, rice field, suburban and urban habitats. Interestingly, the relative abundance of two vector species, Ae. aegypti and Cx. quinquefasciatus, was negatively correlated with mosquito diversity. Furthermore, the microbiota community assembly and diversity of selected vector species were associated with habitat types suggesting that habitat factors differentially affect mosquito microbiota. In this study, mosquito community and vector microbiota diversity varied across a continuum of habitat types in a pattern reflecting habitat change. Moreover, changes in the abundance of important mosquito vectors tended to follow a predictable pattern. Collectively, these findings help illuminate how mosquitoes and their associated microbial communities vary across habitat types and how these dynamics could contribute to emergence of arboviral diseases in Thailand.
STUDYING THE EXTRINSIC INCUBATION PERIOD IN DENGUE VIRUS INFECTED MOSQUITOES

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In the past 20 years, dengue has become the most prevalent arthropod-borne virus affecting humans today. This exponential increase in disease incidence has brought with it significant health, social and economic problems. Vectorial capacity, which is a measurement of the efficiency of vector-borne disease transmission, is influenced by a few key factors. Extrinsic incubation period (EIP), which is the interval of time from the ingestion of an infectious bloodmeal to the time of transmission, is a key determinant of vectorial capacity. Here we have used a repeat sampling technique for saliva to study individual variation in EIP in dengue-infected Aedes aegypti. We show significant heritability (~40%) for EIP and a strong positive correlation between length of EIP and mosquito lifespan. We also examine the effect of the biocontrol agent, Wolbachia piipientis on EIP under different temperature regimes.

RIFT VALLEY FEVER OUTBREAK IN SAUDI ARABIA ANTICIPATED FROM AFRICA OUTBREAKS AND TIME-SPECIFIC SATELLITE DATA

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Rift Valley fever (RVF) is an acute zoonotic viral disease that affects domestic animals and humans in sub-Saharan Africa. More recently, a RVF outbreak was first identified outside Africa in the Arabian Peninsula. We used ecological niche modeling to assess the ability of models to identify areas of disease risk in Saudi Arabia using 8-day composite Land Surface Temperature (LST) and monthly Normalized Difference Vegetation Index (NDVI) data from the Moderate Resolution Imaging Spectroradiometer (MODIS) satellite imagery (1 km spatial resolution) for January to December 2000 (i.e., the time of disease outbreak in Saudi Arabia). The model was calibrated based on records from African outbreaks, and transferred to Saudi Arabia to anticipate suitable sites of RVF across Arabia. Results suggested suitable areas in western Saudi Arabia in Giza region. We evaluated models using occurrence points from the 2000 outbreak in Saudi Arabia. Models calibrated in Saudi Arabia revealed similar spatial patterns. This study revealed the potential of niche modeling approaches to anticipate suitable areas for disease emergence in areas with no previous disease history, opening the possibility of genuine prediction of risk, particularly in future studies that add data on availability of vectors.

UNDERSTANDING DENGUE TRANSMISSION AND RISK FACTORS IN BANGLADESH

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Southeast Asian countries including Bangladesh have remained hyperendemic for dengue due to unplanned urbanization, overcrowding, poverty and health inequalities. In recognition of the need for a multidisciplinary, scientific research on this problem, we applied an “Ecohealth Approach” to understand dengue virus (DENV) transmission and social-ecological risk factors in Dhaka, Bangladesh. Multiple disciplinary aspects were encapsulated by examination of: i) rates of human exposure to DENV by identifying individuals (via a serosurvey in 1200 households and 47 clinical samples) with IgM and IgG antibodies to DENV; ii) abundance of dengue vector during monsoon and dry seasons in the same households; iii) self-risk perception by the community members; and iv) human organizations responsible for interventions. Data included in the analysis are: a) two vector surveys [i.e., pupal surveys conducted in 847 households (monsoon season 2011) and 459 households (dry season 2012)]; b) two serosurveys [i.e., serosurveys conducted in 1128 households (pre monsoon season 2012) and 1130 households (630 paired sera and 500 replacement sera during post monsoon season 2012)]; c) socio-demographic survey of 300 households; and d) 12 focus group discussions and 12 key informant interviews. Competent dengue vectors were detected in >40% and 12% of households during the monsoon and dry seasons respectively. The monsoon and dry seasonal pupal index were 0.40 and 0.33 respectively for the selected 12 wards. Only 8 types of key containers and two types of ecological clusters are responsible for 72% of pupal distribution. More than 80% IgG and nearly 3% IgM were positive during pre- and post-monsoon serosurvey. Among the IgM positives, in-house PRNTs, using a serial dilution of sera mixed with a DENV serotype, are being carried out. There are significant variations in dengue risk perception between lower and higher socioeconomic groups. Also, experts ranked dengue risk at a much lower level than lay persons and experts emphasized the need for stronger institutional measures to control dengue outbreaks. The overall findings of the study will contribute to the advancement of DENV transmission knowledge, forecast the disease burden as well as socioeconomic burden in the City of Dhaka, Bangladesh.

INVESTIGATING THE EFFICACY OF MONOVALENT AND TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE FORMULATIONS AGAINST DENV-4 CHALLENGE IN AG129 MICE

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Dengue virus (DENV) causes a rapidly spreading mosquito-borne human viral disease that has major impact on global health and economics. Currently, there is no licensed vaccine against DENV. We have developed a live attenuated tetravalent dengue vaccine candidate based on an attenuated dengu 2 virus (DENV-2) and three chimeric viruses containing the pre-membrane and envelope genes of DENV-1, -3 and -4 expressed in the context of the attenuated DENV-2 genome. In a mouse model, we investigated the immune responses to monovalent DENV-4, DENV-2 or tetravalent DENV vaccine formulations, and their efficacy against challenge with a newly mouse adapted DENV-4 strain 703. Single doses of the tetravalent or monovalent vaccines elicited neutralizing antibodies and cellular responses to DENV-2 backbone. Monovalent DENV-2 vaccine also elicited cross-neutralizing antibody responses to DENV-4. When tested against a lethal challenge of DENV-4 sixty days post-primary immunization all mock-vaccinated animals (n=15) died, but all vaccinated animals survived except for 1 of 15 DENV-2 vaccinated mice. Investigation of DENV-4 viremias post-challenge showed that only the placebo-treated animals had high viremias on day 3 post-challenge. Overall, these data highlight the immunogenicity and efficacy profiles of our candidate dengue vaccine in the mouse model.
EFFECT OF MIGRATION STATUS ON RISK OF DENGUE VIRUS INFECTION IN PUERTO MALDONADO, PERU

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Dengue virus (DENV) affects more than 100 countries worldwide. DENV has been reported in Puerto Maldonado (population ~80,000) in the Peruvian southern Amazon Basin since 2000. This region also has the highest human migration rate in the country, mainly from non-endemic areas for DENV. The risk of DENV infection may vary between recent migrants (RM) and long-term residents (LTR) due to both biological and sociologic factors. We explored the prevalence of past DENV infection and knowledge, attitudes and practices (KAP) related to dengue disease control and prevention of RM versus LTR, defined respectively as residency in Puerto Maldonado for less than or greater than 5 years. In 2012 we conducted a cross-sectional serosurvey and administered a KAP questionnaire to members of randomly selected households. Sera were screened for antibody to DENV by ELISA and confirmed by plaque reduction neutralization test (PRNT). We created ad hoc indices for KAP (KAPI), household infrastructure and services (CFSI) and assets (Ai). PRNT results were analyzed against migration status and the various indices with an ordered logistic regression. Five hundred and five participants from 309 households provided a blood sample and completed a questionnaire. RM comprised 12% of the study population and were more likely to be DENV antibody negative than LTR on bivariate analysis (42% vs. 56%, p=0.043). However, after controlling for the other variables in the multivariate analysis, KAPI (p=0.032), commercial activities (p=0.023) and CFSI (p=0.017) were associated with antibody positivity, while migration status was not (p=0.226). The higher KAPI is likely consequence of experiencing the disease. Commercial activities and CFSI may be related to the location or infrastructure of areas where participants spend long periods of time. We conclude that risk of DENV infection in Puerto Maldonado relates more to socio-economic status, especially infrastructure and services available in the household, than to migration status per se. These findings should help tailor specific prevention and control strategies for dengue diseases in the area.

HOST CELL RESPONSES ATTENUATE DENV-2 PDK53 BUT NOT DENV-3 PGMK30FRHL3

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The global prevention of dengue is challenging without a vaccine, the development of which has been limited by an incomplete understanding of pathogenesis. In particular, efforts to develop a live attenuated tetravalent vaccine encountered problems in balancing immunogenicity with reactogenicity. Such vaccines have been derived through serial passage of dengue viruses (DENVs) in various primary cell cultures followed by selection of strains that meet specific but empirically-derived phenotypic criteria such as small plaque size. Unfortunately, these empirically-derived criteria do not invariably inform on attenuation. The Mahidol University-developed DENV-2 PDK53 and DENV-3 PGMK30FRHL3 strains both fully met these empirically-derived phenotypic criteria. However, while PDK53 was shown to be safe and immunogenic, PGMK30FRHL3 was reactogenic and vaccine recipients developed symptoms consistent with dengue fever. In this study, we asked if a molecular approach might be able to distinguish a more accurate basis of attenuation. We observed that Huh-7 cells infected with PDK53 upregulated the expression of many genes in the innate immune system compared to wild-type DENV-2 16681 from which it was derived. In contrast, the pattern of expression of these same genes in both PGMK30FRHL3 and the parental DENV-3 16562 were similar and mostly low. Functional studies suggest that the innate immune responses restrict the plaque size of PDK53 while that of PGMK30FRHL3 plaque size is limited by its slower replication. We suggest that a molecular definition could be developed for a more accurate identification of viral attenuation.

DENGUE VIRUS TYPE 3 EVOLUTION AND EPIDEMIC ACTIVITY IN INDONESIA, A HISTORICAL STUDY OF OUTBREAKS FROM 1976-1979

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Dengue viruses are one of today’s most significant vector-borne disease agents threatening human health throughout the tropics and subtropics, infecting hundreds of millions of people annually. Dengue is primarily transmitted by Aedes aegypti mosquitoes. There are four known serotypes circulating in humans (DENV-1 to -4) all of which can cause a febrile illness known as dengue fever that can progress to more severe and potentially fatal disease involving hemorrhage or shock (DHF/DSS). We report here follow up sequence data on DENV-3 strains isolated during epidemics that occurred in Indonesia between 1976 and 1979. The epidemics began with the detection of fatal DHF/DSS associated with DENV-3 in Jakarta in Jan-Mar, 1976. The virus spread to Bantul, Central Java in Oct. 1976, and to Surabaya, East Java and Pontianak, West Kalimantan in 1977. All of these were explosive epidemics with associated severe disease. A smaller outbreak with more sporadic transmission, milder illness and much lower viremia levels occurred in Siemian, Central Java in 1978. Viruses were isolated by one of us (DGJ) from all of these epidemics and stored in infected mosquitoes at -70 C for nearly 40 years. The viruses had not been passaged in mice nor mammalian cell cultures. Full genomic sequence analysis suggests that a single strain of DENV-3 with greater epidemic potential and possibly virulence emerged in Jakarta and spread rapidly along the main transportation routes to Central and East Java, and to Kalimantan. Interestingly, the Siemian DENV-3 viruses were genetically distinct, belonging to a separate clade from the other strains. There were two unique Bantul isolates that also belonged to the Siemian clade, suggesting that the Siemian virus descended from these Bantul viruses. Our findings emphasize the importance of dengue evolution and genetic variation as a contributor to epidemic intensity and severe dengue disease.

ANALYSIS OF HUMAN DENGUE-IMMUNE SERA USING WHOLE VIRUS PROTEOME PEPTIDE ARRAYS

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Dengue, a mosquito-transmitted viral disease, has become a major worldwide public health burden. Four distinct serotypes of dengue virus (DENV1-4) contribute to the challenge of developing a safe and effective tetravalent vaccine. We have exploited high-density peptide arrays for the readout of serum antibodies from dengue patients from ongoing pediatric hospital-based and community-based cohort studies.
in Managua, Nicaragua. First, we selected a set of 3,172 overlapping 15-mer peptides, covering the proteomes of 20 different DENV genotypes (3 DENV1, 7 DENV2, 5 DENV3, 5 DENV4). Then, we generated five peptide arrays applying our novel particle-based peptide synthesis method using a custom-built laser printer, which allows for complete content flexibility on every array. Each array enables simultaneous analysis of a patient’s IgG and IgM antibody reactivity to all four DENV serotypes. We first analyzed acute, convalescent, and 12-month samples from primary and secondary DENV2 cases. The results from the arrays identified the acute infecting serotype and revealed time-dependent waning of antibody responses in patients’ sera. We also observed an increase in reactivity to a few peptides after 12 months, specifically, to peptides in NS1 and NS3. To confirm these results, we are currently analyzing 46 additional serum samples of primary and secondary DENV infection with a set of 2,000 overlapping 20-mer peptides, derived from 361 different DENV strains representing all known Nicaraguan genotypes in DENV1-3 and 9 Colombian DENV4 strains. In addition, to obtain a profile of the immunogenic DENV proteome map, we incubated one peptide array with a pool of 100 patient sera from adult Red Cross donors from Nicaragua who had experienced one or more DENV infections. From this broad polyclonal response, we identified immunogenic pan-serotype protein regions, as well as serotype-specific regions. In contrast to protein arrays, our peptide-based approach allows us to obtain a fine resolution map of the proteome immunogenicity. These results can help to elucidate structural and functional differences among the DENV serotypes and point to possible diagnostic biomarkers and vaccine targets.

ECONOMIC IMPACT OF WORK ABSENTEEISM DUE TO NON-SEVERE DENGUE VIRUS INFECTION IN A CITY IN THE PERUVIAN AMAZON

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Dengue fever is the most prevalent mosquito-borne viral disease globally. The public health impact of this disease is compounded by the fact that it occurs primarily in developing countries, adding a significant burden for local economies and individuals that are already under-resourced, undermining both regional and national development. To assess the economic impact at the community-level in Iquitos, the largest city in the Peruvian Amazon basin, we collected information on work absenteeism and related work absenteeism. Dengue fever was confirmed by PCR, IFA or by a four-fold rise in IgM titer between acute and convalescent blood samples. Housewives were not included in the study, since they are not formally financially compensated. Daily wage estimates were assigned based on the reported occupation (the average of reported salary ranges in the city), then multiplied by the number of work days reported lost due to illness by each case. The effect on the local economy was estimated using data from the Regional Government. Of the 504 enrolled patients who engaged in wage-earning employment and provided absenteeism information, 199 (39%) had laboratory-confirmed dengue fever. Dengue fever patients missed an average of 3.9 work days translating to a mean income loss of US$45. Twenty-six percent of those sampled earned US$151-199 monthly, significantly less than the Peruvian minimum wage of US$283. The average income loss in this group was US $48, representing 24-32% of their monthly income. Despite the fact that these dengue fever cases were not severe because they were all outpatients, in the context of a local economy where many people are independently employed and have subsistence-level income, DENV infection posed a considerable financial burden.

808 IDENTIFYING HOST INNATE IMMUNITY FACTORS CRUCIAL FOR PROTECTION AGAINST DENGUE VIRUS

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Dengue virus (DENV) is a mosquito-borne human pathogen with no existing vaccine. Previous vaccine development efforts have relied on serial passage in cell culture to derive attenuated strains. This approach by Mahidol University led to a tetravalent vaccine with all strains meeting in vitro phenotypic criteria of attenuation, such as small plaque size. In clinical trial, DENV-3 PGMK30FRhL3 caused symptoms consistent with dengue fever, whereas DENV-2 PDK53 did not. As such, the traditional phenotypic criteria are not able to accurately predict human response to vaccines. Defining the molecular basis of attenuation could thus provide for more objective means of assessing candidate vaccine strains. We hypothesized that the PGMK30FRhL3 strain maintains its wild-type ability to evade host antiviral defenses, while the PDK53 strain elicits protective antiviral sensors and interferon signaling. To study host innate immune defenses, we performed RNAi knockdown of key genes of antiviral sensors and interferon signaling cascade--MAVS, IRF3, TRIF, STAT1, and NF-κB—in Huh-7 and BHK-21 cells. Following infection with PDK53 and PGPK30FRhL3, we compared plaque and focus size between cells with normal and impaired antiviral defenses. We then quantified viral spread via plaque size, immunofluorescence, and flow cytometry. As expected, plaque size of attenuated PDK53 was smaller than wild type DENV-2 16681. Upon knockdown of IRF3 or p50, the plaque size of PDK53 but not 16681 increased significantly. Similar observations could be made in the human Huh-7 cell line. These findings indicate that the plaque size of PDK53 is because of its inability to overcome the innate immune response, whereas the replication of wild type 16681 does not benefit from the knockdown of these innate immune genes. Our results suggest that the inability of PDK53 to escape innate immune recognition is the molecular basis of attenuation.

809 THE SPATIAL PATTERNS AND INFLUENCES OF CLIMATE VARIATIONS ON DENGUE OUTBREAKS IN SOUTHERN TAIWAN THROUGHOUT 1998 TO 2012

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Southern Taiwan has been a hotspot of Dengue Fever (DF) transmission since 1998. The incidence of dengue fever in Taiwan shows strong seasonality. Mosquito ecology and the transmission of dengue fever are influenced by multiple environmental factors, especially for climate variations. Thus, interannual variability in climatic conditions could be important drivers for annual outbreaks. This study explored the spatial patterns of dengue outbreaks in Tainan and Kaohsiung city in Southern Taiwan. Multiple climatic indices generated from weather stations at these two study regions were used to develop a model to evaluate the risk of dengue transmission. In view of disease early warning system, the climate variations in the early season is emphasized in the analysis rather than the conditions during the summer time. Non-linear generalized additive models (GAMs) were used to evaluate the influences of monthly weather
variables, including average temperature (TAVG), maximum temperature (TMAX), minimum temperature (TMIN), precipitation (PREC), and relative humidity (RH), on interannual variations of DF incidence from 1998 to 2012. The significant temporal and spatial heterogeneity of large scale DF outbreaks were shown in the two cities. Kaohsiung experienced significant outbreaks in 2002, 2010, and 2011; however, 2007 and 2012 are two outbreaks years in Tainan. The spatial patterns differed in each year for both regions. The best-fitting model highlighted the importance of temperature, especially for TMIN, on the transmission of DF. Warmer TMIN during the preceding winter indicated elevated DF risk for the next summer and fall in both regions (p=0.03 for Kaohsiung, p=0.001 for Tainan). The outbreak of DF in Tainan is also associated with TMIN in February (p=0.007); however, DF outbreaks in Kaohsiung could be determined by the TMIN in April (p=0.009). The different responses to temperature variations in the two connected cities might be affected by other environmental factors, such as urban structures or land use types. Our modeling approach can provide useful information for establishing dengue early warning system in Taiwan.

810 CLIMATE VARIABILITY AND DENGUE EPIDEMICS IN PUERTO RICO

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Inter-annual climate variability is driven by climate systems such as El Niño-Southern Oscillation (ENSO) and the North Atlantic Oscillation (NAO). Warm ENSO events have been found to be associated with dengue epidemics in the Caribbean, whereas the impact of NAO variability on dengue dynamics has not been investigated. These climate systems affect the local climatology (air temperature, rainfall) of Puerto Rico, which in turn may affect dengue virus transmission. This study investigated the influence of inter-annual climate systems at various temporal scales (decadal, yearly, monthly) on local meteorological variables and dengue dynamics in Puerto Rico from 1987-2013. It was found that the impact of El Niño on dengue incidence may be modulated by the phase of the NAO, and that both climate systems can interact to exacerbate or reduce the magnitude of epidemics. For example, the largest dengue epidemic ever registered in Puerto Rico in 2010 occurred after a strong El Niño that peaked in December 2009 - January 2010, bringing anomalous warmer conditions and a strong negative NAO that brought exceptionally wetter conditions. Annual dengue incidence was significantly associated with temperature and dengue incidence during the winter. Warmer winters allow higher than normal dengue virus transmission that may cause epidemics during the rainy season when mosquito populations increase.

811 USE OF HOUSEHOLD CLUSTER SURVEYS TO QUANTIFY UNDER RECOGNITION OF DENGUE DURING THE 2013 EPIDEMIC IN LUANDA, ANGOLA

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Dengue is endemic throughout the tropics and is under recognized in Africa. In March 2013, a dengue epidemic was identified in Luanda, the capital of Angola. In total, 1,214 dengue cases were reported, of which 811 (67%), including 11 fatal cases, tested positive with a dengue rapid diagnostic test (RDT). Only dengue virus (DENV)-1 was identified. To estimate the percent of individuals infected during the epidemic and identify risk factors for infection, we conducted household cluster surveys within a 25-meter radius of RDT(+ dengue case-patients’ and randomly selected households. All participants reported demographic data, medical history, and individual and household mosquito avoidance practices, and provided a serum specimen for dengue diagnostic testing by RT-PCR and anti-DENV IgM ELISA. Of 453 specimens collected, anti-DENV IgM was detected in 41 (9%); none were positive by RT-PCR. Of 173 individuals from 67 households in 21 RDT+ clusters, 16 (9%) were anti-DENV IgM(+). Of 247 individuals from 90 households in 26 random clusters, 25 (10%) were anti-DENV IgM(+). There were no statistically significant differences in frequency of detection of anti-DENV IgM among individuals, households, or clusters. Of the 41 anti-DENV IgM(+), individuals, 13 (32%) reported fever in the past 30 days, of which 5 (38%) and 1 (8%) reported symptoms consistent with dengue with warning signs and severe dengue, respectively. Seven (54%) of the recently infected febrile individuals sought medical care; 1 (14%) was hospitalized, and none were diagnosed with dengue. Factors associated with protection against recent DENV infection identified in this sample of individuals included bed net use in the past 30 days (p = 0.04) and delivery of household water by truck (p = 0.01). Identification of recent DENV infection among 10% of survey participants and lack of accurate diagnosis of participants that sought medical care suggest under recognition and underreporting of dengue. Clinical awareness of dengue should be strengthened to better define the epidemiology of dengue in Angola.

812 COMMUNITY-BASED PARTICIPATORY RESEARCH FOR PREVENTION OF DENGUE FEVER FROM THE APPROACH TO HEALTH COMMUNICATION: THE EXPERIENCE IN THE CARIBBEAN SLOPE OF COSTA RICA

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Dengue fever is the most important tropical vector-borne disease in Costa Rica. In 2013, the country had the largest outbreak in its history when 49,993 new cases were reported. A Community-based participatory research (CBPR) was developed between years 2012-2014 at La Roxana of Pococi, an endemic locality at the caribbean slope. Social, cultural and health public components of two communities (Luis XV and San Antonio) were studied in 2012 with the purpose of promoting community organization for Dengue prevention during 2013. CBPR has demonstrated success in implemented actions. CBPR has demonstrated
the importance of community awareness to increase the success of communication strategies for the prevention of dengue. Also, when CBPR is based on the health needs and interests, it can promote the community self-organization. In the case of La Roxana women’s empowerment and leadership of community groups were considered essential for success.

A NOVEL ALLOSTERIC SMALL MOLECULE INHIBITOR OF INDUCIBLE HSP70 REDUCES DENGUE VIRUS INFECTION

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The inducible protein chaperone Heat shock protein 70 (Hsp70i) is important in maintaining protein folding and cellular homeostasis. Hsp70i is utilized throughout the viral lifecycle for replication and propagation of the virus. Dengue virus, HIV, and rotavirus are a few of the viruses that exploit Hsp70i for infection and replication. However, the complete role of Hsp70i in dengue virus pathogenesis remains unclear. Previous studies have shown that Hsp70 may act as a receptor complex for virus internalization. Additionally, Hsp70 siRNA knockdown reduced dengue virus load, and Hsp70 aids in propagating the virus following internalization. Dengue virus is now endemic in over 100 countries and there are currently no approved vaccines or treatments for dengue virus infection. To date, few Hsp70 inhibitors have been identified and characterized, and their efficacy in clinical settings is unknown. We have identified a novel allosteric small molecule inhibitor of Hsp70i, HS-72, using FLECS (fluorescence linked enzyme chemoproteomic strategy). Inhibition of Hsp70i in a monocyte cell lines reduces dengue virus infection, while maintaining cell viability. Additionally, HS-72 leads to a reduction in the binding and entry of dengue virus in monocytes. Hsp70i is expressed at low levels preceding infection, but intracellular Hsp70i expression is rapidly induced upon dengue virus infection, while surface Hsp70i is unaffected. Furthermore, increasing intracellular Hsp70i expression prior to infection through Hsp90 inhibition, leads to an increase in dengue virus infection. This work highlights an essential role for Hsp70 in dengue virus pathogenesis and identifies a potential therapeutic antiviral agent for dengue virus infection.

NEUTRALIZATION OF DENGUE VIRUSES IN VIREMIC HUMAN BLOOD; MAPPING THE MOST POTENT VIRUS NEUTRALIZING HUMAN MABS

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A better understanding of the characteristics of antibodies that neutralise dengue viruses in vivo, rather than in vitro, is a research priority. We established an experimental human-to-mosquito dengue virus (DENV) transmission system for measuring the potency of human mAbs to neutralise dengue viruses in DENV viremic human blood collected from Vietnamese dengue cases. In this assay we have characterized the potency of a panel of 15 human mAbs that target a range of epitope regions on all four DENV serotypes. The results demonstrated that selected serotype-specific mAbs, recognizing a quaternary epitope, were the only mAbs able to neutralize DENV in the “in vivo” environment of vireemic blood. These results identify a class of antibodies that dengue vaccines should aim to elicit.

ASSOCIATION OF NUTRITIONAL STATUS AND DENGUE COMPLICATIONS IN CHILDREN: RESULTS FROM A PROSPECTIVE COHORT STUDY CONDUCTED IN COLOMBIA

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Undernutrition is an important predictor of complications in communicable diseases; however, the evidence of its role in pediatric patients with dengue remains controversial. We conducted a prospective cohort study (in three recruitment waves between 2003 and 2011) among out-patients aged 5-19 years old from Colombia, who had fever and serologic or virologic evidence of dengue infection. Socio-demographics, warning signs, and nutritional status were ascertained at baseline. Nutritional status was determined by calculating height for age (HFA) and body mass index for age (BMI-for-age) scores using the software WHO-ANTHRO. Patients were followed for complications defined as new-onset of shock (age-specific tachycardia and pulse pressure <20 mmHg or systolic or mean blood pressure below PAHO’s age-specific cut-points for hypotension) or severe hemorrhage (hematemesis, melena, hematochezia or hematuria). We evaluated 330 children free of complications at baseline (mean age: 12.3 years; 57% male; mean disease’s duration: 3.5 days). During a median follow-up of 3 days, 75 patients (22.7%) developed complications: 63 (19.1%) shock, 9 (2.7%) severe hemorrhage, and 3 (0.9%) shock and severe hemorrhage. Baseline HFA and BMI-for-age were lower among incident than non-incident cases: -0.1 vs. 0.3 (p=0.021) and -0.3 vs. 0.1 (p=0.007). After controlling for age, gender, and disease’s duration, an increase in 1 z-score unit of HFA and BMI-for-age were independently associated with 23% (OR=0.77; 95%CI: 0.61, 0.98) and 25% (OR=0.75; 95%CI: 0.61, 0.92) lower probability of complications, respectively. There was no evidence of HFA-by-BMI-for-age interaction (p=0.377). Further adjustment for baseline warning signs did not attenuate associations. Our results suggest that, from a public health perspective, tackling undernutrition in dengue endemic countries might reduce the burden of the disease in the pediatric population.

ESTIMATING CROSS-ENHANCEMENT AND CROSS-PROTECTION OF DENGUE VIRUSES USING TIME SERIES DATA FROM THAILAND

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Using serotype specific case data from Queen Sirikit National Institute for Child Health in Bangkok, previous work estimated the length of cross protection to be 1-3 years. We have extended this work in two directions. First, we have developed models to consider whether there are serotype-specific differences in the cross-protection period and secondly to estimate the population-level impact of any enhancement of susceptibility or symptom severity of secondary infections, concurrently with cross-protection. We estimate whether the length of cross protection depends on the serotype of the primary or secondary infection, or the combination of both. Early results show that it may be difficult to detect any differences in the length of cross-protection by serotype. However our work continues to define what we can estimate, and what the impact of differential infection-to-case ratio or transmissibility by serotype will have on this estimate. We derive estimates of both cross protection and enhancement by combining data from multiple sources, including serotype specific and non-serotype specific case data from multiple locations in Thailand.
EFFECTIVE IMMUNITY IN A MOUSE MODEL

AN EARLY-LIFE DENGUE VACCINE CANDIDATE INDUCES EFFECTIVE IMMUNITY IN A MOUSE MODEL

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Dengue viruses (DENV1-4) cause 50-100 million clinical infections every year, several hundred thousand of which progress to severe hemorrhagic and shock syndromes. Preexisting immunity resulting from a previous DENV infection is the major risk factor for severe dengue during secondary heterologous infections. During primary infections in infants, maternal antibodies pose an analogous risk. At the same time, maternal antibodies are likely to prevent induction of endogenous anti-DENV antibodies in response to current live, attenuated vaccine (LAV) candidates. Any effective early life dengue vaccine has to overcome maternal antibody interference (leading to ineffective vaccination) and poor induction of antibody responses (increasing the risk of severe dengue disease upon primary infection). Here we demonstrate the feasibility of a non-propagating VEE virus replicon vector (VRP) expressing DENV E protein as an early life vaccine platform for dengue. We previously showed that a DENV-VRP vaccine is immunogenic even in the presence of maternal antibodies that otherwise interfere with a live virus vaccination in weanling BALB/c mice. In this report, we observed that a single immunization in 7-day-old neonatal BALB/c mice with a VRP vaccine expressing E ectodomain of DENV induced neutralizing antibody (NAb) titers by 6 weeks, which remained stable until at least 15 weeks post-immunization. DENV-specific cell-mediated immunity was also induced in these immunized mice. Furthermore, the NAb levels induced to each serotype by a tetravalent VRP formulation were equivalent to those of each monovalent vaccine components, suggesting that this vaccine modality can overcome serotype interference. VRP immunization in neonatal mice was durable and could be boosted later in life to further increase NAb and T-cell responses. Although the neonatal immune response was lower in magnitude than responses in adult BALB/c mice, we demonstrate that, both monovalent and tetravalent VRP vaccines generated protective immunity from a lethal intracranial challenge after a single neonatal immunization. In summary, VRP vaccines expressing DENV antigens were immunogenic and protective in neonates, and hence are promising candidates for safe and effective vaccination in early life.
Mombasa Hospital. The surveillance was from 21st June to 30th September, 2013. All persons presenting at the healthcare facilities with symptoms consistent with the case definition of suspect DEN; temperature ≥38.0°C for up to five days AND did not meet criteria for acute respiratory illness, was to be reported using IDSR outbreak report forms. A blood sample was taken where possible from two suspect DEN cases per day for per site for five days a week in the public hospitals and in the private hospitals, as cases were identified. Real-time RT-PCR was performed at the KEMRI/ CDC laboratories. Blood samples were collected from all 67 suspected cases identified. Two-thirds (44/67) were from the public hospitals. Of the 67 cases, 14 (21%) tested positive for DEN, 78.6% from private hospitals and 21.4% from public hospitals. Use of IDSR report forms varied across facilities and partially conformed to the MoH guidelines. We found varying rates of positivity and compliance between the public and private facilities. IDSR is the strategy adopted by WHO-AFRO for disease surveillance and outbreak response in the region; however it needs to be strengthened to ensure that the planned objectives are met while ensuring optimal resource use. In Kenya, IDSR implementation following devolution of government may need to be decentralized to the county level.

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SPATIAL ANALYSIS AND MODELING OF DENGUE SEROPREVALENCE IN VENEZUELA

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Dengue has become one of the most important public health problems of urban areas in Venezuela. Control of dengue and of its mosquito vector has proven challenging in settings of uncontrolled urban growth and unreliable water supply. The ability to identify high-risk areas of dengue transmission can be used to target surveillance and control measures to those locations in a cost-effective manner, particularly in countries where resources are scarce. We used mapping technology and spatial statistics to identify clusters (“hot spots”) of transmission within a community-based cohort of 2012 individuals living in 840 households in the hyperendemic city of Maracay, Venezuela. Two spatial analyses of epidemiological and dengue seroprevalence data were conducted: 1) at house level, and 2) at block level. Risk-maps drawn at a fine scale determined that dengue seroprevalence is highly heterogeneous at the studied spatial scale. We detected significant hot spots (Gri*d >2.79, P<0.05), at each spatial scale in all neighbourhoods. Our results also suggest that dengue transmission is very focal (20-80 meters). To better explain what determines dengue spatial clusters, we did comparative analyses of risk factors within and outside of hot spots areas using logistic regression modelling. Finally, we applied spatial regression modelling to identify which variables (demographic, socioeconomic and environmental) were more relevant to explain local dengue dynamics in Venezuela. Results will be presented.

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LONG-TERM EPIDEMIOLOGICAL ANALYSIS OF PEDIATRIC DENGUE IN VENEZUELA (2000-2011)

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Dengue is a major public health problem in Venezuela. It exhibits a variable epidemiological pattern in time, and associates with a significant annual number of cases, ±50% of which, are seen in children. A retrospective morbidity-mortality and disease burden study of dengue in Venezuelan pediatric population, based on official data from the Ministry of Health and National Institute of Statistics from 2000 to 2011, was carried out. Statistical analysis was performed by a SPSS20 program. Significance tests were performed as required. Burden of disease was estimated as DALYs associated with cases and deaths. Of the 632,066 dengue cases registered in the general population, 316,404 (50.05 %) were seen in children <15 Y/O. Thirty nine per cent of the cases occurred in six epidemic years (2001/2005-2007/2009 and 2010), the latter with a historical record of over 110,000 cases. Whereas the average General Morbidity Rate for the period was 194.87, it was 1.7 higher in children (332.19). The most affected age group was that of 5-9 Y/O (364.98). The <1 Y/O experienced 28,065 cases of severe dengue (56.60% of all severe dengue). Mortality Rate in children was 1.42 times higher than in adults (0.27 vs. 0.19, respectively) (p < 0.05), and in the group under 1 year it was 4.10 times as much (0.78) (p <0.01). Lethality rate was 20% lower in children than in adults (0.08 vs. 0.10, respectively) (p <0.01). However, in <1 Y/O it was 2.4 times higher than in adults and 3.4 times more than in all other pediatric age groups (p <0.01). Of note, during the last half of the analyzed period both mortality and lethality rates significantly increased for all pediatric age groups, especially in 5-9 Y/O. Disease burden was estimated in 3,793.65 DALYs per clinical cases and 9,796.78 per deaths. In Venezuela, dengue exhibits both endemic and epidemic cycles. Epidemics were frequent in the studied period and associated with majority (±70%) of reported cases, reflecting failures in the control and prevention programs. Lethality in children was lower than in adults and that reported internationally. The marked increase in the mortality and lethality during the last lustrum is unexpected, and requires to be explained. Although the most affected groups were the 5-14 Y/O, mortality and lethality rates were much higher in those less than 1 year. These results may be useful to understand better the epidemiology of the disease in the country and improve the effectiveness of disease control.

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THE HUMAN CD8+ T CELL RESPONSES INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE ARE DIRECTED AGAINST HIGHLY CONSERVED EPITOPES AND ARE SIMILAR IN MAGNITUDE AND BREADTH TO THOSE FOLLOWING NATURAL INFECTION

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The incidence of infection with any of the four dengue virus serotypes (DENV 1-4) has increased dramatically in the last few decades, and the lack of a treatment or vaccine has contributed to significant morbidity and mortality worldwide. The recent failure of a candidate vaccine to protect
against disease despite induction of antibody responses against all DENV serotypes in most subjects suggests that better correlates of protection are needed. A recent comprehensive analysis of the human T cell response against wild-type DENV suggested an HLA-linked protective role for CD8+ T cells. Here, we characterize for the first time CD8+ T cell responses after live attenuated dengue vaccination and compare them to responses observed in natural infection with dengue virus. We have collected one-unit blood donations from study participants receiving the monovalent or tetravalent live attenuated DENV vaccine (DLAV), developed by the U.S. National Institutes of Health. PBMCs from these donors were screened in IFNγ ELISPOT assays with pools of predicted, HLA matched, class I binding peptides covering the entire DENV proteome. CD8+ T cell responses in vaccinees were readily detectable with a magnitude and breadth similar to natural dengue infection. Interestingly, while broad responses to structural and non-structural (NS) proteins were observed after monovalent vaccination, T cell responses following tetravalent vaccination were, dramatically, focused towards the highly conserved non-structural proteins NS3 and NS5. Epitopes from these proteins are highly conserved in a vast variety of field isolates and are able to elicit multifunctional T cell responses. Live attenuated vaccines against dengue virus are able to induce a CD8+ T cell response comparable to responses seen in natural infection. Detailed knowledge of the T cell response will contribute to the identification of robust correlates of protection in natural immunity and following vaccination against DENV.

### HISTOLOGIC CHARACTERIZATION OF THE LOCAL IMMUNE RESPONSE TO DENGUE VIRUS INFECTION IN INTRADERMALLY INOCULATED MICE

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Dengue virus (DENV) is an emerging arboviral pathogen transmitted primarily by the mosquito *Aedes aegypti*. Approximately 40% of the world’s population lives in regions that are at risk for DENV transmission. This virus is associated with a high morbidity of febrile disease and in a small percentage of cases, disease can progress to more severe manifestations. Several models of DENV infection have been developed to study the pathogenesis of this disease in various organ systems. While some experiments have examined the role of dendritic cells following intradermal inoculation of DENV, none have attempted to qualify the local inflammatory response and potential differences between viral serotypes in vivo to elucidate the pathogenesis that leads to disease-causing viremia. In this study, mice were intradermally inoculated with either DENV 2 [strain 1232] or DENV 4 [strain 1228] in the hindlimb footpad after allowing mosquitoes to feed at this site. For negative control mice, mosquitoes were allowed to feed, but no viral inoculum was injected. Mice were sacrificed at 3 hours and 18 hours post-inoculation (hpi). The distal hindlimbs, as well as the associated popliteal lymph nodes, were harvested, formalin-fixed and processed for histologic evaluation. At 3 hpi, the dermis was mildly expanded and the inflammatory response consisted predominantly of small numbers neutrophils, fewer mast cells and occasional macrophages. The popliteal lymph nodes contained small numbers of mast cells and fewer neutrophils. There were no observable differences associated with serotype and the inflammatory infiltrates in virus inoculated mice were similar to negative control mice. Histologic evaluations of the 18 hpi mice are pending, as are immunofluorescence assays to correlate location of viral antigen with the inflammatory responses. Characterization of the local immune response following intradermal DENV infection will help elucidate the early pathogenesis of disease and determine any potential differences associated with serotype.

### ASSESSMENT OF INCIDENT DENGUE VIRUS INFECTIONS AMONG FEBRILE PATIENTS IN WESTERN KENYA

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Although dengue occurs in Africa, it is poorly identified and there is uncertainty about its geographic distribution. In many settings, a lack of diagnostic capacity has resulted in use of syndromic guidelines for the management of febrile illnesses. In East Africa, dengue outbreaks have been reported recently and seroprevalence studies suggest dengue virus (DENV) transmission. A retrospective study was conducted to estimate the incidence of DENV infection among febrile patients in western Kenya. Serum was collected from febrile (≥38°C) patients in an ongoing population-based disease surveillance study in Asembo District, Kenya, from Sept.-Nov. 2011 and Mar.-July 2013; rainy seasons when DENV transmission was expected to be high. We excluded patients with alternative clinical or lab diagnoses (e.g., bacteremia, positive urine culture, virus-positive nasal swab), or >5 days of fever. Testing for DENV RNA was performed by real-time RT-PCR and RNA integrity was verified by a human RNAse P control. DENV positive and negative controls performed as predicted. A total of 688 febrile patients met the inclusion criteria and 615 (89.4%) of them had samples available for testing. The median age was 4.5 years and 53% were female. No samples were DENV RNA positive. In this study, no DENV infections were detected using molecular diagnostic testing, despite samples being collected in what would be the viremic period of dengue. Possible reasons include inaccurate reporting of fever onset, a predominantly rural study site resulting in lower vector density than in coastal Kenya where incident dengue has been detected, or a genuine absence of DENV. If subsequent IgM analyses are also negative, the combined results will support the idea of uneven distribution of dengue in East Africa. Such variation, combined with the possible spread of disease in the area, reinforces the need for including dengue diagnostics in febrile illness surveillance to better inform management of fever.

### RISK FACTORS OF BLEEDING IN HOSPITALIZED ADULT DENGUE PATIENTS

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Hemorrhagic manifestations are undesirable clinical outcomes and important criteria of dengue prognosis. Clinical bleeding is required for the classification of dengue hemorrhagic fever. Severe bleeding is a form of severe dengue. However, risk factors for bleeding in dengue patients are not well studied. We conducted a retrospective study of adult dengue patients hospitalized at Communicable Disease Center, Singapore from 2005 to 2008, confirmed by positive PCR or dengue serology with World Health Organization 1997/2009 probable dengue criteria (n=6070). Clinical bleeding was defined as bleeding excluding petechiae and severe bleeding include gastrointestinal bleeding or menorrhagia or requiring blood transfusion. Those with no prior bleeding were included into the multivariate analysis. There were 869 (19.8%) and 148 (3.4%) patients who developed clinical and severe bleeding respectively. Variables with a p-value of <0.2 in the univariate analysis were included into the multiple logistic regression model. The final model was derived using manual backward elimination and adjusted for age, gender,
disease severity, Charlson’s score, hematocrit and aspartate transaminase. Fever on admission (aOR:1.4 [1.2-1.7]), absence of rash (aOR:0.8 [0.7-0.99]), anorexia (aOR:1.2 [1.01-1.4]), neutrophilia (aOR:1.01 [1.008-1.02]), leukopenia (aOR:0.85 [0.82-0.9]) and thrombocytopenia (OR:0.996 [0.993-0.998]) were significantly associated with clinical bleeding. Fever on admission (aOR:2 [1.3-2.9]), neutrophilia (aOR:1.02 [1.005-1.03]) and abdominal pain (aOR:1.44 [1.003-2.05]) were significantly associated with severe bleeding. Our findings warrant further validation in different cohorts, including other countries, children and different serotype outbreaks.

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STUDY OF CROSS-REACTIVE ANTIBODIES AGAINST DENGUE VIRUS ENVELOPE PROTEIN FOLLOWING HETEROTYPIC IMMUNIZATION AND SECONDARY INFECTION

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The four serotypes of dengue virus (DENV) are the leading cause of arboviral diseases in humans in tropical and subtropical regions. After secondary DENV infection, multitypic neutralizing (NT) antibodies (Abs) were developed not only against the previously exposed serotypes but also against the serotypes to which they have never been exposed (“non-exposed serotypes”). These heterotypic NT Abs are believed to contribute to protection against subsequent infection by the non-exposed serotypes. The nature of these NT Abs remains largely unknown. To study cases of well-documented primary and secondary DENV infections, we examined sera from 10 vaccinees, who received two doses of live-attenuated vaccine in a heterotypic immunization study (Durbin et al. J Infect Dis 2011;203:327-334). Serum samples prior to and at 42 days after primary and secondary DENV immunizations were examined by Western blot analysis, virion-ELISA, modified 8M urea-ELISA, and focus reduction neutralization test (FRNT). Binding studies with virion-ELISA and IgG avidity showed stronger recognition of the primary infection serotype compared to other serotypes after secondary DENV infection, which is consistent with the “original antigenic sin”. FRNT revealed multitypic NT Abs to both exposed and non-exposed serotypes. Depletion with West Nile virus antigens resulted in reduction of NT activities, suggesting that group-reactive Abs contributing to NT activities after secondary infection. Similar trend was also observed in sera from patients with secondary DENV infection. Together, these findings resonate with our recently published report of high-avidity and potently NT cross-reactive human mAbs derived from patients after secondary infection.

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DENGUE TRANSMISSION IN A MEDIUM-SIZED CITY FROM BRAZIL AND THE LESSONS WE CAN LEARN TO AVOID IDENTICAL SCENARIOS ELSEWHERE

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Dengue viruses (DENV) have been a public health problem in tropical regions for decades but autochthonous transmission has now been reported in countries like USA, France and Croatia. In 2013, 2.35 million dengue cases were reported in the Americas and Brazil alone was responsible for more than 50%. The country has been presenting outbreaks for more than three decades and important lessons can be learned. Thus, in depth analysis of dengue transmission in Araraquara, a medium-sized city at the central portion of São Paulo, which is the richest state of Brazil and a critical area for DENV transmission, may clarify how random circulation of the virus may evolve to massive outbreaks. DENV has been circulating in the city since 1990s at low incidences. However, the number of cases has increased in recent years and we will be describing the scenario of five years of transmission herein. Official data on dengue reports from 2008 to 2012 were recovered from the Information System on Diseases of Compulsory Declaration. Data from 5,282 reported cases were analyzed. The majority - approximately 60% for the five-year period - was reported in people with up to six years of formal education; this trend is an indication that areas with low living standards may play an important role in DENV dispersion. Another important observation is that dengue transmission has become endemic in the city, with cases being officially reported in all months of the year, with the exception of 2009, which was atypical in the whole state of São Paulo. Severe dengue or dengue with complications was a rare event in the city. The incidence in Caucasians (78.4%) was higher than in other ethnic groups, a pattern that has been described worldwide. Females were more affected (54.5%) than males and further analysis is required to assess this figure. This is part of an ongoing project that is also focused on classical and molecular epidemiology involving the implementation of regular molecular diagnosis in the city, phylogenetic analysis of serotypes in humans and mosquitoes and spatial statistics.

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USE OF ANTIGENIC CARTOGRAPHY TO CHARACTERIZE NEUTRALIZATION OF DIVERSE DENGUE VIRUSES IN THE MONTHS FOLLOWING PRIMARY DENGUE VIRUS INFECTION

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The four dengue virus types (DENV1-4) elicit early neutralizing antibody responses with high titers to homologous as well as heterologous DENV types, which have been observed to narrow to the homologous type in the ensuing months. However, research on changes in the neutralizing response over time often use single representatives of each DENV type, and thus the degree to which changes are due to the quantity of antibodies or the pattern of neutralization is not well understood. Here, we use antigenic cartography to describe how 17 primary-infection African green monkeys (AGM) with antisera drawn one, three and five months after inoculation neutralize a panel of 40 diverse DENV isolates assembled by the Dengue Antigenic Cartography Consortium. The neutralizing antibody response to the DENV panel over the four-month period significantly declined for eight AGMs, was stable for eight AGMs, and dramatically increased for one AGM. We made an antigenic map of the DENV panel titrated against the AGM antisera drawn at one, three and five months, as the fit of the data was comparable to maps made of each time point separately. Like antigenic maps made of each time point, the distance within and between DENV types was comparable: DENV2, DENV3, and DENV4 had similar variation within and between DENV types, while DENV1 isolates were slightly more varied than within type. Antiseras drawn one-month post-infection differed in position on the map by an average of 3.2-fold dilutions from three-month antisera (SD: 2.4-fold), while three-month antisera were only 1.6-fold dilutions from five-month antigenic positions (SD: 1.4-fold). This difference was significant, and suggested moderate changes in reactivity between one and three months, but minimal changes between three and five months. Only four AGMs shifted to the periphery of homologous antigenic cluster, suggesting increasing type-specificity. The remaining antisera moved toward the center of the antigenic map over time, thus transitioning to more cross-reactive responses. We find that only a subset of primary infection antisera become more type-specific over time, and that minimal changes in reactivity are observed between three and five
months after infection. Further, the use of all three time points enables better coordination of the DENV panel, making possible comparison of the relationships between viral genetic and antigenic differences.

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EVIDENCE FOR THE RECENT EMERGENCE OF DENGUE IN BANGLADESH: RESULTS FROM A SEROPREVALENCE STUDY

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Dengue disease is endemic throughout Southeast Asia and has been reported throughout India. Global models of dengue incidence suggest it is widespread across Bangladesh; however, while dengue infections have been reported in Dhaka and other cities, it is unknown if the pathogen has spread to rural communities that make up the majority of the country. To address this gap we conducted a seroprevalence study of dengue in a rural district that borders India in the northwest of the country. We randomly selected 40 villages and visited randomly chosen households within each community. All household residents were asked to provide a blood sample and information about socio-demographics. Indirect PanBio IgG ELISAs were used to identify dengue-specific antibodies as a marker of past dengue infection. In total, 1,497 individuals participated in the study with a median age of 26 (range 0 - 90) years. 18% of the study population had serological evidence of past infection. There was significant spatial heterogeneity with virtually no past exposure detected in the north of the district whereas communities in the south near the district capital had over 60% seropositivity, although we found no differences in seropositivity by age (p-value 0.96). In addition, we found no difference in seropositivity by age (p-value 0.17), suggesting that all individuals had experienced a similar cumulative risk of infection characteristic of recently emergent pathogens. We used a multilevel model to identify risk factors associated with historic dengue infection. Males were 1.4 (95% confidence interval [CI] 1.0-1.9) times more likely to have been infected than females. Having other infected individuals in the household increased the probability of being seropositive by 1.3 (95% CI 1.1-1.5) times. The presence of seropositive individuals in the community (but outside the household) further increased the risk of having been infected by 1.2 (95% CI 1.1-1.3) times. These findings suggest that dengue has only recently emerged in parts of this rural area and underscore the importance of considering rural communities when assessing the burden of dengue. Household and community-specific factors appear key to determining individual risk. Further work exploring differences in the ecological suitability for the vector in this region and the flow of people from dengue endemic communities will help us further understand the observed patterns of exposure.

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ATTITUDES TOWARD HEALTH, VECTOR CONTROL AND DENGUE FEVER IN SEVEN ENDEMIC COUNTRIES: INSIGHTS FROM ETHNOGRAPHIC RESEARCH IN BRAZIL, COLUMBIA, INDONESIA, MALAYSIA, MEXICO, PHILIPPINES AND SINGAPORE

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Perception of dengue and its prevention was assessed in 84 adults from various socio-economic groups and both urban and suburban dengue-endemic areas in 7 countries. Participants aged 18-55y (76 were women), 79 of whom had children <16y, were interviewed at home in 1.5-2h, semi-structured interviews. Prior to interviews, participants prepared scrapbooks to gauge emotional aspects. Interviews were filmed and vector control measures observed. Health was a top priority for all, contributing to family wellbeing and happiness. Work, financial stability and religion contributed to family wellbeing in Asia, and education in both regions. Health prevention was associated with nutrition, hygiene and exercise, and with childhood vaccination in Latin America. Prevention was considered part of hygiene efforts. The top health concerns included: 1/ cancer, heart attacks, and meningitis, 2/ diabetes and obesity, 3/ childhood diseases, 4/ dengue, and 5/ other infectious diseases (IDs). Dengue was spontaneously mentioned in Brazil, Indonesia and Philippines only, and was perceived as a bigger threat than other IDs in all countries. Latin Americans perceived vaccination positively and favored public vaccination, while Asians expressed concerns about vaccines and favored vaccination in private clinics. Knowledge of dengue was high, with gaps around the severity of secondary infection, existence of different serotypes, differences between DF and DHF, and the dangers of self-medication with some classes of NSAIDs. Dengue was associated with negative images, e.g. blood, humidity, pain, disability, dirt, filth, death. Level of concern and intensity of preventive measures were influenced by: personal experience of dengue, time of day, rainy season, national dengue incidence, public awareness campaigns, and concerns about children. Respondents felt safe at home and more exposed outside. They were committed to dengue prevention, yet preventive measures were inadequate and mosquito repellents were often absent. These results shed light on attitudes to dengue and prevention, and may help inform public communication campaigns.

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VIROLOGICAL TEST ALGORITHM FOR DENGUE CASES - OBSERVATIONS FROM A PHASE 2 LATIN AMERICAN DENGUE VACCINE TRIAL

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The CYD tetravalent dengue vaccine candidate is being evaluated for protective efficacy against symptomatic dengue in phase III efficacy trials. The laboratory test algorithm to confirm dengue cases was evaluated prior to Phase III trials. During a Phase II safety and immunogenicity trial in Latin America (Clinicaltrials.gov NCT00993447) a dengue epidemic occurred in the study countries. A total of 72 suspected dengue cases were reported and assessed: virological confirmation comprised qRT-PCR methods and a commercial ELISA kit for NS1 protein (Bio-Rad). The qRT-PCR included a screening assay targeting a dengue-conserved region of the 3’-UTR (Dengue screen assay) followed by 4 individual serotype assays targeting the conserved dengue NS5 genomic region (WT dengue qRT-PCR assays). The NS1 and WT dengue qRT-PCR were the protocol endpoint assays for virological confirmation (PVC). Of the 72 suspected cases, 14 were PVC: 9 by WT dengue qRT-PCR (5 Den-1, 4 Den-3) and 5 positive by NS1 Ag ELISA only. For the 9 PCR positive cases, 8 were also positive by NS1. However, a unique pattern of dengue qRT-PCR results were observed in 5 suspected cases. In these 5 cases, all from Honduras, the dengue screen qRT-PCR assay was positive but both the WT dengue qRT-PCR and NS1 Ag ELISA were negative. To investigate, exploratory data were generated using additional molecular methods: a SYBR Green-based RT-PCR assay sequencing assays directed at the dengue genomic regions covered by the WT dengue qRT-PCR, and a commercial dengue RT-PCR test (Simplex™ Dengue, Focus Diagnostics). The exploratory data confirmed these additional cases as dengue. Results indicated the serotype 2 WT dengue qRT-PCR assay was unable to detect a circulating Latin American strain (DENV-2/N/BID-V608/2006) due to a mutation in the probe-binding region of the isolate. The Simplex™ Dengue RT-PCR test was able to detect dengue in all samples tested except one. On the basis of these results and additional evaluations, the PVC algorithm was modified for the Phase III efficacy trials and future studies.
EVALUATING THE UTILITY OF REACTIVE VECTOR CONTROL FOR DENGUE OUTBREAKS

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Dengue has the highest burden of any viral vector borne disease. A major contributor to this high burden is the unpredictable timing and magnitude of dengue outbreaks that often overwhelm healthcare facilities. While some early warning systems have shown local successes, the standard response to such outbreaks remains reactive. With no effective human prevention or cure, control efforts have to focus on the mosquito population with activities such as fogging of adults or larviciding of juveniles. While reactive measures are important, it has not yet been tested whether the timeliness and effectiveness of currently available vector control interventions are appropriate for controlling dengue outbreaks. Here we use a compartmental SIR model of mosquito and human population dynamics to estimate the effects of interventions on eventual outbreak size given the time delays between dengue virus transmission and intervention implementation. In this analysis we evaluate the effects of several commonly used reactive interventions and calculate the threshold timeliness and effectiveness that would be required for vector control interventions to be more appropriately used as response measures than simply applied at random. This is the first time the time delays at various stages of transmission and subsequent intervention have been incorporated into a dengue transmission model and the resultant outcomes will be important for evaluating and supporting effective public health policies regarding dengue outbreaks.

SEROTYPE-SPECIFIC DENGUE NEUTRALIZING ANTIBODY RESPONSES IN FCγR-EXPRESSING CV1 CELLS

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A Phase 2b efficacy trial (CYD23; Clinicaltrials.gov NCT01134263) of the live attenuated CYD tetravalent dengue vaccine (TDV) showed clinical protection against dengue. Point estimates of vaccine efficacy differed between dengue virus (DENV) serotype and no measurable efficacy was observed against DENV2. This observation contrasted with results of a Vero cell based PRNT50 assay that showed similar levels of vaccine-induced neutralization antibodies for all four serotypes. We investigated the potential of a seroneutralization assay in FcγRIIa-expressing CV1 cells to assess both neutralization and potential enhancement of dengue infection. Parallel assays were performed in Vero and CV1-FcγRIIa cells to evaluate the neutralizing antibody response against all 4 dengue serotypes on serum samples from a naturally dengue-infected cohort and serum samples collected after three injections of CYD-TDV in a clinical trial (NCT01134263) of “dengue-naive” subjects. Neutralizing antibody titer GMT values were 2- to 9-fold lower in CV1-FcγRIIa cells than in Vero cells with lowest relative (Vero titer/CV1 titer) decrease amongst the 4 serotypes seen for the anti-DENV2 response. While the presence of an activating FcγRIIa led to a global decrease in neutralizing capacity, the fact that the lowest relative decrease was against DENV2 suggests that anti-CYD2 responses lead to lesser enhancement of infection relative to the other 3 serotypes. Additional CV1 studies are underway with sera from CYD-TDV vaccinees in dengue-endemic areas (NCT01187433) to determine what role, if any, differential levels of dengue pre-immunity have on the generation of neutralizing antibodies against each serotype. Results of these investigations suggest that despite the absence of clinical efficacy observed against DENV2 in the CYD23 trial, in vitro neutralizing antibody responses against DENV2 elicited by CYD2 in TDV vaccination were not more enhancing than responses against other serotypes.

MULTICENTER CLINICAL EVALUATION OF TWO ELISA AND TWO RAPID FORMAT ASSAYS FOR DIAGNOSING DENGUE

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Extensive prospective evaluations using multiple trial sites, a defensible gold-standard reference testing methodology, and quality systems that provide confidence in the study results, are required for reliable performance assessment of diagnostic tests. In this study, we evaluated the SD Bioline Dengue Duo (NS1/IgM/IgG) and the Panbio Dengue Duo (IgM/IgG) rapid diagnostic tests as well as the Panbio IgM and IgG Dengue ELISAs in a prospective, controlled, multicenter study. Paired samples were prospectively collected from 1021 individuals initially presenting on Days 0-7 at study sites in Peru, Venezuela, Cambodia, and the United States. An additional panel of 135 paired retrospective samples from Thailand was also used. A mix of primary and secondary infections and all four dengue serotypes were captured. Reference testing was performed using an algorithm involving virus isolation, IgM capture ELISA, and plaque reduction neutralization tests, in order to fully characterize the dengue status of each subject. Our primary end-points was positive and negative percent agreements of these devices against the reference methodology, but these numbers were also stratified using several factors known to influence overall accuracy including geography, days post onset of symptoms, infecting serotype, primary or secondary infections, and other demographic features. We determined that the SD Bioline Duo Cassette (NS1/IgM/IgG) had an overall sensitivity of 87% and specificity of 87% over the first 14 days post onset of symptoms. The Panbio Duo Cassette had a sensitivity of 92% and specificity of 59% during days 4-14 post onset of symptoms. This study generated reliable performance characteristics for several dengue diagnostic assays using prospectively collected specimens from both Asia and the Americas. Such results facilitate data-driven healthcare product choices for managing patient care during dengue.

HOST RESPONSES AFTER PRIMARY DENV-2 CHALLENGE IN CYNOOMOLGIS MACAQUES: IMPACT OF STRAIN, DOSE AND ROUTE OF ADMINISTRATION

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Some non-human primates including Rhesus (Macaca mulatta) and Cynomolgus monkeys (M. fascicularis) are sensitive to infection by Dengue viruses and reproducively develop viremia when inoculated by SC route with 4-5 log10 PFU. Viremia is modest, usually several orders of magnitude below human viral load in dengue patient, and animals do not develop dengue clinical signs in this model. However classical dengue hemorrhage was observed in rhesus monkeys showing high viremia after intravenous challenge with 7 log10 PFU (Onlamaon 2010). We here report the results of 2 challenge studies carried out in Cynomolgus macaques with aim to explore parameters associated to high viral loads. In the 1st study, 4 groups of 5 monkeys were challenged by SC route with 5.0 log10 CCID50 of 4 DENV-2 strains presenting different passages history: 2 laboratory strains (DENV-2 16681 and DENV-2 16803) and 2 low-passage isolates. Serum viremia was followed by qRT-PCR, daily from D1 to day 14, then at D28. Viral RNA was detected in all animals (except 1 in the DENV-2 16803 group) on D2 after injection for laboratory strains, and at various time points between D1 and D6 for viral isolates. Peak titers, viremia duration, and AUC were significantly lower in the DENV-2 16803 strain than in other groups. The more homogeneous curves were observed in animals challenged with DENV-2 16881. In the second study, 6 groups of 5 monkeys received 5.0 or 7.0 log10 CCID50 of this virus administrated
by SC, ID or IV route. A clear dose effect was observed whichever the route of administration, with a peak viremia increase of about 1.0 log10. Reduced viremia duration and shorter time to viremia were also associated to dose increase. The IV challenge generated the highest peak titers and the most homogenous viremia curves: peak titer 7.0 ±0.2 on day 2, duration 5.0 ±0.0 days. DENV-2 neutralizing antibody titers and some blood biochemical parameters were also analyzed and will be presented. The implications of these data on the development of new DENV challenge model to measure protective efficacy of vaccine candidates will be discussed.

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COST OF DENGUE VECTOR CONTROL: SYSTEMATIC LITERATURE REVIEW
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As the most important vector-borne viral disease, dengue is a serious and growing global public health problem, with 3.6 billion people at risk of infection. Currently, vector control is the only prevention tool to control dengue transmission. Little is known about the cost and the effectiveness of current dengue vector control measures, which rely heavily on outdoor fumigation and use of larvicides. We performed a systematic literature review, searching for studies on the cost of dengue vector control and found 14 articles and reports. Of these, 4 examined only one specific vector control intervention, such as community mobilization or source reduction, while 10 analyzed comprehensive vector control activities. We then separated these 10 studies according to dengue severity in the study year. Of these, 3 analyzed an epidemic year and 7 a non-epidemic year, consistent with dengue epidemiology. As 2 sites were duplicated, the studies cover 8 locations: Brazil, Cuba, Malaysia, Mexico, Panama, Puerto Rico, Thailand and Venezuela, representing the regions in which dengue is most heavily endemic: Latin America and the Caribbean, and South East Asia. Finally, the costs of these comprehensive programs were compared to the cost of dengue illness in the same country for the same year. Preliminary results show that, on average, comprehensive vector control cost $1.69 per capita and was 52% of the economic cost of dengue illness in these same countries ($3.28). However, this relationship varied widely among countries. For example, Cuba’s per capita cost of dengue vector control activities ($3.03) was 23 times its cost of dengue illness ($0.13). On the other hand, Venezuela’s cost per capita of vector control ($0.57) represented only 8% of the country’s cost of dengue illness ($7.38). If half the population at risk of dengue infection received comprehensive vector control at the current average cost, the global cost would be $6.1 billion annually. Innovative vector control strategies under development include genetics-based sterile insect methods, infection of mosquitoes with Wolbachia, interior residual spraying, auto-dissemination approach (spreading of insecticides by adult mosquitoes), attractive lethal ovitraps, sticky traps, and new pesticides. If any prove more effective than current measures, billions of dollars on current vector control and dengue illness could be saved or redirected.

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EXPERIMENTAL EVOLUTION OF WEST NILE VIRUS IN WILD-CAUGHT BIRDS
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Wild birds are the most important vertebrates in the West Nile virus (WNV) transmission cycle. Several studies have demonstrated that they generally impose purifying selection on the virus, and have suggested that they may select for novel WNV variants. However, the extent to which different important avian species influence WNV at the population level is poorly understood. Therefore, we evaluated whether different wild birds have distinct impacts on WNV populations. Specifically, we serially passed clone-derived WNV five times in wild birds that and experience varying levels of mortality: American crows (Corvus brachyrhynchos), house sparrows (Passer domesticus), and American robins (Turdus migratorius). After passage, we measured virus replication, pathogenesis and fitness in wild birds, chickens and mosquitoes. We also determined levels of intra-host genetic diversity using next-generation sequencing. Crows infected with crow-passed WNV developed higher viremias and experienced earlier mortality compared to birds infected with the unpassed virus. Sparrows developed an earlier peak viremia with the passed compared to unpassed virus, however the mortality and viremia differences compared to the unpassaged virus were insignificant. Passage in birds resulted in the generation of viruses with increased fitness gains in the same species and chicks compared to the unpassaged virus. Additionally, the bird passaged viruses did not lead to a trade-off of decreased competitive fitness in mosquitoes, which was previously observed in our laboratory. Studies in robins are ongoing. We obtained 20,000 to 60,000x WNV sequence coverage and found that intra-host genetic diversity increases in the early crow passages followed by positive selection of potentially adaptive variants. We did not find consensus changes to the crow passaged virus, suggesting that fitness gains may be achieved through rare mutations. Collectively, these results lend insight into the role of wild birds in selecting novel WNV genotypes.

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FLAVIVIRUS INFECTION INDUCES TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 1 (TREM-1): IMPLICATION FOR A ROLE IN INNATE IMMUNE RESPONSES
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Innate immune responses are essential for the control of flaviviruses, including WNV, which has emerged as a significant cause of viral encephalitis in humans. However, the specific mechanisms that regulate the programming of the innate immune signaling pathways remain unclear. Activation of TREM-1 signaling via the adaptator protein DAP12 is important for inflammation and activation of antigen presenting cells, however little is known about its role in viral infections. Here, we investigated the effect of flavivirus infection on the expression of TREMs and its potential role in the production of cytokines. We show that the expression of TREM-1 was markedly increased in dengue virus-infected THP-1 cells, which correlated with peak viral titers. Similarly, WNV infection significantly increased the mRNA levels of TREM-1 in MEFs, BMDMs and BMDCs. In vivo characterization of TREMs in mice demonstrated a significant up regulation in the transcripts of TREM-1, -3 and -4 in the peritoneal cavity cells and brain at day 3 and 8 after WNV infection respectively. Interestingly, serum levels of soluble-TREM-1 also increased significantly at days 2-3 after infection. Further, activation of TREM-1 using an agonist antibody increased mRNA of WNV-induced cytokines such as IFN-α, TNF-α and IL-6 in MEFs, which decreased following blocking of TREM-1. The changes to IFN-α and IL-6 secretion were validated with ELISA and Luminex, respectively. Collectively, our results for the first time document the response of TREMs to flavivirus infection and indicate a novel role of TREM-1 in modulating inflammatory response to WNV. Further studies are ongoing to define role of TREM-1 in WNV disease outcome.
COMPARISON OF THE EFFICIENCY AND COSTS OF WEST NILE VIRUS SURVEILLANCE METHODS IN CALIFORNIA

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Surveillance systems for West Nile virus (WNV) aim to determine the location and timing of viral amplification early enough to direct mosquito control and prevent transmission to the human population. To ensure an optimal surveillance approach, the sensitivity and timing of each component of the surveillance system must be measured against its efficiency and costs. We evaluated each of the most widely used surveillance methods for WNV (testing of mosquitoes and public-reported dead birds, and seromonitoring of sentinel chickens) using data from three vector control agencies in California for the years 2004 through 2012, encompassing the period following WNV’s arrival to California. The methods were compared after standardizing spatial sampling density, frequency of sampling, and costs. At equal spatio-temporal sampling, testing mosquitoes and dead birds typically detected viral activity 2-5 weeks earlier than seromonitoring of sentinel chickens. Viral activity was detected most frequently in mosquitoes during the early season (May-June) and in sentinel chickens during peak season (July-August). Testing dead birds reported by the public was found to be the most cost-effective method of the available methods in areas where corvids and other avian hosts with high disease-dependent mortality were abundant. For a given budget, testing of dead birds or mosquitoes provided the greatest early warning and return on costs during spring and early summer (1.3 and 3.5 WNV-positive samples per $1,000 spent), and serological monitoring of sentinel chickens was of most utility during summer and early fall (2.8 WNV-positive samples per $1,000) as viral activity began to wane.

EVIDENCE OF NEUTRALIZING ANTIBODIES TO WEST NILE AND SAINT LOUIS ENCEPHALITIS VIRUSES IN PERUVIAN HORSES

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Arboviruses are responsible for thousands of human and animal infections worldwide. Saint Louis encephalitis virus (SLEV), West Nile virus (WNV) and Venezuelan Equine Encephalitis virus (VEEV) are zoonotic arboviruses that infect horses and are widely spread in South America. SLEV was first reported in Brazil in 1960, and then reported in human sera in Peru in 1965. Venezuela, Colombia, Argentina, and other Caribbean-bordering countries have reported bird and equine infections. Epizootics and epidemics of VEEV has been reported in many countries throughout Central and South America, including Colombia, Venezuela, Trinidad, Ecuador, Mexico, and Peru. VEEV was isolated in 1942 for first time in Peru. In order to assess the presence of neutralizing antibodies to SLEV, WNV, and VEEV we tested 3470 horse sera samples from 25 locations in Peru. The Peruvian Animal Health Office (SENASA) collected serum samples in 2011. We screened these samples with IgG indirect ELISA at the Naval Medical Research Unit No. 6 (NAMRU-6) in Lima, Peru. Positives were confirmed by solid Plaque Reduction Neutralization Test with 80% reduction (PRNT80). Virus neutralization titers ranged from 1/20 to 1/640. We found 19 (0.5%) positive samples for WNV, mostly from the Cajamarca Region. Fourteen (0.4%) samples were positive for VEEV and 24 (0.7%) for SLEV, mostly from Piura. The samples were equally distributed within ecologically different areas in the coast, highlands, and rainforest. The presence of the neutralizing antibodies in horses against SLEV and WNV suggest prior infections and possible continuing spread of these arboviruses universally throughout Peru. Therefore, continued epidemiologic surveillance in horses is necessary in order to protect human populations against future outbreaks and subsequently confirm the continuing circulation of these viruses.

CONTEXT-DEPENDENT CLEAVAGE AT THE C-PRM JUNCTION BY THE WEST NILE VIRUS NS2B/3 PROTEASE MODULATES THE EFFICIENCY OF VIRUS ASSEMBLY AND RELEASE

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Flavivirus assembly is governed in part by cleavage of the single viral open reading frame orchestrated by host and viral proteases. The sites recognized by flavivirus proteases have been defined principally through the use of small peptide substrates. Prior studies have shown that it is possible to produce infectious viruses composed of the structural genes of one flavivirus and a sub-genomic RNA of a distantly related virus. While the production of “pseudo-typed” virions composed of the structural genes of DEN2V (strain 16681) and a WNV replicon is quite efficient (>106 infectious units/mL), the production of virus particles incorporating the C-prM-E proteins of the DEN2V NGC strain was not possible, despite sharing 97.8% amino acid sequence identity. To understand the underlying mechanism, we constructed chimeras of the structural genes of these two DEN2V strains and identified the capsid (C) protein as the source of incompatibility. Subsequent mutagenesis studies revealed that a single C substitution, T101S, restored the ability to produce infectious virions composed of NGC C-prM-E and the WNV replicon. Residue 101 is the P1 position of the NS2B/3 viral protease cleavage site, and indeed, we found virion production capability mapped to the efficiency of C-prM cleavage. The significant impact of the T101S substitution on the efficiency of cleavage by the WNV protease is surprising in light of published biochemical studies of the requirements for cleavage. We conducted more extensive mutagenesis of the C-prM junction to further define the sequence requirements for cleavage by the WNV and DENV proteases; these studies revealed a context-dependent substrate specificity of the viral protease. Definition of the substrate specificity of the viral protease against the backdrop of the viral polypeptide may facilitate the development of new protease inhibitors and provide insight into associated patterns of drug resistance.

ECOLOGY OF CULEX FLAVIVIRUS DURING WEST NILE VIRUS EPIDEMIC AND INTER-EPIDEMIC YEARS IN SUBURBAN CHICAGO, USA

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Insect-only flaviviruses have been identified in mosquito populations throughout the world. These insect host-adapted viruses co-circulate with medically important arboviruses, but their ecology and effects on their mosquito hosts in nature are poorly understood. Culex flavivirus (CaFV) is an insect-only flavivirus found in Culex species mosquitoes, important
vectors of West Nile virus (WNV) in the United States. CxFV and WNV co-circulate in and co-infect Culex mosquitoes at a WNV “hotspot” in suburban Chicago. We previously identified a positive association between CxFV and WNV in mosquito pools collected in 2006. To further investigate the ecology of CxFV and its association with WNV, we compared the spatial and temporal distribution of CxFV in 2011, an inter-epidemic year for WNV, with its distribution in 2012, an epidemic year for WNV. The overall prevalence of WNV in mosquitoes in 2011 was 0.11% (95% CI: 0.04-0.25), whereas in 2012 it was 0.62% (95% CI: 0.42-0.90). The overall prevalence of CxFV in 2011 was 10.21% (95% CI: 9.28-11.26), whereas in 2012 it was 17.02% (95% CI: 15.37-18.86). Both viruses were significantly more prevalent in mosquito pools in 2012 than in 2011 (Wilcoxon signed rank test, WNV: V=41, p<0.02; CxFV: V=164, p<0.001). CxFV was identified at all 37 trap locations that were repeated in both years. During 2012, the trap location with the highest WNV prevalence (2.83%) was also the trap location with the highest CxFV prevalence (79.96%). Among locations positive for both viruses, a positive correlation between CxFV and WNV was observed in 2011 (r= 4.31, df= 3, p < 0.05), but not in 2012 (r = 1.61, df= 14, p > 0.1). These results demonstrate an association between WNV and CxFV on a fine spatial scale in an urban setting that may be driven by similar responses of the two viruses to common environmental drivers.

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SPATIAL ANALYSIS AND EVALUATION OF 2014 PREDICTIONS FOR A WEST NILE VIRUS EARLY WARNING SYSTEM

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Since the introduction of West Nile virus (WNV) to the USA in 1999, numbers of human cases reported to the CDC have varied from fewer than a thousand cases per year to 5,674 cases in 2012. A national model conditioned on weather and other data, including bird species distributions and human population density, has explained the large variations seen over the 2005-2013 period and a prediction was made for 2014. We found the most significant predictors for a human WNV case in a county are the mean minimum temperature in January, the deviation of this minimum temperature from the expected minimum temperature, the total population of the county, the bird population, and if the county had a case of WNV the previous year. Due to aberrant weather patterns in early 2014, the pattern of human WNV was predicted to be equally aberrant, presenting a genuine test of the model and implications for public health in the face of climate change. Predictions are compared to up-to-date case reports and locally conditioned models are examined for regions where more data are available, providing a start toward regional early warning systems.

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AVIAN SPECIES DIVERSITY AND AMPLIFICATION OF WEST NILE VIRUS IN ATLANTA, GEORGIA

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The dilution effect is the reduction in vector-borne disease risk that occurs through the presence of a diverse set of potential host species, some of which are relatively or completely incompetent as hosts. West Nile virus (WNV) is a mosquito-borne disease that is maintained in various avian host species. In Atlanta, Georgia, substantial WNV presence in the vector and host species has not translated into a large number of human cases. In order to determine whether a dilution effect was contributing to the reduced WNV spillover in the area, we conducted comprehensive multi-season, multi-habitat, characterization of the avian species community as well as longitudinal WNV surveillance in avian hosts and mosquito vectors in urban Atlanta between 2010 and 2011. We measured host diversity in two ways: diversity at-large and diversity as experienced by the pathogen. Regardless of how we measured avian species diversity or whether we considered host infection and vector infection as predictor variables or outcome variables, we did not detect a dilution effect. Instead, we detected an amplification effect, in which increased host diversity resulted in increased rates of infection, the first empirical evidence for this effect in a mosquito-borne system. We suggest that the observed amplification effect may primarily be driven by an over-abundance of moderately to poorly competent host species, such as Northern Cardinals, which may cause optimal hosts to be more rare and therefore to be present primarily in more species-rich areas. Other possible mechanisms driving amplification could be increased vector species richness and innate mosquito preference for certain host species over others. We encourage further research to assess the scale and prevalence of amplification effects in the WNV system, as well as the contributions of various host and vector species to its establishment.

GENETIC DETERMINANTS OF AVIAN PATHOGENESIS OF LINEAGE 2 WEST NILE VIRUS STRAINS

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In 2010, a human outbreak of a West Nile virus (WNV) lineage 2 (L2) in Greece established, for the first time, an association between L2 WNVs and extensive human disease in Europe. Sequence analysis of a L2 WNV virus (NS10) isolated from a pool of infected mosquitoes in Nea Santa, Greece during the 2010 WNV outbreak revealed an NS3-H249P mutation not previously observed in any sequenced L2 WNV strains. Interestingly, an NS3-T249P mutation has previously been demonstrated to increase viremia and virulence of a lineage 1 WNV strain in American crows (AMCRs). In order to assess the virulence potential of NS3-249P substitution in L2 WNVs, AMCRs, thought to be a key reservoir host for WNV in North America, were inoculated with the parental L2 WNV viruses, NS10 and South Africa 1989 (SA89), in addition to mutants generated containing polymorphisms at the NS3-249 site (Pro, His and Thr). The parental NS10 and SA89 strains displayed 100% and 33% mortality with average peak viremias of 9.5 and 7.5 log10 PFU/mL in AMCRs, respectively. The NS10 mutants, NS10 NS3-249H and NS10 NS3-249T, exhibited 80% mortality with peak viremias of 8.7 log10 PFU/mL sera and 20% mortality with peak viremia of 6.0 log10 PFU/mL sera, respectively. The SA89 mutant, SA89 NS3-249H, elicited 100% mortality with a peak viremia of 9.6 log10 PFU/ML and the SA89 NS3-249T resulted in 0% mortality with a peak viremia of 2.6 log10 PFU/mL sera. Viremia and mortality differences in AMCRs between the L2 WNV backbones harboring the same polymorphisms at the NS3-249 site suggest that epistatic interactions of alternative genetic elements are involved in generating the variable phenotypes. The sequence of the SA89 strain was compared with that of NS10 and a total of 16 amino acid differences were identified exclusive to the nonstructural genes. In order to understand the factors related to the emergence of a human disease associated L2 WNV in Europe, and the potential role of alternative genetic factors in epistatic maintenance of the virulence associated with the NS3-249 site, chimeras between NS10 and SA89 were generated and tested in AMCRs and House Sparrows.
**Meteorological Conditions Associated with Increased Incidence of West Nile Virus Disease in the United States, 2004-2012**

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West Nile virus (WNV) is the leading cause of mosquito-borne disease in the United States. Annual seasonal outbreaks vary in size and location. Predicting where and when outbreaks will occur can help direct public health control efforts. Weather can impact several factors associated with WNV transmission, such as mosquito vector and zoonotic host abundance. We developed models for the continental United States to identify meteorological conditions associated with higher incidence of WNV neuroinvasive disease (WNND) from 2004-2012. We used county-level WNV surveillance data reported to CDC and meteorological data from the North American Land Data Assimilation System. Due to geographic differences in WNV transmission, we divided the United States into east and west (defined by ~100 degrees West longitude) and 10 US Environmental Protection Agency regions. Meteorological conditions were evaluated from October of the prior year through September of the given WNV season. For each US county, we calculated standardized z-scores that described annual WNND incidence, average temperature, and total precipitation compared to the mean values for 2004-2012. For WNND incidence, a z-score ≥0.5 was defined as above average. We used fixed effects models to assess independent associations between anomalies in temperature or precipitation and above average WNND incidence within each geographic area. Preliminary results showed warmer than average annual temperature was associated with above average WNND incidence nationally and in all geographic areas. Lower than average total precipitation was associated with higher disease incidence nationally but the effect varied significantly by region. These findings suggest anomalies in temperature and precipitation are associated with above average WNND disease incidence but the overall effects vary by region. Although multiple factors influence WNV transmission, readily accessible meteorological data may be used to develop predictive models to forecast geographic areas with elevated WNV disease risk prior to the coming season.

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**Prevalence and Risk Factors of Human Coronavirus**

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Acute respiratory tract infections (ARI) are the leading cause of morbidity and mortality in developing countries, especially in Africa. However, information on the viral aetiological agents are scanty. We therefore conducted a cross-sectional serological study to determine the prevalence of Human Coronavirus among individuals living in rural areas of Ghana from September 2010 to October 2013. The study areas are Kumawang in the Ashanti Region, Buoyem in the Brong Ahafo Region and Foniorkrom also in the Brong Ahafo Region. A total of 201 subjects were enrolled in the study. Subjects were tested for IgG antibodies to three HCoVs namely; HCoV-NL63, HCoV-OC43 and HCoV-229E. Of the 201 subjects, 97 (48.3%) were positive for all viruses. The most prevalent virus was HCoV-229E (23%), 95% CI: 17.2 - 29.3, followed by HCoV-OC43 (17%, 95% CI: 12.4 - 23.4), then HCoV-NL63 (8%, 95% CI: 4.6 - 12.6). Of all positive HCoV-NL63 subjects, those in Kumawang had the highest sero-prevalence (68.8%). In contrast, HCoV-229E (41.3%) and HCoV-OC43 (45.7%) were much higher in Foniorkrom compared to the other study areas. There was however no statistical difference between living in any of the study areas and being positive for HCoVs. The gender distribution for all three viruses was also similar. The median ages of those positive for HCoV-OC43 (47 years, IQR = 33 - 52.5) and HCoV-229E (40 year, IQR = 27 - 54) were higher than negative subjects. The age difference for HCoV-NL63 subjects were similar (p = 0.994). A comparison of the blood group types between subjects positive for HCoVs and those negative showed no significant statistical difference (p = 0.163). This study demonstrates the occurrence of three types of HCoVs in remote areas of Ghanaian rural populations.

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**The Laboratory as a Tool in the Endgame Poliovirus Eradication Program in Ghana**

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Polioviruses and other enteroviruses cause acute flaccid paralysis, and this is of public health concern. The World Health Organization (WHO) sees the laboratory as being an important tool in the virological investigation of acute flaccid paralysis cases, therefore, the laboratory having a crucial role to play in ensuring that no viruses, especially, wild polioviruses are missed. Until 1999, the Laboratory isolated indigenous wild poliovirus outbreak infections, which led to quick interventions with oral polo vaccines. The country enjoyed close to five years of wild type poliovirus elimination until the laboratory in 2003 and then in 2008 isolated a wild type 1 imported poliovirus in the country. Since the 2008 outbreak, Ghana has been free from wild type poliovirus infections. This paper sought to look at the crucial role the laboratory continues to play in ensuring that no wild poliovirus is missed; even after Ghana, being free of wild poliovirus since 2009. A detailed analyses of data on all AFP stool specimens investigated for a period of 10 years (2004 to 2013), using Epi Info data software; as we look at the performance and progress of the laboratory in polio eradication in Ghana. A total of 4,555 AFP specimens from all ten regions of Ghana were analyzed. Ninety percent(90%) of the stool specimens were from children below the age of 15 years. Males constitute just above half (57.2%) of the total specimens. Over 80% of the specimens were received in good condition. The annual non polio enterovirus isolation rate was above 10%, which was within WHO recommended non polio enterovirus isolation rate of 10% for the laboratory. Two hundred and twenty three (223 - 4.9%) of the specimens were positive for poliovirus; and 8(3.6%) of the 223 were wild-type 1 imported polioviruses. Timeliness of reporting within 14days from date of specimen receipt, annually rated not less than 98%. The laboratory over the period have consistently passed annual proficiency test for virus isolation and also for intratypic differentiation (ITD) of polioviruses. The Laboratory's ability and skills in the delivery of accurate results in a timely manner made it possible for the timely intervention when Ghana recorded the two poliovirus outbreak in 2003 and 2008. Indeed the laboratory's continuous involvement in Polio eradication through virological investigations and timely dissemination of results has brought Ghana into the reality zone of wild poliovirus eradication.

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**Deep Sequencing as a Tool to Identify Pathogens from Pool Reservoir Samples from South/Southeast Asia**

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Emerging and re-emerging respiratory pathogens represent an increasing threat to public health. Asia continues to be the regional epicenter for the emergence of novel pathogens and the site where several pandemics have originated. Etiological determinations during outbreaks have generally relied on clinical information, occasionally accompanied by traditional molecular or serological techniques. Often, the information is inconclusive.
In 2013, the Armed Forces Research Institute of Medical Sciences (AFRIMS) identified one hundred and sixteen nasal-pharyngeal specimens collected from acute influenza-like illness (ILI) patients in several countries in South/ South East Asia which were negative by conventional molecular and culture techniques but demonstrated cytopathic effect (CPE) in cell culture. Groups of 8 to 15 CPE-positive samples were analyzed by the geographic region from where they were initially collected. Deep sequencing was performed on each pool to generate sufficient sequence reads to allow for initial pathogen identification. A total 7.9 Gbases or 22.28 million sequence reads passed quality control with ≥30Q scores. After filtering out host and microbiome background, low abundance sequence reads were analyzed. We were able to identify various respiratory pathogens, which tended to localize in specific regions: (i) parainfluenza 3 in the Bhutan/ Nepal pool; (ii) human metapneumovirus and human coxsackievirus A21 strain in the Cambodian pool; (iii) influenza A in the Philippines pool, (iv) human coxsackievirus A21 strain in the Bangkok, Thailand pool, and (v) parainfluenza 4a in the northern Thailand pool. The pools and individual samples with high viral content were confirmed by singleplex PCR, realtime PCR or conventional PCR. Overall, deep sequencing performed efficiently as an initial identification tool for viral pathogens using pooled respiratory samples of unknown etiology but capable of inducing CPE in cell culture.

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ABOLISHMENT OF INDIVIDUAL N-GLYCOSYLATION SITES WITHIN RIFT VALLEY FEVER VIRUS GN/GC ALTERS INFECTIVITY VIA DC-SIGN

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Rift Valley fever is a mosquito-transmitted zoonosis that is characterized by high rates of abortion and fetal malformations in ruminants and causes severe disease in humans. Recently, DC-SIGN (a C-type lectin receptor) has been demonstrated to be a receptor for phleboviruses, including Rift Valley fever virus (RVFV: genus Phlebovirus; family Phleboviridae). We hypothesized that N-glycosylation of Gn/Gc plays an important role in the infection of RVFV via DC-SIGN. RVFV glycoproteins (Gn/Gc) encode 5 putative N-glycosylation sites: aa. 438 (Gn), 794 (Gc), 829 (Gc), 1035 (Gc), and 1077 (Gc), but their significance in viral infection via DC-SIGN has not been elucidated. Using a reverse genetics system, we generated recombinant MP-12, which lacks one of the five potential N-glycosylation sites of Gn/Gc: N438Q (Gn); N794Q (Gc); N829Q (Gc); N1035Q (Gc); and N1077Q (Gc). To identify which sites are utilized for N-glycosylation, we immunoprecipitated [35S] methionine/cysteine-labeled MP-12 or the mutants in transfected cells. The Gn of N438Q, and Gc N794Q, N829Q, N1035Q, and N1077Q migrated faster than those of the parental MP-12, indicating aa.438, 794, 829, 1035, and 1077 are N-glycosylated. To test the infectivity of each mutant, we measured viral RNA copy number using digital droplet PCR with a Taqman probe specific to the MP-12 L-segment. The ratio of FFI in VeroE6 cells per RNA copy was analyzed. Then, Jurkat cells and those that express DC-SIGN were infected with the mutant viruses at the same RNA copy number, and we analyzed the number of infected cells at 6 hpi by flow cytometry (FACS). We are currently repeating the experiments to conclude the statistical differences of infectivity among the N-glycan mutants. Our findings will be useful for understanding of the pathology of RVFV and the rational design of live-attenuated vaccine candidates.

MOLECULAR ANALYSIS OF INFLUENZA B VIRUSES ISOLATED IN KENYA, 2012

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Introduction: Influenza B viruses belong to two evolutionary lineages (B/Victoria/287-like and B/Yamagata/16/88-like) that continue to co-circulate globally in the human population since 1980s. These viruses do not undergo antigenic shifts but drifts as a result of accumulation of amino acid substitutions especially in the HA1 polypeptide have been confirmed. The evolution of surface glycoproteins occurs over time due to selection pressure exerted by host's immunity. Objective: To investigate molecular evolution of influenza B viruses isolated in Kenya by sequence analysis of HA1 hemagglutinin. Methods: Nasopharyngeal specimens obtained from patients meeting WHO definition criterion for ILI were screened by real-time PCR for influenza A and B viruses. Influenza B virus positive samples were inoculated onto Mardin-Darby Canine Kidney cells and HA protein coding gene of selected isolates sequenced and analyzed. Results: Phylogenetic analysis showed all influenza B viruses isolated in Kenya clustered together with B/Brisbane/60/2008 vaccine strain and other viruses of B/Victoria/2/87-like lineage in other regions of the world. All the Kenyan isolates were characterized with D197N amino acid substitution not present in the vaccine strain. This change occurred within 190-Helix antigenic binding site. Majority of Kenyan isolates (except B/Kenya/242/2012, which had H146A) further had H146V amino acid change within the 150-Loop antigenic site, absent in the vaccine strain. Other mutations occurred stochastically in individual isolates. Most notable was isolate, B/Kenya/239/2012 which had an additional V124I amino acid change within the 120-Loop antigenic binding site. Conclusion: There was limited variation among the Kenyan isolates. Kenyan viruses matched closely with the seasons vaccine strain, B/Brisbane/60/2008 despite having mutations at antigenically significant positions in the HA1 subunit. Molecular analysis of influenza B viruses is important for early detection of strains with epidemic potential.

THE MOLECULAR EPIDEMIOLOGY OF NOROVIRUS IN MILITARY RECRUITS IN IQUITOS, PERU: EVIDENCE OF A NOVEL GENOTYPE

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Norovirus (NoV) is a leading cause of diarrhea in Peru, yet its molecular epidemiology in this region is largely unknown. We therefore explored the genetic diversity of NoV in Peruvian military recruits in Iquitos, a Peruvian city in the Amazon basin. Stools from a random subsample of recruits from a diarrhea surveillance study in Iquitos between 2004 and 2011 were screened for NoV and genogrouped by AgPath-ID One-RT-PCR. The NoV VP1 gene was partially sequenced (330/344 bp, region C) and genotyped using the Noronet webtool. Phylogenetic trees of NoV sequences compared with BLAST and Noronet reference taxa were inferred using the maximum likelihood (ML) method with bootstrapting by RAxML software. 360/4234 (8.5%) participants were tested for NoV, 11.1% (40/360) were positive, including 12 for genogroup GI, 29 for genogroup GII (GII) and one GI/GII dual infection. Genotypic and further analysis was able to be performed in 49% (20/41) of sequences. ML trees demonstrated a wide range of Peruvian GI genotypes with the majority belonging to a GI.4 clade. Spatial clustering of GI Peruvian and Brazilian taxa was noted.
although weakly supported. Genotype diversity of GII NoV in Iquitos was broad with many in GII.4 DenHaag clades. One GII Iquitos sequence was untypable and appears to represent a novel genotype, with a GII.3 Tunisian sequence as the closest typable relative (86% nucleotide similarity) and strong support for clustering with near identical and also untypable Nicaraguan strains. Within most genotype clades there was temporal clustering of Peruvian and reference sequences, consistent with Peru being affected by globally circulating lineages. In conclusion, Noroviruses in the Peruvian Amazon are genetically diverse with evidence of a novel genotype, mixing with global lineages and weak regional spatial structure. Larger studies are needed to clarify the regional phylogeography of noroviruses and confirm and characterise this and perhaps other novel NoV genotypes in Peru.

TIME TRENDS AND CORRELATES OF ROUTINE MEASLES IMMUNIZATION COVERAGE IN ABIA STATE, SOUTHEAST NIGERIA; 2007-2012

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Measles is a vaccine-preventable viral disease associated with high morbidity and mortality. The national target in Nigeria for routine measles immunization coverage is ≥80%. We conducted this study to assess the variations in annual trends of routine measles immunization coverage rates in Abia State and to identify the factors affecting coverage. A time series analysis was performed on administrative measles immunization data collected by Abia State Primary Health Care Development Agency from 2007-2012. Trends in measles immunization coverage, measles vaccine wastage, measles vaccine supply rates, measles vaccine weekly stock and immunization outreach sessions (vaccination points outside health facilities) were assessed in all 17 Local Government Areas (LGAs) and disaggregated by LGA type (urban or rural LGA). The relationship between measles vaccine wastage, supply rates, weekly stock, immunization outreach sessions, LGA type and an outcome of ≥80% measles immunization coverage was assessed and modeled using step-up logistic regression. The annual birth cohort (study population) was 121,107 in 2007, declined to 110,636 in 2009 and rose to 123,034 in 2012. Both measles immunization coverage and immunization outreach sessions increased at a linear rate (p < 0.001) while measles vaccine wastage rate declined linearly (p < 0.001). Measles vaccine weekly stock declined at a rate of 3 days stock per month till 2009, increasing afterwards (p < 0.001). 28% of the LGAs attained ≥80% immunization coverage by 2012; achieved by 46% of urban LGAs. Immunization outreach sessions increased from 1762 in 2007 to 8595 in 2012; 48% were ≥25sessions/month. 58% of LGAs got ≥80% of their measles vaccine. 38% of LGAs had a vaccine wastage rate of <30%. Having <30% measles vaccine wastage (OR=2.2), ≥80% measles vaccine supply (OR=9.8), ≥25 immunization outreach sessions (p=0.001) and being an urban LGA (OR=2.9) was associated with the outcome. The effect of measles vaccine wastage was modified by LGA type (p=0.009). These variables were significant positive predictors for ≥80% measles coverage, following modeling. Routine measles immunization coverage improved over the study period. Only a third of LGAs met the required national target; mostly urban LGAs. Public health resources should be directed at reducing vaccine wastage at service delivery, improving vaccine supply chains and increasing access to immunization, especially in rural LGAs.

EPIDEMIOLOGICAL CHARACTERISTICS OF ACUTE FLACCID PARALYSIS CASES IN LAGOS STATE NIGERIA, 2001 - 2011

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Generally, Nigeria is one of three countries still endemic for indigenous transmission of polio virus. Acute Flaccid Paralysis (AFP) surveillance is one of four strategies recommended for polio eradication. Data from AFP surveillance guides implementation of immunization activities aimed at interrupting polio transmission. In November 2013, we conducted a study to characterize AFP cases in Lagos State, southwest Nigeria. We analyzed secondary data of AFP cases in Lagos State from 2001 to 2011. AFP was defined as recent onset of floppy weakness or paralysis in a child less than 15 years or any paralytic illness in any person in whom a clinician suspects polio. We reviewed the AFP data and performed univariate and bivariate analyses using Epi-info 3.5.4 software. Altogether, 2,896 AFP cases were reported; 1683 (58.1%) were males. Mean age was 2.9 years (+/-2.7). The most affected age group was 0-5 years (86.1%). Of all AFP cases reported, 25 (0.9%) did not receive any OPV (Oral Polio Vaccine); 301 (11%) received 1-2 doses. Over 80% of cases had received 3 or more OPV doses. Adequate stool specimen was collected for analysis in 2815 (97.4%) of the AFP cases. Only 30 (1%) were positive for wild polio virus (WPV). WPV type 1 (WPV1) were 23 of which 1 had no OPV and WPV3 were 7 (all received OPV). Last confirmed human WPV was in year 2009. Polio virus transmission has been interrupted in Lagos state. The high immunization status would have contributed to population immunity and reduced transmission. Government should continue to strengthen and scale-up routine and supplemental immunizations with OPV.

EPSTEIN-BARR VIRUS IN PATIENTS WITH ACUTE FEBRILE ILLNESS IN KENYA

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Epstein Barr Virus (EBV) causes infectious mononucleosis and other lymphoproliferative disorders, including endemic Burkitt’s lymphoma (eBL). EBV epidemiology is best known from serological studies. This study determined the EBV viremia and prevalence in patients with acute febrile illness (AFI) in Kenya. Patients with AFI were enrolled at 8 outpatient hospitals between September 2008 and April 2013. DNA was extracted from whole blood and BALF gene used as target for real-time PCR. Prevalence rates and viremia were determined and correlated by age, region and co-infection with malaria. Of the 2021 patients examined, EBV was detected in 588 (29%) and their viremia ranged from 52 to 7.2 x106 copies/mL (geometric mean 4345 copies/mL). Patient mean age was 5 years (range 1-80 years). Viral prevalence was highest in patients 2000 copies/mL, a cutoff considered clinically important. The <5 year olds constituted the majority (41%) in this group. Regions holoendemic for malaria had the highest prevalence compared to the hypoendemic regions. In addition, patients with EBV/malaria co-infections had higher viremia (geometric mean 5929 copies/mL) compared to those with EBV alone (3793 copies/mL, p = 0.003). The study demonstrates how common EBV is among patients with AFI. That malaria is an important determinant of EBV viremia, reinforcing the possibility that increased viremia in EBV/malaria co-infections could be a precursor to the development of eBL.
ADVERSE EVENTS FOLLOWING IMMUNIZATION WITH NEWLY INTRODUCED MEASLES-RUBELLA VACCINE IN JIRAPA DISTRICT, GHANA

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Background: Vaccines are one of the most cost effective public health interventions. Real or perceived adverse events following immunization (AEFI) can undermine the credibility of a vaccine and an immunization programme. Ghana rolled out a measles-rubella combined vaccine in a mass immunization campaign in September, 2013. We assessed the AEFI associated with the vaccine in Jirapa District to obtain base-line data and appropriately respond to public concerns on safety issues. Method: A risk interval cohort study was conducted. Three hundred and fifty children aged 9 months -14 years followed for twelve weeks. Seventy children in four age groups were selected from each of five communities in Jirapa District using modified EPI coverage guideline. Participants were observed for four weeks before vaccination then eight weeks after vaccination for adverse events in the pre and post vaccination control windows and risk window. An AEFI was defined as any medical incident, which occurred after vaccination with measles rubella vaccine. An AEFI was said to be serious if it was life-threatening and required intervention and/or hospitalization or resulted in disability/incapacity or death. Univariate and bivariate analysis were done using Epi info 3.5. P values less than 0.05 were considered statistically significant. Results: Three hundred and fifty (350) vaccinees, 51.6%(180/350) females and 48.4%(170/350) males were followed for twelve weeks. Overall incidence of adverse events following immunization was 5.1% (95% CI: 3.2-8.2%). Of these fever accounted for 66.7% (12/18), febrile convulsion 5.6% (1/18), headache 16.7% (3/18), skin rash 5.6 (1/18), and pain at injection site 5.6 (1/18). Only two (11.1%) of the adverse events were serious. Three (16.7%) of the adverse events occurred within 24 hours after vaccination while 11 (61.1%) occurred between the first and seventh day after vaccination. Children aged 9months- 3 years were 6.6 times more likely to develop fever than children aged 10-14 years (RR=6.6, 95% CI: 0.83-52.62; P <0.04). Conclusion: The adverse events following immunization with Measles-Rubella Vaccine were few and generally mild. Continued surveillance for adverse events and investigation of serious ones are recommended.

MOLECULAR SIGN OF INFLUENZA A AND B VIRUSES IN CUBA DURING TWO CONSECUTIVE YEARS

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The work contributes to a better understanding of influenza virus circulation in Cuba, sub-tropical country located at the Caribbean area. The objective of this work was to determine circulation and molecular characterization of influenza viruses in Cuba. From January/2012- December/2013, Cuban National Influenza Centre received 7783 clinical samples of individuals presenting influenza-like illness symptoms, severe acute respiratory infection or fatal cases. Samples were tested for seasonal A(H3N2), A(H1N1)pdm09 and B influenza viruses by real-time reverse transcription-polymerase chain reaction. Nucleotide sequences from hemagglutinin HA1 region segment were obtained directly from positive cases clinical samples. Genetic distances were calculated using MEGA v.5.05. Phylogenetic tree was constructed using Mr.Bayes v.3.1.2 software. Potential N-Glycosylation sites were predicted using NetNGlyc server 1.0. Of the 1335 samples positives to influenza virus, 48% were positive to influenza A(H1N1)pdm09, 31% to influenza B and 21% to influenza A(H3N2). Year 2012 was marked by low circulation of H3N2 subtype, with only 23 detections. Sequences obtained directly from clinical samples, belong to the clade 6. In year 2013 circulation of H3N2 subtype was highest, all of them grouped into the clade 3C (3C.2 and 3C.3), related to the vaccine strain signed by lineage A/Texas/50/2012. Circulation of influenza A(H1N1)pdm09 was highest in 2013 (26.2%) respect 2012 year (8,3%). Studied sequences distributes into three distinct clades: sequences from 2012 year belong into the clade 7, sequences from January 2013 belong into the clade 7 mainly and one of them into the clade 6C together with sequences from May 2013, last sequences from November 2013 belong into the clade 6B. Influenza B viruses were detected during the two studied years, characterized by the circulation of lineage B/Victoria in 2012 (20.5% of influenza detections) and lineage B/Yamagata in 2013 (7.4% of influenza detections). Strains of B/Victoria lineage grouped with the vaccine strain B/Brisbane/60/2008, while strains of B/Yamagata lineage belong into the clade 2 represented by the vaccine strain B/ Massachusetts/02/2012. It remains to be defined if these viral variants represent an important antigenic drift that would enable viral evasion and/or affect influenza vaccine effectiveness.

EXPRESSSION OF INNATE IMMUNE GENES IN HUMAN CELLS INFECTED BY BUNYAVIRUS

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The virulence of the pathogenic Bunyaviruses is directly linked to the roles of viral virulence factors and their capacity to counteract the host pathways. These viruses use cellular proteins to promote their own replication/transcription and, in response, host induces transcriptional reprogramming to activate antiviral effects. In order to verify the early steps of Apeu virus (APEV) and Tahyna virus (THAV) induced innate immune system activation, we performed TadMan-based qPCR assays using cDNA obtained from mRNA extracted from A549 cells after 4hs or 8hs of infection with APEV, THAV and VSV virus (ssRNA control virus), besides a mock control. We verified that APEV is recognized by TLR9, differently of THAV, which follows VSV due to TLR7 recognition. However, APEV follows VSV decreasing TICAM1 expression after 4hs of infection. It is also possible to note an early induction of TLR pathway by VSV when compared to APEV and THAV. VSV and THAV, but no APEV, increased expression of IRF5, notably after 8hs of infection. All the viruses were able to increase the expression of TLR3, IRF3 and T. TRAF3 was slightly more expressed (4hs and 8hs) in cells infected by APEV, but not by VSV and TAHV. The TICAM and IRF3 expression levels were normalized after 8hs of infection. We also observed and 8-fold increase of IRF5 expression after 8hs of incubation with VSV. Also, VSV induced IFNβ1 expression just after 4hs of infection, meanwhile TAHV induced IFNβ1 increased levels only after 6hs of infection. At this time, IFNβ1 expression levels in VSV-infected cells started to diminish, but remained higher than the other viruses. Finally, APEV, even after 8hs of infection, was unable to induce a significant increase of IFNβ1 expression. We concluded that these viruses are able to triggers different recognition and intracellular signaling pathways leading to differences in the immune responses and, consequently, determining the pathogenic potential of each tested viruses.
CYCLICAL OUTBREAKS OF Rift Valley Fever IN EAST AFRICA: WHY THEY PERSIST AND POSSIBLE SOLUTIONS TO PREVENT OR CONTAIN ITS SPREAD

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Rift Valley fever (RVF) caused by Rift Valley Fever virus (RVFV) is a zoonotic viral disease primarily of domesticated animals that also can cause disease in humans. RVFV belongs to the genus Phlebovirus, family Bunyaviridae and was first isolated during epizootics in Rift Valley Province, Kenya in 1930. RVF outbreaks have occurred in periodic cycles of 4-15 years in East Africa. During 2006-2007, an RVF outbreak that started in Kenya involving 684 cases (case fatality rate (CFR) = 49%), spread to Somalia (114 cases; CFR = 45%) and extended to Tanzania (309 cases; CFR = 46%). In 2008, the outbreak recurred in Sudan (698 cases; CFR = 31.8%) and Mozambique (412 cases, 17 deaths). The outbreaks have been associated with flooding from unusually high precipitations in many flood-prone habitats and with significant increases in vegetation cover. Flooding of the dambos (shallow depressions) usually induces the hatching of transovarially infected Aedes mosquito eggs that are dormant in the soil. The hatched eggs produce infected adult females which are capable of transmitting RVF virus to high population of domestic animals and/or human (amplification hosts). Culex mosquitoes subsequently colonize these flooded dambos, feed on the amplifying hosts and produce large populations of infectious mosquitoes which efficiently transmit the virus to non-infected domestic animals and immunologically naïve humans within the environment. Due to these unique ecological and geographical factors involved in RVF transmission cycles, RVF outbreaks may be feasibly predicted and prevented. We review the epidemiological factors associated with outbreaks and their predictability, methods of estimating infection rate based on confirmed or suspected cases, and the importance of entomological/sero-surveys. Ultimately, we conclude that RVF outbreak prevention and outbreak impact mitigation requires timely implementation of appropriately phased activities during inter-epidemic, prediction and outbreak periods.

DEVELOPMENT OF A TISSUE CULTURE INFECTIOUS DOSE (TCID50) ASSAY AS METHOD FOR QUANTIFYING INFECTIOUS UNITS IN NON-CYTOPATHIC EFFECT CAUSING VIRUSES

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For cytopathic viruses, the gold standard for quantitation of infectious viral particles is the plaque assay; however this method can yield inconsistent results due to variability in plaque quality and the subjective nature of plaque counting. For non-cytopathic viruses, plaque assays are not an option. We report the development of a novel tissue culture infectious dose at 50% assay (TCID50) which is simple to perform, can be utilized for both cytopathogenic and non-cytopathogenic viruses, and does not have the shortcomings of the plaque assay. The TCID50 assay is performed by adding virus dilutions to cells seeded in a 96 well plate. After viral replication in cells, a solution of Presto BlueTM dye (Life Technologies, Grand Island, NY) is added and incubated for 30 min at room temperature. The dye is enzymatically reduced in living cells proportional to the concentration of NADH and NADPH present, causing a color change proportional to cellular changes in metabolism. The results can be either visually read or spectrophotometrically measured. By monitoring of the colorimetric change and comparing this to the non-infected cells, infectious viral concentrations can be determined. Multiple cytopathic and non-cytopathic strains of Crimean Congo Hemorrhagic Fever (CCHF) virus were used to develop this technique. TCID50 assays were tested concurrently with plaque assays and ELISA antigen detection assays that correlated the change in color with the presence of antigen. The assay was also evaluated using E bola virus, Marburg virus, Middle Eastern Respiratory Syndrome Coronavirus, and Lassa virus. The TCID50 assay developed is a useful tool for the quantitation of both cytopathic and non-cytopathic infectious viruses.

ARBOVIRUS SURVEILLANCE IN BATS IN UGANDA

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Arboviruses including Rift Valley fever (RVFV), Yellow fever (YFV), West Nile (WNV), Chikungunya (CHIKV) and Zika (ZIKV) viruses have been isolated or detected serologically from various East African bats, however the role of bats in arbovirus transmission cycles is poorly understood. The aim of this study was to investigate the exposure history of Uganda bats to arboviruses as well as attempt virus isolation from bat tissues. Blood, tissues, or both were obtained from 1067 bats from Uganda between 2009 and 2013. Liver/spleen samples were mechanically homogenized in tissue culture media and virus isolation was performed on Vero cells. Virus isolates were identified by either RT-PCR using virus group-specific primers, or next generation sequencing. Serum samples were tested for specific neutralizing antibodies against WNV, YFV, Dengue 2 (DENV2), CHIKV, O’nyong-nyong virus (ONNV), Babanki virus (BABV), ZIKV and RVFV by plaque reduction neutralization test. Rousettus aegyptiacus from Maramagambo forest in western Uganda had specific neutralizing antibodies against CHIKV (3/303), ONNV (32/303), YFV (3/303) and WNV (1/303). R. aegyptiacus from Mt. Elgon in eastern Uganda also contained neutralizing antibodies against YFV (1/445). Epomorphus labiatus from the Entebbe/Kampala area demonstrated specific neutralizing antibodies against BABV (3/52), DENV2 (1/52), and WNV (2/52). DENV2 antibodies were also present in Chaerephon pumila (3/123) and Mops condylura (1/36) captured around Entebbe, and Nycteris spp. (2/10) from Mt. Elgon. One C. pumila also had a high neutralizing titer against ONNV. Virus isolates to date include Entebbe bat virus (Flaviviridae: Flavivirus) from C. pumila in Entebbe. Testing is still in progress and complete results will be presented. Serological and virological evidence suggest that multiple species of fruit and insectivorous bats from Uganda are exposed to and are potential amplification hosts for arboviruses.

OPTIMIZATION OF A RIBOSOME PROFILING-BASED PIPELINE TO MEASURE GLOBAL CHANGES IN GENE EXPRESSION IN RESPONSE TO VIRAL INFECTION

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Ribosome profiling is a new, powerful technique that enables direct measurement of protein expression at the whole cell level. The technique is based on the deep sequencing of ribosome footprints generated during nuclelease digestion of extracted polysomes. During digestion, individual ribosomes protect discrete 30bp fragments (referred to as footprints) that reflect the various positions of ribosomes along any actively translated...
mRNAs. Aggregation of footprint sequence data generates all the information needed to build a comprehensive understanding of how global gene expression may delineate particular phenotypes. Ribosome profiling was first developed by Ingolia et al. to run on the Illumina platform. We have adapted the original ribosome profiling strategy to run on the Ion Proton platform, and have optimized it to monitor virus-induced changes in gene-expression profiles. The main modification to the workflow includes a complete re-design of primers and adapters to include the A and P1 sequences required for Ion Proton workflows. Final library lengths were about 151pb (as opposed to 175pb for Illumina libraries) and 13pm of each library were amplified by emulsion PCR. Once amplified, libraries were sequenced using a PIv2 chip. P1 chips contain 165M (million) wells. Assuming a 60-70% bead deposition it is expected that runs will generate 99-115M reads. We have achieved deposition rates of up to 93%. We routinely obtain runs of around 145M reads, which after initial quality control processing (elimination of clonal beads, etc) result in runs of about 105M usable reads. These then enter bioinformatics pipelines that make use of Botvieve, Cutapad, Tophat and Bioconductor in order to specifically filter process the data and perform differential analyses of expression. To date, we have successfully adapted ribosome profiling for the study of dengue-induced changes in gene expression profiles. Further studies of a variety of infectious diseases caused by various pathogens, including bacteria and parasites, will also be possible provided minor modifications to the already established ribosome-profiling pipeline.

**DISTRIBUTION OF INFLUENZA ANTI-VIRAL RESISTANCE IN SOUTHEAST ASIA IN 2012-13**

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The Department of Virology at ARFIMS conducts influenza surveillance in SE Asia. Over 400 acute respiratory specimens collected from Thailand, Bhutan, Nepal, Cambodia and the Philippines in 2012 and 2013 and found positive for influenza virus by RT-PCR were selected for genotypic anti-viral analyses by pyrosequencing. Of these, close to 200 were further analyzed using a functional cell-free neuraminidase inhibition assay to determine their oseltamivir inhibitory Concentration (IC50). Pyrosequencing analyses found a wide-spread prevalence of genome markers associated with resistance to M2-inhibitor adamantane derivatives in pdmH1N1 and H3N2 specimens. Resistance to M2 inhibitors, associated with the S31N mutation, was present in 100% of 93 pdmH1N1 and 153 H3N2 specimens tested. However, pyrosequencing showed widespread susceptibility to neuraminidase inhibitors (NAI) among influenza A, with 98.9% of all tested pdmH1N1 lacking the NAI resistance-associated H275Y marker. Only one pdmH1N1 specimen was found to carry the H275Y marker. Of the H3N2 specimens tested, 78% were found to be sensitive to NAIs. One H3N2 specimen had the E119V NAi resistant marker. Thirty-three H3N2 specimens had indeterminate results since they showed mixed populations at the D151N mutation. Two of these H3N2 specimens also showed the E119V mutation with varying distribution. There were no NAI-associated R292K or N294S mutations among the tested H3N2 specimens. NAI-associated resistance markers were more common among the 168 influenza B specimens tested, with nearly 20% displaying the E117A and R374K resistance-associated mutation at varying degrees. Phenotypic testing of NAI resistance to oseltamivir carboxylate showed widespread susceptibility to oseltamivir, most of which correlated with lack of genotypic NAI resistance-associated markers. However, several influenza A and B specimens showed reduced susceptibility to oseltamivir (several-fold higher IC50 than negative controls), some of which did so despite lacking NAI resistance-associated genotypic markers.

**COMPARISON OF MICROSCOPY, NESTED AND REAL-TIME PCR-BASED ASSAYS WITH HIGH-THROUGHPUT POOLED SAMPLES FOR SCREENING ASYMPTOMATIC MALARIA CARRIERS FROM ENDEMIC AREAS OF MYANMAR**

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Asymptomatic infection is an important obstacle for controlling disease in malaria-endemic countries. Because asymptomatic carriers do not seek treatment for their infection, asymptomatic carriers can have high levels of gametocytes and constitute a reservoir available for new infection. Herein, we employed a sample pooling/PCR-based molecular detection strategy for screening malaria infection in residents from endemic areas of Myanmar. Blood samples (N = 1,552) were collected from residents in three malaria-endemic areas (Kayin State, Bago and Tanintharyi regions) of Myanmar. Two nested PCR and real-time PCR assays showed that asymptomatic infection was detected in about 1.0%-9.4% of residents from surveyed areas. The sensitivities of the two nested PCR and real-time PCR techniques were higher than that of microscopy examination (100% vs. 26.4% sensitivity; kappa value = 0.2-0.5). Among the three regions, parasite-positive samples were highly detected in subjects from the Bago and Tanintharyi regions. Active surveillance of residents from regions of intense malaria transmission would reduce the risk of morbidity and mitigate transmission to the population in these endemic areas. Our data demonstrate that PCR-based molecular techniques rather than microscopy are more efficient for nationwide surveillance of malaria in endemic countries.
10.13-134.00) were positively associated with awareness of MDS. Overall, 2011(67.9%) children had received any PHT, 121 children (41.0%) at patent medicine stores. Of the 31(10.5%) FUS diagnosed with malaria and 264 (89.5%) without malaria diagnosis, 13 (41.9%) and 65 (24.6%) have had PHT with CQ respectively. Awareness of MDS remains low. Treatment of FUS against malaria is predominantly inappropriate within the community despite widespread deployment of affordable ACTs. There is a need to sensitise caregivers and health staff on use of ACTs and adherence to confirmatory malaria diagnosis.

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IMPROVING MALARIA DIAGNOSIS THROUGH MICROSCOPY COMPETENCY ASSESSMENT PROGRAMS IN RESOURCE LIMITED SETTINGS

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Malaria remains a major public health problem in Uganda. Early case detection is fundamental for the reduction of mortality and morbidity. Following the WHO recommendation for Parasitological confirmation of suspected cases, Uganda adopted this guideline with scale-up of Microscopy and Rapid Diagnostic Tests. Although microscopy-based diagnosis remains the standard, its quality is frequently inadequate to ensure good treatment outcomes and accurate epidemiological and surveillance data. Our aim in this program was to identify expert blood smear readers through WHO competency assessment scheme that could be utilized to improve Microscopy capacity in Uganda. A total of 33 experienced microscopists working as trainers and performing routine blood smear examination were selected to participate in the competency assessment. Participants were subjected to read standardized blood slides from the WHO slide bank under an “examination” environment. Scoring and grading was done for parasite detection, species identification and counting in accordance with WHO guidelines. Performance data was analyzed to generate sensitivity, specificity, level of agreement and if there was improvement at significant level of 5%. Participants were aged 20-50 years with 5 to over 10 years of experience in malaria microscopy. The mean score was 79%, 32% and 19% for parasite detection, species identification and parasite counting respectively in the baseline pretest and scores were 83%, 52% and 27% for parasite detection, species identification and parasite counting respectively in the final assessment. Performance improved significantly between pre-assessment baseline and final assessment 79% to 83 %, p=0.006 for parasite detection, 43% to 65%, p=0.003 for species identification and 28% to 27%, p=0.648 for parasite quantification. The Mean sensitivity at baseline and final assessment were 80% and 88.4%, p=0.314 and specificity 67.5% and 87%, p=0.817 respectively. Based on results obtained in this assessment, performance for parasite counting and species identification was below the WHO recommended levels of >50% and 90% respectively. Performance in parasite detection was better for all participants. In light of these results, we recommend that competency assessment schemes be conducted for all persons involved in microscopy training, reference expert slide readers and those involved in clinical trials and therapeutic efficacy trials.

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C-REACTIVE PROTEIN IN DIAGNOSIS OF MALARIA IN HYPERENDMIC RURAL AREA OF LAKE VICTORIA IN UGANDA AND TANZANIA

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There is increasing discussion on the role of C-reactive protein (CRP) in differentiation of malaria from other (bacterial) infections especially in regions with hyperendemic malaria. The aim of this study was to assess if CRP is confirmative to the classic microscopic diagnosis of malaria and if it helps in differentiating malaria from other infections. Altogether, 68 patients with positive blood smear for malaria from two rural hospitals (Kibara, Tanzania and Buikwe, Uganda) close to Lake Victoria in hyperendemic malaria region were assessed for CRP levels during acute phase of infections. All of the 68 patients had CRP levels measured within 48 hours of onset of symptoms when seeking medical advice. Their levels were within 8-184 mg/l. Only 7 patients (10,3%) had CRP values >100 (high level of CRP patients - HLP) and 2 (2,9%) had even more than 200 mg/l, with suspicion of additional bacterial super-infection (e.g. meningitis). However, 33 cases (48,5%) had values below the reference level (8-10 mg/l). According to our results, CRP values can neither predict, nor exclude malaria in 51% of all microscopically positive cases which had CRP less than reference level (< 8 mg/l). Given this, also negative CRP does not exclude acute falciparum malaria, however positive CRP with levels >100 mg/l may suggest cerebral malaria or severe bacterial infection. Therefore, the yield of CRP detection in hyperendemic malaria region of great lakes in sub-Saharan Africa remains to be low.

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LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) - EVALUATION OF A NEW KIT FOR DETECTION OF ASYMPTOMATIC LOW-DENSITY MALARIA INFECTIONS IN ZANZIBAR

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Loop mediated isothermal amplification (LAMP) provides an opportunity for improved, field-friendly detection of malaria infections in low-endemic settings but data on its accuracy for detection of asymptomatic low-density parasitemias in pre-elimination settings are lacking. We therefore evaluated the performance of a commercial LoopampTM MALARIA Pan/ Pf detection kit (Eiken Chem., Japan) compared with PCR using DNA extracted from dried blood spots from 465 asymptomatic individuals in Zanzibar. All samples were analysed both for Pan (all Plasmodium species) and Pf (P. falciparum) specifically. The amplification was performed at 65° and results were interpreted after 40 minutes in a real-time turbidimeter. A total of 49 (10.5%) and 38 (8.2%) samples were Pan and Pf-LAMP positive, respectively, whereas 54 (11.6%) were positive by PCR, i.e. 33 P. falciparum and 13 P. malariae mono-infections and 8 mixed P. falciparum/P. malariae infections (mean parasite density 10/µL, range 0-4972). The sensitivity of Pan-LAMP for P. falciparum mono-infections (33) was 97% (95%CI 84.2-99.9) and PF-LAMP for all P. falciparum infections (33+8) was 92.7% (95%CI 80.1-98.5), respectively. The sensitivity of Pan-LAMP for P. malariae detection was 76.9% (95%CI 46.2-95) (range 0-5 µg/µL). The corresponding specificities were 100% for both Pan and PF-LAMP. The LoopampTM MALARIA Pan/Pf kit was further evaluated in a field pilot study of 1026 asymptomatic individuals in three malaria hot
ARE EXIT INTERVIEWS RELIABLE? ANALYSIS OF THE HAWTHORNE EFFECT IN A STUDY OF ADHERENCE TO MALARIA TREATMENT GUIDELINES IN TANZANIA

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Interviewing patients exiting health facilities is a commonly used way to assess consultation practices. It is however unclear if health professionals change their practices when they are aware of such interviews taking place, possibly paying more attention to follow recommended practices. This so-called “Hawthorne effect” could have important consequences for interpreting research and for monitoring program performance, but has rarely been assessed. A cluster-randomised trial of interventions to improve adherence to national guidelines for the use of anti-malarial drugs was conducted in Tanzania. As part of the evaluation, patient interviews were conducted outside participating health care facilities on two randomly-selected days per week for a one year period. Health workers in these facilities were also routinely documenting each consultation in their ledgers. A possible Hawthorne effect was investigated by comparing routine data recorded in ledgers on days when exit interviews were conducted with data from days when no exit interviews were conducted. Routine data were collected in 34 facilities on over 38,000 consultations. No statistically significant differences were found on survey versus non-survey days on any of three pre-specified primary outcomes, after adjusting for geographic region and season. The odds of having a rapid diagnostic test (RDT) result was 7% higher on survey days (Odds Ratio 95%CI: 0.94-1.21, p=0.31), 11% lower for prescribing an anti-malarial drug to a RDT negative patient (0.70-1.14, p=0.36), and 10% lower for prescribing anti-malarial without an RDT result (0.71-1.14, p=0.38). We found no strong evidence of a Hawthorne effect in a study using exit surveys of primary care clinics with data collected from locally trained assistants. This is likely to support such methods in other studies.

OVER-DIAGNOSIS OF MALARIA USING A RAPID DIAGNOSTIC TEST IN A HIGH MALARIA TRANSMISSION SETTING IN UGANDA

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The introduction of rapid diagnostic tests (RDTs) has provided a means for improving the diagnosis of malaria so as to minimize overuse of treatment and thereby delay development of resistance to ACTs. RDTs based on Plasmodium falciparum histidine-rich protein 2 (PfHRP2) have been rolled out across Uganda and several types of RDTs are now available, both in the public and private healthcare sectors. However, important questions remain as to whether all approved RDTs perform well compared to microscopy. The accuracy of SD Bioline Malaria Ag Pf (HRP2/PLDH) RDT was assessed in an area of high malaria transmission in Uganda. The study was conducted at Malaba Health Centre where children aged 0-12 years with a history of fever/axillary temperature ≥37.5°C in the past 48 hours were recruited. Those with recent antimalarial use were excluded. A total of 217 febrile children were tested using the RDT and the results compared to microscopy as the gold standard. Patients were treated on the basis of the RDT results alone and follow up was done on day 3 and subsequently at 7-day intervals for 28 days. Ninety-four of the 217 patients tested had a positive blood smear for asexual forms of P. falciparum. An additional 45 patients tested RDT positive and received antimalarial treatment. Malaria Ag Pf (HRP2/PLDH) RDT had an overall sensitivity of 97%. However the specificity was significantly low at 63%. A negative predictive value (NPV) of 97% and a positive predictive value (PPV) of 68% were observed. A proportion of positive HRP2-based test results that were categorized as false-positive when compared with microscopy may have been due to the presence of subpatent parasitemia and thus PCR testing is being carried out. Reports on specificity of RDTs should be interpreted with caution as there may be wide variations in these measurements depending upon endemicity, season and the age group of patients studied. As RDTs become increasingly available there is a need in Uganda to recognize that ‘one size does not fit all’.
PCW during routine work were receiving a new stock of RDT, and wanting to check on RDT stock quality. Initial field reports of negative RDT with PCW were not confirmed upon repeat testing. Data on RDT usage and adherence to RDT results will be available in May 2014. PCW training was effective and in general, PCW appear to improve HW confidence in RDT results.

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PREVALENCE OF ASYMPTOMATIC MALARIA AND GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN WESTERN PROVINCE, SOLOMON ISLANDS

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The Solomon Islands has experienced a significant decrease in malaria prevalence over the last three decades. In Central and Temotu Provinces, the estimated malaria prevalence by microscopy is up to 5% while PCR-based diagnostics have indicated local prevalences between 4 and 40%. In Temotu Province, more than 84% of the malaria-positive cases were asymptomatic, with Plasmodium vivax being the predominant species. The prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Isabel and Guadalcanal Provinces were up to 20.3%. Malaria prevalence and G6PD deficiency in other provinces is not described in the literature. The objective of this study is to determine the prevalence of malaria and G6PD deficiency in Western Province, Solomon Islands, toward baseline data for malaria intervention studies and characterizations of the province for the Solomon Islands’ malaria elimination program. A total of 3,837 blood spots on filter paper were collected from 19 selected villages located in five island regions of Western Province between August and October 2013. G6PD deficiency was assessed with a NADPH-fluorescent-based diagnostic kit. A total of 2.4% (range 0-6.4% by village) and 9.3% (range 1.9-24% by village) of the population have the deficient and intermediate G6PD phenotype. The samples were then screened for malaria parasites based on amplification of the 18S rRNA gene using a direct PCR approach (sensitivity of 1.6 parasites/µL). Based on successful genus-specific PCR, the average malaria prevalence was 16% (range 5.1-44%) by village. In contrast, when Plasmodium species-specific primers were used, the total prevalence was 5% (range 0-13%) by village, with P. vivax accounting for 95% of cases, and 99% of malaria-positive subjects being asymptomatic. Genus-specific positives that did not amplify with species-specific primers are being sequenced to identify species. We will discuss our results in the context of the performance of malaria diagnosis PCR in areas with extremely low parasitemia and implications for the malaria elimination program in the islands.

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ASSESSMENT OF A THREE-BAND (HRP-2/plDH) RAPID DIAGNOSTIC TEST FOR THE IDENTIFICATION OF SEVERE MALARIA AT A PERIPHERAL HEALTH FACILITY IN WESTERN UGANDA

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The use of rapid diagnostic tests (RDTs) for the diagnosis of malaria has rapidly proliferated at peripheral health facilities in resource-limited settings where there is often no laboratory capability. In this observational study, we examined the utility of a three-band, HRP-2/plDH (SD Bioline FK60) RDT as a tool for the early identification of patients at risk for severe malaria. A total of 1,509 patients underwent RDTs with 637 (42%) positive for malaria. 326 (21.6%) exhibited a single HRP-2 band, 307 (20.3%) exhibited both HRP-2 and plDH bands, while only 4 (0.3%) exhibited a single plDH band. The rate of three-band positive results was twice as high among patients <15 years compared those ≥15 years of age (26.3% vs. 13.2%, χ²=39.9, p<0.001). Notably, the absolute number and proportion of three-band positive RDTs declined over the study period with the transition from the dry to rainy season. The trend of declining three-band positivity in the setting of relatively stable HRP-2 positivity was significant (OR 0.51, 95% CI 0.39 to 0.66). The vast majority (92.1%) of smears from patients with three-band positive RDT results demonstrated Plasmodium falciparum mono-infections. The mean parasite density was approximately 42,000/µL (95% CI 6,827 to 77,349) for HRP-2 positive RDTs and 72,300/µL (95% CI 54,859 to 89,727) for three-band positive RDTs (p=0.072). The mean hemoglobin (Hb) was 8.9g/dL (95% CI 8.3 to 9.3) in patients with a negative RDT, 7.3g/dL (95% CI 6.6 to 8.1) in patients with a HRP-2 positive RDT, and 6.3g/dL (95% CI 5.6 to 7.1) in patients with a three-band positive RDTs. The difference in Hb levels between HRP-2 and three-band positive RDTs, however, was not significant (p=0.17). These results require further investigation, but suggest that a HRP-2/plDH RDT may help identify patients with higher parasite densities and more severe anemia, both risk factors for severe malaria.

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MALARIA IN PREGNANT WOMEN LIVING IN AREAS OF LOW TRANSMISSION OF THE SOUTHEAST BRAZILIAN COAST: MOLECULAR DIAGNOSIS AND HUMORAL IMMUNITY PROFILE

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Pregnant women and children are the main groups under risk of acquiring malaria worldwide. Although Plasmodium falciparum in pregnant women has been widely addressed in the literature, the interaction of this cohort with P. falciparum and P. malariae was poorly explored to date and requires a more comprehensive approach. In Brazil, 99% of the infections occur in the Amazon Region. Although malaria is not considered endemic outside this region, autochthonous cases are registered in areas covered by the Atlantic Forest biome. Studies related to malaria in these low transmission areas have acquired scientific and epidemiological relevance, since they suggest continued transmission and potential outbreaks. The Southeast of Brazilian coast has been focus of studies on the occurrence of Plasmodium, with reports of asymptomatic cases. In this region, where the transmission of P. vivax was established from several studies, our group detected for the first time the occurrence of P. malariae, using molecular tools. Data on the occurrence of the disease or presence of antiplasmodial antibodies in pregnant women living in this area of low endemicity had not been described previously. This study monitored quarterly the circulation of Plasmodium in pregnant women attended in five health facilities located in Juquitiba, State of São Paulo. We performed diagnosis by thick blood film and sensitive molecular protocols for parasite gDNA detection, as well immunological assays in order to investigate humoral immune parameters. For the first time P. vivax and P. malariae were detected in pregnant women living in this low endemicity area, with positivity (95% CI) of 5.6% (1.7-9.0). It was possible to detect the two species through sensitive molecular tools, once the cases were asymptomatic. We also found a high prevalence of IgG antibodies showing a significant exposure of this
population to *Plasmodium*, with 44.0% (35.6-52.7) for ELISA-Pv and 18.4% (12.6-26.1) for IFA-Pm. In regions with a similar profile presented in this study, the diagnosis of malaria might be indicated in prenatal care.

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**A QUANTITATIVE NANOPARTICLE-BASED HISTIDINE-RICH PROTEIN 2 ASSAY FOR THE MALARIA PARASITE *PLASMODIUM FALCIPARUM***

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Diagnosis of severe malaria is particularly important in highly endemic regions since most of the patients are positive for parasitemia. Accurate diagnosis is increasingly important to avoid overprescribing antimalarial drugs, minimize drug resistance, and minimize costs. Microscope does not reflect the pathogenic-sequestered parasite burden. HRP2 levels associated with severe malaria are typically greater than 100 ng mL⁻¹. Rapid diagnostic tests (RDTs) are qualitative and because of that they cannot be used for diagnosis of severe malaria. Here we report on a magnetic bead - quantum dot (MBQD) assay for measurement of levels of HRP2 antigen. The assay is relatively straightforward with magnetic beads for capture and concentration of the target protein, and quantum dots for efficient quantitative detection. Magnetic beads containing surface epoxy groups were coupled to a mouse IgG monoclonal antibody anti-HRP2 (clone 3A4). Human urine samples spiked with HRP2 were incubated with antibody-bead conjugates and then analyzed by Western Blot. Western Blot shows detection of captured protein down to a concentration of 5 ng mL⁻¹. Without concentration, the detection limit was about 100 ng mL⁻¹, suggesting a protein concentration of about 20-fold. To demonstrate the complete sandwich assay, magnetic beads conjugated with antibody were incubated with different amounts of HRP2 spiked in serum samples. After magnetic isolation and washing, the magnetic beads were resuspended and incubated with Quantum Dots 525 coupled to the same monoclonal antibody. Using this assay, we were able to detect HRP-2 concentrations in serum as low as 1 ng per test. The correlation between intensity and antibody. Using this assay, we were able to detect HRP-2 concentrations and incubated with Quantum Dots 525 coupled to the same monoclonal antibody. The assay is relatively straightforward with magnetic beads for capture, and quantitative detection of HRP-2 using magnetic beads and quantum dots that can be easily adapted for point-of-care diagnostics.

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**ADVANCED QUANTITATIVE MICROSCOPY FOR *PLASMODIUM FALCIPARUM* DIAGNOSIS DURING PFSPZ CHALLENGE AND OTHER CONTROLLED HUMAN MALARIA INFECTION STUDIES: RESULTS OF AN AFRICAN TRAINING WORKSHOP**

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Sanaria has developed aseptic, purified, cryopreserved infectious *Plasmodium falciparum* (PI) sporozoites (SPZ) called PFSPZ Challenge as a tool for Controlled Human Malaria Infections (CHMI) to study protective efficacy of anti-malarial drugs and vaccines, to allow refinement of the method of administration of the highly protective PFSPZ Vaccine, and to study innate and acquired immunity to Pf. A critical component of the CHMI studies with PFSPZ Challenge is the diagnosis of malaria parasites in the blood. Diagnosis needs to be highly sensitive in order to detect parasites before the onset of major clinical symptoms, and needs to be highly specific in order to prevent misdiagnosis (i.e. false positive results), which could alter the outcome of the study. False positive results must be avoided in PFSPZ studies where misdiagnosis after vaccination would have potential safety implications and where misdiagnosis after CHMI would alter the estimates of protective efficacy. The European and Developing Countries Clinical Trials Partnership has funded a 7 country African consortium of institutions working with PFSPZ Challenge to optimize CHMI studies in Africa. Technical staff from across the network were hosted by the Ifakara Health Institute in Bagamoyo, Tanzania, for a 1 week course to develop expert level technical expertise in Advanced Quantitative Microscopy for rapid, sensitive, and specific diagnosis of Pf. Here we describe the results of the intensive training sessions and the subsequent establishment of a quantitative thick smear microscopy certification center at KEMRI, Nairobi, Kenya.

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**STIMULATING A PRIVATE SECTOR MARKET FOR MALARIA RAPID DIAGNOSTIC TESTS (RDTs): BASELINE RESULTS FROM KENYA, MADAGASCAR AND TANZANIA**

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Key baseline results and formative insights from a multi-country project to stimulate the creation of a private sector market for malaria Rapid Diagnostic Tests (RDTs) will be presented and discussed. Since 2010 the WHO has recommended confirmatory diagnostic testing for suspected malaria, followed by treatment with ACT for positive cases. However, in the private sector, where over 40% of the population in endemic countries seeks care and treatment for febrile illness, RDTs are either non-existent or no cheaper than ACT. The UNITAID Private Sector RDT Project aims to increase both access to and demand for quality-assured RDTs, while improving private providers’ fever case management skills and implementing a public-private roadmap that will guide policy and regulation. The Project is led by Population Services International, together with its collaborating implementers, Foundation for Innovative New Diagnostics, Malaria Consortium and the World Health Organization; it is being implemented in Kenya, Madagascar, Nigeria, Tanzania (mainland) and Uganda. In 2011, RDTs were available in fewer than 1 in 10 private health facilities in Kenya and Nigeria, and in Kenya the median price for a test was approximately $1.00, much more expensive than (subsidised) ACT treatments for children ($0.46). Although RDTs were available in over 90% of public facilities in Madagascar, in 2011 only 9% of private doctors and clinics had tests available. Within this context, results will be presented from the 2013 household survey of over 1,300 fever cases on the Kenyan coast, and exit interviews among private-sector fever patients in Kenya, Madagascar and Tanzania (results due July 2014). Operational insights from qualitative research with participating providers will also be shared. The project will contribute to the ongoing discussion on how best to scale-up access to affordable, quality-assured RDTs offered by trained and supervised providers who are incentivized to correctly manage febrile illness.
MALARIA TESTING AND TREATMENT IN TANZANIA AFTER INTRODUCTION OF RAPID DIAGNOSTIC TESTS IN ACCREDITED DRUG DISPENSING OUTLETS

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In Tanzania, the private sector provides 30% of all treatment for febrile illness to children under 5 years receiving it, but the availability of diagnosis is more limited than in the public sector. The reliance on presumptive malaria treatment in this sector means that many patients receive an incorrect diagnosis and inappropriate treatment. One challenge is that drug outlets comprise 70% of the private sector, but are not allowed to sell or perform malaria tests. This study assessed how patient testing and medication-purchasing behaviors evolved following a pilot study that allowed Accredited Drug Dispensing Outlets (ADDOs) in two districts to sell and perform malaria rapid diagnostic tests (RDTs). A baseline survey was completed in March 2013 to assess testing practices, RDT availability, and medication sales in ADDOs in Morogoro Region. In May 2013, dispensers in 270 intervention ADDOs were trained on how to properly stock and perform RDTs, and given access low cost RDTs. Dispensers from 91 control ADDOs were not given access to the RDTs. Over the next year, ADDO patient register books were used to evaluate the proportion of febrile patients that received an RDT and an artemisinin combination therapy (ACT). Surveys found RDTs were available in 77.5%-87.0% of ADDOs in the intervention region following training. Of 12,730 patients that sought treatment for fever or malaria in the accredited ADDOs during the nine months following training, 79.4% (95% CI: 78.7%-80.2%) elected to purchase an RDT. Of those for whom an RDT test result was recorded (n=9,872), 57.0% (95% CI: 56.5%-58%) tested positive, and 79.1% (95% CI: 77.9%-80.1%) of those who tested positive received an ACT. Only 3.1% (95% CI: 2.5%-3.7%) of those who tested negative purchased an ACT. In this study, introducing RDTs in ADDOs resulted in high availability and uptake of testing, as well as adherence to test results. The study highlights the potential for improving appropriate use of anti-malarials and preventing overtreatment with ACTs by placing RDTs in ADDOs and training staff in their use.

EVALUATION OF THE EFFECTIVENESS OF A TRAINING PROGRAM FOR SCHOOL TEACHERS IN PERFORMING AND INTERPRETING MALARIA RAPID DIAGNOSTIC TESTS SAFELY AND ACCURATELY IN ZOMBA, MALAWI

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With increasing levels of attendance, schools present a pragmatic opportunity to improve the access of school children to timely diagnosis and treatment of malaria, increasingly recognised as a major health problem for this age group. The expanded use of malaria rapid diagnostic tests (mRDTs) by community health workers has led to an interest in whether teachers can provide similar services for school children. We investigated the ability of school teachers to constitute safe, accurate and acceptable providers of malaria diagnosis and treatment using mRDTs and artemisinin-based combination therapies (ACTs) following training, as well as the retention of these skills during implementation of a school-based malaria case management programme. A comprehensive, skill-focused, pilot training was conducted in Zomba District, Malawi. Teachers were trained in the use of first aid kits including instruction on the principles and use of mRDTs by facilitators from the Ministry of Health. The four-day training workshop consisted of theoretical and practical sessions, with manuals and job aids adapted from a range of developed materials pre-tested with community medicine distributors and health surveillance assistants. Feedback from this pilot was used to design the full seven-day training workshop conducted prior to implementation of the intervention in schools. We present results on both the effectiveness of the pilot and full training workshops, in relation to increased knowledge and skill sets, and the retention of these through pre and post evaluation questionnaires, and checklist evaluations. Additionally, we report on the acceptability to teachers of carrying out such a role, and their confidence in providing this service, assessed through focus group discussions. To our knowledge, this is the first study in which teachers have been trained to use mRDTs. The results provide important evidence on the feasibility of using teachers to diagnose malaria using mRDTs in terms of safety, accuracy and confidence and to make appropriate treatment decisions based on the results.

MALARIA DIAGNOSTICS QUALITY IMPROVEMENT AND ASSURANCE PROGRAM FOR TANZANIAN MILITARY HEALTH FACILITIES

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Malaria is responsible for over 100 million reported cases annually and 1-2 million deaths, especially in children. Within Tanzania, malaria diagnostics continues to be challenging, especially in resource-challenged settings. Amethyst Technologies LLC (ATL) partnered with the US Army, Tanzania People’s Defense Force (TPDF), and Tanzania National Service Program (JKT) to develop and implement a malaria diagnostics quality assurance program to support the Tanzanian military’s malaria measurement and control efforts at 21 TPDF/JKT training camps throughout Tanzania in 2011-2013. The approach of the strategy was to 1) develop Quality Improvement tools, which included assessment checklists (for evaluating camp health facilities), standardized laboratory SOPs, and a malaria diagnostic training course, 2) conduct baseline assessments of the 21 camps using the QI tools and provide malaria diagnostic training at all sites, 3) develop a QI and lab strengthening plan based on individual site assessments, 4) provide feedback to the health facilities and execute QI plans, and 5) execute a quality management plan to provide on-going quality improvement of malaria diagnostics. The QI assessment checklists evaluated sixteen quality criteria, including laboratory safety, human resources, personnel training, supply/stock management, results recording, results reporting, implementing of quality assurance procedures, external quality assessment (EQA), electrical supply, and staining capabilities (thick or thin smears). The quality management plan involved continuing quarterly re-assessment visits using QI tools, performing quality assurance of malaria diagnostics (by collecting malaria blood smears for cross-checking by expert microscopists), and supporting corrective actions. An overall improvement in the diagnostic services performed and strong increases in initial site assessment scores were observed, though sustaining the quality improvements proved challenging. We will present data captured from the baseline assessments and reassessments, showcase areas of success and challenges, demonstrate the utility of the QI tools, and discuss the sustainability of the program.
MALARIA MICROSCOPY QUALITY ASSURANCE AND SLIDE CROSSCHECKING AT RUVI KJT LABORATORY OF TANZANIA

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The purpose of this exercise was to provide malaria microscopy External Quality Assurance (EQA) at KJT Ruvi where Walter Reed Program - Tanzania (WRP-T) is conducting malaria attack rate study. This EQA consist of 3 elements of (1) On-site Evaluation to assess the performance of the laboratory against the available SOPs, (2) Proficiency testing, and (3) Slide cross-checking. This exercise was also to assess the effect of microscopy error on estimating the malaria attack rate. Overall the performance at KJT Ruvi is satisfactory on the parameter of documentation, accommodation, electrical power supply, laboratory safety, equipment, stain reagents, and training. However the performance of blood film preparation and staining need to be improved as well as re-estimating the workload. Of 583 slides that had been randomly selected, 28 (5%) were judged to be unreadable, 67 (11%) were readable with difficulty due to artefacts, 22 (4%) were of poor quality due to poor preparation, 136 (23%) had poor staining quality, and 330 (57%) were in good quality and easily readable. The technician serving as microscopist at the study site was categorized as expert malaria microscopist for reading Plasmodium falciparum. The sensitivity and specificity scores of the microscopist were 90% and 95% respectively. The agreement of initial results between microscopist and expert confirmation is 99.14%. Agreement on positive slides was 95% (76/80), and for negative slides 99.8% (502/503). Those disagreements are due to poor preparation and workload. In conclusion, the commonest cause of inaccurate results was the quality of the slides, correction of which is likely to be achievable within existing Standard Operating Procedures on site. An expert malaria microscopist may report inaccurate results when the workload is too high. The laboratory workload should be measured, recorded and monitored. Microscopy error (false positives, false negatives, and species identification errors) may mislead estimation of malaria attack rate results.

CLINICAL PERFORMANCE EVALUATION OF THE FYODOR MALARIA URINE TEST (UMT)

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Effective case management of malaria requires prompt diagnosis and treatment within 24 hours. Despite current policy guidelines that mandate confirmed parasitological diagnosis before treatment, access to diagnostic testing remains low in sub-Saharan Africa. Today, malaria diagnosis is only by blood-tests (microscopy and rapid diagnostic tests, RDTs), which are invasive, multistep and therefore relatively complex to perform, require technical expertise, and not available in most public and private sector healthcare settings where more than 65% of the population seek care. Here, we report the results of a multicenter pivotal clinical trial of Fyodor Urine Malaria Test (UMT) - a simple (one-step, no blood, no reagents, no equipment) dipstick test that detects Plasmodium falciparum parasite proteins shed in the urine of febrile malaria patients. A total of 1,893 participants (≥2 years) with fever (axillary temperature ≥37.5°C) or history of fever in the last 48 hours were enrolled at 6 primary healthcare centers in rural and suburban communities in Lagos State, Nigeria, over a 7-month period that covered both rainy and dry seasons. Matched patient urine and fingerprick blood samples were tested using the UMT, Binax NOW (Inverness) (HRP-2/pLDH) test, and microscopy. A total of 358 participants (18.9%) had confirmed malaria by microscopy; Fyodor UMT, 450 (23.8%); Binax NOW (pLDH), 386 (20.4%) and Binax NOW RDT (HRP-2, 731 (38.6%). Statistical data analyses to determine test performance characteristics are ongoing and will be made available within a month. The UMT has the potential of expanding access to malaria diagnosis especially in settings where blood test is not possible.

MALARIA INFECTION IN CHILDREN AGED 0 TO 5 YEARS IN THE KASSENA NANKANA DISTRICT, NORTHEASTERN GHANA. A GROUP BASED TRAJECTORY ANALYSIS

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Information about progression of Plasmodium falciparum risk infection in children over time is limited. This study aimed to assess the pattern of infection events over time in a cohort of children under five years, and to explore the association to maternal factors such as education, ethnicity or preventive care behavior during pregnancy. A cohort study analysis of health surveys with on-site temperature, thick blood smear and hemoglobin measurements. Subjects were from Kassena-Nankana District of northern Ghana, who were previously enrolled at birth into a five-year prospective study. Assessment visits were done twice a year in high and low seasonal transmission periods. A Based Group Trajectory Analysis approached was used to allow identification of sub-groups over time, and a multinomial logistic regression to assess risk factors. A total of 2,107 children were recruited at birth, 4,892 events of infection were identified. Individuals belongs to three patterns of infection, a low-risk group “LoR” (47.5%) which had a prevalence of infection <20% over the whole observation, a intermediate risk “InR”, ascending group (44.0%) which reached to 50%-60% of prevalence in the 3-4 years of age, and a high risk group “HiR” (8.5%) with a rapid ascending pattern reaching over 80% of prevalence by the third year of age. The risk of infection increased with less years of mother education especially in those under elementary school (InR OR 1.70: HiR: OR 3.10); and decrease with: each additional antenatal care visit (InR: OR 0.94; HiR: OR 0.80), use of bed net during pregnancy (InR: OR 0.69: HiR: OR 0.60), and receiving antimalarial drugs during pregnancy for the HiR group (OR 0.59). Populations are not homogeneous in patterns of infection over time, subgroups can be identified based in social characteristics which may allow preventive health intervention in those individuals.
ECONOMIC EVALUATION OF INTERVENTIONS TO IMPROVE HEALTH WORKERS’ PRACTICE IN DIAGNOSING AND TREATING UNCOMPROMLICATED MALARIA IN CAMEROON

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Malaria rapid diagnostic tests (RDTs) are a valid alternative to malaria testing with microscopy and are recommended for testing of febrile patients before prescribing an antimalarial. There is the need for interventions to support the uptake of RDTs by health workers. This study evaluates the cost-effectiveness of introducing RDTs with basic or enhanced training in health facilities where microscopy was available, compared to current practice. A three-arm cluster randomized trial was conducted in 46 facilities in Centre and North-west Cameroon. Basic training had a practical session on RDTs and lectures on malaria treatment guidelines. Enhanced training included small-group activities designed to change health workers’ practice and reduce consumption of antimalarials among test-negative patients. The primary outcome was the proportion of febrile patients correctly treated: febrile patients should be tested for malaria, artesiminin combination therapy should be prescribed for confirmed cases, and no antimalarial should be prescribed for patients who are test-negative. Individual patient data were obtained from facility records and an exit survey. Costs were estimated from a societal perspective using project reports and patient exit data. Results showed that the incremental cost per febrile patient correctly treated was $8.40 for basic and $3.71 for enhanced arms. Upon scale-up it was estimated that RDTs with enhanced training would save $0.75 per additional febrile patient correctly treated. Introducing RDTs with enhanced training was more cost-effective than RDTs with basic training, when each was compared to current practice.

IMPROVED TARGETING OF ANTIMALARIAL TREATMENT IN COMMUNITY-BASED MANAGEMENT OF MALARIA: EVIDENCE FROM CLUSTER-RANDOMIZED TRIALS IN UGANDA

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Universal access to diagnostic testing for malaria is now recommended by WHO, to encompass all levels of health care, including community-based treatment programmes. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in locations lacking electricity and qualified staff, however data on the impact of diagnostic testing on treatment and referral practices by community health workers remains limited. A cluster-randomised trial to evaluate the impact and cost-effectiveness of RDTs in community case management was conducted in two areas with contrasting malaria transmission in Rukungiri District, Uganda. A total of 120 communities [379 community medicine distributors (CMDs)], were randomised to training either in use of RDTs or presumptive diagnosis of malaria. All CMDs were trained on how to give antimalarial treatment with ACTs, rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness, and close support supervision to CMDs for the first six months of implementation, after which supervision was scaled back to mimic levels typically seen in health systems in rural Africa. Nonetheless, adherence to RDT results by CMDs remained high, with over 95% of ACT treatments given being consistent with the RDT test results. When treatment decisions by providers were validated by expert microscopy on a reference blood slide collected at the time of consultation, the proportion of patients receiving appropriately targeted treatment was significantly higher in villages where community health workers used RDTs, compared to presumptive treatment: 79% vs 31% (p<0.001) and 90% vs 8% (p=0.001) in the high and low transmission areas respectively. Data on the impact of RDTs on referral practices will also be presented. In conclusion, diagnostic testing with RDTs in community case management can reduce over-diagnosis and substantially increase the proportion of patients receiving appropriately targeted malaria treatment.

DISPENSER PERFORMANCE ADMINISTERING MALARIA RAPID DIAGNOSTIC TESTS IN THE PRIVATE RETAIL SECTOR OF TANZANIA

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In 2010, the World Health Organization (WHO) recommended that all suspected malaria cases be tested. In Tanzania, the low confirmatory diagnosis rate (approximately 25% for children under 5) means that many febrile cases receive clinical diagnoses and potentially inappropriate treatment. While approximately 30% of febrile cases seek treatment in the private sector, informal shops comprising 70% of this sector are not allowed to stock or perform malaria tests. We evaluated whether the gap in accessibility to testing could be addressed by allowing Accredited Drug Dispensing Outlet (ADD0) shop dispensers to sell and perform malaria rapid diagnostic tests (RDTs). Dispensers from 270 ADDOs were trained on RDT administration and safety in Morogoro Region in May 2013. If they passed the training, they were certified and allowed to purchase RDTs at a negotiated low or subsidized price. Certified dispensers were monitored quarterly from May 2013 to 2014. RDT safety, administration, and interpretation were assessed using a list of indicators adapted from the checklist published by the WHO in “Universal Access to Malaria Diagnostic Testing: An Operational Manual”. Data on ADDOs’ safety and hygiene practices were collected. RDTs were stocked in 87% of enrolled shops at the first and second visits and 78% of shops at the third visit (p=0.006 for change across surveys). At least 83% of dispensers performed an RDT correctly on all of the 17 indicators. More than 98% of dispensers correctly interpreted results at each visit. Regarding ADDOs’ safety and hygiene practices, over 95% of ADDOs kept the area around the shop clean and free of used RDT products across surveys. However, the fraction passing the test in a private sector decreased between the first and third visits from 31% to 19% (p<0.001 for change across surveys). Following training, ADDO dispensers competently stocked and safely administered RDTs, demonstrating that placing RDTs in certified private shops may be a feasible solution to increase malaria diagnostic access.

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FATTY ACID SYNTHESIS AND PYRUVATE METABOLISM PATHWAYS REMAIN ACTIVE IN DIHYDROARTEMISININ INDUCED DORMANT RING STAGES OF PLASMODIUM FALCIPARUM

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Artemisinin (ART) based combination therapy (ACT) is used as the first line treatment of uncomplicated falciparum malaria worldwide. However, despite high potency and rapid action there is a high rate of recrudescence associated with ART monotherapy and recrudescence is not uncommon even when ACT is used. This is independent of the recent observation of ART resistance. ART induced ring stage dormancy and recovery has been implicated as possible cause of recrudescence; however, little is known about the characteristics of dormant parasites including whether dormant parasites are metabolically active. We investigated the transcription of 12 genes encoding key enzymes in various metabolic pathways in Plasmodium falciparum during dihydroartemisinin (DHA) induced dormancy and recovery. Transcription analysis showed an immediate down regulation for 10 genes following exposure to DHA, but continued transcription of 2 genes (Rho-mediated) in apicoplast and mitochondria. Transcription of several additional genes encoding enzymes in pyruvate metabolism and fatty acid synthesis pathways, were also maintained. Additions of inhibitors for biotin acetyl CoA carboxylase and enoyl-acyl carrier reductase of the fatty acid synthesis pathways delayed the recovery of dormant parasites by 6 and 4 days, respectively following DHA treatment. Our results demonstrate most metabolism is down regulated in DHA induced dormant parasites. In contrast fatty acid and pyruvate metabolic pathways remain active. These findings highlight new targets to interrupt recovery of parasites from ART-induced dormancy and to reduce the rate of recrudescence following ART treatment.

EFFECT OF TRANSMISSION SETTING ON THE SULFADOXINE-PYRIMETHAMINE RESISTANT HAPLOTYPES AND SELECTIVE SWEEP CHARACTERISTICS IN MALAWI

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The decline of antimarial resistant parasites, and the re-expansion of drug sensitive parasites, after the removal of drug pressure, raises the possibility that previously abandoned drugs might once again find clinical utility. Here we investigate the role of transmission setting on the dynamics of sulfadoxine-pyrimethamine (SP) resistance alleles and the characteristics of their associated selective sweeps. High transmission settings are associated with more clinically immune hosts who may serve as a reservoir for drug-sensitive parasites. High transmission intensity leads to higher parasite recombination, with greater genetic diversity. Samples from patients who presented to health care facilities and were found to have malaria were collected from three transmission sites in Malawi: urban (Ndirande), rural-high transmission (Chikwawa), and rural-low transmission (Thyolo). Pyrosequencing was used to determine the predominant haplotypes of dhfr and dhps in each infection. A ~96% prevalence of dhfr 511/59R/108N, and ~96% prevalence of dhps 437G/540E was found at all three sites. There was no significant difference in haplotype prevalence found between any of the transmission settings, regardless of season. A single SP-sensitive parasite was found in Thyolo. Neutral and flanking microsatellite analysis was used to calculate expected heterozygosity (He) and estimate diversity ratios between the three sites. At markers flanking dhfr and dhps significant differences in He were found between Thyolo and Chikwawa and between Thyolo and Ndirande, however no significant difference in diversity ratios was found between Ndirande and Chikwawa for either dhfr or dhps. Our data indicate that the differences in transmission between these sites, given the high level of SP resistance, were not sufficient to effect change in haplotype prevalence. Differences in sweep characteristics between Thyolo and the other transmission settings will be pursued in future analyses.

CHANGES IN OCCURRENCE OF DRUG RESISTANCE POLYMORPHISMS TO SULPHADOXINE - PYRIMETHAMINE IN THE PLASMODIUM FALCIPARUM IN SOUTHERN ZAMBIA

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Malaria remains one of the major global diseases affecting humans and drug resistance is a major concern for effective treatment. In Zambia, a change from Sulphadoxine Pyrimethamine to artemether lumefantrine as first line treatment for malaria was made in 2004 due to evidence of increasing levels of resistant parasites. However, it is still used for intermittent preventative treatment in pregnancy. Mutations in the dhfr and dhps genes are associated with resistance to pyrimethamine and sulphadoxine. Plasmodium falciparum antifolate drug resistance polymorphisms are detected using nested PCR and restriction enzyme digestion. This study aimed to determine and compare the prevalence of dhfr and dhps polymorphisms in a low endemic area at two time points 2008-2009 and 2012-2013. Finger-pricked blood samples (Dried Blood Spots) were collected on filter paper from 993 consenting participants from communities in Macha, Choma District between 2008 and 2009, and 1303 consenting participants between 2012 and 2013. Parasite DNA was extracted and a nested PCR run on these samples from which the positives were genotyped for dhfr and dhps mutations. Restriction enzyme digestion was done on the positives and restriction fragments analysed by gel electrophoresis and visualized under UV transillumination. The number of individuals with P. falciparum resistance mutations in the dhfr gene and dhps was 11 for 2008 and 2009 and 5 for 2012 and 2013 respectively. Malaria infections in Southern Zambia have declined due to combined interventions and efforts. The prevalence of mutations in the dhfr and dhps genes has decreased from 2009 to 2013, resistance alleles are still present in the general population. The impact of resistant parasites on the efficacy of SP for IPTp needs to be assessed.

FORWARD GENETIC CHEMICAL PROFILING OF PIGGYBAC MUTANTS OF PLASMODIUM FALCIPARUM REVEALS NEW DRUG TARGETS AND INSIGHTS INTO MECHANISMS OF RESISTANCE TO ARTEMISININ

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The emergence and spread of Plasmodium falciparum multidrug resistance highlights the urgency to discover new targets and chemical scaffolds with reduced potential for emergence of resistance. Achievement of this goal will be enhanced by developing a better understanding of mechanisms of action and resistance of available drugs and inhibitors on vulnerable metabolic pathways. In this study we conducted a novel forward genetics, chemical profiling screen of 80 P. falciparum piggyBac (pB) mutants to the
identify, validate and prioritize anti-malarial drugs, targets, and resistance mechanisms. Mutants carrying a single transposon insertion were profiled for altered responses to standard antimalarial drugs and inhibitors of known metabolic pathways. The results of the screen provide proof of concept and yield new insights into mechanism of action and resistance. For example we found that drugs targeting the same pathway have a significantly higher connectivity to each other than to drugs that inhibit other pathways. In addition, we made novel observations about important drug resistance mechanisms. One of the mutants profiled in our study contains an insertion in the intergenic region between the kelch protein 13 (K13) gene implicated in artemisinin resistance (PF3D7_1343700) and a gene encoding a conserved protein of unknown function (PF3D7_134800). This mutant exhibited 2-7 fold increased susceptibility to artemisinin drugs and was one of 10 pB mutants that based upon hierarchical cluster analysis had similar enhanced responses to artemisinin and other inhibitors. We demonstrate that K13 and 3 other genes (PF3D7_0727100, PF3D7_0619800 and PF3D7_1126100) in its chemogenetic cluster are tightly linked in co-expression networks. This implies that these genes are functionally related to K13 and mediate in vitro artemisinin response. Our data demonstrate that chemical-genetic profiles can reveal unexpected drug relationships and connect them to gene functions, including hypothetical genes in the malaria parasite.

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NOVEL K13 PROPELLE (KELCH PROPELLE) MUTATIONS IN BANGLADESHI PLASMODIUM FALCIPARUM CLINICAL ISOLATES

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The K13 propeller protein of Plasmodium falciparum is associated with artemisinin resistance. K13 propeller is a kelch motif containing protein, related to KEAP1 protein of its human host. Its functional role in artemisinin resistance is unknown. On the other hand, few clinical studies carried out in Bangladesh have not concluded any case of artemisinin resistance. In the current study, Plasmodium falciparum positive blood samples (n=238) were collected from seven endemic districts of Khagrachari (n=131), Rangamati (n=3), Cox’s Bazar (n=68), Bandarban (n=14), Mymensingh (n=2), Netrokona (n=9) and Moulvibazar (n=11) in Bangladesh. K13 was bidirectionally sequenced. ClustaW and Jalview analysis have revealed five different mutations present in these clinical isolates. Two of these were synonymous mutations originating from Myanmar and Rangamati. A578S mutation was found in two different samples collected from Khagrachari district and W470C and Y604H were found in Rangamati. Mutations observed in this study are different to those reported from Cambodia. W470C and Y604H mutations are located in the highly conserved β-sheet structure. These mutations may alter the integrity of the sheet. A578S is located in the loop structure close to the C580Y mutation - the major one in Cambodia associated with treatment failure and resistance. We report several novel mutations in Bangladesh in the K13 gene associated with artemisinin resistance. Further clinical studies are required to confirm any relationship to delayed parasite clearance.

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RAPID TURNOVER OF PVMDR1 HAPLOTYPES IN PLASMODIUM VIVAX POPULATIONS

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Plasmodium vivax is the most widely distributed malaria parasite and causes a serious public health burden. The emergence of chloroquine (CQ) P. vivax resistance - the first line treatment in most of the world - represents a hurdle to malaria control. Due to difficulties to in vitro P. vivax culture systems, a data base of the mutations in target genes could help to set a baseline for CQ drug-resistance surveillance. For this purpose, 97 P vivax blood samples collected from patients attending for PCR diagnosis at the Brazilian Ministry of Health Extra-Amazonian Malaria Reference Laboratory, between 2010 and 2013, were direct DNA sequencing from PCR products containing pvmdr1 Y976F and F1076L SNPs. We observed that, between 2010 and 2012, the great majority of the 64 samples tested presented a single mutation, showing the FF (62 / 97%) or the FL (1 / 1.5%) profiles, while only one sample presented the FL double mutant (1.5%). Interestingly, this pattern of haplotypes inversely changed when samples collected in 2013, where analyzed: in this case all the samples presented double mutants showing the FL profile, and single mutations were not detected anymore. Thus, this first report showing the turnover of pvmdr1 haplotypes in P. vivax parasites could supply a baseline to monitor P. vivax CQ-resistance. The turnover of P. vivax parasites may reflect the introduction of new parasites carrying pvmdr1 alleles associated to drug-resistance by mosquitoes. To explain this finding additional studies on molecular epidemiology are required.

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EFFECTICACY OF ARTEMETHER-LUMEFANTRINE AND AMODIAQUINE-ARTESUNATE FOR TREATMENT OF UNCOMPPLICATED MALARIA IN CHILDREN: RANDOMIZED CLINICAL TRIAL AT THREE SITES IN UGANDA

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Plasmodium falciparum resistance to artemisinin derivatives is emerging in Southeast Asia, and resistance to artemisinin partner drugs may be increasing. In Uganda, artemether-lumefantrine (AL) is the first-line therapy and artesunate-amodiaquine (AS/AQ) the alternative for the treatment of uncomplicated malaria. We are conducting a randomized, single-blind trial comparing AL and AS/AQ to treat uncomplicated falciparum malaria at 3 sites in Uganda with different transmission intensities and a range of malaria intervention coverage. A total of 600 children aged 6-59 months will be enrolled (100 per treatment arm per site), randomized to treatment with AL or AS/AQ, and followed for 28 days. The primary outcome is the risk of treatment failure unadjusted and adjusted by genotyping at day 28. Recruitment and follow-up have been completed at two sites (Apac and Mubende), and are ongoing at the third site. No serious adverse events have been reported to date. Preliminary results show no early treatment failures. The uncorrected 28-day risk of treatment failure was significantly lower for children treated with AS/AQ than for those treated with AL at Apac (15.0% vs 31.2%; p = 0.008) and Mubende (33.6% vs 53.7%; p = 0.003). Two recrudescences were observed in Apac in the AL treatment arm compared to none in the AS/AQ arm (corrected: 2.1% vs. 0%; p = 0.16). Parasite clearance was rapid at Apac (37.0 vs. 37.6 hours; p = 0.74) and Mubende (42.6 vs. 39.4 hours; p = 0.06) in AS/AQ and AL treatment arms, respectively. Our results show that the corrected treatment success rates were not different and were very high for both AS/AQ and AL. However, AS/AQ appeared to have a better prophylactic effect.
PLASMODIUM FALCIPARUM GENOTYPES FROM GAMBIAN CHILDREN WHO FAILED TREATMENT WITH ARTEMETHER-LUMEFANTRINE; RECURRENCE OR RE-INFECTIONS?
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The growing risk of resistance to artemisinin derivatives calls for regular monitoring of the efficacy of artemisinin-based combination therapy locally used in endemic countries. Artemether-lumefantrine, the first line ACT in the Gambia shows high but variable efficacy in sites across the country. We conducted a 28-day follow up study to investigate the consistency of this variability in public health facilities in the country. DNA was extracted from filter paper blood spots of eight participants with treatment failures (by microscopy) using the QIAamp DNA blood kit (Qiagen) with DNA concentrations determined on the nanodrop. Each isolate was genotyped by PCR amplification and analysis of MSP1 and MSP2 gene loci and amplified products separated by capillary electrophoresis. Band sizes were determined against a 50-800bp DNA ladder. Isolates were further analysed in duplicates in two sequential assays by MSP1 & MSP2 amplification with Dye labelled primers and fragment analysis employing the GenScan1200LIZ size marker for size calling. Fragment size analysis was done with GeneMarker software (Softgenetic). PCR band and fragment sizes were compared between consecutive PCR runs and scored according to WHO/Malaria Warning prescriptions for distinguishing recrudescence from re-infection. This is based on the presence of at least one PCR band with identical size between samples from different time points and band sizes scored to be similar within a sensitivity margin of ±5 basepairs. Five of the eight samples analysed showed presence of identical-size PCR fragments for MSP1 and MSP2 either by Qiaceq capillary electrophoresis, Fluorescent based fragment analysis or both. Two samples did not yield Plasmodium specific amplification products for the Post Day 0 samples and hence remain undetermined, and one sample was likely a re-infection as all loci analysed between Day 0 and subsequent timepoints were different. Further analysis will help verify the genetic identities of the five samples that indicated possible treatment failure.

THERAPEUTIC EFFICACY OF DIHYDROARTESINISIN-PIPERAQUINE FOR TREATING UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA AND K13-PROPELLER POLYMORPHISMS ALONG THE CHINA-MYANMAR BORDER
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The World Health Organization (WHO) recommends Artemisinin-based Combination Therapy (ACT) for treating uncomplicated Plasmodium falciparum malaria, but resistance to artemisinin derivatives in Southeast Asia threatens malaria control and elimination activities worldwide. Mutations within a P falciparum kelch protein, K13, are associated with both in vitro and clinical measures of artemisinin-resistant malaria. Validation and mapping of this marker throughout the malaria endemic world will be helpful to track the emergence and spread of artemisinin resistance. Yunnan, China’s only province with endemic P falciparum malaria transmission, borders Myanmar, Vietnam and Laos, and is the key focus of the national malaria elimination program. Patients from two sites (Yingjiang and Tengchong) bordering Myanmar received antimalarial treatment with either dihydroartemisinin-piperaquine or artesunate by directly observed therapy as part of therapeutic efficacy studies conducted from 2009 until the present. After a 28 or 42-day follow-up, treatment efficacy was estimated according to the WHO protocol for assessing and monitoring antimalarial drug efficacy. The P. falciparum K13 gene was sequenced in samples collected from approximately 400 patients including almost 120 clinical trial participants, using Sanger sequencing. The prevalence of K13 mutations was estimated by study site and year. Linear regression was used to assess the association between K13 mutations and parasite clearance half-life, while adjusting for confounding variables. Haplotype networks of SNPs surrounding the K13 gene were used to assess origins of K13 mutations. Preliminary results indicate a high prevalence (34%) of the K13 F446I mutation, which was significantly associated with the presence of parasitemia 72 hours after treatment. These results suggest that K13 mutations are responsible for artemisinin resistance in Yunnan Province, China, although the predominant K13 mutation is different than in other areas of Southeast Asia, suggesting independent emergence rather than spread of resistance.

A BETWEEN-HOSTS AND WITHIN-HOST COMBINED MODELING FRAMEWORK FOR THE EVOLUTION OF RESISTANCE TO ANTIMALARIAL DRUGS
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The emergence and spread of drug resistance in Plasmodium parasites has long been and is still a significant problem for chemotherapeutic approaches to malaria control. Evolution of drug resistance in mosquito-borne parasites is a complex process, influenced by transmission dynamics between hosts and vectors as well as within-hosts competition among parasite strains. We present here a theoretical model of the evolution of drug resistance that combines both between-host and within-hosts scales, aiming to understand how the dynamics at each level and the interaction between scales influences drug resistance evolution. Our model combines an epidemiological model of malaria transmission between hosts and vectors and a within-hosts model of the course of infection for multiple competing strains. The latter includes the effects of immunity, treatment and cost of resistance. The epidemiological level can reflect various epidemiological settings and transmission intensities. The model shows that in high transmission environments, where co-circulation of sensitive and resistant strains is more frequent, resistant strains are less likely to spread, particularly when within-hosts costs of resistance are high. We illustrate how treatment impacts the spread of resistance, and show a general trade-off between disease prevalence reduction and resistance management. We show however that treatment coverage has a stronger impact on disease prevalence, whereas treatment efficacy primarily affects resistance control. We conclude therefore that a primary focus on coverage over efficacy would have the strongest impact on disease control while minimizing selection for resistance. More generally we underline the importance of modeling the evolution of drug resistance across biological scales for a better understanding of the evolutionary dynamics in a variety of eco-epidemiological settings, providing valuable insights for both disease control and drug resistance management.
Efficacy, Safety and Pharmacokinetics of Artemether-Lumefantrine for the Treatment of Uncomplicated Malaria in HIV-Positive Adults Receiving First-Line Antiretrovirals in Muheza, Tanzania

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Although there is concern of drug interactions between artemisinin-based combination treatment (ACTs) for malaria and antiretrovirals (ARVs) for treating HIV/AIDS, very limited information is available about the clinical importance of such interactions. The aim of this study was to examine the efficacy and safety of artemether-lumefantrine (AL), the most commonly used ACT, for the treatment of uncomplicated falciparum-malaria in HIV-positive adults receiving first-line ARVs in Tanzania. The InterACT Study was conducted from July 2009 to September 2012 at Muheza District Hospital in northern Tanzania. HIV-positive adults (>15 years of age) receiving either nevirapine- or efavirenz-based ARVs were enrolled and followed-up for 42 days using WHO standard protocols. Three additional groups of patients were included for comparison: 1) HIV-positive malaria patients not receiving ARVs but treated with AL, 2) HIV-negative malaria patients treated with AL, and 3) HIV-positive patients receiving ARVs but without malaria. Blood levels of lumefantrine were measured on day 7 in all patients receiving AL. A total of 17,269 patients were screened for malaria, amongst whom 385 HIV-positive patients with confirmed malaria were enrolled into the study and followed-up successfully for 42 days. The therapeutic efficacy of AL after parasite PCR-correction was 99% in HIV-positive patients receiving ARVs (total n=193; 106 on nevirapine, 87 on efavirenz), 100% in HIV-positive patients not on ARVs (n=43) and 98% in HIV-negative patients (n=149). Rates of malaria re-infection within 42 days of AL treatment was low and did not differ significantly between the groups. Mild adverse events were commonly recorded in all four patient groups. Severe adverse events were more commonly observed in HIV-positive versus HIV-negative patients, regardless of receiving ARV treatment or not. Day 7 levels of lumefantrine were found to be elevated in patients receiving nevirapine, but were reduced in patients receiving efavirenz. Our results confirm the presence of drug interactions between AL and nevirapine- and efavirenz-based ARVs; however, these interactions were not found to be clinically significant. Our findings thus support the current treatment guidelines for malaria and HIV co-infection in adults.

Validation of a Tool for Predicting Anti-Malarial Drug Mechanism of Action

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Malaria mortality has decreased over the last decade, but these gains are threatened by the emergence of drug resistance to artemisinin in South East Asia. There remains an urgent need to develop new classes of effective anti-malarial drugs. High-throughput screens have identified many drugs and drug-like compounds with anti-malarial activity. However, the mechanisms of action of these potential new drugs as well as for many established anti-malarial drugs is unknown or poorly understood. Systems biology tools that could predict the mechanisms of action of candidate drugs would be valuable for prioritizing drugs for further development. Work in our lab indicates that drug perturbations of Plasmodium falciparum provoke discernible transcriptional signals. These signals contain information about the pathway(s) that a drug targets and can be used to relate drugs by the extent to which their targets overlap. To validate and expand on our capacity to detect subtle drug-specific response signatures in the face of broad biological and experimental variation, transcription profiles were generated for parasites in which the purported pathway targets are genetically disrupted by piggyBac transposon insertions. Transcriptional profiles of a drug-perturbed parent line were compared to lines carrying a genetic perturbation of a related target. We find that genes that are differentially expressed in each genetic perturbation relative to the wild-type control are significantly enriched for biological pathways related to the perturbed genes. Transcriptional signals obtained from drug perturbations of a given pathway overlap with signals observed in genetic perturbations of the same pathway, indicating that a genome wide effort to catalog and relate drug target(s) by their transcriptional response profiles is feasible.

Refining a Tool to Predict Antimalarial Drug Mechanisms of Action

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High-throughput screens have identified many drugs and small molecules with antimalarial activity, usually with no knowledge of their molecular targets. Cost-effective prioritization of these potential drugs would be valuable to avoid pathways that have already developed drug resistance and to highlight compounds with novel mechanisms of action. We measured gene expression profiles in Plasmodium falciparum to construct a transcriptional response database for 31 drug perturbations. New drugs of interest can be queried against these gene expression patterns, identifying shared targets by similar response signatures. So far, successfully identifying drug-specific response signals has required that each gene’s value be normalized across many drug perturbations from the same experiment to compute a drug’s specific ‘response index’ from myriad other experimental and biological sources of transcription variation. However, this is a cumbersome task, and we are therefore exploring modifications to our approach that minimize experimental complexity while still filtering out nonspecific responses. We find that conventional, one drug vs control experiments with replication do not allow for normalization of nonspecific drug responses; consequently, we developed new protocols that can leverage existing data to specify a generalized stress response signature to be used as a standard normalization with each candidate drug. Deeper analysis of the 31 drug response gene expression profiles identified a small subset of drugs with highly diverse mechanisms of action. Normalization with this panel allowed removal of nonspecific culture and perturbation stress and accurate prediction of drug mechanism of action. By optimizing this analysis, we hope to build a standardized community web-based search tool for predicting drug mechanism of action.
PREVALENCE OF THE DHFR AND DHPS MUTATIONS AMONG PREGNANT WOMEN IN RURAL BURKINA FASO SEVEN YEARS AFTER THE INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE

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The spread of drug resistance is one of the major challenges for malaria control in endemic areas. Intermittent Preventive Treatment of malaria in pregnancy (IPTp) with Sulfadoxine-pyrimethamine (SP) is currently recommended by the World Health Organization for preventing the adverse effects of malaria during pregnancy in mothers and their offsprings. In order to assess the evolution of SP resistance in Burkina Faso, we analyzed the prevalence of dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) mutations among pregnant women in samples collected in 2010 and compared our results with retrospective data from 2003. Asymptomatic and symptomatic pregnant women attending Antenal Clinics (ANC) in Nanoro District were invited to participate in the study. All enrolled women were interviewed and examined clinically before taking a blood smear for microscopy examination and a filter paper blood sample for genotyping. Mutations at codons 51, 59, 108 and 164 of the Pf dhfr gene, and at codons 437 and 540 of the Pf dhps gene were examined using PCR-RFLP. Logistic regression was used to calculate odds ratios and confidence intervals for the comparison between 2003 and 2010. The dhfr and dhps genes were successfully genotyped in most samples, respectively 99.6% (255/256) and 90.2% (231/256). The dhfr C59R mutation was the most prevalent (61.2%=156/255) followed by the S108N mutation (55.7%=142/255). The 59R mutant was not observed in the period of 2000-2010 when compared to 1980-1990. In relation to artemisinin (ART) resistance candidate mutations, DNA sequence analysis of the pfdhfr gene showed previously described mutations. Two novel mutations were observed in the pfatp2-µ gene. A new molecular marker for ART resistance (Kelch-13 propeller) is being sequenced in several isolates. No association between the polymorphisms studied and in vivo or in vitro responses to mefloquine, quinine and ART derivatives was observed. This study established the genetic profile of P. falciparum regarding resistance-associated mutations over twenty-seven years, when the parasite was exposed to the selective pressure of several therapeutic schemes adopted in Brazil.

PARASITE CLEARANCE TIME AND IN VITRO SENSITIVITY OF PLASMODIUM FALCIPARUM TO ARTEMETHER-LUMEFANTRINE IN SOTUBA, MALI

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In Mali, previous studies show high frequency (30-40%) of recurrent parasite after treatment with artemether-lumefantrine. We hypothesize that recurrent parasites after treatment have a lower sensitivity to artemether-lumefantrine. We conducted a longitudinal study with 250 field isolates. In vitro field isolates sensitivity to Artemether and Lumefantrine was assessed using hypoxanthine isotopic test. A total of 250 P. falciparum isolates were successfully cultured in vitro and the sensitivities of 25% are available to date. All of the isolates tested in vitro were 100% sensitive to Artemether and Lumefantrine. The mean IC50 values were 3.08 nM and 3.54 nM for Artemether and Lumefantrine, respectively. Malian field Plasmodium falciparum isolates are sensitive in vitro to Artemether and lumefantrine.

THE POLYMORPHISMS IN KELCH AND FALCIPAIN-2 ASSOCIATED WITH ARTEMISININ RESISTANCE ARE NOT PREVALENT IN PLASMODIUM FALCIPARUM ISOLATED FROM UGANDAN CHILDREN

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Artemisinin resistance, manifested as delayed clearance of Plasmodium falciparum following treatment with artemisinins, has emerged in Southeast Asia. SNPs in the PF3D7_1343700 kelch propeller (K13) domain were recently associated with artemisinin resistance after in vitro resistance selection and in clinical isolates, providing a molecular marker to monitor the spread of resistance. The cysteine protease falcipain-2
(FP2; PF3D7_1115700) contributes to artemisinin action via hemoglobin degradation, and it also was mutated after in vitro selection with artemisinin. Although delayed parasite clearance after artemisinin therapy has not yet been noted and artemisinin-based combination therapy remains highly efficacious in Uganda, it was important to characterize the diversity of these genes. We therefore sequenced the K13-propeller domain and FP2 genes in 104 samples collected in 2011-2012 from Ugandan children with malaria emerging after recent exposure to ACTs for treatment (within 28 days of treatment with artemether/lumefantrine) or chemoprophylaxis (monthly DHAPiperaquine). Using 3D7 as the reference genome, we identified polymorphisms resulting in 5 amino acid substitutions in the K13 gene, none of which were among the markers of resistance seen in Asian isolates. No single SNP was found in more than 2 isolates. For FP2, we identified polymorphisms resulting in amino acid substitutions at 24 loci, 17 in the pro and 12 in the mature domain of the protease; these did not include the SNP reported after in vitro selection for resistance. The prevalence of K13 and FP2 polymorphisms did not increase over time, and no SNPs were associated with malaria episodes in which parasite clearance was relatively delayed (persistance ≥2 days after the onset of treatment). These results indicate that the K13-propeller and FP2 coding polymorphisms associated with artemisinin resistance are not prevalent in Uganda. Thus, we see no evidence of artemisinin resistance in Ugandan parasites at present, but continued surveillance for resistance-mediating genotypes is warranted.

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RELATIONSHIPS BETWEEN K13-PROPELLER ALLELES AND ARTEMISININ SUSCEPTIBILITY IN CAMBODIAN AND SENEGALESE PLASMODIUM FALCIPARUM ISOLATES

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Plasmodium falciparum resistance to artemisinin and its derivatives (ART) has emerged in Southeast Asia. ART resistance manifests in vivo as a long parasite clearance half-life after ART monotherapy or ART-based combination therapy (ACT), and in vitro as increased survival of young ring-stage parasites in the ring-stage survival assay (RSA). In Cambodia, several mutations in the PF3D7_1343700 kelch propeller domain (K13-propeller) were recently associated with ART resistance in vitro and in vivo (Anrak et al., 2014). To investigate the role of these and other K13-propeller mutations in ART resistance, and to screen for emerging ART resistance in Africa, we have initiated studies of Cambodian and Senegalese parasite isolates. In collaboration with the Tracking Resistance to Artemisinins Collaboration (TRAC), we are presently adapting Western Cambodian isolates (half-life range, 1.7 – 11.8 h) to in-vitro culture, PCR re-sequencing their K13-propeller domains, and testing them in the RSA as previously reported. Preliminary data from this study have identified parasites carrying the CS080Y, Y493H, R539T, IS43T, and D584V mutations, and have associated these K13-propeller mutations with long half-life and elevated % survival values in the RSA. Among Senegalese samples, we have identified a parasite isolate carrying a novel K13-propeller V637I mutation which has not yet been observed in Southeast Asia. We are now developing genotyping and sequencing assays to screen for these and other K13-propeller mutations in Africa.

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TRENDS IN ANTIMALARIAL MEDICINE AND MALARIA DIAGNOSTIC AVAILABILITY IN CAMBODIA BETWEEN 2009 AND 2013

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The advancement of artemisinin resistance (AR) in the Greater Mekong Subregion threatens the international community’s ability to combat malaria. Ensuring consistent and accurate diagnostic testing, better access to artemisinin-based combination therapy (ACT), and removal of oral artemisinin monotherapy (oAMT) are key activities to contain AR. As such, a thorough understanding of the anti-malarial market is a pre-condition for guiding containment efforts. In 2009, 2011 and 2013, ACTwatch conducted nationally representative outlet surveys in Cambodia. Data from the 2013 survey is currently being analyzed and will be presented at the ASTMH conference. Preliminary results indicate that among outlets stocking antimalarials, nearly all public sector outlets had ACT available and very few had OAMT available in 2009 (ACT: 96%, OAMT: 2%), 2011 (ACT: 97%, OAMT: 0%) and 2013 (ACT: 99%, OAMT: 0%). In comparison, antimalarial availability has been less stable in private sector outlets stocking antimalarials, with an increase in ACT availability (63% to 83%) and a decrease in OAMT availability (20 to <1%) between 2009 and 2013. The percentage of outlets stocking rapid diagnostic tests (RDTs) has increased by 20% in public sector outlets (75% to 95%) and 26% in private sector outlets (37% to 63%) between 2009 and 2013. Logistic regression will be used to compare the rate of change for antimalarial and malaria diagnostic availability between outlet types, urban/rural status and artemisinin tolerance zones. Additional analysis will be performed to identify factors associated with changes in ACT, OAMT and RDT availability in private sector outlets in order to better understand the improved private sector market profile. Several interventions are being implemented in Cambodia to contain artemisinin resistance. The current study demonstrates increasing ACT and decreasing OAMT availability in the private sector, suggesting that the regulation of antimalarial medicine sales and other AR containment efforts may have been successful in improving the antimalarial market profile.

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POOLED SEQUENCING OF MALARIA PARASITES FOR IDENTIFICATION OF DRUG RESISTANCE GENES

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The rapid spread of drug resistance genes through malaria parasite populations distorts the allele frequencies of flanking mutations. Such “selective sweeps” can be rapidly uncovered by pooled polymerase chain sequence, but this approach has yet to be applied to malaria parasites. We explored the potential of pooled sequencing to swiftly and economically identify selective sweeps due to emerging artemisinin (ART) resistance in a South-East Asian malaria parasite population. ART resistance is defined by slow parasite clearance from the blood of ART-treated patients and mutations in the kelch gene (chr. 13) have been strongly implicated to play a role. We constructed triplicate pools of 70 slow-clearing (resistant) and 70 fast-clearing (sensitive) infections collected from the Thai-Myanmar border and sequenced these to high (~150-fold) read depth in an Illumina HiSeq lane. Allele frequency estimates from pools

astmh.org
showed almost perfect correlation (Lin's concordance = 0.98) with allele frequencies at 93 SNPs measured directly from individual infections, giving us confidence in the accuracy of this approach. By mapping genome-wide divergence (FST) between pools of drug resistant and drug sensitive parasites we identified three large (>150kb) regions (on chr. 11, 13 and 14) and 18 smaller candidate genome regions. To identify individual genes within these genome regions we re-sequenced an additional 38 individual parasite genomes (22 slow and 16 fast-clearing) and performed rare variant association tests. These confirmed kelch as a major molecular marker for ART resistance (p=6.03x10^-6), and provide suggestive associations for the involvement of several other genes. This two-tier approach is powerful because pooled sequencing rapidly narrows down genome regions of interest, while targeted rare variant association testing within these regions can pinpoint the genetic basis of resistance.

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SEQUENTIAL EVOLUTION OF DRUG RESISTANCE: EVIDENCE THAT EPIGENETIC REGULATION PRECEDES GENETIC ADAPTATION IN THE ACQUISITION OF HALOFUGINONE RESISTANCE

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Understanding mechanisms of drug resistance is essential for the design of future antimalarial therapies and rational design of drug combinations. To understand the multi-step nature of drug resistance acquisition in Plasmodium falciparum, we performed metagenomic sequencing of six time points in two in vitro drug resistance selections over 60 generations. For these experiments we used the P. falciparum cytoplasmic prolyl tRNA synthetase (cPRS) inhibitor halofuginone. We found that non-genetic adaptation to halofuginone precedes mutation or amplification of the target cPRS gene. Part of this non-genetic adaptation occurs through regulation of cellular amino acid homeostasis. Upon exposure to halofuginone, P. falciparum increases cytosolic proline and overexpresses PSAC determinants cytoadherence-linked asexual gene (clag) 2 and clag 3.2. Using an allelic exchange approach, we found that both cPRS halofuginone resistance mutations HFGRI (L482H) and HFGRII (L482F) only confer halofuginone resistance when clag genes are also overexpressed. Furthermore, by tracking the evolution of two drug resistance selections by whole genome sequencing, we demonstrate that the cPRS locus accounts for the majority of genetic adaptation to halofuginone in P. falciparum. Thus, we provide evidence for a three-step model of multi-locus evolution of drug resistance via genetic and non-genetic adaptations in P. falciparum: first, non-genetic adaptation predominates and permits later acquisition of target-site mutations; second, either target-site mutations or amplifications develop; and third, wild type target-site amplifications take over and out compete less fit target-site mutations. This sequential model of drug resistance evolution has greater implications for malaria drug-resistance surveillance and combination drug development.

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A COLLECTION OF CLONED PARASITES FROM PLASMODIUM FALCIPARUM INFECTIONS SHOWING SLOW OR FAST CLEARANCE FOLLOWING ARTEMISININ TREATMENT

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A collection of laboratory adapted parasites with carefully defined clearance rates following artemisinin (ART) treatment would provide a valuable resource for understanding the mechanism of ART-resistance, the role of kelch, the involvement of other loci and for understanding the costs of resistance. We therefore dilution-cloned 45 infections from hyper parasitaemia patients visiting clinics run by the Shoklo Malaria Research Unit on the Thailand-Myanmar border (2008-12) that showed either very slow (T1/2 > 5) or very fast clearance (T1/2 < 3), determined by 6 hourly measures of parasite density following ART-treatment. The clones isolated were genotyped using 93 polymorphic SNPs to verify their matches to the original infections. Clones identical to the original infection were obtained from 33/45 infections. These 33 genotype-verified parasite clones from a single location, and from the extremes of the clearance rate distribution, will be made available to the research community.

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RESISTANCE MARKER PREVALENCE IN PLASMODIUM FALCIPARUM IN RESPONSE TO RECENT INTRODUCTION OF AL ANTIMALARIAL DRUG

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Due to increasing drug resistance in the malaria parasite Plasmodium falciparum, chloroquine (CQ) was replaced by sulphadoxine-pyrimethamine (SP) in 1998 as first-line treatment for uncomplicated malaria in Kenya. However, after less than a decade following the policy changes, artemisinin-based combination therapies (ACTs) have replaced SP as the most effective anti-malarial option due to widespread SP-resistance. In 2006, the new malaria policy implemented arteether-lumefantrine (AL) as the first-line treatment for uncomplicated malaria and quinine for complicated and severe malaria. Monitoring and understanding the genetic factors underpinning drug resistance is imperative to maintaining effective antimalarial drug administration policies. This study aims to examine the resistance marker prevalence of P. falciparum and determine if there is a transition toward widespread P. falciparum resistance to the recent introduction of AL as compared to previously used drugs in Western Kenya. Sequences of five candidate genes were compared among P. falciparum samples to examine targeted mutations associated with resistance. They are (1) the chloroquine resistant transporter (crt) and multidrug resistance 1 (mdr1) genes for CQ/quinine resistance; (2) the dihydrodipropionate synthase (dhps) and dihydrofolate reductase (dhfr) genes for SP resistance; and (3) the sarcoplasmic reticulum Ca2+-ATPase (atp6) gene for AL resistance. Two comparisons were made to investigate the level of selective pressure on the various resistance genes. The first was between high and low transmission areas. In areas of high malaria transmission, we expect that selective pressure could be greater in parasite populations due to frequent use of drugs. This would be reflected by a higher frequency of the selected codon as compared to parasite populations in low transmission areas. The second compared different resistance genes based on parasite samples from the same area. This comparison allows us to infer the degree of resistance based on genetic
PERSISTENT RESISTANCE TO DISCONTINUED ANTIMALARIAL AGENTS IN PLASMODIUM FALCIPARUM CLONES OF SOUTHWEST INDIA

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Malaria control in India comes with significant challenges, including variations in accuracy of differential diagnosis, the frequent co-occurrence of Plasmodium falciparum and P. vivax, varying drug-treatment preferences for the different species in different localities, and migrations of people across the sub-continent. The impact of this, both on the emergence as well as persistence of drug resistance in this environment, is of interest.

As a part of the US NIH International Center of Excellence for Malaria Research (ICEMR) program activities, we have collected and culture-adapted malaria parasites at Goa Medical College, Bambolim, Goa. This is a tertiary care center with patients presenting uncomplicated as well as severe malaria. This site is of particular interest because Goa stopped using chloroquine for P. falciparum treatment, as national policy favored artesunate-derived combinations about seven years ago. Five years ago, Goa stopped using the nationally recommended first line artesunate-pyrimethamine-sulphadoxine combinations to treat P. falciparum in favor of artesunate-mefloquine. Given the well-known fitness costs of chloroquine-resistance and fansidar-resistance, we expected emergence of sensitivity to these traditional antimalarials. Our studies from 2012-2014, based on DNA sequencing of drug-resistance markers as well as direct phenotypic assays of freshly cultured parasites, point to persistent and frequent occurrence of chloroquine and fansidar resistance. This suggests that there must be unintended but sustained drug pressure from old antimalarials on modern parasite populations in this locality. So far, resistance is not seen to mefloquine and artesunate, the preferred drug combination in Goa. Similar background surveys are planned across multiple ICEMR sites across India, with patient parasite samples collected over a period of five years.

MONITORING ANTIMALARIAL DRUG RESISTANT PARASITES IN WESTERN KENYA

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Artemether-lumefantrine was implemented as first-line therapy for treatment of uncomplicated Plasmodium falciparum malaria in Kenya in late 2006. Armodiaquine-artesunate is registered as an alternative treatment. In vitro drug sensitivity assay and molecular surveillance for markers associated with resistance are useful surrogates to monitor trends in the susceptibility to P. falciparum parasites to anti-malarial drugs. We report findings from a study conducted in hospitals in western Kenya between 2010 and 2013. A total of 204 samples were enrolled. In vitro sensitivity to chloroquine (CQ), mefloquine (MQ), amodiaquine (AQ), artesunate (ART) and dihydroartemisinin (DHA) was determined. Samples were also genotyped for single nucleotide polymorphisms (SNPs) associated with CQ, MQ, AQ, and sulphadoxine-pyrimethamine (SP) resistance. No significant changes in the mean IC50 response to ART or DHA were observed during this study period. A significant decline in the prevalence of the CQ resistant CVR1 genotype and the Pfmdr1 B6Y mutation (both associated with CQ and AQ resistance), was observed between 2010 and 2013. A decrease in the mean IC50 to these drugs was also observed. No increase in the copy numbers of the Pfmdr1 gene and mean MQ IC50 both associated with MQ resistance, was observed. On the contrary, the prevalence of parasites with the combined Pfdhfr/Pfdhps quintuple mutation (51V/59R/108N and 437G/540E), associated with SP resistance, was above 90%. The rare 164L and 581G mutations were observed in 4/204 and 3/204 samples, respectively and a 2.3% increase in the prevalence of the previously described 436H mutation was recorded during this study period. The prevalence of the Pfdhps 436H/437G/540E genotype is on the rise in this region of East Africa. Further microsatellite analysis around the Pfdhps gene demonstrates that it is under selection. In conclusion, the in vitro drug sensitivity pattern for ART and DHA is consistent with in vivo drug trials data conducted by other groups in this region indicating that artemisinin-based combination therapies are efficacious in western Kenya. CQ sensitive strains are increasing, likely due to decreased use of CQ. High prevalence of SP resistant quintuple mutations in this population is an important concern, because SP remains the only recommended drug for intermittent preventive treatment in pregnant women.

EVALUATION OF DIHYDROARTESININ-PIPERAQUINE WITH AND WITHOUT SINGLE-DOSE PRIMAQUINE: AN OPEN-LABEL RANDOMIZED, CONTROLLED TRIAL IN ANLONG VENG, CAMBODIA

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Evidence of dihydroartemisinin-piperaquine (DP) resistance has recently emerged outside of western Cambodia. We are currently evaluating the safety and therapeutic efficacy of DP for uncomplicated Plasmodium falciparum or mixed malaria infections, as well as its transmission blocking potential in combination with single dose primaquine (PQ) in northern Cambodia. Up to 150 volunteers, including those with mild-moderate G6PD deficiency, are being enrolled in a randomized, open-label clinical trial of 3 consecutive daily oral doses of 120/960mg of DP with or without a single 45mg dose of PQ. Transmission blocking is assessed with a mosquito membrane-feeding assay while cardiac safety is evaluated with serial electrocardiograms and time-matched drug levels. In the 107 patients completing follow-up to date, the 42-day per protocol PCR-unadjusted failure rate was slightly higher (45%) that the previously reported PCR-adjusted rate of (36%) among the first 50 volunteers. Mean piperaquine drug levels in the terminal elimination phase were lower than mean in vitro parasite piperaquine IC50 among treatment failures. Mean piperaquine QT-interval prolongation was lower (428ms) than those we previously reported with a compressed 2-day course of therapy (455ms) at Cmax (4 hours after the last dose). Gametocytemia among P.f.-infected patients was uncommon, though limited data suggests that 45mg single-dose primaquine was effective in preventing transmission based on oocyst counts. DHA-piperaquine is rapidly failing in Northern Cambodia, though single dose primaquine (45mg) may help to halt the spread of resistant parasites and should be implemented where adequate safety assessment is
feasible. The standard 3 day course of DP appears to carry a lower cardiac safety risk than a 2 day course and is consistent with previously reported safety data.

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DIHYDROARTEMISININ-PIPERAQUINE TREATMENT FAILURES IN PLASMODIUM FALCIPARUM IN NORTHERN CAMBODIA ARE NOT MEDIATED BY KNOWN ACT RESISTANCE MARKERS

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Dihydroartemisinin-piperaquine (DP), one of the last remaining effective drugs for multidrug resistant Plasmodium falciparum, has been the first line artemisinin combination therapy (ACT) in Cambodia since 2012. However, clinical trials in northern Cambodia in 2010 and 2013 show rising rates of DP failure, reaching a 42-day PCR-corrected recrudescence rate greater than 40%. With nearly 60% of patients remaining parasitemic at 72 hours and increasing piperaquine IC50, treatment failures are likely due to decreased susceptibility to both artemisinin and the partner drug. We are investigating whether these failures are associated with polymorphisms in known or candidate molecular markers of antimalarial resistance. Isolates from approximately 140 Cambodian patients with P. falciparum infections treated with DP are being analyzed. Preliminary analysis of over half of the isolates shows fixation of the CVIET haplotype at pfcr, near fixation of the 184F mutant (96%) in pfmdr1, and wild type alleles only at codons 86, 1034, 1042, and 1246 of pfmdr1. Increased Pfmdr1 copy number (CN) is present in 14% of isolates and is associated with greater in vitro sensitivity to piperaquine (mean PPO IC50 18.3nM vs. 36.7nM in samples with CN>1 and CN<1, respectively, p=0.02). Sequencing of the recently described kelch propeller gene (K13) associated with artemisinin resistance reveals that, as in western Cambodia, mutant K13 alleles are highly prevalent, with only 7.5% showing the wild type allele. Finally, we measured CN of the Xr5 locus, where a duplication on K13 alleles are highly prevalent, with only 7.5% showing the wild type allele. Increased CN at four genes within the Xr5 domain. In sum, none of the molecular markers analyzed correlate with DP treatment failure in these patients. Converely, areas with a disproportionate number of sub-patient infections are likely to represent sink areas where these lingering old infections have little vector contact, and contribute little to transmission. Hence, detection of these infections is of little relevance for elimination programs. The use of more sensitive diagnostic methods that lower the limit of detection of asexual blood stage parasitemia would not address the key limitations of mass screening and treating as a strategy for long-term reduction in malaria transmission. These limitations are the short term impact of the intervention, and inefficiency owing to lack of targeting in space and time of infections that are particularly likely to transmit.

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COSTS OF ELIMINATING MALARIA AND THE IMPACT OF THE GLOBAL FUND IN 34 COUNTRIES

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International financing for malaria increased more than 18-fold between 2000 and 2011; the largest source came from The Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund). Countries have made great progress, but achieving elimination requires sustained financial resources to interrupt transmission and prevent reintroduction. Since 2011, global financing for malaria has declined, fueling concerns that progress in reducing malaria may be impeded, especially for the 34 malaria-eliminating countries that face a particular risk of malaria resurgence if programs are disrupted. This study aims to 1) assess past total and Global Fund funding to the 34 malaria-eliminating countries, and 2) estimate future funding needs to achieve malaria elimination and prevent reintroduction through 2030 in the 34 countries. Historical funding is assessed against trends in country-level malaria annual parasite incidences (APIs) and income per capita. Following Kizewski et al. (2007), program costs to eliminate malaria and prevent reintroduction through 2030 are estimated using a deterministic model. The cost parameters are tailored to a package of interventions aimed at malaria elimination and prevention of reintroduction. The majority of Global Fund-supported countries experiencing increases in total funding from 2005 to 2010 coincided with reductions in malaria APIs and also overall GNI per capita average annual growth. The total amount of projected funding needed to achieve elimination and prevent reintroduction through 2030 is approximately US$8.5 billion, or about $1.84 per person at risk per year (PPY) (ranging from $2.51 PPY in 2014 to $1.43 PPY in 2030). Although external donor funding, particularly from the Global Fund, has been key for many malaria-eliminating countries, sustained and sufficient financing is critical for pursuing global malaria elimination. Projected estimates of costs through elimination should help countries identify funding gaps and mobilize resources to obtain adequate financing to achieve their goals.
A CASE STUDY OF MALARIA ELIMINATION IN THE ECUADOR-PERÚ BORDER REGION

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In recent years malaria has been successfully controlled in the Ecuador-Perú coastal border region. The incidence of disease declined from a high of 2,117 per 100,000 in 1999 in El Oro Province, of southern Ecuador, to 0 as of 2011. The rate declined concomitantly in Pirua Province of northern Peru during this same period. Our aim is to document this control effort and identify the best practices and lessons learned that are broadly applicable to malaria control and other vector borne diseases.

We conducted a proximal outcome evaluation of the robust elimination program in Ecuador, using El Oro Province as a case study. We conducted semi-structured group discussions with public health experts who played central roles in the elimination effort, reviewed epidemiological records by the Ministry of Health, and reviewed national policy documents to produce a detailed timeline of events, a list of the crucial programmatic and external factors of success and the barriers faced, and to identify the important lessons learned. We found that the binational Ecuador-Peru collaboration was the most important component of the elimination program. This unique relationship created a trusting, open environment, allowing for flexibility, rapid response, innovation and resilience in times of crisis, and ultimately a sustainable control program. Strong community involvement, an extensive microscopy network and horizontal, intersectoral collaborations at the local level were also identified as key programmatic strategies. The results of this study provide key principles of a successful malaria control program that can be looked to by other nations and regions currently working to control malaria infection. These principles should be disseminated to the next generation of public health professionals in the region and serve as a guide to ongoing and future control efforts of other emerging vector borne diseases in this region and elsewhere.

ACTIVE CASE DETECTION OF MALARIA AND URINARY SCHISTOSOMIASIS IN PUPILS OF KOTTO BAROMBI, SOUTHWEST CAMEROON USING THE CYSCOPE® FLUORESCENCE MICROSCOPE

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Malaria and urinary schistosomiasis (U.S.) remain important public health issues in Kotto Barombi, South-west Cameroon even though the village benefits from free distribution of treatment of both diseases by health authorities. Accurate diagnosis and prompt treatment of malaria and U.S. are necessary for their elimination. Novel tools for rapid and mass diagnosis of malaria and other parasitic infections have recently been developed. This study was aimed at assessing their use in the field to determine the prevalence of malaria and U.S. in Kotto Barombi. Blood and urine samples were collected from 94 boys and 122 girls aged 3 to 14 years. Malaria and U.S. were diagnosed with a rapid test using pre-stained slides for fluorescence microscopy (CyScope®, Partec GmbH, Goerlitz, Germany). Furthermore, Malaria parasite detection and speciation were done using Giemsa-stained blood films. Lugol direct examination was performed for U.S. Performance characteristics of CyScope® for malaria and U.S. were determined. Overall prevalence of malaria was 19.0% and 41.2% for light and fluorescence microscopy respectively (X2=15.33, P=0.00019). Malaria prevalence and density were similar in the different age groups, sexes, socio-economic class (SEC) and nutritional class. Overall prevalence of anemia was 18.5%. Sensitivity was 68.3% and specificity was 64.9% for malaria. Overall prevalence of U.S. was 43.4% and 48.5% for light and fluorescence microscopy respectively. Many cases of co-infection (19.9%) were recorded. Mean intensity of US was 8.1±27.02. Prevalence and intensity of U.S. were significantly higher in the Kotto Barombi Island (78.3%, 33/42) than mainland (33.8%, 52/154). U.S. prevalence was similar in the different age groups, sexes and SEC. Sensitivity of the test was 90.6% and specificity was 83.8%. The CyScope® could be a good tool for active case detection of both diseases especially in areas that lack electricity since it can be battery operated. Drastic measures need to be taken for the elimination of these diseases.

IDENTIFICATION OF THE MOST EFFICIENT INTERVENTION PACKAGES ACROSS DIFFERENT EPIDEMIOLOGICAL STRATA: AN APPLICATION TO PLASMODIUM FALCIPARUM MALARIA TRANSMISSION AND MORBIDITY IN AFRICA

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Reducing the burden of major endemic infectious diseases is a global priority, but financial constraints require available resources to be allocated rationally to maximise impact. This poses particular challenges
MATING COMPETITIVENESS OF STERILE MALE ANOPHELES COLUZZI IN SEMI-FIELD CAGES

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The renewed interest in the development of control strategies using sterile insects raises hopes of being able to control the disease by cutting down the high reproductive rate of mosquitoes. Specifically, investigations into factors that account for male mating competitiveness are critical to the development of genetic control strategies. In this study, we assessed the effects of partial irradiation with 75 Gy on Anopheles coluzzii sexual competitiveness when allowed to mate in different ratios of sterile/fertile males for 2 nights in field cages. Moreover, to determine the dynamics of this competition, competitiveness was compared between males allowed 1 night vs 2 nights of contact with females. Sterilized (S) and fertile (U) males between 4 and 6 days of age were released in field cages (1.70mx1.70mx1.70m) with females (F) of similar age and left for 2 nights at the following ratios (S:U:F): (100:0:100) (100:100:100) (0:100:100). Each treatment was replicated 3 times. Competitiveness was determined by assessing the hatching rate of eggs laid en masse and the insemination rate, determined by dissecting recaptured females. An additional experiment with a ratio of (500:100:100) has been done with a mating period of either 1 or 2 nights. For the first experiment, the egg hatching rate was significantly affected by the release ratio and we thus observed that the Fried competitiveness index of sterile males was between 0.29 and 0.55. A similar insemination rate was recorded after 2 nights of contact in experiment 1, while significant difference was observed in the (S:U:F) (100:0:100) ratio between the males left to mate for 1 and 2 nights. However, a similar hatching rate was observed when mating occurred for 1 or 2 nights. The results suggest a release ratio of at least 2 sterile males for 1 fertile male and that An. coluzzii mating competitiveness experiments in field cages should be run for 1 instead of 2 nights.
health facility malaria incidence before and after the scale-up of malaria diagnostic and treatment services at the community level in Southern Province, Zambia. We controlled for environmental drivers of malaria incidence using remotely sensed variables, and adjusted for temporal autocorrelation. After accounting for environmental factors, implementing CHW case management of malaria was associated with an 8.2% (95% CI = 4.1% - 12.2%) reduction of outpatient attendance at the health center. When combining the additional malaria cases actively and passively identified through CHW case management with monthly confirmed health center incidence, implementation of CHW case management was associated with a 45.5% increase in monthly confirmed malaria incidence (95% CI = 33.5% - 58.6%). The implementation of CHW case management has both shifted the burden of outpatient care away from the health center into the communities as well as expanded the access to malaria treatment.

REDUCING MALARIA AMONG MIGRANTS AND MOBILE WORKERS IN THE GREATER MEKONG SUB-REGION BY BROADENING OPPORTUNITIES FOR MALARIA SERVICES AND PREVENTION

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Migrant and mobile populations are a difficult to reach population and often coined a ‘hot population’ in malaria control programs in the Greater Mekong Sub-region. Engaging in construction and agricultural production in remote areas where malaria is common, these workers often lack malaria knowledge or immunity, and information on and access to preventive measures and curative services. They could serve as a source of infection introducing malaria to historically low- or non-transmission areas. The PMI Control and Prevention of Malaria (CAP-Malaria) Project has implemented innovative approaches to reach migrant and mobile populations in remote border areas of Thailand, Cambodia, and Myanmar where malaria is concentrated. The project utilizes a multi-pronged approach that provides malaria information and services at multiple points that migrants typically visit or pass through while in the region and returning home. For instance, malaria information is provided by transportation services taking migrants to their employment destinations. Migrants may also obtain information and services during arrival or departure at malaria posts located at border crossings or border malaria clinics. Mobile malaria workers (community volunteers) and mobile clinics with medical staff frequent locations with high concentrations of migrants. Large multinational companies as well as smaller agricultural employers in both Cambodia, Thailand and Myanmar are participating in a lending scheme through which migrants can borrow a long-lasting insecticidal net (LLIN) for the duration of their employment. In Cambodia, a radio show reaches residents and mobile populations in remote areas, providing malaria information and promoting a malaria hotline for one-on-one malaria discussions. Engagement of taxi and bus drivers in providing information on malaria prevention and health seeking behavior are vital for mobile and migrant populations prior to reaching highly malaria endemic zones. During the past 2 years, the project has reached over 50,000 migrant workers in Cambodia, 439,345 in Myanmar, and 3,600 in Thailand.

USING INCIDENT MALARIA CASES TO FIND ASYMPTOMATIC MALARIA INFECTIONS IN SOUTHERN PROVINCE, ZAMBIA: WHICH CASES INCREASE THE PROBABILITY OF FINDING INFECTIONS?

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As malaria transmission declines it becomes heterogeneous, characterized by pockets of sustained transmission. Finding and removing the pockets of sustained malaria transmission is of utmost importance to malaria elimination programs. Incident malaria cases may be indicative of malaria transmission, and returning to the household of incident malaria cases may be one way in which to target pockets of residual malaria transmission. In Southern Province, Zambia reactive case detection (RCD) has expanded to included >1500 community health workers (CHWs) operating out of >260 health centers. CHWs return to the household of a rapid-diagnostic test (RDT) confirmed incident malaria case and screens individuals within 140 meters for a malaria infection using RDTs, treating those positive. Detailed records of each reaction have been kept by CHWs in Southern Province, and are currently being digitized for further data analysis. These records contain information on the incident malaria case demographics as well as the demographics of individuals screened during reactions. Preliminary analyses of aggregated RCD data suggest that RDT positivity during reactions exceed RDT positivity during passive case surveillance more often in the dry season, when mosquito habitat as measured through remotely sensed enhanced vegetation index is limited, and when the ambient temperature as measured through remotely sensed land surface temperature is lower. These results suggest that incident malaria cases during the dry season may be more indicative of pockets of asymptomatic malaria infections than incident malaria cases during the wet season. Further analyses with the digitized CHW records will investigate what effect different demographics of incident malaria case affect the ability to find pockets of asymptomatic malaria infections using each individual reaction as the unit of analysis. A more robust analysis of the environmental factors affecting the probability to find pockets of asymptomatic malaria infections will also be conducted. Full analyses will be completed by June 2014.

SPATIO-TEMPORAL CHARACTERISTICS OF MALARIA INCIDENCE IN LUSAKA, ZAMBIA: TOWARDS MALARIA ELIMINATION IN AN URBAN ENVIRONMENT

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Malaria elimination may be more feasible from within urban areas than rural areas, due to differences in Anopheles habitat. Lusaka, Zambia has documented zero malaria infections in children under the age of five in Malaria Indicator Surveys conducted in 2010 and 2012 suggesting elimination of malaria transmission from within Lusaka is near. However, health facilities in Lusaka during this time period continue to record confirmed malaria incidence. Enhanced surveillance of incident malaria cases in Lusaka, Zambia was initiated in 2011. All confirmed incident malaria cases are questioned about history of travel outside Lusaka and previous malaria episodes. Case investigations are undertaken for incident malaria cases without a history of travel, wherein all members of the 9 adjacent households are screened with a rapid diagnostic test.
and treated with artemether-lumefantrine if positive. We describe the temporal characteristics of health facility malaria incidence with and without a history of travel in relation to remotely sensed estimates of mosquito habitat. We use spatial scan statistics to estimate the location of spatial clusters of health facility malaria incidence without a history of travel within the catchment areas of 5 health centers in Lusaka using remotely generated population-weighted controls. Eighty percent of confirmed incident malaria cases report a history of travel outside Lusaka in the previous month, with travel being most common in December and January. Incidence rates of malaria without a history of travel follow the suspected seasonal pattern and are highly correlated with those of malaria reporting a history of travel (rho = 0.82). Significant clusters of malaria incident cases were found within each health center catchment area assessed. In all but 1 of the catchment areas, greater than 75% of incident cases without travel were located within clusters. The heterogeneous spatial pattern of malaria incidence in Lusaka is clear - identifying and targeting clusters of malaria transmission will be critical in the pursuit of elimination.

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IMPLEMENTATION AND APPLICATION OF A MULTIPLEX ASSAY TO DETECT MALARIA ANTIBODIES: A PROMISING TOOL FOR ASSESSING MALARIA TRANSMISSION IN PRE-ELIMINATION AREAS

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In countries that move towards pre-elimination, malaria is concentrated in hotspots. To identify these hotspots for targeted and effective interventions it is crucial to improve the understanding of the effectiveness of malaria control tools. However, in areas with a low prevalence and incidence of malaria, such as Cambodia, detection of parasitological indicators is very insensitive. Seroprevalence is based on the detection of antibodies against antigens of malaria parasites, which offers an advantage as anti-Plasmodium antibodies can persist for months after infection. Therefore, a multiplex assay has been recently developed for the simultaneous detection of multiple antibodies. An assay based on 14 Plasmodium specific peptides, 1 peptide specific for Anopheles gambiae salivary protein and 5 Plasmodium specific recombinant proteins was developed for the MAGPIX system and applied on blood spots from 2000 individuals collected in the Ratanakiri province, Cambodia. This project fits within the framework of a research project that aims to evaluate the effect of mass use of safe and effective mosquito repellents on the malaria transmission, in addition to the use of impregnated mosquito nets (MalaResT). For all antigens the assay performed equally well in monoplex and in multiplex formats (p<0.001). High specific antibody levels and a high seropositivity were detected for antibodies against Pf-LSA3-RE, Pf-CSP, Pf MSP1-19, Pf-GLURP, Pf-SALSA2 and Pf-GLURP-R2 with the comparison between the different serological markers. Blood samples positive for malaria by PCR showed a higher response to some of the antigens as compared to PCR negative samples. An increase in seropositivity was observed with increasing age. Differences in seropositivity were observed between different districts, indicating the heterogeneity of malaria transmission within the most endemic province of Cambodia.

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MALARIA ELIMINATION - NOT YET ACCOMPLISHED IN ZANZIBAR

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Zanzibar represents a unique case study for potential malaria elimination in a previously high endemic area in sub-Saharan Africa. The Zanzibar Malaria Elimination Programme (ZAMEP) has implemented modern malaria control interventions since 2003. These interventions included combined vector control with long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) as well as improved malaria case management with rapid diagnostic tests (RDT) and artesinisin-based combination therapies (ACT) in all public health facilities. We have studied temporal trends of different malaria indices such as incidence of parasitologically confirmed malaria infections in public health facilities, crude child mortality and community parasite prevalence in two districts of Zanzibar, North A and Micheweni, between 1999 and 2013 in a population of about 200,000 people. Malaria transmission declined already after introduction of ACT and dropped further after addition of intensified vector control. Health facility data showed a 96% reduction in positivity rates (from 38.2% to 1.6%) of parasitologically confirmed malaria cases in the two districts between 2002 and 2012 with a relative shift towards older age groups and an increasing seasonability of the malaria incidence. In North A district, all cause <5 years mortality rate decreased by 70%. A population survey in 2013 revealed a malaria prevalence of 0.3% determined by RDT representing a 97% reduction compared to 2003 (10.3%). However, the parasite prevalence as determined by PCR was 10-fold higher, indicating a remaining reservoir of asymptomatic low-density parasitemias. Moreover, signs of minor increase of reported malaria infections from public health facilities occurred in Micheweni district in 2012 compared with 2010-2011. Malaria control in Zanzibar has reached a level equivalent to malaria pre-elimination. However, a new malaria epidemiological context has emerged necessitating new tools and strategies as well as re-orientations of ongoing control activities in order to further reduce malaria transmission.

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STOCKS AND SHOPS: THE IMPORTANCE OF HEALTH SYSTEMS STRENGTHENING WITHIN PRIVATE SECTOR OUTLETS FOR THE DELIVERY OF ARTEMISININ COMBINATION THERAPIES (ACTS) TOWARDS MALARIA CONTROL AND ELIMINATION

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There remains debate regarding the inclusion of Informal sources of antimalarials, ranging from drug shops and pharmacies to general village stores, within national malaria control planning. The Affordable Medicines facility for malaria (AMFm) has been effective in increasing stocks of subsidised quality-assured Artemisinin Combination Therapies...
(ACTs) within drug shops but the impact of this initiative remains unclear. We extended an existing mathematical model of malaria transmission to include health systems factors: access to sources of ACTs in the private, public and tertiary sectors, and quality of care for malaria and non-malarial febrile illness (NMFI). Data from the IMPACT 2 study in Tanzania was used to parameterise the model. Our aim was to estimate the impact of interventions in the private sector on malaria mortality and parasite prevalence (i.e. transmission risk). We compared the effect in three settings, which differed by epidemiology and prevailing health systems. Model outcomes suggest in areas where there is high private sector preference, strengthening of public facilities has less impact than anticipated. Improving stocks of ACTs in the private outlets, as per AMFM, was predicted to be more effective than in public clinics in all three regions considered, with a greater relative impact on parasite prevalence than mortality; likely due to high levels of NMFI overtreatment thereby opportunistically treating asymptomatic and sub-patent infections. Modelling the rollout of diagnostic led therapy in drug shops reduced missed cases and thus mortality without increasing prevalence, but only if adequate stocks of ACTs were present. At low and medium transmission settings, pharmacy accreditation schemes to limit the diversity of informal private sources, and improve ACT stock and dispensing practices may have the potential to interrupt transmission, reducing parasite prevalence by up to 86% in scenarios with private sector preference. However investment would be required across the spectrum of case management to improve the quality of care delivered. Innovative methods need to be found to harness the private sector cost-effectively in the push for control and the quality of care delivered. Innovative methods need to be found to

929 IDENTIFYING AND CHARACTERIZING ASYMPTOMATIC MALARIA INFECTIONS IN ZANZIBAR: THE RESIDUAL PARASITE RESERVOIR

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Asymptomatic Plasmodium infections are an important reservoir for continued malaria transmission and must be targeted to achieve malaria elimination. Sensitive molecular methods have shown that asymptomatic infections are often of very low-density in low endemic settings, frequently falling below the detection limit of both rapid diagnostic tests (RDT) and microscopy. This study aimed to identify and characterise asymptomatic low-density malaria infections in Zanzibar by active case detection employing community-based screening for Plasmodium infections by real-time PCR. Dried blood spots on filter paper (Whatman 3mm) were collected from asymptomatic individuals participating in cross-sectional surveys conducted in 2005, 2009, 2011 and 2013. For each respective year 548, 2324, 2978 and 3038 samples were screened for parasite DNA using a highly sensitive Cytochrome B SYBR green real-time PCR for Pan-Plasmodium detection and restriction fragment length polymorphism assay for species determination. The PCR determined asymptomatic parasite prevalence was 25.7% (95%CI 21.7-29.0), 3.3% (2.6-4.0), 2.2% (1.6-2.8) and 2.3% (1.7-2.8) in 2005, 2009, 2011 and 2013, respectively. The corresponding yearly microscopy/RDT positivity rates were 10.9% (95% CI 9.2-12.7), 0.0% (0.0-0.3), 0.7% (0.4-1.3) and 0.3% (0.13-0.54). Children 5-15 years and young adults 15-25 years had higher prevalence of asymptomatic malaria (range 2.7-52%) and 3.7-13.7%, respectively) as compared to children <5 years and adults (range 0.5-24.5% and 1.7-8.6%). P. falciparum remained the predominant species (2.0-25.4%) followed by P. malariae (0.3-3.1%); no cases of P. ovale or P. vivax were identified. Although the asymptomatic malaria prevalence has declined since 2005, this study revealed a substantial remaining reservoir of low-density, sub-microscopy/RDT but PCR detectable parasitaemias among asymptomatic individuals in Zanzibar. The results highlight the need for sensitive molecular methods for identifying and targeting the residual parasite reservoir in malaria elimination programmes.

930 CLONAL OUTBREAK OF PLASMODIUM FALCIPARUM IN EASTERN PANAMA

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Reemerging parasite populations threaten to undermine efforts of global malaria eradication. Identifying the source of resurgent parasites is therefore paramount to strategic and successful intervention for malaria elimination. Genetic tools such as the malaria barcode to “fingerprint” parasite lineages are anticipated to help identify sources of parasite outbreaks and track their spread. Panama has been notably successful in reduction of Plasmodium falciparum with only one reported case in 2011. Although malaria incidence in Panama has remained low over the past two decades, a major outbreak from 2001 to 2005 resulted in more than 60 percent of all cases reported over the past 35 years. We hypothesized that parasites from this epidemic might be highly related genetically and exhibit a largely clonal population structure. We thus tested the relatedness of Plasmodium falciparum parasites from this outbreak using informative single nucleotide polymorphisms and also examined drug resistance loci from among these parasites. We found the parasites to be clustered into three clonal subpopulations (AMOVA, 96%, p = 0.001) among eastern Panamanian isolates and shared genetic relatedness with parasites sampled from Colombia. Structure analysis revealed there was most likely two populations (ΔK = 108), however given the clone-corrected clusters and AMOVA results, we found the second most likely number clusters (ΔK = 61) revealed additional sub-population structure. Two clusters of Panamanian parasites from the epidemic shared identical drug resistance haplotypes, and all clusters shared a chloroquine resistance genotype matching the pfcrtp haplotype found among parasites of Colombian origin. Our findings suggest these resurgent parasite populations are highly clonal and likely resulted from epidemic expansion of imported or vestigial cases. Outbreak investigation using genetic tools can illuminate the relatedness and potential sources of epidemic malaria cases and guide strategies to prevent further resurgence in areas of malaria elimination.

931 THE AGRICULTURE-MALARIA RELATIONSHIP AND THE CONTRIBUTIONS OF DELOS LEWIS VAN DINE TO THE MALARIA PROBLEM IN THE SOUTHERN U.S.

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Human malaria has a strong association with agricultural activity. Increased food production via cultivation and irrigation often increases malaria risk in malaria-endemic zones, creating a conflict between agricultural enterprises and health. Conversely, malaria stultifies agricultural productivity by causing illness in laborers at critical times. This presentation highlights the efforts of USDA entomologist Delos Lewis Van Dine to quantify the impact of malaria on cotton production in Louisiana in 1914 and argues that it is was the first effort to connect malaria and agriculture fiscally. The study, conducted on the Hecla Plantation in the Mississippi delta, showed high malaria prevalence in black tenant farmers beholden to white plantation owners and that lost profits of $3.98 per acre could be attributed to malaria. It further demonstrated that impoundment of bayou waters and

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water level manipulation eliminated Anopheles breeding near tenant farmers’ shacks. Overall, this poorly known but seminal project provides an early, effective demonstration of intersectoral malaria control well before drugs and insecticides were available and that investment from the agricultural sector was key to malaria reduction.

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SUBMICROSCOPIC PARASITEMIA IN RURAL AND URBAN SOUTHWESTERN NIGERIA

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Epidemiology of submicroscopic parasitemia will become more relevant in determining malaria control measures in the era of elimination. This study evaluates the performance of microscopy and qPCR in detecting and quantitating malaria parasites in an urban and a rural sites in South West Nigeria. Cross-sectional surveys were conducted in hospital laboratories in rural Ijede and urban Lekki, spanning the period 2008-2010. Malaria parasite detection and quantitation using microscopy and qPCR were compared for these two sites. Prevalence at the urban site was 29% (330/1158) by PCR and 11% (111/997) by microscopy while at rural Ijede corresponding values were 30.2% (197/652) and 25%(168/658) respectively. The differences between the prevalence values at both sites were not significant by PCR (χ²=0.52, P=0.47) but very significant by microscopy (χ²=57.6, P<0.0001). Overall composite sensitivity for microscopy(41%) was lower compared to PCR (95%) while their respective specificities were 98% and 95%. At individual study sites, sensitivity of microscopy was better at the rural site with 98% than at the urban site 30% and specificities at both sites were 99% and 98% respectively. Sub-microscopic parasitaemia at the rural site was less, 74/197(37.5%), compared with the urban 211/336(62.2%), (χ²=30.34, P<0.0001). This was reflected in variance in their geometric mean parasite density(GMPD) observed-6999 pg/µL at the rural, and 2701 pg/µL at the urban sites by microscopy and corresponding values of 4693 pg/µL and 1100 pg/µL by qPCR (Cohen’s Kappa agreement 0.48, Spearman’s rank’s correlation coefficient was 0.5, CI 0.37-0.59, mean difference of log units in the Bland Altman plot of 0.87, and 95% limits of agreement(mean ± 2SDs) between the two methods, 1.5 to 3.1.In spite of apparent lower prevalence of malaria parasites by microscopy in rural Ijede compared to urban Lekki, the actual prevalence when PCR technique is employed shows that parasite prevalence are similar in both study sites. Result from this study highlights a possible role for more sensitive diagnostic method in urban and other apparent regions of diminished parasitemia load and prevalence, over rural settings.

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ON THE ESTIMATION OF MALARIA TRANSMISSION INTENSITY USING SEROLOGICAL DATA: POWER CALCULATIONS AND ESTIMATION BIAS

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Parasite prevalence and entomological infection rate are two popular measures of malaria burden in a given population. An alternative measure is the so-called sero-conversion rate that can be estimated from sero-prevalence data of specific anti-parasite antibodies in the serum using a series of reverse catalytic models. In theory, sero-conversion rate is the frequency by which individuals convert from a sero-negative state into a sero-positive one upon parasite challenge. In practice, sero-conversion rate has proven to be strongly correlated with the underlying force of infection, suggesting its routine use in malaria epidemiology. Statistically speaking, little is known about the expected behavior of the corresponding estimates under different estimation methods, sampling schemes, and disease dynamics. This knowledge is particularly instrumental in helping the design of future field studies. In this work we perform a comprehensive theoretical study using simulated data from typical cross-sectional, health-facility and school surveys. Specifically, we generate sero-prevalence data assuming either a constant sero-conversion rate over time or assuming a change in it after a field intervention. We assess the underlying estimation bias and statistical power in order to calculate the minimum sample size required to (1) estimate sero-conversion rate with a given precision, (2) or to detect any change in disease transmission after an intervention with a given probability. We also suggest different statistical strategies to reduce estimation bias introduced by using convenience sampling or the maximum likelihood estimation method. Finally, we present a new R package for sero-prevalence data analysis that can be easily used by malaria epidemiologists.

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THE POTENTIAL TO TEST AND TREAT MALARIA IN NIGERIA: RESULTS FROM NATIONAL OUTLET SURVEYS (2009-2013)

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The WHO T3 initiative: Test, Treat and Track promotes effective management of suspected malaria cases through diagnosis, effective treatment and accurate surveillance. In Nigeria, children with fever are taken to private (46%) or public sector providers (18%) for treatment. We assessed readiness of these outlets to provide malaria testing and treatment and the cost to the consumer. Nationally-representative antimalarial medicine outlet surveys were conducted in 2009, 2011 and 2013 as part of the ACTwatch project. A comprehensive census of all outlets with the potential to stock antimalarials within selected clusters was conducted. Outlet types were classified as either public/not-for-profit or private for profit. The primary monitoring indicators were the weighted proportion of outlets offering/stocking any malaria diagnostics or Quality Assured Artemisinin-based Combination Therapy (QAACT). Data on the cost QAACTs to the consumer were also collected. Among outlets that had any antimalarial medicines in the public sector, 22% (2009) and 31% (2011) reported having malaria blood testing available on the day of interview. This compares with 2% (2009) and 4% (2011) of outlets in the private sector. Availability of QAACTs in the public sector remained stable at 44% (2009) and 48% (2011). In the private sector availability remained low at 7% (2009) and 10 % (2011). In the private sector, QAACT prices decreased from $3.94 (2009) to $1.40 (2011). 2013 survey findings will be reported at the conference. Across survey rounds, availability of malaria blood testing and QAACTs is lower in the private sector compared to the public sector. While readiness for malaria case management is improving in the public sector, nearly half of all children with suspected malaria in Nigeria are taken to the private sector where most of these providers are not equipped to appropriately test and treat cases. Improving readiness of the private sector for malaria case management in Nigeria may be an important strategy to improve effective coverage.
FORECASTING THE BURDEN OF MALARIA IN UGANDA USING CLINICAL AND ENVIRONMENTAL PREDICTORS

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Malaria thrives in poor tropical and subtropical countries where local resources are limited. Accurate disease forecasts can provide public health and clinical organizations with the information needed to implement targeted approaches for malaria control that make effective use of limited resources. Previous malaria forecasting work has not considered clinical predictors, such as antimalarial treatment, which is an important predictor of malaria burden in a population. The objective of the research was to identify the environmental and clinical predictors that produce the most accurate forecasts of malaria at six different health facilities in Uganda. Malaria forecasting models were developed using health facility data collected by the Uganda Malaria Surveillance Project and satellite-derived rainfall, temperature, and vegetation estimates. Short-term (4-week) and long-term (52-week) weekly forecasts of confirmed malaria from June 1, 2012 to May 31, 2013 were developed for each health facility using multivariate transfer function models. The model with the most accurate forecasts varied by site. Clinical predictors were retained in the most accurate models across all facility sites with the exception of one model. The average short-term error ranged from 20% to 96% over the forecasting period. The long-term models performed best for predicting the cumulative cases with error ranging from 2% to 22%. Study limitations included not knowing if the confirmed malaria cases were incident or recrudescent as well as measurement errors associated with the remote sensing and clinical data sources. Incorporating clinical predictors such as type of antimalarial treatment, improved the forecasting accuracy of several of the models. These results demonstrate the utility of using clinical predictors in conjunction with environmental predictors to forecast malaria. With the mounting cost of the global fight against malaria and the drive towards elimination in many countries, accurate forecasts of malaria remain essential.

FETAL GROWTH RESTRICTION IS A MAJOR FACTOR INVOLVED IN GESTATIONAL MALARIA IN BENIN

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Malaria in pregnancy (MiP) leads to low birth weight, which is mainly due to intra-uterine growth restriction in Africa. However, when and how malaria impacts on fetal growth is still unknown. We investigated this issue using data on 774 Beninese pregnant women from the STOPPAM cohort. They were screened for malaria (using blood smears) every month during pregnancy until delivery and had 4 ultrasound scans at 20, 26, 30 and 36 weeks of gestation, allowing repeated measurements of growth within the same pregnancy. For the analysis, birth weights (BW) and estimated fetal weights for gestational age were converted into Z-scores using Tanzanian sex-specific charts as reference. First, BW Z-scores were compared between women infected and uninfected with malaria during pregnancy. Then, the effect of malaria on fetal growth, measured as a change in Z-scores between two consecutive measurements, was assessed. Both analyses were adjusted for potential confounding factors such as maternal undernutrition, maternal anemia and gravidity. More than 40% of women had at least one malarial infection during pregnancy. We showed that women infected in the first and at least one subsequent trimester of pregnancy had significantly lower BW Z-scores (-0.40 [-0.78; -0.01]) than uninfected women during pregnancy. We did not find that malaria limited to either the 1st trimester or the 2nd/3rd trimester was associated with BW Z-scores, suggesting a cumulative effect of malaria on fetal growth. A decrease in Z-scores during pregnancy was significantly associated with malaria infections that occurred several weeks before the decrease (Z-scores decrease in women infected with malaria compared to non infected women: -0.23 [-0.40; -0.05]), but not with recent malaria infections. In conclusion, we confirmed the effect of malaria on fetal growth in Africa. Our results suggest both a long-term and cumulative effect, starting from the 1st trimester onwards.

USING GENOME-WIDE SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) TO TRACK MALARIA SPREAD AMONG LOCAL COMMUNITIES IN THE MYANMAR-CHINA BORDER AREAS

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Border areas are reservoirs of malaria because of frequent and recurrent parasite introductions via human movements. Areas along the international border of Myanmar and China have reported high malaria incidence in recent years. In Myanmar, civil unrest and establishment of internally displaced persons (IDP) camps along the Myanmar-China border have impacted much of the malaria transmission in the region. The growing IDP populations raise deep concerns about health impact on local communities. Moreover, in remote countryside of poor accessibility and general negligence, and where the ethnic minorities reside, over 60% of the populations are potentially at high risk of malaria. This study used genome-wide Single Nucleotide Polymorphisms (SNPs) and microsatellite markers to examine the source and spreading patterns of malaria parasites (Plasmodium falciparum and P. vivax) between IDP camps and surrounding villages in Myanmar, as well as villages/towns in the border areas of Myanmar-China. We compared genotypic composition of Plasmodium samples collected in 2011, 2012, and 2013 from the same area and examined demographic history with the goal to determine whether recent infections were caused by the same or different parasite genotypes. In addition, we tested if IDP camps are the source of malaria infections particularly concerning border-malaria cases based on genetic diversity differences among localities. Broadly, we tested the hypothesis of whether P. vivax is genetically more diverse than P. falciparum under the same environmental and temporal settings at a local scale. In-depth knowledge and information on the extent of malaria spread are keys to target disease control efforts in high-risk areas such as those near international borders and remote countryside. This is of particular relevance when most other parts of Southeast Asia are entering the malaria elimination phase.
USE OF GPS DATA LOGGERS TO DESCRIBE SPATIO-TEMPORAL HUMAN MOVEMENT PATTERNS IN AN AREA OF EFFECTIVE MALARIA CONTROL IN SOUTHERN ZAMBIA

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The role of human movement in malaria transmission dynamics, particularly in pre-elimination settings, has not been fully elucidated. GPS data loggers allow for micro-scale estimates of human movement in rural areas of sub-Saharan Africa, which can aid in explaining the micro-epidemiology of malaria importation and transmission in these areas. Participants currently enrolled in a longitudinal cohort study of the impact of malaria control measures in a region of declining malaria transmission in southern, Zambia (one of three Southern Africa International Centers of Excellence for Malaria Research sites) were invited to participate in a population movement study using GPS data loggers. Approximately 10 participants at a time were asked to carry the loggers for a one-month period. Data will be collected over 12 months to account for seasonality in movement patterns. Enrollment began in October 2013 and is ongoing. Serial numbers of GPS data loggers were matched to participant IDs and geographic position logged every two minutes. Movement data from the GPS loggers were imported into ArcGIS for pre-processing and analysis. The movement tracts were used to determine the cumulative amount of time spent in different areas, derived from the frequency of visits and amount of time spent during each visit to different areas. An intensity map was created to display the cumulative amount of movement in different areas. An analysis of time spent in and around the residence was conducted. The distribution of time spent in and around the household and time spent at varying distances from the household was analyzed, and the cumulative amount of time spent in areas of high and low malaria risk was calculated. An analysis to determine whether time spent inside an area of high malaria risk is due to residence in a hotspot or routine travel to a location of high malaria risk was conducted.

PLASMODIUM VIVAX MALARIA IS NOT A MAJOR THREAT IN MADAGASCAR

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Published reports on malaria in Madagascar in the last seven decades showed that Plasmodium vivax, P. ovale and P. malariae are present; however, P. falciparum remains the predominant species. Effort has been achieved with the introduction of artemisinin-based combination therapy (ACT) and the scaling-up of the vector control since 2006. The last two malaria index surveys among children in Madagascar from 6 to 59 months of age (n = 6154 in 2011 and n = 5504 in 2013) showed that malaria transmission is highly variable in different areas. Global malaria prevalence ranged from 1% in the highlands to 15% on the rainy eastern coast. P. falciparum infection was responsible for >99% of the cases. In those two surveys, only one P. vivax infection (1/863) and two P. malariae infections (2/863) were detected. It was shown recently that P. vivax is capable of breaking through the Duffy-negative barrier in Madagascar, leading some policy makers to consider introducing P. vivax specific treatments. Available data, however, indicate that at this time, P. vivax is not a real threat in Madagascar. With its local population of both Asian and African origin, Madagascar is an ideal place to investigate the fundamental aspects of the P. vivax biology including the molecular mechanisms underlying red blood cell invasion. The key issues in malaria elimination in Madagascar are the recurrent shortage of stocks of ACT, the interruption of insecticide indoor spraying and the irregularity in insecticide treated bed net distribution. Nonetheless, the impact of the intervention against malaria in Madagascar on P. vivax between 1940 and 2000 will be also discussed in our presentation.

MALARIA IN BORDERLANDS: A CASE STUDY FROM THE THAILAND-MYANMAR BORDER

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The central plains of Thailand have been mostly malaria free now for several decades. However, malaria continues to persist in the borders connecting the Thai Kingdom with Laos, Cambodia, Malaysia, and Myanmar. The reasons for this persistence are complex. These are areas with high historical, ethnic, and linguistic diversity. These borderlands have been exposed to political instability that has existed across the borders in Cambodia, Laos, and Myanmar. The common logic on the ground is that the malaria situation on the Thai side is a spill-over effect of the malaria situation on foreign soil. In this research, we take a micro, spatial-epidemiological approach to understanding border malaria, using a study site along the Thai-Myanmar border. The underlying goals of this work were to empirically test for demographic factors (including migration) as risks for malaria infection; in the potential for asymtomatic carriers to exist in a region that is considered to be a “low transmission” area; and in mapping space-time patterns in malaria cases in households. We find that a large proportion of cases are missed by microscopic diagnosis, that migration does not appear to be a significant risk factor for individuals or household members who live with migrants, and that individuals without
citizenship (either in Myanmar or Thailand) exhibit a significantly higher risk of malaria infection when compared to any other demographic group. Finally, the spatial distribution of malaria cases clusters tightly around year-round water sources within the village during the dry season, but expands throughout much of the village during the wet season. Taken together, these factors indicate that the border malaria situation is much more complex than generally considered. While this research does not rule out the potential of malaria importation from Myanmar, it does show that such importation isn’t necessary for continued persistence of malaria on Thai soil.

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**USING GEOSTATISTICAL METHODS TO ANALYZE SPATIO-TEMPORAL CHANGES IN CHILDHOOD MALARIA FOLLOWING SCALE-UP OF CONTROL EFFORTS IN CHIKWAWA DISTRICT, MALAWI**

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Geostatistical methods are increasingly used to support disease control efforts and analyse spatial variation in disease prevalence. These methods are especially useful in settings where disease registries are non-existent or geographically incomplete, and data on disease prevalence must be obtained via cross-sectional surveys of the population of interest. In order to obtain timely, accurate and (sub-)district estimates of prevalence, however, continuous prevalence surveys could provide a more powerful approach for identifying local hotspots and guiding more targeted control efforts. Applying novel model-based geostatistical methods for malaria mapping, we estimated changes in the spatial distribution of malaria prevalence over time using data from a continuous Malaria Indicator Survey conducted within a 20 x 20 km area of Chikwawa District in Malawi, from May 2010 to June 2013, a period of district-wide scale-up of malaria control. We developed a statistical model that accounts for temporal and spatial variation in prevalence, and over-dispersion within households. This approach allows us to quantify the uncertainty in prevalence estimates more accurately than standard statistical methods that ignore the temporal and spatial correlation induced by unmeasured risk factors for malaria and, as a result, give an exaggerated impression of regression relationships. To model seasonality accurately we used a linear combination of sinusoidal curves at different frequencies. The resulting time-series of malaria prevalence maps show how the model can provide an evaluation of control progress. The method can also be used to predict changes in prevalence that would result from different control progress scenarios, by running the model with different values for the input variables. Finally, visualizations using animations of spatio-temporal prevalence allow for a more intuitive interpretation by end-users that can guide more targeted control efforts towards hotspots.

### 943

**DETERMINING THE CHANGE IN MALARIA ACROSS AFRICA FROM 2000 TO 2012**

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Decreases in malarial prevalence over the past decade have been mainly associated with the expansion of vector control measures such as insecticide-treated nets (ITNs) and changes in environmental and socioeconomic factors. Here we seek to formally evaluate the determinants and patterns of the change in malaria prevalence from 2000 to 2012 across all of Africa. We first build Bayesian spatial models of malarial prevalence using a large assembly of geopositioned parasite rate surveys, along with new dynamic environmental and socioeconomic covariates varying through time. Then, using a combination of Bayesian compartment and spatial modelling we build models of ITN usage through time. Using this rich data set of prevalence, environmental factors, socioeconomic factors and ITN usage, all modelled continuously at a 5km by 5km resolution across all of Africa, we characterise the change that has occurred in the last decade.

### 944

**AIR TEMPERATURE SUITABILITY FOR PLASMODIUM FALCIPARUM MALARIA TRANSMISSION IN AFRICA 2000-2012**

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Air temperature affects multiple aspects of the malaria transmission cycle, including vector survival and sporozoite development. These factors have been combined within a biological model to produce a single metric (temperature suitability) that is useful as a predictor variable in modeling and mapping malaria endemicity. In this study we improve on previous research endeavors by creating a dynamic temperature suitability product for Plasmodium falciparum in Africa, from 2000-2012, with a 1km spatial resolution, and a monthly temporal resolution. The temperature suitability product is generated using land surface temperature data derived from satellite imagery, which is converted to air temperature using an approach we develop, and then ingested into an improved version of an established temperature suitability model. Results indicate that the dynamic temperature suitability product is an improvement over earlier synoptic products, particularly with respect to its ability to characterize spatio-temporal patterns in areas with seasonally variable infection rates.

### 945

**CONSENSUS FORECASTS OF CLINICAL DISEASE BURDEN FROM PLASMODIUM FALCIPARUM: A STOCHASTIC MODELING APPROACH**

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Mechanistic transmission models for “micro-simulation”—the stochastic, computational realization of disease dynamics at the individual level within a mock human population—represent a key tool for forecasting the burden of clinical disease from community prevalence estimates. These models reveal a complex, non-linear relationship and age-dependence to the parasite prevalence—clinical incidence relationship, owing to the effects of exposure-driven immunity, which in turn depends on a combination of the historical mean entomological inoculation rate and its seasonality profile. In this study we examine the predictions of multiple model variants and forge consensus forecasts of Plasmodium falciparum disease burden on national and continental scales.
MALARIA TRANSMISSION, INFECTION AND DISEASE AT THREE SITES WITH VARIED MALARIA TRANSMISSION INTENSITY IN UGANDA: IMPLICATIONS FOR MALARIA CONTROL

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Intensification of control interventions has led to reductions in malaria burden in some, but not all settings. To describe the malaria epidemiology in Uganda, we conducted entomological surveys and cohort studies at 3 sites with varying transmission intensity. All households were enumerated in 3 sub-counties: Walukuba (peri-urban), Kihii (rural) and Nagongera (rural). In each sub-county, 100 houses were randomly selected for measures of malaria transmission, infection, and disease over 24 months. Annual entomological inoculation rate (AEIR) was estimated from monthly collections using CDC light traps. The incidence of malaria diagnosed, only 9 (0.3%) met criteria for severe disease with no cases of cerebral malaria or deaths. The prevalence of moderate (Hb<10) and severe (Hb<7) anemia were 8.5% and < 0.4%, respectively, and did not vary by transmission intensity. In the setting of LLINs and prompt effective treatment, the risk of complicated malaria and anemia was very low. However, the burden of malaria remains high and increased at two rural sites, suggesting that additional malaria control interventions are needed in Uganda.

THE RELATIONSHIP BETWEEN THE PREVALENCE OF MALARIA IN PREGNANT WOMEN AND THE PREVALENCE OF MALARIA IN CHILDREN AND NON-PREGNANT WOMEN IN SUB-SAHARAN AFRICA

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In malarious areas, malaria is more frequent in pregnant women than in age-matched non-pregnant women and the magnitude of the excess risk varies with age and exposure to malaria in previous pregnancies. Pregnant women attending for their first antenatal clinic visit are a potential pragmatic sentinel group to track malaria transmission intensity, however the relationship between malaria infection prevalence in asymptomatic pregnant women and other transmission indices, such as the prevalence of malaria infection in children obtained from population based cross-sectional surveys, a commonly used indicator for malaria transmission intensity, is not yet known. To determine if pregnant women can provide an alternative source of transmission intensity information, we evaluated the relationship between the prevalence of malaria among asymptomatic pregnant women and a) asymptomatic non-pregnant women and b) asymptomatic children (0-59 months) in the same area. Studies in sub-Saharan Africa were obtained using the malaria in pregnancy library (January 2014) and national surveys. We used random effects meta-analysis and meta-regression. The summary risk ratio (RR) of the malaria prevalence among pregnant vs non-pregnant women was 1.45 (95% CI 1.33-1.59), I-square 68% among all gravidae (51 studies in 32 records), and 2.10 (1.82-2.43), I-square 74% among primigravidae (19 locations). Information from 58 studies (18 records) was available for the comparison between pregnant women and children aged 0-5 years. The prevalence was highest in children: compared to all gravidae RR 1.45 (1.31-1.60), I-square 80%, and compared to primigravidae RR 1.13 (1.00-1.28), I-square 72% (5 studies). The malaria prevalence among primigravidae at the first antenatal booking visit may be a good approximation of the prevalence of malaria in children obtained from household surveys.

STATISTICAL INFERENCE OF PLASMODIUM FALCIPARUM TRANSMISSION NETWORKS BASED JOINTLY ON EPIDEMIOLOGICAL AND GENETIC DATA

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Decisions about how to best allocate finite resources for malaria surveillance and control depend on a range of factors, including epidemiological quantities such as the proportion of local versus imported cases, the origin of imported cases, and variation in local transmission potential. Measurement of these quantities is difficult, however. For a variety of viral and bacterial pathogens, recent advances in genetic sequencing and statistical inference methodology have enabled the estimation of transmission linkages between small geographic areas or groups of infected hosts, and in some cases between individual hosts. Methods for making such estimates currently depend on the assumption that ample genetic variation is generated by neutral mutations on a timescale that is fast relative to that of transmission. Because this assumption is likely violated for Plasmodium parasites, which undergo a sexual reproductive phase and have a lower mutation rate than viruses, existing methods to build transmission networks from genetic and epidemiological data cannot be applied to malaria. To overcome these obstacles, we have developed bespoke statistical methodology for making inferences about transmission linkages between human malaria cases. This methodology makes use of the multi-locus genetic composition of an individual’s parasites, the individual’s home location, and the date when the infection was detected. We make explicit assumptions about the processes that generate and erode genetic variation, including superinfection, the importation of parasites from source populations, and the stochastic loss of alleles over the course of multiple transmission events. Using this mathematical framework for epidemiological dynamics and parasite evolution, together with Bayesian statistical techniques, we made inferences about transmission networks using simulated data and data sets from Zanzibar and Swaziland. These networks can be used to determine both population and individual-level parameters, such as $R_0$ and the probability of a given infection being imported, respectively. We furthermore used simulated data to explore the robustness of this method to assumptions about the proportion of cases detected, mechanisms of inheritance, and epidemiological heterogeneities. Our results demonstrate the potential for this method to estimate key epidemiological parameters for malaria surveillance and elimination.
VARIABLE MALARIA PREVALENCE ON ISLANDS IN LAKE VICTORIA, WESTERN KENYA

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Kenya launched its second National Malaria Strategy with a notably ambitious vision for a “malaria free Kenya”, but malaria remains a major health problem among communities in Lake Victoria. We conducted two malariometric surveys during the dry (January) and wet (August) seasons in 2012 to determine the prevalence, geographical and seasonal variation of malaria among communities in the coast (Ungoye: population ~2,000) and on islands of Lake Victoria (Mangano [large island]: population ~25,000; Takawiri, Kibuogi, and Ngodhe [small islands]: population ~700 each). Overall, parasite rates (PRs) as determined by microscopy (18.9% vs 14.5%), rapid diagnostic test (36.9% vs 31.9%) and PCR (31.1% vs 25.8%) were higher in the dry season than in the wet season (P<0.01) with characteristic age distribution. The highest prevalence by RDT was observed in Ungoye (dry season): 54.4% in age group 0-5 years old, 68.4% in 6-10, 55.3% in 11-15, 13.2% in 16-30 and 11.3% in >30, while the lowest was in Takawiri: 13.1%, 21.5%, 11.5%, 7%, 1%, respectively. Species-specific prevalence by PCR was 29.3% for Plasmodium falciparum, 8.5% for P malariae, and 2.1% for P ovale in the dry season, and 24.5%, 6.0%, and 2.1%, respectively, in the wet season. No P vivax was detected. Prevalence of mixed infections was 8.3% and 5.8% in the dry and wet seasons, respectively. In both seasons, PRs were highest in the coast, followed by large island and lowest in the small islands, with significant fluctuations in islands but not the coast. Fluctuations in PRs among island settings were significant only in children and young adolescents but not in adults. PRs were correlated with prevalence of fever in both seasons (P<0.05), however they were correlated with enlarged spleen in the dry season only (P<0.01). Paradoxically, PRs were either not correlated (wet) or negatively correlated (dry) with rates of anaemia. Overall prevalence of G6PD deficiency was 12% in male and 2% in female. No significant correlation between the deficiency rates and PRs was observed among islands and the coast. Variation in malaria prevalence reflected the different dynamic of malaria transmission between the islands and the coast of Lake Victoria. Our results provide baseline data for the planned feasibility study of malaria elimination on islands in Lake Victoria.

MALARIA AND ANEMIA PREVALENCE TWO YEARS FOLLOWING MASS NET DISTRIBUTION IN PLATEAU STATE, NIGERIA

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Nigeria suffers the world's largest malaria burden and in 2009 it launched a plan to provide two long-lasting insecticidal nets (LLINs) to every household via state-level mass distribution campaigns. In Plateau State (pop. 3.6 million) in central Nigeria, a baseline survey was conducted in September 2010 prior to mass net distribution of 1.4 million LLINs in December 2010. Two years later (October 2012), a modified malaria indicator survey was conducted to compare changes in net ownership, utilization, IRS coverage, Plasmodium prevalence and childhood anemia. In each survey >1,300 households in at least 58 clusters were sampled. Household ownership of at least one net increased from 35.1% in 2010 to 74.0% in 2012 (P<0.001), while ownership of two or more nets increased from 14.5% to 50.1% (P<0.001). In 2012, households had a mean of 1.6 nets per household, 0.62 nets per sleeping space and 0.31 nets per person. Overall reported net use the night before the survey among all individuals, children <5 years, and pregnant women was 49.0%, 59.0% and 60.5%, respectively in 2012 among all households (all P<0.001 versus 2010) and 64.5%, 77.6% and 79.1%, respectively, among households owning at least one net (all P<0.001 versus 2010). IRS coverage remained low (<1% in both surveys). Between 2010 and 2012, crude Plasmodium prevalence by microscopy decreased by 58% from 47.7% (n=4,209) to 19.9% (n=3,911; P<0.001). However, parasite prevalence by rapid diagnostic test (RDT) in 2012 (36.7%) was not significantly lower compared to 2010 RDT prevalence (40.5%, P=0.16) using the same test (CareStart Pf/PAN). Prevalence was highest among children 5-9 years old. Plasmodium malariae accounted for 3.7% of infections diagnosed by microscopy. Anemia in children ≤10 years was equally prevalent in 2012 (57.8%) and 2010 (57.1%). These results, believed to be the first state-level report of the impact of mass net distribution in Nigeria, document significant increases in net coverage and usage that correspond with a decrease in parasite prevalence as diagnosed by microscopy, but not RDT.

DETERMINANTS OF SOCIO-ECONOMIC STATUS AND RISK OF MALARIA INFECTION IN PANAMA (2009-2012)

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In the Americas malaria remains a major problem in areas with low socioeconomic-status (SES). In order to achieve elimination, current strategies must have a multi-sectorial approach that addresses social and environmental determinants of malaria infection. The current focal low incidence of malaria in Panama presents a unique opportunity to start an elimination phase. In this study we examine the epidemiology of malaria in Panama with special reference to social and environmental determinants of malaria infection at the corregimiento level (smallest political division), analyzing census demographic and epidemiological data for 2,295 malaria cases between 2009-2012 in 621 corregimientos. Our analysis indicated that the burden of P vivax infections by health region was higher among Amerindian reservations (where the incidence of Plasmodium falciparum and vivax infections was estimated between < 1 % to 8.4 % respectively), and female individuals less than 15 years old. In order to test the hypothesis that those corregimientos with the highest proportion of type 2 households were more likely to be infected, we performed a multivariate logistic regression analysis to evaluate the association between risk of malaria infection and type 2 houses, controlling for other predictors of low SES such as dirt floor, lack of potable water, lack of electricity, lack of sanitary facilities, unemployment and illiteracy. Results indicated that those corregimientos with the highest proportion of type 2 households were more likely to be infected with malaria (OR = 43.24 (2009), 821.20 (2010), 1359.23 (2011) 729.52 (2012) ( p < 0.05 ), all other predictors held constant. Pairwise correlations indicated a protective effect of type 1 households (p < 0.05), while risk of malaria infection was positively correlated with determinants of low SES such as type 2 houses, dirt floors, illiteracy and lack of electricity but was not with lack of potable water, sanitary facilities or unemployment. In conclusion, risk of malaria infection was associated with corregimientos having the highest proportion of type 2 households. We expect that this data will help implement a multi-sectorial approach for the elimination of malaria based on determinants of SES in Panama.
DETERMINANTS OF EFFECTIVE DELIVERY OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) IN SUB-SAHARAN AFRICA: A MULTI-COUNTRY ANALYSIS

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Malaria infection during pregnancy leads to adverse health outcomes for both mothers and infants. IPTp of at least two doses of sulphadoxine-pyrimethamine (SP), administered at antenatal care (ANC) visits, is an effective malaria prevention intervention. Despite increasing investment in IPTp programs over the past decade, and despite high rates of attendance at ANC visits, use of IPTp remains low. To identify bottlenecks in IPTp delivery, service effectiveness analyses were performed on data from 16 Demographic and Health Surveys (DHS) and Malaria Indicator Surveys (MIS) conducted between 2007 and 2011 in malaria-endemic countries in SSA. Multi-country, pooled, multivariate logistic regressions were used to identify determinants of IPTp delivery. Distributions of key determinants were compared for lower IPTp coverage countries (<20% IPTp use) and higher IPTp coverage countries (≥20% IPTp use). IPTp was effectively delivered for only 18% of targeted women. Access to ANC services was not identified as a major reason for this low rate. In fact, 83% attended ANC at least once and 97% of those receiving one dose of SP attended ANC twice. However, levels of SP delivery to those attending ANC was lower: 42% of those attending one ANC visit received one SP dose, and 57% of those attending two ANC visits received two SP doses. Determinants of IPTp use included number of ANC visits, receipt of other maternal health interventions, and malaria transmission level. Effectiveness of IPTp delivery systems varied substantially between higher and lower IPTp coverage countries. Women in higher coverage countries made fewer ANC visits, attended ANC for the first time earlier in gestation, and were more likely to use ANC services at public or religious facilities than were women in lower coverage countries. Results show that most pregnant women are obtaining ANC services at sufficient frequency and appropriate timing to permit IPTp delivery, but the intervention is not being effectively delivered in these settings.

ACCESS TO MALARIA CONTROL INTERVENTIONS AMONG SCHOOL-AGE CHILDREN IN MALAWI

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In Malawi, school-age children have the highest rates of malaria illness at health facilities and asymptomatic infections in the community. Whether this is due to age-related biological susceptibility or difference in access to malaria control interventions is unclear. We used community-based surveillance data to assess interventions, including bednet use and prompt diagnosis and treatment, in school-age children (6-15 years) compared to younger children (0-5 years) and pregnant women, groups that are typically the target of anti-malaria interventions. During cross-sectional surveys in September 2012 and May 2013, we enrolled 7653 participants. School-age children were less likely to report using bednets (64.5/1130, 57%) than younger children (600/790, 76%, p<0.001) and pregnant women (57/74, 77%, p<0.001). Among school-age children, the likelihood of bednet use was not affected by gender or rural vs. urban setting, but they were more likely to sleep under a net in the rainy season and when the ratio of household members to nets was lower (2.6 vs 4.5 people per net, p<0.001). Effects of bednets in this age group will be determined by comparing rates of anemia, microscopic and submicroscopic parasitemia. Fever in the last two weeks was reported in fewer school-age children (163/130, 14%) than younger children (200/790, 25%; p<0.001). Among those with fever, there was no difference between the groups in seeking treatment or duration of fever prior to seeking treatment. School-age children were most commonly taken to a shop for treatment compared to younger children, who were most commonly taken to a government health facility. By parent report, the two groups were equally likely to be tested for malaria and to receive antimalarial drugs. In Malawi, school-age children have less access to antimalarial interventions than younger children and pregnant women. This may partially explain their high rates of disease and infection. New interventions targeting this group or strategies to increase access to existing programs may decrease both disease burden and transmission.

MALARIA IN CHILDREN UNDER-TWO IN RURAL SINDH, PAKISTAN - RESULTS OF A COMMUNITY BASED ACTIVE SURVEILLANCE STUDY

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Burden of malaria is not clearly defined in Pakistan, and is believed to be sporadic and limited to certain high risk areas. These high risk zones have not been clearly defined. With the MDGs and Roll Back Malaria’s targets approaching in 2015, it is important to characterize this malaria burden better. We aimed to determine the incidence of malaria in children less than two years old in a rural community typically considered at low risk of malaria. Prospective surveillance was carried out in children between 0 - 24 months over a period of two years from October 2011 - November 2013 in the district of Matiari, in Sindh, Pakistan. Children of parents and / or guardians able to give informed consent were recruited as newborns, and underwent routine fortnightly active surveillance for two years. Children meeting the World Health Organization’s Integrated Management of Childhood Illness’ criteria for febrile illness and severe pneumonia were tested for malaria using Malaria Pf/Pan rapid immunochromatographic tests. Positive malaria cases were confirmed by light microscopy. Febrile children had weekly follow-ups and were treated or referred. 817 children were followed and total child-years of follow-up were 1374 after adjusting for children lost to follow-up or missed on surveillance visits. 409 (50.1%) were male. Malaria incidence rate was 9.5 cases per 1000 child years (95% CI 5.0 - 16.1). No cases of neonatal malaria were detected. Clinical presentation was nonspecific and overlapped with pneumonia. There were no mortalities due to appropriate treatment and follow-up. Malaria in rural Sindh in Pakistan is a more common occurrence than previously recognized. This has implications for diagnostic and management algorithms used for young febrile children in the community.

THE TRIPLET METHOD: A QUICK AND RELIABLE METHOD FOR DELIVERING PARASITE INFECTION CLEARANCE AND DETECTION SENSITIVITY ESTIMATES

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Malaria infection and clearance rates can be estimation by analysis of longitudinal molecular typing data. In general, infections appear and disappear over short time intervals, but these highly dynamic patterns
are mainly the result of imperfect detection owing to samples that fall below the detection limit of PCR. Several different statistical techniques, such as hidden Markov models, have been used to estimate infection and clearance rates allowing for imperfect detection, but these can be difficult to implement. We present improvements on a simple method for estimating force of infection for Plasmodium falciparum from longitudinal typing data. The longitudinal sequence of observations for any one genetic type of parasite is evaluated as overlapping triplets observations, at time steps zero, one and two in order to estimate the true clearance rate between times steps zero and one. Counts from the set of triplets beginning with a positive measurement at time step zero are evaluated as realizations of a multinomial distribution conditional on the true clearance and detection sensitivity. We amend the original method to estimate the force of infection, clearance, and detection rates, as part of the same Bayesian model. The triplet method is straightforward to calculate, does not require extensive statistical modelling, and produces estimates comparable to more involved approaches for longitudinal data. Missing data are naturally accommodated as a category of the multinomial. We provide a program that calculates the carriage prevalence for each genotype, estimates of infection incidence, and the clearance rate, all corrected for imperfect detection. The method applied to the Albina directory cohort (ages five to nine) of the TransEp study in Papua New Guinea produces overall Plasmodium falciparum parasite mean daily clearance rate of 0.142 [0.005, 0.743] and annual incidence of infection 4.011[3.837, 4.093] (median[95% CI]); both the recovery rate and the infection rate are higher than those found for younger children in Tanzania and Ghana using similar methodology.

INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED WITH RAPID DIAGNOSTIC TEST (RDT)-POSITIVITY IN A HIGH MALARIA TRANSMISSION SETTING OF NORTHERN ZAMBIA, 2012-2013

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While malaria transmission has declined substantially throughout parts of Zambia, some areas continue to experience high transmission levels despite deployment of malaria control efforts. Understanding factors associated with continued malaria transmission in these areas may inform control efforts. Household malaria surveys were conducted in Nchelenge District, Luapula Province, Zambia. Households were enumerated based on satellite imagery and randomly chosen within selected grid cells. Households were enrolled into cross-sectional (one visit) or longitudinal (visits every other month) cohorts; analyses were restricted to cross-sectional and the first visit to longitudinal households. During study visits, adults and caretakers of children were administered a questionnaire and a blood sample was collected for a malaria rapid diagnostic test (RDT). Individual and household level factors associated with RDT positivity were analyzed using logistic regression models. A total of 1,201 individuals from 339 households were enrolled. Over the study period, 43% of participants were RDT positive. Over half of RDT positive individuals were between the ages of 5 and 17 years, and half of RDT positive individuals had visited a health center or health post for malaria in the past 6 months. In the multi-variable logistic regression analysis, RDT positive individuals were over twice as likely to be between the ages of 5 and 17 years as compared to children younger than 5 years (Or=2.06, 95% CI: 1.23, 3.44), over half as likely to report a fever within the past two weeks (Or=1.57, 95% CI: 1.04, 2.37), and 73% more likely to live in a household using an open well as the main water source (Or=1.73, 95% CI: 1.15, 2.6). RDT positivity was highest among children and adolescents between the ages of 5 and 17 years. RDT positives were more likely to experience symptoms and have sought care. Open wells may be a breeding site for mosquito vectors, potentially contributing to malaria transmission.
years tested (HE = 0.8 - 0.85). Differentiation between Anhui and Jiangsu was modest (FST = 0.1). Several clusters of isolates with identical multilocus haplotypes were observed across both Anhui and Jiangsu. Linkage disequilibrium was strong in both populations and in each year tested (IAS = 0.2 - 0.4) but declined two- to four-fold when identical haplotypes were accounted for, indicative of occasional epidemic transmission dynamics. None of 5 imported isolates shared identical haplotypes to any of the Central Chinese isolates. The population genetic structure of P. vivax in Central China highlights unstable transmission, with limited barriers to gene flow between the central provinces. The challenge of imported cases and risks of resurgence emphasise the need for continued surveillance to detect early warning signals. Although parasite genotyping has potential to inform the management of outbreaks, further studies are required to identify suitable marker panels for resolving local from imported P. vivax isolates.

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PLASMODIUM VIVAX MORBIDITY AFTER RADICAL CURE: A TWO-YEAR COHORT STUDY IN THE PERUVIAN AMAZON

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The current national treatment guidelines for vivax malaria in Peru include a 7-day primaquine (0.5mg/kg/day) course together with chloroquine (25mg/kg, 3days). In order to evaluate the efficacy of this protocol a cohort of P. vivax infected patients was treated and monitored for 2-years. The study was conducted between 2008 and 2011 in 29 communities around Iquitos city. Plasmodium 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 303 P. vivax infected individuals were recruited and treated, and 270 (89%) of them completed follow-up. At baseline, males and females were equally represented and the median age was 20 years [IQR: 11-38]. Two late parasitological failures, both at day 28, were detected, though at the same time point 16 participants (5%) had a PCR detectable P. vivax infection. Almost half (144) of the participants had P. vivax recurrent infections most of them (70%) repeatedly (median=3, range [2-11]). The incidence of P.vivax recurrent infections by microscopy and by PCR was analyzed using negative binomial regression; and time to event was analysed by survival analysis and cox regression; and time to event was analysed by survival analysis and cox regression. Results will be compared to a sister cohort study carried out in Vietnam. In conclusion, after radical treatment, a high number of P. vivax recurrent infections were observed, most of them asymptomatic and submicroscopic. Our results show that a substantial amount of the P. vivax transmission occurs silently in the Peruvian Amazon, a finding that calls for improved diagnosis and treatment if elimination is to be achieved.

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SEIZURE OCCURRENCE DURING TWO-YEAR FOLLOW-UP OF PEDIATRIC MALARIA PATIENTS: PRELIMINARY OBSERVATIONS

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Seizures are common following cerebral malaria (CM). Previous studies have demonstrated that epilepsy develops in 5-10% of survivors of CM. Less is known regarding the incidence of provoked and unprovoked seizures in other forms of severe malaria. At Mulago Hospital, Kampala, Uganda we conducted a prospective cohort study of pediatric malaria between 2008 and 2013. Children with cerebral and severe malarial anemia (SMA) were enrolled, with community children (CC) without a history of seizures or neurodisability enrolled as controls. The children’s caretakers were asked whether the child had seizures during follow-up at 6, 12 and 24-month visits. 218 children with CM, 180 children with SMA and 182 CC were enrolled. Seizures occurred before admission in 205 children with CM (94%) and 3 children with SMA (1.7%), and during admission in 125 children with CM (57%) and 2 children with SMA (1.1%). 182 children with CM, 164 children with SMA, and 171 CC completed 2-year follow-up. Among children who completed follow-up, 5 children with CM (2.7%), 11 children with SMA (6.7%) and 2 CC (1.2%) had febrile seizures during the follow-up period, while 4 children with CM (2.2%), 1 child with SMA (0.6%) and 0 CC (%) had unprovoked seizures during follow-up. 3 children with CM (1.6%), 1 child with SMA (0.6%) and no CC met the definition of epilepsy (two separate incidents of unprovoked seizures). In this cohort of children with CM or SMA, febrile seizures were reported, but epilepsy was infrequent, occurring in <2% of the cohort.

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NEXT GENERATION DURABLE WALL LINERS, A NEW STRATEGY FOR MALARIA CONTROL: BASELINE RESULTS FROM A CLUSTER RANDOMIZED TRIAL

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Despite widespread adoption of long lasting insecticide nets (LLINs) and indoor residual spraying for vector control, malaria remains a major source of morbidity and mortality. Pyrethroid insecticide treated wall liners (ITWL) have been efficacious in reducing malaria prevalence in small-scale studies, however the increasing prevalence and distribution of pyrethroid resistance poses a threat to many pyrethroid-based vector control strategies. A new generation ITWL made of polymer based fabric impregnated with a mixture of non–pyrethroid insecticides has been developed. ITWLs act as a physical barrier to vectors and they release insecticide gradually, killing vectors that come into contact with them. They are expected to last several years before needing to be replaced. Like IRS the efficacy of ITWLs does not depend as heavily on human behaviour as LLINs do. A three arm cluster randomized controlled trial is underway in Muheza district, north-eastern Tanzania to measure and compare the efficacy and cost effectiveness of (1) LLIN (reference group), (2) IRS + LLIN, and (3) ITWL + LLIN. We present baseline findings collected prior to the randomisation of clusters into study arms. The study area was mapped and a census was performed. Sixty clusters were created, and 15 to 20 houses were randomly selected from each. All consenting household members were
asked demographic and behaviour questionnaires. Blood samples were drawn for malaria diagnosis, anaemia testing, and immunochromatographic testing (ICT) for *Wuchereria bancrofti*. Entomological evaluations by WHO cylinder tests were performed. A total of 92,538 individuals from 24,198 households in the study area were enumerated, and 3208 people from 954 houses were sampled in the baseline epidemiological survey. Malaria parasitemia by mRDT was 21.6% (95% CI: 20.2-22.1%) and 15.7% (95% CI: 14.4-17.2%) were positive for *W. bancrofti* by ICTs. Anaemia prevalence for under fives (haemoglobin <8g/dL) was 4.3% (95% CI: 2.7-6.0%). Malaria infection was more common in 5-12 years olds 37.2% (95% CI: 33.5-40.9%) compared with < 5 children 17.8% (95% CI: 14.6-21.0%) (p = 0.001). WHO cylinder tests showed reduced susceptibility to pyrethroids of *An. gambiae s.l.* 24 h post exposure ranging from 51 to 90%. When completed, this study will provide important information to National Malaria Control Programs and international agencies to guide future malaria control strategy and allocation of resources.

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**FACTORS ASSOCIATED WITH DECREASED PLASMODIUM FALCIPARUM INFECTION RISK IN MALIAN CHILDREN**

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Malaria control efforts remain suboptimal for many reasons including knowledge gaps in malaria epidemiology and immunology. To better understand the epidemiologic and immunologic factors associated with risk of *P. falciparum* infection and clinical malaria, we conducted a longitudinal cohort study of 695 individuals aged 3 months to 25 years in the rural village of Kalifabougou, Mali. We did bi-weekly active surveillance for *P. falciparum* infection by PCR and weekly active clinical surveillance for clinical malaria. Nearly all adults and children over four years of age became infected during the malaria season at a rate that was independent of age (log-rank test, p = 0.37), indicating that sterile immunity to *P. falciparum* infection is not acquired through natural exposure; and as expected, the risk of clinical malaria decreased with increasing age (log-rank test, p = 0.0038). Surprisingly, we observed that children under 4 years of age were less likely to be infected with *P. falciparum* compared to older subjects (p <0.0001), and indeed, 24% of children under 4 years of age remained PCR negative throughout the intense 6-month malaria season. Exposure was measured by antibody response to gSG6, an Anopheles gambiae specific salivary protein, and results indicate that uninfected children were less likely to have serologic evidence of exposure to the mosquito vector over the course of the malaria season. Self-reported bed net use was not different between infected and uninfected children. Because evidence of decreased exposure did not fully explain decreased infection risk in young children, we are taking several approaches to test the hypothesis that uninfected children have enhanced pre-erythrocytic immunity and/or that developmental differences render young children less permissive to *P. falciparum* infection. Findings from this study may help inform strategies to prevent *P. falciparum* infection in malaria endemic areas.

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**INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED MALARIA INFECTION AS DETERMINED BY PASSIVE AND REACTIVE CASE DETECTION OF MALARIA IN CHONGWE DISTRICT, ZAMBIA**

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In moderate and high malaria transmission areas, current surveillance systems rely solely on passive detection methods. As malaria transmission in Zambia declines, it will become important to identify continued foci of infection via active case detection to bolster malaria control efforts. Individuals seeking care at the Chinyunu clinic within Chongwe District, Zambia with a chief complaint of fever were administered a questionnaire, a rapid diagnostic test (RDT) and a blood sample was obtained for microscopy. Field teams were dispatched to the homes of RDT positive individuals where an additional survey and RDTs were administered to members within the household. GPS coordinates were recorded for each household. Descriptive statistics and multi-level modeling methods were implemented to compare index cases to RDT positive household contacts, and compare RDT positive to RDT negative household contacts. A total of 472 index cases were identified between June 2012 and June 2013, with 1,621 household contacts investigated and 731 (45%) testing positive for malaria. Index cases were significantly more likely to report symptoms (fever, headache) and be of younger age than RDT positive household contacts (p<0.05 for all comparisons). The mean age of passively detected cases was 12.35 (SD=15.82), the mean age of RDT positive household contacts was 14.74 (13.86), and for RDT negative household contacts was 22.16 (19.88). The proportion of index cases with fever and headache was 97% and 85%, the proportion for RDT positive household contacts was 2.7% and 54.5%, and RDT negative household contacts was 1.1% and 19.3%, respectively. In conclusion, almost half of household contacts of index cases were identified as RDT positive for malaria during the 1-year study period, suggesting passive surveillance underestimates the malaria prevalence for the clinic catchment area. Age and symptoms were the most important factors in seeking care at the clinic. RDT positive household contacts were less likely to report symptoms and slightly older than those that sought care.

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**MODELING LONG-TERM MALARIA TRANSMISSION CHANGES IN A TANZANIAN VILLAGE USING CROSS-SECTIONAL DATA ON AGE SPECIFIC PREVALENCE AND LEVELS OF ANTIBODIES**

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Robust estimates of *Plasmodium falciparum* transmission intensity are imperative for planning, implementation and evaluation of malaria control interventions. Seroconversion rates (SCR) to asexual blood-stage antigens can provide estimates of transmission intensity that correlate with entomological inoculation rates. We study past transmission trends in a rural village and evaluate new models to improve SCR based methods by analysis of antibody levels from multiple cross-sectional serological surveys. The study was conducted in Nyamisati, Rufiji, Tanzania, where parasite
prevalence decreased from 65 to 18% from 1999 and 2010. A single intervention with ITNs was performed in 1999. IgG levels to recombinant *P. falciparum* antigens (MSP-1, MSP-2, MSP-3, AMA-1) and *An. gambiae* salivary protein gSG6 were measured in children (1-16y) sampled in cross-sectional surveys in 1999 and 2010. SCR and rates of antibody decay were estimated by fitting mathematical models to data from the two cross-sections, assuming three profiles of exposure: (i) stable; (ii) stepwise decrease; or (iii) continuous decrease. Results suggest an average 66% decrease in malaria transmission intensity and an 89% reduction in *Anopheles* exposure. Transmission trends were best described by a stepwise decrease model with a reduction predicted to occur shortly after distribution of ITNs. The new models provide estimates of the duration of antibody responses under this transmission decline. MSP-1, seropositive individuals were estimated to convert to seronegative with a half-life of 12 (95% CI 7-20) years due to antibody decline with a half-life of 3 (95% CI 2-6) years. The reduction in transmission may in part be attributed to reduced anopheles exposure following the introduction of ITNs, but is not likely to be explained by ITNs alone. Despite reduced parasite prevalence many children remained seropositive to blood-stage antigens. The new sensitive models using antibody levels enabled detection of reduced exposure among seropositive children and provided estimates of both antibody and transmission dynamics.

**MALARIA AT NATIONAL UNIVERSITY HOSPITAL, SINGAPORE**

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Globally, malaria affects 300 to 500 million people each year. It is endemic to several countries in South East Asia. Singapore has maintained its malaria free status since 1982 despite occasional clusters due to local transmission. However, travel related malaria is seen in hospitals here. We describe the current epidemiology of malaria at our institution. The National University Hospital (NUH) is a 1000 bed multi-speciality, tertiary teaching hospital in Singapore. Laboratory surveillance for malaria is part of the infectious disease surveillance at NUH since 2004. A retrospective study, describing the epidemiology of malaria cases presenting between January 2009 and mid-April 2014, was conducted. A total of 44 cases were analyzed. 39 (88.6%) of them were male, 25 (6.8%) were Indian, 6 (13.6%) Chinese, 1 (2.27%) Malay and 1 (2.27%) Caucasian. 28 (63.6%) of the 44 patients had travelled to a malaria endemic area. All 9 Singaporeans with malaria had preceding travel history. The most common species of malaria was *Plasmodium vivax* (n=31, 70.5%). Of the other species, 7 (15.9%) were *P. falciparum*, 4 (9.09%) were *P. knowlesi* and 1 (2.27%) had a mixed *falciparum* and vivax infection. 17 of the 44 patients had traveled to India. 15 of the 17 patients who traveled to India were infected with vivax. Amongst the 4 patients with *P. knowlesi*, 3 had traveled to Malaysia. In particular, they had visited forested areas for recreation or training. Other travel destinations included Indonesia, Thailand, Hong Kong and Ghana. 39 of 44 patients were admitted and their mean length of stay was 4 days. 4 (9.0%) patients required ICU care and all 39 were discharged well. The global disease burden, modern travel dynamics and emergence of *P. knowlesi*, a zoonotic malarial species, contribute to the continued presence of cases of malaria in Singapore. The existence of *Anopheles* mosquito vectors on the island warrant ongoing vigilance to limit the risk of local transmission.

**EARLY DETECTION OF MALARIA RESURGENCE IN THE PERUVIAN AMAZON REGION USING SEROLOGICAL MARKERS**

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In the past decade, increased support from international donors, e.g. the Global Fund-PAMAFRO Project (2005-2010), allowed for the scale-up of comprehensive malaria control strategies in the Amazon Region. During this period, malaria declined drastically in Peru from 87,805 reported clinical cases in 2005 to 29,355 and 23,075 cases in 2010 and 2011, respectively. Since 2011, malaria control activities are mainly supported by the MoH budget, prioritizing passive and reactive case detection and treatment of confirmed infections. Since 2012, several malaria outbreaks have been detected in diverse Amazonian areas, a phenomenon that had not been observed since 2007. In order to assess recent changes the malaria transmission intensity (MTI), a cross-sectional survey was conducted during November 2012 in eight peri-Iquitos villages using molecular and serological tools. After a full census of the study villages, each household was visited and all available children<7 years plus one randomly selected individual>7 years were enrolled. A total of 651 survey participants were interviewed, clinically examined and a blood sample taken for the detection of malaria parasites (microscopy and PCR) and antibodies to *Plasmodium vivax* (PvMSP119, PvAMA1) and *P. falciparum* (PfGLURP, PfAMA1) antigens by ELISA. Age-specific seroprevalence was analyzed using a previously published catalytic conversion model based on maximum likelihood for generating seroconversion rates (SCR). Overall parasite prevalence by microscopy and PCR were low, i.e. 1.8 and 3.9%, respectively for *P. vivax*, and 1.5 and 6.7%, respectively for *P. falciparum*, while seroprevalence was much higher, 23.3% for PfGLURP and 18.0% for PfAMA1. Most of infections were asymptomatic (79.2%) and sub-patent (71.6%). Likelihood ratio tests supported age seroprevalence curves with two SCR for both *P. vivax* and *P. falciparum* indicating a significant increase in MTI since 2011. Additional data including antibody responses to two antigens for each species and a risk factor analysis for malaria infection and exposure will be presented. In conclusion, this sero-epidemiological analysis allowed for an in-depth characterization of the current malaria transmission pattern as well as for the identification of a recent increase in MTI in the peri-Iquitos area of the Peruvian Amazon following a reduction of control efforts since 2011.

**ROLE OF AUTOPHAGY AND POLYMORPHIC VARIATION IN AUTOPHAGY GENES IN CONDITIONING CLINICAL OUTCOMES IN CHILDREN WITH MALARIAL ANEMIA**

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*Plasmodium falciparum* polymerizes free heme into hemozoin (PHz) as a byproduct of hemoglobin (Hb) digestion. Phagocytosis of PHz by leukocytes promotes dysregulation in the immune response and enhanced pathogenesis. Autophagy is a process that eliminates intracellular components to maintain homeostasis. Although no studies have investigated autophagy in malaria, inactivation of autophagy is associated...
with enhanced susceptibility to infectious and inflammatory disorders. To investigate the role of autophagy in malaria, we genotyped autophagy genes [ATG9 i.e., -2896C/G, -4970T/C, -6561A/G] and ATG10 (-2442G/C, -4322T/A, -7723G/T) in parasitemic children (n=1220; 3-36 mos.) and determined the association between variation and severe malarial anemia (SMA, Hb <5.0 g/dL). Regression analyses, controlling for covariates of anemia, revealed that carriage of CCA (-2896C/-4970T/-6561A) and GTA (-2896C/-4970T/-6561A) haplotypes (ATG9) were associated with reduced risk of developing SMA (Avg: OR, 0.26; 95% CI, 0.08-0.66; P=0.001). Similarly, carriers of CTG (-2442C/-4322T/-7723G) and GTG (-2442C/-4322T/-7723G) in ATG10 had decreased susceptibility to SMA (Avg: OR, 0.52; 95% CI, 0.18-1.05; P=0.070). Analysis of intragenic haplotypes revealed that carriage of CCAGCT, CCCTGT, CCACCTG, GTAGCT, and GTTCCT were correlated with a reduced risk of developing SMA (Avg: OR, 0.27; 95% CI, 0.05-0.94; P=0.001). Analysis of gene expression profiles showed increased levels of autophagy genes in children with SMA (ATG9, 1.57 fold, P=0.022; ATG10, 2.9 fold, P=0.001). In addition, treatment of cultured PBMCs with PFH enhanced autophagy, as illustrated by elevated LC3-II (P<0.05). Consistent with previous studies showing the importance of inflammatory mediators in autophagy, analysis of malaria-associated cytokines/chemokines revealed that haplotypes associated with disease susceptibility had dysregulation of IL-1Ra, IL-1β, IL-8, and IFN-γ (P<0.05).

Collectively, these studies suggest that autophagy plays an important role in conditioning clinical outcomes in children with malaria.
ASCERTAINING THE DIVERSITY AND RATE OF EVOLUTION OF THE COMPLETE MITOCHONDRIAL GENOME IN HAEMOSPORIDIAN PARASITES

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Our understanding about the origin of human malaria has been invigorated by the discovery of many new species in non-human primates. Many of those species, however, have been described solely using molecular data. Furthermore, zoonotic malaria species have been misidentified due to the poor resolution of some morphological characteristics. Thus, the traditional view that relies solely on morphology to define malaria parasite species has been challenged. There are few systems that allow contrasting morphological and molecular data, one of those are the avian haemosporidians. In this study, we compare the rate of evolution of mitochondrial genes using standard molecular clock methods in both mammalian and avian parasites. We include newly described species belonging to three Haemosporidians genera: Leucocytozoon, Haemoproteus and Plasmodium. We contrast the results of single mitochondrial gene approaches with those from complete mitochondrial genomes and test both the potential and limitations of mitochondrial genes, including complete Cytb sequences, as ways to delimit species in malarial parasites that have been identified using morphology. Overall, we found that Cytb allows the correct differentiation of morphologically distinct species. However, the Cytb cannot, by itself, reliably uncover many recent phylogenetic relationships of species that radiated at a scale of 2-7 million years ago. Our conclusion is that single gene approaches do not provide enough information to properly differentiate or estimate species in avian malaria parasites. To evaluate the applicability of our assays in pooling samples covering all ages. The highest gain in Pf prevalence was observed from samples containing as little as 0.00034 parasites/µl blood. The sensitivity of TARE-2 and varATS assays reliably detected all pools containing a Pf sample. Both TARE-2 and varATS assays can be used to screen sample pools for high-throughput and cost-effective detection of Pf infections.

RHOPTRY PROTEINS ARE INVOLVED IN SPOROZOITE INVASION OF THE SALIVARY GLAND

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It has been shown that rhoptry proteins are secreted and localized to the tight junction, during Plasmodium merozoite invasion of erythrocytes. One of rhoptry proteins, Rhoptry Neck Protein 2 (RON2) is known to form complex with RON4 and RONS. The interaction between RON complex and Apical Membrane Protein 1 (AMA1) at the tight junction confers the invasive motility on merozoites. Previously, we reported that RON2 is also localized to the rhoptries in sporozoites and that RON2 is involved in sporozoite invasion of the salivary glands by generation of sporozoite stage-specific ron2 silencing transgenic parasites. Here, we investigated whether RON complex is also formed in oocyst-derived sporozoites or not, by co-immunoprecipitation assay. Sporozoites were collected from infected Anopheles mosquito midguts at day 19th post-feeding and lysed in 1% CHAPS buffer. Solubilized proteins were then precipitated with anti-RON4 antibodies with protein G Sepharose beads. Western blotting showed that RON2 and RONS were co-precipitated with RON4, indicating that RON complex is formed in oocyst-derived sporozoites. Next, the functions of RON4 and RONS were examined by sporozoite-stage specific gene silencing system. The efficiency of salivary gland invasion was significantly decreased in ron4 or ron5 gene repressed sporozoites. These data suggested that sporozoite rhoptry proteins, RON2, RON4 and RONS, are involved in invasion of the salivary glands in a coordinated manner.
suggest that the genetic diversity of *P. falciparum* populations is affected by transmission. More in depth analyses including sequencing are in process to clarify more in detail the dynamics of the genetic diversity of the *msp2* gene in this setting.

**FUNCTIONAL CHARACTERIZATION OF RNA REGULATORS IN MALARIA TRANSMISSION**

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The malaria parasite has a complex life cycle with two bottlenecks that occur during its transmission between mosquitoes and mammals. These transmission events require translational repression to ensure that only the proper proteins are being expressed, while allowing the parasite to prepare the mRNAs it will need for the next developmental stage. Pu2 is a RNA-binding protein and translational regulator, which when knocked out, causes the parasites in the salivary gland to become less infectious over time. In these Pu2 parasites, the mRNA abundance of two putative deadenylases (CCR4, CCR4L) increases substantially. CCR4 is a deadendylase with the same functional domain as CCR4L. CCR4 is well characterized in yeast and mammals as a major translational regulator, and initiates the degradation of ribonucleic acids as part of the CCR4-Not complex. These proteins have yet to be well characterized in the malaria parasite, but the lack of a leucine-rich region in CCR4L may indicate that it does not interact with the CCR4-Not complexes suite of proteins and thus may have a specialized function. We have produced transgenic parasites that either knock-out the CCR4 or CCR4L genes, or express epitope-tagged versions of these proteins in order to compare their functions, localizations, binding partners and essentiality. Localization data in blood stage suggests that there is expression of CCR4L in schizonts but little to no expression in ring and trophozoite stages. Characterization of these proteins in the remainder of the life cycle may show that they are potential drug targets and by disrupting their function one might prevent parasite transmission, halt the infection, or reduce the infectivity of the parasite.

**ENHANCING LONGEVITY OF PLASMODIUM VIVAX AND *P. FALCIPARUM* SPOROZOITES DISSECTED FROM MOSQUITO SALIVARY GLANDS**

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Many unknowns still exist regarding the liver stage forms of *Plasmodium* species that cause malaria. To investigate the liver stage invasion and development, malaria parasites need to be preserved in the infectious sporozoite form by preventing maturation during mosquito dissection. Mimicking salivary gland conditions with a dissection buffer comprised of insect media may aid in the preservation of sporozoites, by creating a more stable transition out of the mosquito. Our goal is to determine which buffer variation prolongs life and viability of sporozoites over time as measured by gliding motility. Variations of Hank’s Balanced Salts and Grace’s Insect Media were compared to RPMI 1640, the current standard dissection buffer. *Plasmodium vivax* and *P. falciparum* sporozoites were harvested from *Anopheles* mosquitoes into each test buffer, and gliding assays were performed at time 0, 4, 8, and 24 hours post dissection. Gliding percentages were compared for statistical difference between buffers and time points within three experimental groups. *P. vivax* experiments were split into two groups based on whether or not sporozoites were harvested from mosquitoes that were internationally shipped after blood feeding, and *P. falciparum* experiments composed the third group. At time 0 hours, RPMI and Grace’s both showed strong gliding percentages in all groups (RPMI: 31-57%, Grace’s: 33-57%), but by time 4 hours, RPMI consistently had the lowest gliding percentage (0-35%). Grace’s has statistically higher (p<0.001) or equivalent gliding percentages compared to all other buffers at time 4, 8, and 24 hours (4 hours: 12-56%, 8 hours: 9-56%, 24 hours: 5-22%). Based on gliding percentages, our variation of Grace’s preserved sporozoites over time better than both Hank’s variations and the standard dissection buffer RPMI. Using a buffer variation such as Grace’s that is more similar to the salivary gland environment, increases access to sporozoites for essential liver and pre-erythrocytic stage studies. *Rapatborn Patrapuvich and Alison Roth contributed equally.*

**ANALYSIS OF THE GENETIC PROFILE OF PLASMODIUM FALCIPARUM ISOLATES FROM URBAN AND RURAL AREAS FROM GABON**

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*Plasmodium falciparum* malaria is a major public health problem in Africa. Indeed, in many African countries, new malaria control strategies recommended by the World Health Organization (WHO) have been adopted during the last decade. In Gabon, the deployment of these was followed by a decrease and a rebound in malaria prevalence, a change in the age of populations at risk, suggesting a changing epidemiology. It is therefore important to assess the impact of these strategies on the genetic diversity of circulating parasites. The aim of the study was to analyze and to compare the allelic diversity of *Plasmodium falciparum* isolates in Gabon. Febrile children and adults were recruited in 2011 at Oyem, Port-Gentil and Libreville. All patients had a malaria diagnosis based on microscopy. Peripheral blood samples were collected from and *msp1* and *msp2* were analysed by nested-PCR in malaria positive samples. The allelic family Ro33 was the most frequent (> 50%) in isolates from all sites. The highest diversity was found in the K1 allelic family with a total of 14 alleles while 10 and one Mad20 and Ro33 alleles was identified, respectively. The majority of 307 alleles was detected in Libreville and Oyem whereas most of the *Pc27* alleles was found at Oyem. Among isolates, 42 *msp2*-type alleles were detected, with nearly half belonging to each allelic family. The complexity of infections was the highest with *msp1* gene: 1.95 in Port-Gentil, 1.91 and 1.66 in Oyem and Libreville. With the *msp2* gene it was 1.33 in Port-Gentil, Libreville 1.24 and 2.15 Oyem. A significant allelic diversity was found in all isolates. The complexity of infections and the different genetic profile of the detected parasite strains varying according to the site suggest a heterogeneous transmission in the different sites. However, strategies for malaria control appear to have a limited impact on the diversity of *Plasmodium* strains.

**GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM IN HAITI: INSIGHTS FROM MICROSEMITATE ANALYSIS**


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Haiti is the only Caribbean island with endemic malaria, and the majority of malaria cases, all due to *Plasmodium falciparum*, are reported in Haiti. Recently, there have been renewed discussions on malaria elimination for Hispaniola, with the focus on interventions in Haiti. Effective antimalarial treatment policies are crucial to successful malaria elimination programs. Presently, Haiti employs chloroquine + primaquine regimen for patients diagnosed with malaria. Although antimalarial resistant *P. falciparum* is not present on a wide scale in Haiti, the emergence of chloroquine resistance remains a threat in Haiti.
Previous studies investigating *P. falciparum* evolution in Haiti in response to widespread antimalarial use have focused on specific genes associated with antimalarial resistance. The present study takes a broader approach to understanding *P. falciparum* evolution by measuring genetic diversity using microsatellite loci located on multiple chromosomes. Eighty-nine samples were collected on blood spot cards from Terre Noire, Leogane, Jacmel, Chabin, Nippes, North Cap Haitien, and Hinche between 2010 and 2013. DNA was extracted and amplified for 12 putatively neutral microsatellite loci. Based on analysis of five loci (TA1, T460, POLYa, ARAZ, and Pfg377), we identified multiple infections in 6.3% of our samples. Expected heterozygositites for these loci ranged from 0.55 to 0.72, suggesting a highly diverse *P. falciparum* population in Haiti. Future analysis of all 12 loci will compare the level of diversity across multiple geographic sites and assess population structure within Haiti. Overall genetic diversity and geographic distribution of parasite diversity can aid in understanding present malaria transmission and impact of antimalarial drug use in Haiti.

**GENETIC DIVERSITY OF PLASMODIUM FALCIPARUM IN HAITI: INSIGHTS FROM MICROSATellite ANALYSIS**

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Haiti is the only Caribbean island with endemic malaria, and the majority of malaria cases, all due to *Plasmodium falciparum*, are reported in Haiti. Recently, there have been renewed discussions on malaria elimination for Hispaniola, with the focus on interventions in Haiti. Effective antimalarial treatment policies are crucial to successful malaria elimination programs. Presently, Haiti employs chloroquine + primaquine regimen for patients diagnosed with malaria. Although antimalarial resistant *P. falciparum* is not present on a wide scale in Haiti, the emergence of chloroquine resistance remains a threat in Haiti. Previous studies investigating *P. falciparum* evolution in Haiti in response to widespread antimalarial use have focused on specific genes associated with antimalarial resistance. The present study takes a broader approach to understanding *P. falciparum* evolution by measuring genetic diversity using microsatellite loci located on multiple chromosomes. Eighty-nine samples were collected on blood spot cards from Terre Noire, Leogane, Jacmel, Chabin, Nippes, North Cap Haitien, and Hinche between 2010 and 2013. DNA was extracted and amplified for 12 putatively neutral microsatellite loci. Based on analysis of five loci (TA1, T460, POLYa, ARAZ, and Pfg377), we identified multiple infections in 6.3% of our samples. Expected heterozygositites for these loci ranged from 0.55 to 0.72, suggesting a highly diverse *P. falciparum* population in Haiti. Future analysis of all 12 loci will compare the level of diversity across multiple geographic sites and assess population structure within Haiti. Overall genetic diversity and geographic distribution of parasite diversity can aid in understanding present malaria transmission and impact of antimalarial drug use in Haiti.

**DISTINCT ESSENTIAL FUNCTIONS FOR POLYAMINES BIOSYNTHESIS ENZYMES IN MALARIA PARASITE BLOOD AND MOSQUITO STAGES DEVELOPMENT**

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Polyamines are positively charged organic molecules that play important roles in cell cycle regulation, cell proliferation, senescence and death of eukaryotic cells. Polyamine analogues have been considered and applied in cancer therapy. Moreover, DFMO (α-Difluoromethylornithine), one of the main drugs targeting African Trypanosomiasis, is an inhibitor of Ornithine Decarboxylase, an essential enzyme in the biosynthesis of polyamines. Despite the importance of this pathway as possible target for multi-stage malaria intervention, little is known about the cellular functions of polyamine biosynthesis enzymes for *Plasmodium* development. We applied gene-targeting techniques for *Plasmodium yoelii* to target enzymes of this pathway for deletion. Our results indicate that the bifunctional Ornithine Decarboxylase/ S-Adenosylmethionine Decarboxylase (ODC/ SAMDC) enzyme is not essential for sexual and asexual blood stage (BS) development. However, BS growth was reduced in odc/samdc(-) and male gamete exflagellation was completely abolished with no development of mosquito stages. Spermidine Synthase, the downstream enzyme of ODC/ SAMDC, was shown to be essential for BS development by knock-out/knock-in approach. These results indicate alternative essential roles for the polyamine biosynthesis enzymes during BS and early mosquito stages development. This validates the enzymes of this pathway as multi-stage drug targeting candidates.

**EUPATHDB: AN ONLINE GENOMICS RESOURCE FOR EUKARYOTIC PATHOGENS**

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The Eukaryotic Pathogen Database Resource (EuPathDB) is a family of free online databases that integrate genomic data with functional genomics and clinical/field isolate data for numerous eukaryotic pathogens within Amoebazoa, Apicomplexa, Diplomonadida, Microsporidia, Trichomonadida, Kinoplastida. EuPathDB also integrates data informing upon host-parasite interactions from datasets that analyze mixed parasite-host samples such as human cells infected with parasites. An interactive data exploration platform, EuPathDB provides data mining and visualization tools for discovering meaningful relationships between
The databases are updated and expanded bimonthly with data ranging from genome sequence and annotation to expression data, to parasite field isolates, to host data in response to infection. Despite the breadth of data (140 genomes, 150 functional datasets), it is easy to mine, visualize, download and browse different data types. Data is mined using the Strategy System to search within and between datasets, developing in silico experiments that identify features with similar biological characteristics. Search strategies and results can be downloaded, saved and shared with a colleague. Data may be visualized in the context of the genome sequence and annotation using an interactive and configurable browser. Individual record pages that compile all available data for a feature (e.g. gene, isolate, genomic sequence) provide a comprehensive view of the feature. Our extensive user-support system includes video tutorials, a rapid-reply email question hotline, and hands-on workshops at locations worldwide. Attend our poster or exhibit booth for an overview of this NIH/NIAD-funded resource. Or visit one of our sites: AmoebaDB.org, CryptoDB.org, EuPathDB.org, GiardiaDB.org, MicrosporidiaDB.org, PyroplasmaDB.org, PlasmoDB.org, ToxoDB.org, TrichDB.org, TriTrypDB.org, OrthoMCL.org, and HostDB.org.

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DETECTION OF PLASMODIUM FALCIPARUM IN THE BLOODMEAL OF ANOPELES GAMBIAE USING QUANTITATIVE NUCLEIC ACID SEQUENCE BASED AMPLIFICATION (QT-NASBA)

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Control of the human-to-mosquito transmission of Plasmodium is being increasingly explored through drugs against sexual parasite forms in the human and mosquito, transmission blocking vaccines, and mass drug administrations. Successful implementation of these measures will require a greater understanding and quantitation of stage-specific infections of mosquitoes from humans. Past work on the human to mosquito portion of the Plasmodium life cycle has primarily focused on dissection and staining of wild blood fed mosquito midguts for early sexual forms and later stage oocysts, or examination of human infectiousness by direct skin feeds of wild blood fed mosquito midguts for early sexual forms and later stage oocysts, or examination of human infectiousness by direct skin feeds. As an alternative, we present a method of detecting the acquisition of Plasmodium falciparum in the bloodmeals of field caught Anopheles gambiae utilizing the modern molecular technique of Quantitative Nucleic Acid Sequence Based Amplification. QT-NASBA was performed on individual and pooled RNA extracted from fresh bloodmeals preserved on FTA cards for detection of Pf155/25 transcript. We were able to detect Plasmodium falciparum in 5 of 6 RNA pools and 5 of 6 individual mosquitoes. Negative controls of RNA extracted from bloodmeals taken from uninfected individuals were all negative. Future experiments will focus on discrimination of this technique and parasite quantification from laboratory infected and naturally-infected mosquitoes. We see a variety of uses for this approach, including the correlation of human gametocytemia detected by QT-NASBA on human blood spots to the presence of early sexual forms in mosquitoes that bite upon these same individuals, performing sensitive spatial and temporal microepidemiology, and investigating biting tendencies of wild mosquitoes as they relate to human gametocytemia in a natural setting. These measures should lead to a more complete understanding of Plasmodium transmission, and may become an important measure to validate transmission-blocking interventions.

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DEVELOPMENT OF A SINGLE-CELL GENOMICS PLATFORM FOR MALARIA INFECTIONS

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Mixed- or multiple-clone malaria infections are commonplace in areas of high endemicity, are a major outcome in drug and vaccine efficacy trials, and can confound most parasite genetic studies. Though, surprisingly, their composition is still poorly understood. A major roadblock in exploring multiple-clone infections is that current genome sequencing tools only allow us to examine infections in bulk, providing little information on individual parasite genomes within an infection. To address this we have developed a single-cell genomics approach to dissect multiple-clone infections. By combining cell sorting and whole genome amplification we are able to capture single malaria parasite-infected red blood cells and generate sufficient high quality material for genomic analysis. We optimized our approach by assaying >260 single cells across fourteen experimental conditions. To quantitatively, we created artificial mixtures of Plasmodium falciparum laboratory lines (HB3/0D2/0D7) and obtained highly accurate (>99.9%) single cell genotypes. We genome sequenced 4 single cell amplifications obtained from these mixtures (HB3 (n=2), 3D7 (n=2)), confirming 99.29% of the 196,332 SNP calls made across these 4 sequencing reactions. We saw no contamination from other genomes present in the mixtures in single cell genome sequences. This single cell genomics platform can be extended to malaria parasite species where long-term culture is not possible, precluding the direct dissection of infections through dilution cloning. We performed single cell genotyping and single cell sequencing of P. vivax infections obtained directly from patients, and obtained comparable accuracy and coverage to P. falciparum assays. These methods open the door for large scale analysis of within-host variation of malaria infections at single cell resolution.

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POPULATION GENETICS AND NATURAL SELECTION OF CANDIDATE MALARIA VACCINE ANTIGENS IN MALIAN PLASMODIUM FALCIPARUM ISOLATES

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Vaccination, in combination with other control measures, is an essential strategy for malaria eradication. However, the highly polymorphic nature of many candidate vaccine antigens can result in allele-specific immune responses that limit vaccine efficacy against diverse strains found in nature, as has been observed with vaccines based on apical membrane 1 antigen and merozoite surface protein 1. Little is known about the genetic diversity in field isolates of the next generation of vaccine antigens even as vaccines based on these antigens are entering Phase 1 clinical testing. Some of these candidates include the liver and blood stage antigen merozoite surface antigen 5 (MSP5), the blood stage antigens glycosylphosphatidylinositol-anchored micronemal antigen (GAMA), and the RfH-interacting protein (PfRipr). We estimated the haplotype prevalences and genetic diversity within vaccine antigen-encoding genes in 91 specimens collected from asymptomatic infections and clinical malaria
episodes experienced by children in Bandiagara, Mali. We hypothesized that field isolates of at least some of these candidate antigens would exhibit low genetic diversity that would predict cross-protective efficacy against heterologous strains found in endemic areas. We estimated heterozygosity of the full-length malaria vaccine antigen sequence by the parameter \( r \), estimate \( D^* \) and \( F^* \) to detect significant departures from the neutral model with Plasmodium reichenowi, the chimpanzee malaria parasite as the out-group, and test for balancing selection using Tajima's D test. Preliminary results indicate that MSP5 and PFRipr are well-conserved while GAMA is highly polymorphic. The results from this study will be used to down select conserved candidate antigens and antigenic variants for possible inclusion in a broadly cross-protective, multivalent malaria vaccine.

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A NEW MALARIA VACCINE CANDIDATE, GPI PROTEIN TRANSAMIDASE RELATED PROTEIN SHOWN TO HAVE GOOD PROTECTIVE IMMUNE RESPONSE IN MOUSE MODEL

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Following a genome-wide search for a blood stage malaria candidate with GPI anchor motif using bioinformatics, and DNA-based vaccine screening and mouse malaria model, our study identifies PyGPI8p-transamidase related protein (PyTAM) as hypothetical protein with a protective immune response (Shuaibu et al., 2010). The homology search of PYTAM molecule using Conserved Domain Architecture Retrieval Tool (CDART) revealed that it's belonging to Cysteine proteases C13 family. Cysteine peptides of parasitic protozoa have been implicated in a variety of biological events including invasion of host cells, immune evasion, pathogenesis and virulence and proteolytic degradation of hemoglobin and therefore some have also been validated as drug targets. Interestingly, we have found by immunofluorescence staining and immune-electron microscopy that the molecule is cytosolic at ring stage, and then located finally onto the parasitophorous vacuole at the late schizonte. Also, we successfully demonstrated the vaccination efficacy of PYTAM candidate as DNA vaccine formulated with Nanoparticle delivery system in controlling malaria in mouse model, as previously reported. Although the protection enhancing mechanism is not clearly understood. It is clear that antibody levels, was not significantly increased but Th1-mediated antigen-specific immune responses (INF-γ producing CD4 and CD8 T cell) was significant. Currently, we are working on molecular characterization of PYTAM and its mechanism of protective immune responses. These data indicate that PyTAM could a promising malaria vaccine candidate for further development.

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IMMUNOGENICITY AND EFFICACY OF CHAD63-MVA ME-TRAP PRIME-BOOST VACCINATION AGAINST PLASMODIUM FALCIPARUM INFECTION IN HEALTHY ADULTS IN SENEGAL

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Malaria transmission is in decline in some parts of Africa, which is partly due to the scaling up of control measures. Previous attempts at malaria elimination ended with mixed success. It is currently agreed that additional control measures including vaccination will be required. Recent studies using viral vectors with prime-boost approach to deliver ME-TRAP (multiple epitopes thrombospodin related adhesion proteins) showed promising safety, immunogenicity and significant efficacy in sporozoite challenge studies. Our study reports on the efficacy and immunogenicity of the prime-boost vaccines in peri-urban area of Dakar West Africa. We conducted a single-blind, randomised controlled phase IIb efficacy trial of 120 healthy men aged 18-50 years, living in a malaria endemic area of Senegal. Eligible study participants were randomised to receive either the active vaccine ChAd63 encoding the pre-erythrocyclic antigen ME-TRAP as prime vaccination, followed eight weeks later by Modified Vaccine Ankara also encoding ME-TRAP as booster or two doses of anti-rabies vaccine as comparator. They were all followed up for eight months. The immunogenicity was determined by ELISPOT to quantify T cells responses to TRAP, and ELISA to identify the specific antibodies. We used finger-prick thick blood film to evaluate parasitemia throughout the study follow-ups. We determined time to first P. falciparum infection and re-infection by Real time PCR. Prior to intensive PCR survey, all study participants received a 3-day malaria treatment with atoquaquine-proguanil and artesunate at the end of the vaccinations to clear all traces of parasitaemia. We also assessed the reactivity using all adverse and serious adverse events which occurred during follow-ups. This pre-erythrocyclic malaria vaccine is safe and induces high immunogenicity with a mean T-cell response at 1266 SU/106 PBMCs compared to 84SU/106 PBMCs for the control group. qPCR Data analysis is ongoing and efficacy results will be presented at the meeting Vaccine efficacy against infection in adults may be rapidly assessed in peri-urban areas using this efficient trial design.
relevance to understand the molecular mechanisms mediating the severity of malaria infection in pregnant women and indicate interesting ways for potential optimization in the ongoing effort to develop a VAR2CSA based vaccine.

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INTRODUCTION OF MALARIA VACCINE IN NIGERIA: STATUS AND PROGRESS UPDATE

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Malaria is endemic in Nigeria. About 97% of the population is at risk and it accounts for 25% under-5 mortality. Artemisinin-based Combination Therapy and Intermittent Preventive Treatment are current curative and preventive interventions. Clinical trials using malaria vaccine were conducted in Enugu and Jos, Nigeria. The RTS,S/AS01 coadministered with Expanded Programme on Immunization (EPI) vaccines provided modest protection against both clinical and severe malaria in young infants. Meetings were held in-country to discuss the pros and cons, in preparation for the adoption of malaria vaccine in Nigeria. Nigeria officials held Stakeholder and advocacy meetings with facilitators from MVI PATH in 2011 and 2012. Key informants were interviewed about national policy decision making processes on adopting new malaria control interventions and new vaccines in Nigeria and the readiness of the health system in adopting the candidate vaccine. The results were analyzed by content analysis. Progress is being made with adoption of malaria vaccine in Nigeria. There was no National Immunization Technical Advisory Group/Committee for all vaccine preventable diseases in Nigeria, and no standard documented guideline for decision making process for the adoption of new vaccines. The existing public-private partnership for the adoption of Human Papilloma Virus vaccine and Expanded Programme on Immunization in Nigeria was proposed for Malaria vaccine. Challenges foreseen with the decision-making process include large population size, weak health system, resistance to change by health staff, inadequate trained staff, high cost of implementation, budgetary deficits, inadequate cold chain and storage supply. A Ministerial memo has been sent to the National Council of Health and the Federal Executive Council. A proposal presentation would be made to the Technical Working Group on Malaria and relevant stakeholders. Lessons will be learnt from neighboring countries with functional health system. Funding will be harnessed from World Health Organization and Global Alliance for Vaccine and Immunization. A proper cost benefit analysis would be done to ascertain cost-effectiveness. High level advocacy will be carried out at all levels.

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EVIDENCE OF SELECTION IN POLYMORPHIC PLASMODIUM FALCIPARUM MEROZOITE ANTIGENS DURING THE RECOVERY OF CHILDREN FROM MALARIA

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Several Plasmodium falciparum merozoite invasion genes are highly polymorphic, potentially allowing the parasite to evade the host’s immune responses and utilise alternative invasion pathways. The extent to which immune evasion shapes the parasite’s population dynamics in natural infections is unknown. The Reticulocyte binding homologue (Rh), erythrocyte binding antigen (EBA), merozoite surface protein (MSP) 3-like gene families, apical membrane antigen (AMA) 1 and MSP1 have been shown to be involved in invasion as antibodies to these proteins inhibit invasion in vitro. Fifteen of these merozoite genes were sequenced from parasite DNA isolated from children, under 5, with uncomplicated malaria recruited into a drug trial between 2007 and 2008, which consisted of pre (samples at recruitment)- and post (malaria slide positive sample during 84 days of weekly follow up)-treatment samples. The AMA1, MSP142, EBA175, MSPDBL1, MSPDBL2 and Rh2b loci were 100% heterologous genotypes in pre- and post-treatment parasite pairs. In contrast, 70% of the pre- and post-treatment parasites were homologous genotypes at the Rh5 locus. An analysis of the proportion of individual alleles revealed that the proportion of certain alleles in MSP142, EBA175, EBA181, Rh5 and AMA1 loci were significantly different (p<0.05) pre- and post-treatment. These changes in allele frequencies reflect the highly polymorphic nature of these antigens and suggest there may be a selective mechanism potentially allowing the parasite to evade immune responses, by allele-specific immunity. The allele proportions of MSP3, MSP6, MSPDBL2 and Rh1 remained similar pre- and post-treatment, suggesting that they may generate cross-reactive immune responses and thus there is no distinction between the multiple allelic types. In summary, the antigens which showed allele frequency changes may require a multi-allelic approach for vaccine development. Also, the antigens that showed 100% heterologous parasite pairs are good candidates for discriminating between recrudescence and new infections in antimalarial drug trials.

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IDENTIFICATION OF THE NOVEL TRANSMISSION-BLOCKING VACCINE TARGET EXPRESSING ON THE SURFACE OF MALE GAMETES

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Plasmodium transmission from mosquitoes to mammals requires sexual stage parasite development and fertilization in mosquito midguts. After ingestion of gametocytes by mosquitoes, fertilization occurs to form zygotes, which develop into invasive forms, oocyt es. These stages could be promising targets for transmission blocking strategy, which intends to break malaria life cycle inside the mosquito vector. Despite large research effort on screening for parasite molecules expressing on the surface of gamete, zygote or ookinete, the number of the candidate antigens of transmission-blocking vaccine is still limited. Previously, we reported that a novel male specific protein, designated PyGM75, is localized to the osmophilic bodies of male gametocytes then transported to the surface of microgametocytes in Plasmodium yoelii. Furthermore, we demonstrated that pygm75 disrupted parasites impaired the exflagellation ability. In this study, we investigated whether PyGM75 can be a novel transmission-blocking vaccine target. First, to examine if anti-PyGM75 antibodies can prevent parasite transmission to mosquitoes, we mixed the specific antibodies and parasitized RBCs, then let them feed on Anopheles mosquitoes using membrane-feeding system. As the result, the numbers of oocysts formed on the mosquito midgut were greatly reduced by adding anti-PyGM75 antibodies, as a dose dependent manner. In addition, the antibodies strikingly impaired the motility of microgametocytes in vitro. Taken together, our data demonstrates that anti-PyGM75 antibodies have potential to prevent the malaria transmission, presumably by interfering fertilization occurred in midguts. Since PyGM75 is conserved among Plasmodium species, including P. falciparum and P. vivax, this study strongly suggests that GM75 is a novel candidate target of transmission-blocking vaccine.

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INVESTIGATING THE ROLE OF GILT ON IMMUNE RESPONSES TO A MALARIA VACCINE ANTIGEN

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Malaria ranks fifth as leading cause of death from infectious diseases. Our overall research goal is focused on the development of a malaria transmission-blocking vaccine (TBV). One such vaccine target (Pfs48/45 protein) is based on antigens expressed on male and female gametocytes, which establish infection in the mosquito vector. The targets of blocking antibodies are disulfide bond constrained conformational epitopes. As Pfs48/45 has not been crystallized, precise location of the disulfide bonds...
and the topology of epitopes are unknown. The disulfide bonds have been shown to greatly influence the ability of an APC to process and present epitopes, and elicitation of an appropriate immune response. GILT, a thioreductase constitutively expressed in APCs, mediates endocytic reduction of antigens and display of peptides on MHC class I and II. Using recombinant Pf48/45 as the model antigen, this project seeks to identify mechanisms involved in presentation of relevant epitopes to T and B cells leading to effective antibody responses and generation of the memory B cell pool. We hypothesize that reduction of disulfide bonds in Pf48/45 will dramatically impact the generation of T cell epitopes, and thus influence downstream B cell stimulation and protective antibody responses. We have conducted immunogenicity studies in wildtype and GILT-/− mice using both non-reduced and reduced forms of Pf48/45 and analyzed the responses using full length and five overlapping (~100aa long, spanning full-length Pf48/45) sub-fragments and 39 peptides by ELISA, western blotting, ELISPot and T cell proliferation assays. Results from these studies have indeed revealed significantly different patterns of recognition of putative B and T cell epitopes. These ongoing studies have revealed the presence of immunodominant B cell and T cell epitopes in the sub-fragment 2 (aa 108-200) and sub-fragment 5 (aa 341-420), respectively. We are now initiating studies to investigate the influence of immune epitopes in functional responses and transmission-blocking protection. Results of the study will impact vaccine considerations.

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IDENTIFICATION OF CORRELATES OF DISEASE AND PROTECTION IN A CONTROLLED HUMAN MALARIA INFECTION MODEL

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Long-lasting protection against homologous Plasmodium falciparum infection can be induced in malaria naïve subjects taking chloroquine prophylaxis during immunizations with bites of infected mosquitoes followed by a Controlled Human Malaria infection (CHMI). However, identification of correlates of disease and protection, which could be prospectively explored as biomarkers for host protective immunity to malaria, is still critically limited. Here, we monitored the kinetics of the human immune response at the transcriptomic and cellular level during immunizations and controlled infection. Direct ex-vivo whole blood gene expression profiling and cell subset analysis was performed using dual-color Reverse-Transcriptase Multiplex Ligation-dependent Probe Amplification (dRT-MLPA) and polychromatic (16-color) flow cytometry, respectively. Volunteers from three CHMI trials were included in this study: (1) a mosquito/sporozoite dose titration CHMI using chloroquine prophylaxis during immunizations, (2) a non-inferiority CHMI comparing the efficacy of chloroquine to mefloquine prophylaxis during immunizations, and (3) a heterologous challenge of previously (under chloroquine prophylaxis) immunized and homologously protected volunteers. Genes differentially expressed between protected and unprotected volunteers were identified by the Global test at each time point during vaccination and challenge. Biomarker signatures for disease and protection were generated using Lasso and Ridge regression analysis. Critical signalling networks that discriminate between protective and non-protective immune responses will now be generated by pathway analysis. In addition, key cellular changes, both quantitative and qualitative, have been analyzed by multi-parameter flow cytometry. This will be followed by integration of the molecular and cellular datasets to correlate data to protective immunity and/or disease.

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PROFILING THE HUMORAL IMMUNE RESPONSES TO PLASMODIUM VIVAX INFECTION AND IDENTIFICATION OF CANDIDATE IMMUNOGENIC RHOPTRY-ASSOCIATED MEMBRANE ANTIGEN (RAMA)

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Completion of sequencing of the Plasmodium vivax genome and transcriptome offers the chance to identify antigens among >5000 candidate proteins. To identify those P. vivax proteins that are immunogenic, a total of 152 candidate proteins (160 fragments) were expressed using a wheat germ cell-free system. The results of Western blot analysis showed that 92.5% (148/160) of the targets were expressed, and 96.6% (143/148) were in a soluble form with 67.7% of solubility rate. The proteins were screened by protein arrays with sera from 22 vivax malaria patients and 10 healthy individuals to confirm their immune profile, and 44 (27.5%, 44/160) highly reactive P. vivax antigens were identified. Overall, 5 candidates (rhoptry-associated membrane antigen [RAMA], Pv-fam-a and -b, EXP-1 and hypothetical protein PVX_084775) showed a positive reaction with >80% of patient sera, and 21 candidates with 50% to 80%. More than 23% of the highly immunoreactive proteins were hypothetical proteins, described for the first time in this study. One of the top immunogenic proteins, RAMA, was characterized and confirmed to be a serological marker of recent exposure to P. vivax infection. These novel immunoproteomes should greatly facilitate the identification of promising novel malaria antigens and may warrant further study.

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IMMUNOGENICITY OF A SYNTHETIC VACCINE BASED ON THE PLASMODIUM VIVAX DUFFY BINDING PROTEIN REGION II

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Molecules that play a role in Plasmodium merozoite invasion of host red blood cells represent attractive targets for blood-stage vaccine development against malaria. In Plasmodium vivax, merozoite invasion of reticulocytes is mediated by the Duffy binding protein (DBP), which interacts with its cognate receptor, the Duffy antigen receptor for chemokines (DARC) on the surface of reticulocytes. The DBP ligand domain, known as region II (DBPII), contains the critical residues for receptor recognition making it a prime target for vaccine development against blood-stage vivax malaria. In natural infections DBP is weakly immunogenic and DBPII allelic variation is associated with strain-specific immunity, which may compromise vaccine efficacy. In a previous study, a synthetic vaccine termed DEKnull that lacked an immunodominant variant epitope in DBPII induced functional antibodies to shared neutralizing epitopes on the native Sal1 allele. Anti-DEKnull antibody titers were lower than anti-Sal1 titers but produced more consistent, strain-transcending anti-DBPII inhibitory responses. In this study, we further characterized the immunogenicity of DEKnull, finding immunization with rDEKnull produced an immune response comparable to native recombinant DBP alleles. Further investigation of DEKnull is necessary to enhance its immunogenicity and broaden its specificity.
TARGETING MALARIA PARASITE INVASION OF RED BLOOD CELLS: HOST-CELL ENGAGEMENT AND ANTIBODY NEUTRALIZATION

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Host-cell invasion is a critical step in the pathogenesis of malaria. Erythrocyte Binding Like (EBL) ligands mediate critical interactions during RBC infection by Plasmodium falciparum and P. vivax, and are targets of antibodies that play a role in naturally acquired immunity. However, the structural basis and mechanisms of host-cell engagement, as well as the neutralization mechanisms of antibodies that prevent parasite growth have not been defined. Lack of this knowledge has hampered the effective rational design of agents to disrupt invasion. We will present structural, mechanistic and functional studies of EBL ligands from both P. falciparum and P. vivax in association with receptors and antibodies. These studies demonstrate that multimeric assembly of receptor-ligand interactions is crucial for host-cell invasion, and highlight critical functional regions that can be exploited for targeted disruption, including receptor-binding pockets and multimeric assembly interfaces. We found that potently neutralizing antibodies target the assembly interfaces and receptor-binding residues, while non-neutralizing antibodies target decoy epitopes far removed from functional regions of ligands. These results explain why only a subset of antibodies that recognize EBL ligands are neutralizing. The results reveal the complex nature of EBL-RBC interactions and highlight new approaches to target the molecular mechanism of invasion.

Vaccine efficacy may be improved by targeting critical functional regions and protective epitopes of EBL ligands, while avoiding decoy-epitopes identified by these studies.

A NEXT GENERATION GENETICALLY ATTENUATED PLASMODIUM FALCIPARUM PARASITE CREATED BY TRIPLE GENE DELETION

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A malaria vaccine could dramatically reduce the tremendous morbidity and mortality due to Plasmodium parasites. Immunizations with live-attenuated sporozoites in animal models and humans have shown the feasibility of a pre-erythrocytic malaria vaccine that confers complete and protracted protection against infection. Genetic engineering offers a versatile platform for controlled and consistent design of pre-erythrocytic genetically attenuated parasites (GAPs) as vaccine candidates. We previously generated a GAP by deleting the P. falciparum P52 and P36 genes (Pf p52-/-p36- GAP) preclinical assessment of which indicated an early liver stage growth defect. However, human exposure to >200 P. falciparum-infected mosquito bites caused peripheral parasitemia in 1 of 6 safety trial volunteers, revealing that this GAP was severely but incompletely attenuated. We modeled this phenotype with rodent malaria P. yoelii p52-/p36- GAP induced substantial human immune responses including antibodies that blocked in vitro hepatocyte infection by sporozoites. We have now created a triple gene deleted GAP by additionally removing SAP1 (Pf p52-/p36-/sap1- GAP). SAP1 deletion alone was sufficient to completely attenuate P. yoelii in Balb/cByJ mice. Deletion of genes whose encoded proteins perform distinct biological functions (as do P52, P36 and SAP1) should improve the robustness of attenuation and greatly reduce the possibility of compensatory changes. Pf p52-/p36-/sap1- GAP and wildtype parasites were indistinguishable in blood and mosquito stages of development. Using an improved humanized mouse model transplanted with human hepatocytes and erythrocytes, we demonstrate that despite a high dose sporozoite challenge, Pf p52-/p36-/sap1- GAP did not transition to blood stage infection and appeared completely attenuated. We also used FLP/FRT recombination to remove all drug selectable markers from Pf p52-/p36-/sap1- GAP. Preparations are underway to test this next generation GAP in a human phase I clinical trial.

IMMUNOSCREENING OF THE TARGET PROTEINS CONTRIBUTING TO GROWTH INHIBITORY ACTIVITY OF HUMAN IGG AGAINST PLASMODIUM FALCIPARUM

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Residents in malaria endemic area acquire the protective antibodies against the disease by continuous infection with Plasmodium falciparum. It has been shown that a part of the antibodies have growth inhibitory activity (GIA) against in vitro cultured P. falciparum. We then hypothesized that identification of responsible antigens to GIA may support the discovery of novel blood-stage vaccine candidates. The GIA of individual IgG purified from a Malian immune adult was measured (n=51). To identify the antibody responses correlating with GIA, we established a modified AlphaScreen system for high-throughput detection of antigen-antibody reaction. Genome-wide expressed 1,848 parasite proteins by a wheat germ cell-free system were screened with the Malian adult IgGs. Known malaria vaccine candidates were also included in the 1,848 proteins. First, we selected approximately 900 immunoreactive proteins with the Malian adult IgGs (giving the AlphaScreen count more than negative control). Of those, we selected antigens which showed significant positive correlations between their AlphaScreen counts and GIA. As a result, the ratio of the known malaria vaccine candidates among the finally selected antigens was significantly higher than that in the original 1,848 proteins. The result suggests that this screening system would be effective to discover novel malaria blood-stage vaccine candidates. In addition to the analysis of the individual antigens, we are now analyzing which combination of antigens will better explain the GIA of total IgG. The results of this analysis will be discussed in this presentation.

CHARACTERIZATION OF PLASMODIUM FALCIPARUM MAS170 AS NOVEL MALARIA BLOOD-STAGE VACCINE CANDIDATE

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A novel 170-kDa merozoite apical surface protein of Plasmodium falciparum (PfMAS170) was characterized as a novel malaria blood-stage vaccine candidate in this study. The PfMAS170 is conserved among Plasmodium spp. and is predicted as 170-kDa protein with a signal peptide at the N-terminus. We expressed recombinant protein corresponding to the C-terminal region of PfMAS170 using a wheat germ cell-free system to obtain anti-PfMAS170 sera. Western blot analysis detected approximately 170-kDa signal corresponding to the full-length of PfMAS170 in late blood-stage parasites, and PfMAS170 fragments from approximately 120-kDa to 30-kDa were released in the culture medium. Immunofluorescence assay of the conditioned culture medium showed that PfMAS170 localizes on the surface of apical end of merozoite. Erythrocyte binding assay of the conditioned culture medium showed that the secreted 30-kDa fragment of PfMAS170 binds to erythrocyte
Evaluation of 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.

Immunogenicity of DNA vaccines encoding Pf48/45, 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 Pfs48/45 orthologs 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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DEVELOPMENT OF PLASMODIUM FALCI PARUM 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 (PFRH240). Recombinant PFRH240 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 PFRH240 with cGMP specifications for its clinical development. Thus, PFRH240 is a major candidate antigen under the ICGEB portfolio for the development of a new generation combination based blood-stage malaria vaccine.

1003

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 DNAalkylating agents to chemically attenuate blood stage parasites which will be used as vaccines. We have demonstrated that attenuated Plasmodium chabaudi or P. yoelli 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. Irradiated 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 PLSA1 and PLSAP2 as more protective than FCSP or PFRAP 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.

1005

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 SP2, 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 PSpZ 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) SP2 administered SC or ID as compared to IV to achieve similar protection in BALB/c mice. Subsequently, a clinical trial involving IV administered PSpZ Vaccine demonstrated protection in 6/6 subjects against controlled Pf malaria infection. Many think it would be optimal if PSpZ 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 2x10^1 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 SP2. However, the data indicate that a systematic approach has the potential to significantly increase the efficiency of administration by non-IV routes.

1006 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 result 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+CD11a+), which are known to play a role in liver-stage immunity.

1007 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 defined 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.

1008 REVERSIBLE CONFORMATIONAL CHANGE IN THE PLASMODIUM FALCI PARUM

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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 NAP repeat domain and C-terminus comprised of a thrombospondin-like type I repeat (TSR) domain. RTS,S which contains numerous NAP 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. 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.

1009

DOUBLE-BLIND DOSE-ESCALATING RANDOMIZED CONTROLLED PHASE 1 STUDY IN MALARIA EXPOSED ADULTS OF THE SAFETY AND IMMUNOGENICITY OF PF525-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, Pf525-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 Pf525-EPA/Alhydrogel® or control) have received 2 doses (Days 0 and 56) and 100 (high dose of 47µg Pf525-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 Pf525-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 Pf525-EPA/Alhydrogel® and Euvax B/Hepatitis B vaccine (control) groups. In the group who received the high dose of 47µg of Pf525-EPA/Alhydrogel®, antibody responses to Pf25s increased with each subsequent dose of vaccine given, but dimished quickly following vaccination. Overall, Pf525-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 x 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 insecticite 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 96 % 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.
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 stagnant 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% of the community health workers were trained and over 200 members of 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.

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 CO₂ 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.

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.

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 affected 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. gambiae 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.
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.

## 1019

### AN ASSOCIATION BETWEEN THE 1014F KDR ALLELE AND PLASMODIUM FALCIPARUM INFECTION IN ANOPELE S 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 since 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.

## 1021

### 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 (CO2) gas is recognized as an important stimuli mediating host attractiveness to mosquitoes. Currently, the main sources of CO2 for mosquito sampling include dry ice, sugar-yeast fermentation and bottled industrial CO2, 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 CO2 for sampling mosquito vectors in Tanzania. Mosquito trapping experiments were conducted in the field by using Ifakara OBS baited with 3 different sources of CO2 and an unbaited Ifakara OBS as control. In each trap, the CO2 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 fermentation caught significantly more An. gambiae mosquitoes than traps baited with industrial CO2 (P≤0.01) and unbaited traps (control) (P≤0.6). The trap baited with industrial CO2 had higher mosquito catches than the traps baited with molasses CO2, but was less attractive compared with conventional industrial CO2 yeast-sugar fermentation. We also assessed whether CO2 generated from molasses fermentation can enhance the attractiveness of synthetic human-derived cues to mosquitoes. Traps baited with CO2 obtained from molasses-yeast fermentation caught significantly more An. gambiae mosquitoes than traps baited with yeast-sugar CO2 (P≤0.01) and unbaited traps (control) (P≤0.05). The trap baited with CO2 had higher mosquito catches than the traps baited with molasses CO2, but was not statistically significant (P≤0.75). Addition of molasses derived CO2 to traps baited with human odour significantly increased trap catches. There were no significant differences on the catches between the traps baited with molasses derived CO2 and traps baited with industrial CO2 (P≤0.6). Yeast-molasses fermentation is an effective, locally available and cheap source of CO2 for mosquito vector studies. The CO2 could potentially be used to replace yeast-sugar CO2 in odor-baited devices for mosquito sampling, as so as to maintain monitor disease vector and control in low income communities.
at least once. In the first study, all traps except HLC were baited with experimental days, each trap type had been to each of the five locations in a latin-square design in which the different traps were randomly allocated. The study was conducted in rural southern Tanzania, known as M-Trap, as an outdoor surveillance tool for disease-transmitting mosquitoes attracted to their legs, unfortunately remains the standard labour-intensive method requiring use of human volunteers to collect mosquitoes. In recent years alternatives to HLC have been tried including Ifakara tent trap for sampling host-seeking mosquitoes outdoors and indoors. In recent months, new interventions have been introduced 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 assessed 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.

**1023**

**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 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 >300m 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 from 18:00hrs 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 performances 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.

**1024**

**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 $28.95\pm8.1$ 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.
1025

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 an 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 (Cbfm), 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 MS 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 infection: 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 = 0.0004) 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, underestimate 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 spot announcements, 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 and of 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 channels of antenatal care and child immunization services alone was 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 and of 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 nighttime 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 ADHEDENCE 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 insecticidal net 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 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, hence 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/m², and with pirimiphos methyl (an organophosphate), at an application rate of 1g/m² 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.

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/sulphamethaxazole (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/clavulante, ciprofloxacin, nitrofurantoin, ciprofloxacin, gentamicin, tetracycline and trimethoprim/sulphamethaxazole. 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.

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 mannotit 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 oxacinil (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.

BRUCELLA MELITENIS METHYONYL-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 disproportionately 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 enzymes 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 (BruMetRS) and this allows for structure-based drug development. Furthermore, we have developed a working assay to screen for compounds that inhibit BruMetRS 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 BruMetRS 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 BruMetRS inhibiting E. coli growth of bacteria expressing BruMetRS but not E. coli 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 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 leptospirosis in 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 leptospirosis infection (odds ratio [OR] = 18.24; P = 0.003) whereas exposure to flood water more than 3 hours per day was significantly associated with only leptospirosis 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 leptospirosis 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 leptospirosis 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 leptospirosis infection.

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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 MELOIDIOISIS CASES REVEALS REGIONAL ENDEMICITY - PUERTO RICO, 2010 AND 2012

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Meliodosis 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
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.

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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 (Mbogath 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/

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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 (0.5%), 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 rrs and pathogen-specific Lipl32 (and including an internal control), with a previously described qPCR targeting rrs, using serum anduffy 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, rrs-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, rrs-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 rrs-Lipl, p=0.44; for rrs PCR, p=0.86). Mean C1 values were lower for BC (suggesting higher bacterial loads) but not significantly (for rrs-Lipl, p=0.21; for 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, rrs-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.

**1047**

**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 which 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 leptospirosis 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 Leptospiara. 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.

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 diarrheal 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, Tumus, 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 diarrheal 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.

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.

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 antimarial 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.

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 gametocyctical 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 arteether-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 situations 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 anaemia 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 parasitaemia 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 anaemia 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 transmisiones 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 6.7% (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 Pivxix, and 1 was mixed. With respect to the controls, BS were Pfalciparum, 32 were Pivxix 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), jaundice 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 opportuntic 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 opportunists in microscopy and pathogens were typed by PCR methods. In hospital 246 children were treated for malnutrition out of which 43 children had diarrhea. Opportunistic pathogens were frequent with mainly yeast in abnormal quantity (51%), microsporidia (21%), Cryptosporidium hominis (7%) and mixed co-infections. 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 (Sx increase in risk p=0.004), non-exclusive breast-feeding, and hygiene (absence of soap). Cyclospora cayetanensis 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 cryptosporidias, whereas microsporidias 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 Leptospira 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 lept-IGG prevalence of 4.5%. Subjects from households with positive zebus 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 LEPTOSPIRA 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 165 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 165 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.

**1059**

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 index (BMI) were recorded according to the WHO classification of BMI in 3 groups: normal weight, overweight and obese. Radiological grading was assigned according to Kellgren and Lawrence 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 ABSCESES IN A RETURNING TRAVELER

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Amebic liver abscesses (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 102°F, 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 minimal 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 mass 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.
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, 1,499 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: 4.5-5.5 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, Lymphocytes: 1.90-3.17 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 most of the people suffer from at least 1 type of intestinal helminth, the data obtained can contribute to defining more locally-relevant ‘normal laboratory values’.

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 included two amoebae, 13 viruses, 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 assessed 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.

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, Sphylitis and rotavirus, 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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**1067**

**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.

**1068**

**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 Seenah 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 HEMARCHIASIS 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 trichiuriasis. 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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Podocnosis 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 podocnosis 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 podocnosis 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 podocnosis 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 podocnosis 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 podocnosis, and possession of a functional radio were significantly associated with higher odds of WTP. The considerable WTP estimates showed that podocnosis treatment could improve sustainability and service utilization. A subsidized cost recovery scheme could reduce treatment costs and more feasibility integrate podocnosis 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 helminths (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 (KIs) 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 non-acceptance 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.

1073
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 gambiæ 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 anthelmintic 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.

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.

NEGLIGENCE 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 and journalists 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.

ASSESSMENT OF SUPPLY CHAIN MANAGEMENT (SCM) SYSTEMS FOR NEGLIGENCE 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 children 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 Ouaké (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 NEGLLECTED 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 925 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, MDA 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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COENCEMIBILITY 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 Kamanya dam in Rachuonyo district, Katumani, Osani and Wachara pond in Homabay district, and along the Ongoch River in Migori district. The prevalence of S. haematobium monoinfection (7.2%, 95% CI = 6.4-8.7%), 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.

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.
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 (≥214 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 a NTD 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.

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; and at least 3 health centers at 5 or more health facilities. 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.
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 Morrupa 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, Acanthic lumbricoides and Plasmodium falciparum. Antibody responses to antigens for LF (Wb123, Bm14, Bm33NS), trachoma (ppg3, 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 pathogens were 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.

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 parasitites Trypanosoma brucei brucei GVRS3 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 GVRS3 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 in 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.

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.

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 positive results. In this work, we present a non-conventional 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 Phycoceryrin-fluorescent positive parasites (PPP) 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 (PFP>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 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 gambiensae 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 (t1/2 > 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 (ECPScr) did not have any effect. In rats, co-administration of ECP1 (50 mg/kg) with eflornithine (100 mg/kg) orally increased plasma Cmax and AUC of eflornithine by 40-60%, compared to vehicle or ECPScr. 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 EXTRACT 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-52". They shared the same side chain and two of them had same functional group. FAC5 analysis of the Nexin assay revealed that ML-2-3 and ML-52 induced apoptosis in T. b. brucei (GUTat3.1 strain), whereas ML-2-2 did not. FAC5 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-52-treated trypanosomes 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-52 suppressed the expression of both α-tubulin and PFR-a in parasites, while ML-2-2 showed no effect. ML-2-3 and ML-52, 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 purposes.

1094

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 in vitro activity of various NP and also 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 A590 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.

1095

PCR-BASED DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN TWO ENDEMIC VILLAGES FROM PERU AND BOLIVIA: AN ARTICULATED STRATEGY FOUND 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 Leishmann 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.

1096

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), Hippastrum reticulatum c3 (0.9 µg/mL), Psychotria berteroa c4 (0.98 µg/mL), Mentungia calabura c7 (0.57 µg/mL), Eucalyptus robusta c3 (0.92 µg/mL) and Myrsine coriacea c140.84 µg/mL were further analyzed for QC-UPLC/MS/MS data and showed several new anttrypanosomal drug leads with novel pharmacophores.

1097 LEISHMANIA IN SURINAME - NEW INSIGHTS INTO A NEGLLECTED 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 ongoing. 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.

1098 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 fulfills 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.

1099 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 Haell. 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’-GACGGTGCTGCTGCTTACCTCAA-3’) (Reverse 5’-CCGCCCATGCTGCTGATC3’). 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.

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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.

1100
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 Guinea with a sensitivity of 92% (95% CI: 78.9-97%) and a specificity of 96% (95% CI: 86.8-99%). 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 INBIO 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: GAM 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: GAM 23.3% (19.9-26.6%) of severe acute malnutrition (SAM); age 6-59 months: 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 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 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 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:

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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 above. Measurement parameters of the immune response were: production of IFN-γ and IL-13 as markers of Th1 and Th2 response respectively, by peripheral 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.

1105

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 shown 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.”

1106
NANOTECHNOLOGY TO DNA DETECTION OF TYPANOSOMA 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 specificity 100%. We consider the use of microparticles could be as biomarker through DNA in urine samples

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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 significantly associated 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 SP. 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 determining genes. The presence of ssaT, sseB, sseG, sseC genes was (100%) and (93.3%) sseD, sseF 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.

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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 (Limbike, 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.

**1110**

**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, only 16 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 Epinfo 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 (AOR=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.
SEROPREVALENCE OF RIFT VALLEY FEVER, Q-FEVER AND BRUCELLOSIS IN RUMANITANTS 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 x 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.

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 Mycobacteria 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.

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-typed 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 especially 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 sequencing could reveal characteristics of their pathogenicity, relationship and origin.

INCREASED MORBIDITY AND MORTALITY IN DOMESTIC ANIMALS FED GROUND AND BITTEN FRUIT IN BANGLADESI 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 astmh.org
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.

**1117**

**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 associated to HAM/TSP.

**1119**

**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...
immunoglobulin. 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, 11-150 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.

**1121 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 smear 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.

**1122 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 pre-morbid 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.

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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 estimates 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.

1124

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 x-ray 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 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.

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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.

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

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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 sweating, 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 store 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.

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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 naive 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.

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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 rich 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 Entamoeba; 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 dysentery.

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 diarrhea. 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 diarrhoeal 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 (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 13 months, 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.
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, Remell) 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 discrepant 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.

**1133**

**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, amebae 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 amebic 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.
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. bangladesi 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.

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 intestinal parasites was 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 OFPROTOZOANS 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 most challenging mechanisms of disease identification, however it also allows for a rapid epidemic pathway of diagnosis of parasitic disease. Histopathological evaluation of all submitted tissues revealed a wide range of nematodiasis, trematodiasis, and protozoal diseases and parasites 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 ascariasis, multiorgan spirochiasis, 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 naive 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
Cryptosporidium. positive diarrhoea Cryptosporidium immunoassay. We described the prevalence of sample to identify enteropathogens, including Cryptosporidium generated information on socio-demographic, water, sanitation and community within two weeks of recruitment of cases. A questionnaire presenting at health centres, along with 1-3 controls matched for age, sex with both MSD and less severe diarrhoea (LSD) (Nov. 2011-Nov. 2012) children enrolled in GEMS from an enumerated population in rural Cryptosporidium diarrhoea among epidemiology and risk factors for severe diarrhoea (MSD) in children age <5 years. We investigated the Cryptosporidium prevalence was similar between season (188/231, 81%) diarrhoea cases (198/231, 86%) Cryptosporidium 231 (11.9%) diarrhoea cases and 141 (4.7%) controls were positive for and specificity of the methods will be discussed along with identification of unusual species.

1139 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 diarrhea (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.

1140 DETECTION OF NAEGELIERA 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.
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.

1142 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 laboratory, 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 peaks were detected in 5% 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 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.

1143 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’an city, 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 careucrcement management systems in the displaced person settlements 3) carrying out snail control (mollusciciding or black plastic in high-risk environments, 4) conducting health education, 5) increasing patient monitoring and 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 Schistosomiasis cases in humans, but the average density of Oncomelania hupensis snails was higher than observed before the earthquake (4.1snails/m² vs. 0.3 snails/ m²). 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.

1144 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.
Chronic schistosomiasis has been associated with reduced physical fitness, while MICHAEL O. OGUTU 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.

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 anthelmintic 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 (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.

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.
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=2033); 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.

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.

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.

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 benefited 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 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.
Institute of Tropical Medicine, Antwerp, Belgium, 2Institut National Liliane Mpabanzi MATERIAL RESOURCES IN ENDEMIC REGIONS. CONGO: PRE-ASSESSMENT OF STAFF PERFORMANCE AND SCHISTOSOMIASIS IN THE DEMOCRATIC REPUBLIC OF THE ROAD TOWARDS SUSTAINABLE CONTROL OF CHARACTERISTICS OF ABNORMAL BLOOD VESSELS

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1University of Oslo, Oslo, Norway, 2Oslo University Hospital, Oslo, Norway 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.

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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1Institute of Tropical Medicine, Antwerp, Belgium, 2Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo, Kinshasa, Democratic Republic of the Congo 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.

HIGH PREVALENCE OF SCHISTOSOMA 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.

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 (COPPT) 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, COPPT, 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 COPPT, 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 study reached 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 (Zaire 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.
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.

**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 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.

**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 improved 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.

**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.

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.

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.
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.

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.

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.

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. While the value to human health of ecosystem services is well established, 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 of 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 portaline 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. 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.
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.

PROFILING OF AGENTS AGAINST BABESIA DIVERGENS

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Babesiosis is a tick-borne 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 cytoidal profiles of various agents as well as characterizing interactions between different drug combinations.

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.

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, 10^4 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 enterogastric Escherichia coli strain 042. The ampicillin, neomycin, vancomycin cocktail depleted stool microbiota 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–d14). Duodenal burden at 15 days post-infection by RT-PCR revealed similar burden of parasites irrespective of antibiotic treatment. In G. lamblia-enterogastric E. coli 042 co-infection models, growth faltering was worse in the co-enteroparasite infection (p<0.05 dB, dB10) 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%. Infection 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 Δ 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 SCHISTOSOMIASIS 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 haematobia 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 haematobia. 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/PD 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 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 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 mollusciciding 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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EFFECTS OF THE KDR RESISTANCE MUTATION ON THE SUSCEPTIBILITY OF WILD ANOPHELES GAMBAE 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 sporozoites 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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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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GENETIC DIVERSITY PATTERNS IN THE MAJOR MALARIA VECTOR, ANOPOHELES 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 Malawian 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 Pyrethroid 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.

A SIGNIFICANT ASSOCIATION BETWEEN THE VGSC-L1014S KDR MUTATION AND INFECTION WITH PLASMODIUM FALCIPARUM IN ANOPEHELES 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.s. 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 (ψ² = 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. (ψ² = 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.

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 mechanisms have been suggested to be essential in the adaptation of mosquitoes to insecticides and temperature. 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 to examine 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.

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 transfuslin. 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 repelents. 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 INSSENSITIVE ACETYCHOLINESTERASE (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-1R 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 kdr 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. 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 farmgate 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 infected with neither control nor NCC patient groups,suggesting that observed differences. Moreover, the sera samples from idiopathic seizures increased to >0.2 suggesting that physiologic processes accounted for the 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 patients 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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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 patients 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 cisticercosis. 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 G* 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 pancytopenia and 8 bicytopenia. All infected dogs had normocytic normochromic anaemia, leukopenia and/or thrombocytopenia. In myelogram, we observed dysgranulopoiesis (100%), dyserthropoiesis (100%) and dysmegakaryopoiesis (53.8%). VL dogs presented an increase in the myeloid:erythroid ratio compared with non-infected dogs and infected pancytopenic dogs had a greater erythroid precursor:mature erythroid ratio when compared with infected bicytopenic 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:erytroid 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. Anemia 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 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 SYSTEMATICALLY 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 L. 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-L. 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 L. 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 L. 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 L. 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 L. intermedia saliva. In exposed individuals, we observed higher (p<0.01) concentrations of IL-10, IL-13, IFNγ, 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 L. intermedia saliva and CL development, after a 3 year follow-up. We conclude that natural exposure to L. intermedia saliva polarizes the immune response to a regulatory phenotype, predisposing development of CL caused by L. braziliensis.
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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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 (IFN-β) 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 IFN-β and 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, IFN-β 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.

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 PDL1+CD4+ T cells. To evaluate PDL1 expression on DC, we performed in vitro infection with promastigotes and amastigotes. L. amazonensis infection occurred in 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, JIK, JIKIII 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.

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.

DOXYCYCLINE TREATMENT SHOWS A STRONG EMBRYOSTATIC EFFECT AND CLEARANCE OF PERSISTENT MICROFILARIAE IN THE SKIN OF ONCHOCERCALISIS 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 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 (Achtaechelornaenia vitea, 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 microfilariaemia. 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
association is based on a causal link or is confounded by nutritional, socio-
birth outcome. However, at this stage it remains unclear whether this
Unadjusted analysis suggests an association of loiasis with adverse
women in Gabon and that microfilariae commonly invade the placenta.
These findings suggest that loiasis occurs at high prevalence in pregnant
into the intervillous space of the placenta. No signs of further pathological
statistical significance. Out of 186 women with microfilariae in peripheral
analysis demonstrated that preterm birth was more prevalent in infected
the likelihood ratio test (p=0.056 and P=0.008, respectively). Univariate
recruited, 194 (16.4%) presented with microfilariae of
in peripheral
L. loa
infection and those without infection. 1184 pregnant women were
histopathological specimens were compared between women with
infections were
were invited to participate in this cohort study.
preventive treatment of malaria in pregnancy (MiPPAD, NCT 00811421)
clinical trial assessing mefloquine as an alternative drug for intermittent
HIV-negative pregnant women participating in a randomised controlled
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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 microfilariaemia, 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 microfilaremic 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.

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-infecting 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, Wuchereria 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 onconege 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.

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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, mosquitoes, fleas). The low incidence and curious presentation in Lao, a country otherwise endemic for flea-borne rickettsioses, 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.

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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 reinfection. 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 reinfection 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 reinfection. 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 reinfections 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.

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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.

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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. fels 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. fels and uninfected fleas in feeding capsule), co-fed (R. fels-infected and uninfected fleas in same feeding capsule), and cross-fed (R. fels-infected and uninfected fleas in separate feeding capsules). Bleb and co-fed bioassays were also performed with uninfected rat fleas (Xenopsylla cheopis) and R. fels-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. fels 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. fels to flea salivary glands by microscopy. Delineation of transmission mechanisms of R. fels 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 infected 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 sequelae.

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EVIDENCE OF NON-SYSTEMIC BORRELLIA 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 ASEMOBOENSI FROM KENYAN FLEAS

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Candidatus Rickettsia asemboensis was detected molecularly in fleas collected during 2009 from Asemo, 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. fels 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 from round/elongated forms with the presence of double membranes and electron lucent “halos”. Genome sequencing of DNA prepared in TEM were found to be heavily parasitized, and the rickettsiae appeared during 2 weeks of culture.

**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 occurring 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.

**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 monitored 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 included 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 parasitolological 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 patient 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.

**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 high 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 seropositive 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 RNAlater™ (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 (X², 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
A COMPARISON OF HEMOLYTIC POTENTIAL OF THREE ANTIMALARIALS 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 or relative 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.

NOVEL SERO-EPIDEMILOGICAL TOOL FOR ASYMPOTOMIC LEISHMANIASIS 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 asymptotically 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.

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 Biomerieux), 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 immunosuppression 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 DISPERSEL 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.
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.

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

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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 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.

### 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 jointpoint 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 jointpoint 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.

### 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 S80Y and the S74L 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.

### ARTEMISININ RESISTANCE AND THE EMERGENCE OF “SOFT” SWEETS 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 P. falciparum parasites using 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, R359T 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.
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 resistance to chloroquine resistance in 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. Cameroon, 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 resistance to chloroquine, 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 resistance in South-East Asia.

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 the chloroquine sensitive (CQS) parasite line Dd2. Strikingly, dose-response to the compound is significantly reduced in chloroquine resistant (CQ') parasite line Dd2. Seven further refinements of this signal with GSAS analysis of 40 culture adapted clinical isolates indicated a role for PfCRT haplotypes in CQ' response. Independent experiments lead to the generation of an IDI-3783 resistant 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 chemotype in drug resistance. Interestingly, the IDI-3783 resistant mutants also demonstrated a marked reduction in CQ EC50 rendering them susceptible to the drug despite retaining an otherwise CQ' 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.

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.

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

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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.

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/m2 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 overall 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.

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 monoxygenase; P450) on the X chromosome. The selected CYP9K1 allele (cyp-) 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.
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.

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) have. 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 blood feed 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.

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 to develop other alternative chemical classes 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 - lambda-cyhalothrin 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 lambda-cyhalothrin 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 lambda-cyhalothrin. Exit 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.

ADAPTATIONS OF ANOPHELES GAMBIAE TO BREEDING IN POLUTTED 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...
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: 0.25 in 2000 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.

INCREASING HUMAN RESOURCES FOR MALARIA PROGRAM IMPLEMENTATION IN LOW RESOURCE SETTING: THE IMPACT OF A MALARIOLGY 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 malariaology 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 malariaology, 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 (DHPAQ) 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 DHPAQ was superior to AL from both the clinical and economic perspectives for treatment of uncomplicated childhood malaria in high transmission
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 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 Bugarasera 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.

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 and KABC-II neuropsychological testing of 40 Congolese children (21 with konzo and 19 presumably healthy controls, overall mean age (SD): 9.3 (2.2) years). Cyanogenic exposure was ascertained by levels thyrocyanate (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 0.6 to 8 (isoprostane-II, 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 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 (β = -32.36 [-51.59 to -13.03 95% CI; P<0.001]. A regression model including age, gender, motor proficiency impairment, serum albumin and triacylglyceride 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 poisoning 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.

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 anaemia, 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 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 varis 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 inactive 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 suggests 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-2R Null (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-2Rnull (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 demonstrated 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 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.
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.

**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.
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.

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

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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.

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.
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 1.2 fold/year (p<0.05), respectively. When neutralizing antibody responses were analyzed in the same subjects following subsequent second infections, the second-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.

DENGUE VIRUS-SPECIFIC T CELL RESPONSES IN THE GENERAL POPULATION OF NICARAGUA VARY AS A FUNCTION OF THE INFECTING SEROTYPE AND ARE DOMINATED BY HLA-B35-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, 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 target NS proteins, especially NS5, 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.

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 26 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 22 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.
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 host human 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.

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.
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.

HIGH PREVALENCE OF PNEUMOCYSTIS JIROVECCI INFECTIONS AMONG MOZAMBIAN 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 (Pi) 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 Pi infection were performed on all NPA samples using a tri-sequential PCR strategy, assessing two multiplex mitochondrial genes (mtLSU y mtSSU) and a third unicon gene, linked to sulfia drug resistance (DHP5). 77/835 (9.2%) of the patients tested positive for at least one of the Pi genes. 32.5% (25/77) patients showed triple (mtLSU, mtSSU and DHP5) 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 comitant IBI (6 cases of S. pneumoniae, and 5 other bacterial). 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 R. 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.
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 helmhnt infections may blunt control of mycobacteria, and epidemiologic data suggest an association between helmhnt infections and tuberculosis disease. Our objective was to determine whether there is an association between parasite infections and positive tuberculin skin test results (TSIs). 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 TSIs, stool ova and parasite examinations, complete blood counts with differential, and serologic testing for helmhnts (done if eosinophilia was found) and looked for an association between TS 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 helmhnt infection depending on the model).

Among 7220 participants, 2843 (53%) were male, mean age was 25 years (range 1-88), and 3355 (46.4%) had positive TSIs. Individuals with positive TSIs 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). Helmhnt infections were found in 393 (5.4%) including Trichuris trichura (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%). TS positivity was not associated with helmhnt 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 TS positivity. Unmeasured confounders (HIV infection, poverty, malnutrition, etc.), undetected parasites, pre-departure parasite treatment and other reasons for false negative TSIs may have obscured an effect. More sensitive methods for MtB detection and a study of the effect of individual parasite species on TSs are needed to confirm this lack of association.

CHALLENGES OF DETECTING RESISTENCE 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-naive 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 MTBDRplus assay and GeneXpert. A subsample of isolates selected randomly for geographic variability was also tested with the MTBDRs 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 MTBDRs, 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.

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.
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 undernourishment 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 overhydration in resource-limited settings.

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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Podocioniosis is a 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 for podocioniosis were under strong genome-wide association with podocioniosis 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 Pygmies. 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 podocioniosis.

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 weight-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.

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 ≥2 staff on call (24-hour coverage), and an ambulance and phone for referring patients, were considered to provide high quality EmNC. Hospitals with ≥2 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 ≥2 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.

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 geographic 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.

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.

1297

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 $19 (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.

**1298**

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.

**1299**

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, bronchiolitis; 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.

### Mentorship for Global Health Researchers

Hazel E. McCullough, Donald C. Cole, The Canadian Coalition of Global Health Research International Mentorship Working Group

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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 LMICs 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 LMICs; and who are well situated to contribute to the emergent literature in GHR mentorship.

### 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.
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.

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 (UF) and hemorrhagic fever syndrome (HFS). From June to December of 2013, patients ≥2 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., Coxiella burnetii, CCHF virus, hantavirus, Spotted Fever Group (SFG), Scrub Typhus group (STG), and Typhus group (TG) Rickitttsiae. 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 UF 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.

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 pharmacopeial analyses in August 2014. The overall results are expected by October 2014 and will be presented.

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.

**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.

**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.

**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.
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 qualitative 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. 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.
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 Ibon 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 Ibon 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 intervention packages and organized workshops for local government health care in the MOHs. Then these state teams developed their own 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.

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.

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.

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-colonizing 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 1/4 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.

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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 as 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.

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NOVEL DETECTION OF CARDINIUM ENDSYMBIONT 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 as 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 transfection 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 Cardinium 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.

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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 (GiURs) and metabotropic glutamate receptors (miGRs) that mediate impulse transmission across synapses. The iRs, are expressed in antennal coeloconic sensillae neurons, while GiURs and miGRs are expressed on post-synaptic membranes in central nervous system. In order to identify the Glossina morsitans morsitans iRs, GiURs and miGRs, 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 GMOVY1.1. Phylogenetic relationships among Glossina iRs, GiURs and miGRs 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, GiURs and miGRs were established using RNA-seq data of adult female fly. Overall, 40 putative glutamate-gated receptor loci comprising 19, 15 and six iRs, GiURs and miGRs respectively were recovered in Glossina. The Glossina iRs, GiURs and miGRs 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 GiURs. Relative to D. melanogaster, annotation of Glossina revealed lower numbers of iRs, but certain loci had multiple related copies. The iGRs numbers were similar, while miGR-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.
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.

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 calcitrans) 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.

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.

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. 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. 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. 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. 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. 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.
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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 Panama. 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. 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 trapaidai 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).

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 (R0 ± 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 R0 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 Trypanosoma cruzi is transmitted by the bug Triatoma dimidiata. While Triatoma 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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ONCHOERCIASIS 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 simulid 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 SCABIOUS 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 animals. 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. quinquefasciatus the primary vector for Wuchereria bancrofti and Ae. 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. quinquefasciatus the primary vector for Wuchereria bancrofti and Ae. 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. quinquefasciatus the primary vector for Wuchereria bancrofti and Ae. 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. quinquefasciatus the primary vector for Wuchereria bancrofti and Ae. aegypti abundance was measured to determine weather's influence on mosquito population.

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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.

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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 QIME 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 Thorsella (67.6%) and Propionibacterium (9.08%). Further studies will investigate the effects of varying levels of commonly administered antimicrobials on the gut microbiota of the An. gambiae gut microbiome 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 SYMBIOTS 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 sand 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.

HYPOTHESIS TESTING CLARIFIES TRIATOMA DIMIDIATA (LATELLE, 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.

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 hemisynthrophic 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 CONSERVATION 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 conditions while on and off the vertebrate host. To counteract 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 (TrxR) 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 feeding 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 RNA 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 months. 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 papatasii, 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 hypothesis 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 a habitat for ticks 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.
Distribution of common polymorphic inversions positively correlates with the density of simple repeats, suggesting their high rates of evolution in the X chromosome. Anopheles stephensi is characterized by relatively low repeat content compared with that of An. gambiae. 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.

Silencing caspase decreases DENV-2 infection of the mosquito vector Aedes aegypti

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, Drorc, has been previously shown to be an essential component of the core apoptotic pathway. This caspase showed higher expression in vitro 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 Drorc decreased virus titer and infection in resistant mosquitoes. This reveals that Drorc may be important for affecting DENV-2 infection in A. aegypti. Furthermore, we investigate whether silencing of Drorc effects non-apoptotic processes influencing DENV-2 infection.

Physical mapping reveals chromosome-specific genomic landscapes in Anopheles stephensi

Anopheles stephensi type form is the key vector of malaria on the Indian subcontinent and the Middle East. Anopheles 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.
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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¹Institute of Tropical Medicine - Nagasaki University, Nagasaki, Japan; ²University of the Philippines, Manila, Philippines; ³Aedes 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 mosquitoes 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.
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 individuals, 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. Interestingly, a detailed analysis of the genomic locus of chromosome 2 (2R:57.6-2L:4.0MB) that has fewer SNPs and largest genome. Interestedly, detailed analysis of the genomic locus Meantime, we discovered that several large LD blocks were clustered in A. gambiae genome. Interestingly, a detailed analysis of the genomic locus of chromosome 2 (2R:57.6-2L:4.0MB) that has fewer SNPs and largest genome. Interestedly, detailed analysis of the genomic locus

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 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.

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.

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

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mikrobiërs 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 larva, 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 1.5 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 larva and pupa were similar and resembled to the microbiomes of the larval habitats. Proteobacteria, Bacteroidetes 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.

**DISSECTING GENETIC AND MICROBIAL FACTORS OF AEDES AEGYPTI FIELD POPULATIONS WITH DISTINCT SUSCEPTIBILITY TO 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 mosquitoes 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.
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.

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN IMMUNE RESPONSE PATHWAYS GENES, AND SUSCEPTIBILITY/RESISTENCE 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 Plasmodium 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 Plasmodium 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 Plasmodium 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.

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.

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 1950s. 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.

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 zoonoses 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 assessed 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 (Lambdacalothrin 0.05%, Permethrin 0.75%, Deltamethrin 0.05% and Etofenprox 0.5%) and Bendiocarb 0.1% was respectively found out in eight (25%), 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 ENZYMOTOLOGICAL INOCULATION RATE IN NASARAWA STATE, NORTH CENTRAL NIGERIA

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The President’s Malaria Initiative | Africa Indoor Residual Spraying project, executed as second 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 circumsporozoite 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. ELISA 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 ($\chi^2=8.696$, p<0.0032, df=1). At baseline (pre-intervention), EIR was found to be 1.31 infective bites/person/night (bpm) in Doma, 0.16 in N’Eggon has bed 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 bpm. 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.
DYNAMIC RELATIONSHIPS BETWEEN MOSQUITO MICROBIOME AND VECTOR COMPETENCE
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The holobionome theory of evolution proposes that natural selection of an organism is also driven by its symbiotic microorganisms. Research on the insect holobionome (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 holobionome 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 transmission capability. However, the function and utility of those microbes are virtually unknown, especially in mosquitoes that transmit viruses. We compared microbiomes of two phenotypically distinct colonies of Anopheles gambiae, constituting 11.3% the control (S-form) samples. A. gambiae s.s. was 0% and An. gambiae s.s. were found positive. After IRS, density 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 An. arabiensis species ratio with 0% 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 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.

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 these 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 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.

POPLATION 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. and An. gambiae s.m. 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 An. 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 PomPA 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 transstadial 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 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 ANOPELEHS 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 repellents. 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 I trial of a Plasmodium falciparum transmission-blocking vaccine (Pf5s25), 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, Aedes 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.

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OCCURRENCE OF PAROUS FEMALES IN COPULA IN NATURAL SWARMS OF ANOPELEHS GAMBIAE S.L.: EVIDENCE FOR REMATING 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.

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COMPARATIVE ABILITIES OF MICROFILAREMIC VERSUS NON-MICROFILAREMIC BIRDS TO INFLICT 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 microfilaricar 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 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 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 microfilaricar 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 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 microfilaricar 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
the rate of spread was strongly seasonal and greatest during late spring.

We also show that to be much greater for introductions of eggs in containers compared to for reproduction and spread. We found the probability of establishment on this landscape using modeling with remote sensing and surveillance data from the study area.

invasions occur. Here, we consider the ongoing invasion and establishment surveillance paradigms and detailed data on the real landscapes where biology with Ae. albopictus is understood because even intensive surveillance systems yield limited results.

is also well-studied, but the fine-scale vector of several zoonotic arboviruses. As a result of its importance, important dengue and chikungunya viruses, and a potential bridge to human health, being a day-biting pest, a competent vector for globally distributed 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 m² to one per 300 m². 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.

The mosquito, Ae. 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.

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. punctatus 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.

Pre-clinical evaluation of a combined live attenuated (LAV) and subunit (DEN-80E) prime-boost vaccine approach against dengue

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Merk & 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 naive 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 titters 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.

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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.

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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.

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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: 5.753) 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 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 it 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 fatal 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.
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.

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.
SPATIAL CLUSTERING OF DENGUE AT THE HOUSEHOLD LEVEL IN A HIGHLY URBAN SETTING
Katherine Anders¹, Tran Van Ngoc², Nguyen V. Chau², Nguyen Duc Tho³, Cameron Simmons¹
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EPIDEMIOLOGY, IMMUNOGENICITY AND PROTECTION OF INACTIVATED DENGUE VIRUS ANTIGENS FORMULATED WITH A NOVEL BIOLOGICAL ADJUVANT IN RHESUS MACAQUES
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Dengue viruses (DENV-1-4) 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 (GV30000/3A). 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.

THE DETECTION OF ANTI-DENGUE VIRUS IgM IN URINE AS A POTENTIAL 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 illness. Acute 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 95%CI 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.

DEVELOPMENT OF DENGUE VIRUS PRM-REACTIVE ANTI-BODIES 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 stoichiometric insufficiency 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 DENGGUE 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 (hosDF) and dengue hemorrhagic fever (DHF) over the period. Talled 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, hosDF, 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.

1389

INNATE IMMUNITY AND TRANSCRIPTOME PROFILING AFTER ADMINISTRATION OF TAKEDA'S LIVE ATTENUATED DENGGUE VACCINE CANDIDATE IN FLAVIVIRUS-NAIVE HUMAN VOLUNTEERS: ASSOCIATION OF GENE EXPRESSION WITH DEVELOPMENT OF NEUTRALIZING ANTIBODY RESPONSES TO DENGGUE

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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 DENGGUE 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.
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.

1393

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 exsorpatated 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 exsorpatated, DENV, and the vertebrate host environment.

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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
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.

### 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.

### Dengue and Efforts to Leverage Data for Real-Time 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.
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.

1398

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.

1400

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.

1401

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 costs 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 available for 1168 (81%). For direct medical costs, we observed an increased cost gradient depending on patient age. Dengue was associated with: leukopenia, rash and joint pain (p < 0.003) in adults aged ≥20 years. Knowledge of predictors can be used to direct anticipatory guidance.

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-identifier 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: retroorbital 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.

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**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 to the presence of the vector and previous DEN 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 Dengue Duo). 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 Somgandé) and DENV4 (Dapoya, Gounghin and Somgandé). 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, the presence of DENV 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 (SEDDS) 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 2014 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 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 N53 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 DENV3, 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.

**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 infants 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, morbidity 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 N53 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 DENV3, 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) and 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 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.

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 USA 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.

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 both countries. 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 preemptively.

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ESTIMATING CROSS-IMMUNITY INTERACTIONS OF DENGUE SEROTYPES USING LONGITUDINAL SEROLOGICAL DATA

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Dengue, a mosquito-borne disease whose incidence and geographic 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. 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, metapopulation 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.

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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.

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MOLECULAR CHARACTERIZATION OF INFLUENZA A AND B VIRUSES IN CUBA DURING 2006-2010, IMMUINOLOGICAL 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 CCR5 and TLR-2, and the presence of seasonal 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.

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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.

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CYTOKINE RESPONSE OF RHESUS MACAQUES EXPOSED TO LIVE EBOLA ZAIRE VIRUS CHARACTERIZED WITH MAGPIX PARAMAGNETIC BEAD TECHNOLOGY

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Viral Hemorrhagic Fevers (VHF) are serious, frequently fatal illnesses characterized by fever and unusual susceptibility to bleeding. VHF 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.
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 nuceloprotein 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.

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 (M1) gene and in-house designed primers and probes to detect the influenza B virus M1 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.
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.

**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 Ballet, 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 Ballet 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.

**COVERAGE DURING AN IMMUNIZATION CAMPAIGN PROVIDING INACTIVATED AND ORAL POLIO VACCINES IN REFUGEE CAMPS AND HOST COMMUNITIES, KENYA - DECEMBER 2013**

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Polioymelitis 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.

**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 feverviridae, active and passive development should be high on the agenda for the availability of rapid diagnostic tests for viral hemorrhagic feverviridae, active and passive immunizations for infected patients and the passive immunization of communities at risk.

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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 samples collected before the OPV administration (day 0) and on days +4, +11, +18, and +25 after administration. Of the 1350 samples examined (270 infants x 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 3.37x10^4 ± 1.22x10^4 versus 3.88x10^3 ± 1.45x10^3, 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 53 (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.

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-Como 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/ΔR2) which illustrates the additive effect of genomic regions in MPXV pathogenicity. Deletions of MPXV regions in the highly virulent MPXV-Como genome hindered cell culture growth and significantly reduced morbidity, replication, spread and mortality in infected CAST/EiJ mice. Thus, parental MPXV-Como/Luc+ caused 100% mortality while all mice infected with recombinant MPXV/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.

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 meningocencephalitis 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.

**1429**

**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 virus evolution.

**1430**

**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 4.4% 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) is a 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
baboos 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 non-human 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.

1433
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 zero-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 breast milk 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 quintile 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 flexibility to detect trends in skewed data.
**1435**

**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-nitrosogluthionate 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.

**1436**

**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: 0.18-0.50), 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.

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 cathelicidin 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 malarian 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.

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 to 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 glycoporphin 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.

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.
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 of 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 infections by semi-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 neumaminidase, trypsin, chymotrypsin or a combination of these enzymes, in the first round of invasion following thawing but prior to adaption to culture. DNA 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 GAMETO CYTOGENESIS**

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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 erythrocyte 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.
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 neighboring 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 ~350 km 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.

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 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.

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 (Kd = 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 (Kd = 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-Lexo) 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 extractable, indicative of integral and peripheral membrane proteins, respectively.
Preliminary analysis of co-immunoprecipitation and proteomics analysis confirmed direct association of PICSA-L and VAR2CSA on the surface knobs of Pf-E. These findings suggest that PICSA-L interacts with VAR2CSA on surface knobs of Pf-E, where they contribute to the CSA-binding phenotype. We are attempting to immunize rats with recombinant PICSA-L and VAR2CSA DBL2X complexes to generate antibodies against neo-epitopes that may be capable of blocking Pf-E binding to CSA. As a highly conserved protein of small size (~25 kDa), PICSA-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 EBL-1/Glycoporphin B (GPC) invasion pathways, 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 (GBP) invasion pathway because polymorphisms in GBP are prevalent in malaria-endemic regions, suggesting selection from malaria pressure. Through bioinformatic analysis, we have also recently identified considerable variation in GBP transcript levels in individuals from Benin. To elucidate the relative importance of the EBL-1/GBP 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, GBP 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 invasion in patients with HbAS, we quantified parasite cellular phenotypes while cultivating in vitro 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 paralyzed 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 oxygenation 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 vs. CM day 3: 1.37[1.18-1.50] % O2 saturation/second).
second, $p = 0.176$ vs. CM admission; CM day 3: $1.07(0.98-1.33)$% $O_2$ 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: $1.99(0.59-2.74)$% $O_2$ 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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RETNAL 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 (MSP-1) 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 (Fy1/Fy1 = 2197 ± 608.15 vs Fy2/Fy2 = 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.

**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, which is responsible for determining imputability, judged that imputability was possible in 18 cases, improbable in 1 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.

**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, 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 quintile. Use of AR 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.

**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, 24 to 8 years, and adults, respectively]. For the artesminins (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.

**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 QA QACT, 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 (63% vs. 41%), although it was a strong predictor of both outcomes (p<0.001 for both). The type of co-paid QA QACT being stocked (ASAQAQ/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.

**EFFECTIVENESS AND TREATMENT ADHERENCE TO ARTEMETHER-LUMEFANTRINE UNIT DOSED BLISTER-PACKS VERSUS STANDARD BLISTER-PACKS IN THE TREATMENT OF UNCOMPROMICATED 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 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.

**BIOLOGICAL STABILITY OF DIHYDROARTESMININ 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 culture 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 heterocyclic 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 pyrimethamine 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, and 3) Determine the frequency of chorioitis 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 ninth 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. Difference 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 complex treatment regimes 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 regimes, 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 Malarialogy, Parasitology and Entomology (NMPE), has funding from the Global Fund to implement DOT in areas where multi-drug resistant malaria is emerging. In coordination with NMPE, 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.
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. Chemo-genetic 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-parasitic 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 chemogenetic 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.

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 (FGDs) 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 parasitaemic are then given treatment.

IN VIVO EFFICACY AND SAFETY OF ARTEMETHER/ LUMEFANTRINE VS. DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPPLICATED 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 Kitgum 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.

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 mepracrine, 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 mepracrine, 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.

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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 premonition 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 University 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, GRHK and GRHM. 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 PPiR, 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 GHC 1.50. 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 artemisinin therapies from 34.2%, and 6.9% to 9.5% in the most recent censuses. 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 (QA ACTs) 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 QUAACTs 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 QUAACTs 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 gametocytomia in patients treated for uncomplicated malaria. Gametocytes carriage and density were estimated by PfS25mRNA amplification using QF-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 gametocytomia. 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 gametocytomia 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 recrudescence 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.

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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-naive 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-naive 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-naive 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-naive 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.
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 antimarial 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 artmether/lumefantrine 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. Artmether 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 widespread use of artmether/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.

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 F. 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.

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 Thies 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 included 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 artmether + 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 artemether 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 time of recurrence with different genetic markers (using the SNP-based barcode).
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 sulfadoxine-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 plasmodial 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 IPT 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.

PREGNANCY LOWERS THE EXPOSURE OF DIHYDROARTESMININ: 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 dihydroartesminismin is decreased during pregnancy after oral administration of dihydroartesminisin, artesunate and artetherm. 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 artesminisin 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 dihydroartesminisin, artesunate and artetherm in pregnant women with uncomplicated Plasmodium falciparum malaria. Simulations indicated that it was possible to achieve similar dihydroartesminisin 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 dihydroartesminisin 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.

SIMULATIONS TO INVESTIGATE NEW INTERMITTENT PREVENTIVE THERAPY DOsing REGIMENS FOR DIHYDROARTESMINisin-Piperquine

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A fixed-dose combination of dihydroartesminisin (DHA) and piperquine (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 dosing dose strategy in a year. Clinical trial simulations were applied to investigate the necessary sample size to confirm the predicted advantage with weekly dosing 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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failures and piperaquine IC50s relative to our treatment study conducted in the 4 halted volunteers (Spearman ρ (Spearman ρ = 0.6706, p-value < 0.0001), with strong correlation pre-specified safety endpoint of >500 ms QTcF prolongation. Two-day DP blind, placebo-controlled study evaluating 2-day DP as a monthly malaria treatment, the latter used by the Cambodian military for malaria treatment, clinical studies to explore the cardiotoxicity risk of administering DP for 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 ρ = 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 IC50 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 of 47, 76.6%) relative to 3-day DP (13 out of 50, 26%). Mean plasma piperaquine Cmax 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). 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.

PKPD RELATIONSHIPS BETWEEN PLASMA PIPERAQUINE LEVELS AND CARDIAC QTc PROLONGATION IN MALARIA PATIENTS ADMINISTERED DIHYDROARTESININ - 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 first-line 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 ρ = 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 IC50 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 of 47, 76.6%) relative to 3-day DP (13 out of 50, 26%). Mean plasma piperaquine Cmax 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.
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 UNCOMPPLICATED 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 sublingual (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 of 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 of 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, sublingual 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.

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 8/89 [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.
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 ± 2230, 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 (N2) via nasal cannula (INOPulse, TM, Ikaria, USA) for at least 24 hours. All patients received IV artesunate and were monitored continuously for changes in methHb% 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 N2 group, and 4 (8.7%) patients died in the INO group. Five patients in the N2 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 ± SD). There was no difference in the baseline methHb% levels among patients in both study arms. There was an increase in methHb% in patients treated with INO, up to 4.1 ± 2.3% (N=33, mean ± SD) at 12 hours, which remained at safe elevated levels up to 72 hours. MethHb levels were unchange throughout the treatment period in the N 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.

**1488**

**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 pruritus, 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. Gametocyteemia 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 Malaria Infection (CHMI) studies are assuming an increasing place in evaluation of interventions as they can lead to accrual of pivotal efficacy trials. 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 pruritus, 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. Gametocyteemia 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.

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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 serocorrections were detected nor were any additional confections 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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Piperazine (PQP) is a 4-aminouquinoline 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 escalation clinical trial of this drug in the induced blood stage infection malaria (IBSM) system, with simultaneous measurement of drug levels and parasitaemia, 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 = 79 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 parasitaemia 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 parasitaemia 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 parasitaemia 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.

1491

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 artemunate 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, Sulphadoxine-pyrimethamine, Amodiaquine and Sulphadoxine-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 hematocrit 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.

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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/IV 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 ≥26 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/ml (405.7 to 948.6 ng/ml; n=343) and 697.15 ng/ml (491.69 to 967.93 ng/ml; n=135). There was, however, an increase between first and third episodes (789.3 ng/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 recrudescence primary infections from new infections) and drug plasma concentrations.

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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 VANECCAM project, and were randomised to receive either artemether-lumefantrine, dihydroartemisinin-piperazine, 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.

1494

STUDY ON EFFICACY OF ARTESUNATE-MELOQUINE COMBINATION THERAPY FOR TREATMENT OF UNCOMPPLICATED 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 at baseline 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 Kankanaburi 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 antimalarials 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.

1495

PRELIMINARY RESULTS: PHASE 2 RANDOMIZED PROOF OF CONCEPT STUDY COMPARING AN INVESTIGATIONAL AMINOQUINOLINE ANTIMALARIAL (AQ-13) TO COARTEM IN ADULT MALIAN MALES WITH UNCOMPPLICATED 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 artemisins 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 (sexual 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.

**1496**

**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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**1497**

**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 (10/10) 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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**1498**

**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-RBCs 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=0.0010 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.

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 artemisinin 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.

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 geospatial 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 target appropriate and aggressive response measures to support malaria elimination in areas of global significance will be presented.

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 restratification 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...
malaria transmission, area at risk for reintroduction of malaria, low (>0-1/1000), medium (1-5/1000), or high (>5/1000)). The current levels of malaria endemicity using historical passive case detection data will be determined by September 2014. Using available data, greater precision of where malaria transmission is occurring and populations at risk will also be estimated. Enhanced methods to collect these data prospectively will be developed. Additionally, data on all prior malaria interventions for the last 5 years will also be collected and entered into a database. These will be analyzed to estimate the impact of prior malaria control interventions in an operations research model to help select which methods should be continued or reassessed. Methods to prospectively assess the impact of new interventions in an on-going and iterative fashion will also be developed. We will present the new 2014 restratification data and compare and contrast it with the 2009 data. We will illustrate how these new data will be used to better target interventions. The plan to collect more precise prospective information, as well as the status of the analysis of intervention impact, will also be presented.

1502

**LAMP AS DIAGNOSTIC TOOL FOR DETECTION OF SUB-PATENT ASYMPTOMATIC MALARIA INFECTIONS IN PRE-ELIMINATION SETTINGS IN NORTHERN NAMIBIA**

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The global map of malaria is shrinking with 34 out of 99 malaria endemic countries embarking on eliminating the disease. Transmission in Namibia has declined dramatically from 477,786 cases in 2000 to 1546 in the 2013 malaria season. Namibia is now in the pre-elimination phase of malaria and is targeting elimination by 2020. A new challenge facing the elimination campaign is the detection of asymptomatic malaria cases with low levels of parasitaemia. These infections are difficult to detect as they are below the threshold of routinely used Rapid Diagnostic Test (RDT) kits, yet can result in onward transmission. Molecular tools based on DNA amplification such as PCR are sensitive and specific enough to detect low parasitaemia but their routine use requires expensive, highly technical equipment and expertise. Loop-mediated isothermal amplification (LAMP) is a tool based on DNA amplification and has the advantages of PCR yet is requires less expertise and equipment. This study was conducted to determine the usefulness of LAMP as a diagnostic tool to detect asymptomatic, sub-patient infections found during reactive case detection in Engela district in Northern Namibia. All RDT confirmed malaria cases reported in the Engela district and members of their households as well as occupants of the four surrounding households were recruited into the study. RDTs and dried blood spots (DBS) of all subjects were collected and DNA was extracted from both using the chelex method. LAMP was run using DNA extracted from all the collected samples and results detected as fluorescence under a UV light. Preliminary results from 416 RDTs and DBS collected during follow up of 11 index cases, showed 11 individuals positive by RDT and 18 positive by LAMP. Thus 7 additional secondary malaria cases associated with index cases (a 1.6 fold increase) were detected by LAMP over RDTs. This shows LAMP could be a useful tool to detect sub-patient asymptomatic malaria infections at low transmission and may be a suitable diagnostic tool for use in pre-elimination settings.

1503

**BASELINE EPIDEMIOLOGICAL CHARACTERISTICS OF PARTICIPANTS ENROLLED IN A TRIAL OF INTERMITTENT MASS SCREENING AND TREATMENT FOR MALARIA IN WESTERN KENYA**

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The prevalence of malaria infection remains high in western Kenya despite over 30 years of control efforts. Community parasite prevalence in children <5 years of age was estimated at >80% in the early 1980s, and dropped to 26% by 2008. Prevalence rose to 43% in 2011. Individuals with asymptomatic parasitaemia account for 90% of infections in specific age groups and may be sustaining the continued high level of transmission. Mathematical models suggest that strategies targeting the asymptomatic population could reduce malaria transmission. We have initiated a multi-year two-arm cluster randomized controlled trial to evaluate the impact of intermittent mass screen and treat (iMsaT) campaigns for malaria in Siaya County, western Kenya. We describe baseline cross-sectional survey results. Twenty compounds were randomly selected from each of 20 clusters during peak malaria transmission season in July-August, 2013. All consenting individuals within houses of selected compounds were asked demographic, behavioral and symptom-based questions by community health workers using personal digital assistants. Blood samples were tested for malaria using a combination HRP2/pLDH rapid diagnostic test (RDT), light microscopy (LM), and polymerase chain reaction (PCR). A total of 1,987 persons living in 605 households from 359 selected compounds were interviewed. Of these, 1,402 consented for both RDTs and LM. Baseline malaria infection prevalence was 47.1% (95% Confidence interval [CI] 43.9-50.2) and 36.6% (CI: 33.3-39.8) by RDT and LM, respectively. RDT positivity was strongly associated with age; 64.9% (CI: 57.6-72.2), 70.8% (CI: 65.1-76.4), and 27.8% (CI: 24.5-31.1) of persons aged <5 years, 5-15 years, and older than 15 years were RDT positive (P < 0.0001), respectively. Only 25% who were RDT positive reported a fever in the prior 24 hours, and 45% reported a fever in the previous two weeks. Overall, history of fever in the previous 2 weeks was not associated with RDT positivity, PR 0.94 (CI: 0.84-1.04). Of persons reporting a fever in the previous 2 weeks, RDT positive individuals were as likely to seek care as those who were not, PR 1.04 (CI: 0.93-1.16). PCR results and multivariable analyses are pending. The large proportion of infections that were not associated with fever or care-seeking behavior suggests that strategies targeting the asymptomatic population may be beneficial for reducing malaria transmission in western Kenya.

1504

**COMMUNITY-LEVEL MALARIA SURVEILLANCE IN SOUTHERN PROVINCE, ZAMBIA - AN ANALYSIS OF PERCEPTIONS, PRACTICE AND PROGRESS IN AN AREA TARGETING ELIMINATION**

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Since its inception in 2011, the training techniques and personnel associated with the program have evolved and matured using anecdotal feedback from community health workers (CHWs) trained to deliver the program, their supervisors and district-level staff. We have recently conducted a systematic qualitative program review to determine how malaria is perceived by the program implementers and beneficiaries, how the community received the surveillance platform, and solutions to key
operational challenges that could improve implementation. A criterion-based sampling framework based on training regime and performance level of facilities offering the program was used to select six rural health posts, three per district, and a representative from associated stakeholder groups (community health workers delivering the program, their clinic-based supervisors, regional-level staff, and community members).

Individual interviews and focus group discussions were then held in these selected sites. Service providers, supervisors, program administrators and community members all credited the program with helping to reduce the number of malaria cases. Barriers to fuller implementation of the program included transportation (e.g. ensuring all CHWs had working bicycles), communication (e.g. providing CHWs with working cell phones and “talk time” to transmit data by phone) and supplies (e.g. ensuring adequate number of RDT kits to test for malaria in clinics and communities, artemether-lumefantrine to treat uncomplicated malaria cases, and antipyretics for malaria-negative patients to encourage future visits to rural health centers). Results from this review will be used when developing plans to scale-up the program for delivery in other parts of Zambia.

1505
REACHING MIGRANT AND MOBILE POPULATIONS THROUGH A PRIVATE SECTOR INITIATIVE: MALARIA BED NET LENDING SCHEME IN CAMBODIA

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While tremendous strides have been made toward eliminating malaria in Cambodia, pockets of risk persist in border areas that are remote and have high population mobility. Lacking knowledge of malaria, migrant and mobile populations (MMPs) are particularly at risk. The PMI/USAID Control and Prevention of Malaria (CAP-Malaria) Project in the Greater Mekong Sub-region works with private sector employers to increase access to and use of long-lasting insecticidal nets (LLINs) by their migrant workers. Employers range from small farm owners to large companies that manage rubber, cassava plantations, and hydroelectric dam construction. Employers receive a stock of LLINs and malaria educational materials for their employees. The employers then lend the LLINs to their workers, retrieving them prior to their departure for reuse with other migrant employees. An evaluation of the lending model was conducted during the harvesting season in late 2013. The study assessed access to and utilization of LLINs by migrant workers, and explored reasons for non-use. Interviews were conducted with 207 farm owners and 712 workers. Results showed that farm owners were generally satisfied with the LLIN lending model. Some employers (28%) ran out of nets. LLIN uptake among the workers was high, most (93%) had a bed net at their residence, and almost all (96%) reported sleeping under a bed net the previous night. Half of the workers (58%) had received an LLIN from their employer.

Some employers (28%) ran out of nets. LLIN uptake among the workers was high, most (93%) had a bed net at their residence, and almost all (96%) reported sleeping under a bed net the previous night. Half of the workers (58%) had received an LLIN from their employer.

1506
MODELING PHARMACOKINETICS AND PHARMACODYNAMICS OF ANTIMALARIAL DRUGS IN THE EPIDEMIOLOGICAL MODELING (EMOD) MODEL WITH IMPLICATIONS FOR TRANSMISSION REDUCTION

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Modeling approaches can predict the effect of large-scale drug deployments on reducing morbidity over several years of campaigns, leading to better understanding of the role of antimalarial drugs in eradication. The Epidemiological Modeling (EMOD) simulation program includes individual-level modeling of vectors and within-host dynamics, providing an ideal environment in which to study the interplay of antimalarial drugs and host immunity in clearing both asexual parasites and transmission-stage gametocytes. We modeled the pharmacokinetics (PK) and pharmacodynamics of two artemisinin-based combination therapies (ACTs), artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP), and one gametocytocidal drug, primaquine, using age-based dosing and weight-dependent PK. We show that current dosing regimes, especially current fixed-dose recommendations for DP, significantly underdose children. We also find that asexual-stage immunity alone is insufficient to explain low gametocyte prevalence in populations with endemic malaria; host physiological responses are likely to modulate prevalence of the sexual stage. We identify a maximum EIR above which co-dosing ACTs with primaquine has little effect on reducing prevalence, and we demonstrate that a minimal level of individual compliance is necessary for mass drug treatments to impact transmission. Pharmacological modeling of antimalarials can guide community decisions in drug administrations and alert administrators to the most likely and deleterious modes of drug failure.
provides an evidence base for informing the design and simulation of elimination strategies by identifying regions afforded protection from malaria elimination. These data were also combined with Plasmodium falciparum and P. vivax malaria transmission maps and a global population dataset to identify the likely principal sources and destinations of imported cases. Results indicate that certain groups of countries and regions within countries are much more strongly connected by high levels of population movement than others. The mapping of these communities provides an evidence base for informing the design and simulation of malaria elimination strategies globally.

PHASE 2A DOSE ESCALATION STUDY OF SAFETY AND EFFICACY OF LOW SINGLE-DOSE PRIMAQUINE FOR GAMETOCYTICidal ACTIVITY AGAINST P. FALCIPARUM IN SUB-SAHARAN AFRICA

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Primaquine is the only currently available drug with strong gametocytocidal activity against Plasmodium falciparum and P. vivax malaria. While its deployment has been limited due to safety concerns, we conducted a Phase 2a dose escalation study of safety and efficacy of low single-dose primaquine in non-deficient G6PD male in Ouessolessebougou Mali. The first 50 participants aged 5 to 50 years with Plasmodium falciparum gametocyte at blood smear were randomly allocated to one of the following treatment groups with primaquine at 0, 0.125 mg/kg and 0.5 mg/kg. All participants received standard dose dihydroartemisinin-piperaquine. Subjects were positive by microscopy and q-PCR, respectively. On the last survey, 32.8% (95% CI 22.9%, 45.4%) of 151 subjects were positive by microscopy and q-PCR, respectively.

GENOME-SCALE PROTEIN MICROARRAY ANALYSIS OF PLASMODIUM FALCIPARUM AND P. VIVAX SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS IN A REMOTE VILLAGE OF THE PERUVIAN AMAZON

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The Peruvian Amazon is hypoendemic for Plasmodium falciparum (Pf) and P. vivax (Pv) malaria. To assess, at a population level, the proportion of people infected by the endemic malaria parasites, a prospective cohort study was done in Santa Emilia, a remote-rural village in the Peruvian Amazon in 2013 where the main seasonality for malaria relates to river height rather than rainfall. Light microscopy and an aldolase gene-specific qPCR assay that detects both Pf and Pv were used to assess the presence of parasitemia. Plasmas were obtained for genome-scale protein microarray analysis using a combined Pi500/Pv500 chip containing a down selected list of the 500 of the top most sero-reactive antigens for each Pf and Pv. At baseline, 33(22%) and 77(51%) of 151 subjects were positive by microscopy and q-PCR, respectively. On the last survey, 3(2%) and 8(5%) of 157 subjects were positive by microscopy and q-PCR, respectively. Asymptomatic parasitemia detected by microscopy ranged from 22% to 40%, while for q-PCR ranged from 41% to 60%. A significant proportion of infections detected by q-PCR, 39% and 47% for Pf and Pv, respectively, were undetected using microscopy. Comparison of proportions of negative subjects at each of the eleven surveys revealed a significant increase of negative subjects during September to December surveys in comparison to March surveys. Protein microarray analysis was done with 324 plasma samples: 132 matched paired samples from the two time points and 60 unpaired samples. The top 200 most sero-reactive antigens were selected for comparison. Seroreactivity increased with age and in response to documented malaria infection. Seroreactivity was lower in September than in March for all age groups, paralleling mosquito abundances (related to river height, not rainfall). While there was a gradual increase in seroreactivity associated with age, the differential seroreactivity between March (high) and September (low) was greatest in youngest and this difference decreased with age. Prior to embarking on an elimination strategy, monitoring changes in transmission intensity and identification of malaria foci is mandatory for best intervention efforts. This population-based study of a malaria-endemic population identified new serological markers of infection using
Antonio M. Quispe

COUNTRIES

AT THE COMMUNITY LEVEL IN MALARIA ENDEMIC ELIMINATION INTERVENTIONS AND OUTBREAK RESPONSES

A FREE SURVEILLANCE APP FOR PLANNING MALARIA control interventions to ensure sustained gains in reduction of disease area. Success of the campaign depends on continued coverage of vector with MDA. The optimal implementation strategy for MTAT/MDA will vary depending on local conditions. Simulation results indicate a high proportion of low density infections prevalence. The addition of an endectocide, such as ivermectin, which acts as a gametocide, to the drug regimen did not further reduce parasite prevalence during the peak transmission month following the campaign, compared to the currently-implemented strategy. Simulation results suggest that the most important determinant of success in reducing prevalence is the coverage of the population achieved in the campaign. However, even with high coverage with mass drug administration (MDA) in areas with a pre-intervention all-age parasite prevalence of less than 10%, simulations suggest that elimination would require more than one year of campaign implementation. Including single low-dose primaquine, which acts as a gametocide, to the drug regimen did not further reduce prevalence. The addition of an endectocide, such as ivermectin, resulted in lower simulated parasite prevalence and warrants further investigation. Simulation results indicate a high proportion of low density infections were missed by rapid diagnostic tests that would be treated and cleared with MDA. The optimal implementation strategy for MTAT/MDA will vary by background level of prevalence and rate of infections imported to the area. Success of the campaign depends on continued coverage of vector control interventions to ensure sustained gains in reduction of disease burden.

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Malaria elimination requires reducing both the potential of mosquitoes to transmit parasites and the infectious reservoir of parasites in humans, including asymptomatic infections. To achieve this goal in Southern Province, Zambia a mass test and treat (MTAT) campaign using artemether-lumefantrine was conducted from 2011-2013 to complement high coverage of long lasting insecticide-treated nets. In order to identify factors likely to increase campaign effectiveness, a modeling approach was applied to investigate the simulated effect of alternative operational strategies for MTAT in Southern Province. OpenMalaria, a discrete-time, individual-based stochastic model of malaria, was parameterized for the range of transmission intensities observed in the study area to simulate antimalarial administration for interruption of transmission. Simulations were run for scenarios with a range of artemisinin-combination therapies (ACTs), proportion of the population reached by the campaign, targeted age groups, frequency of campaign rounds, Plasmodium falciparum test protocols, and the addition of drugs aimed at preventing onward transmission. Scenarios were evaluated based on the reduction in all-age parasite prevalence during the peak transmission month following the campaign, compared to the currently-implemented strategy. Simulation results suggest that the most important determinant of success in reducing prevalence is the coverage of the population achieved in the campaign. However, even with high coverage with mass drug administration (MDA) in areas with a pre-intervention all-age parasite prevalence of less than 10%, simulations suggest that elimination would require more than one year of campaign implementation. Including single low-dose primaquine, which acts as a gametocide, to the drug regimen did not further reduce prevalence. The addition of an endectocide, such as ivermectin, which acts as a gametocide, to the drug regimen did not further reduce prevalence. The addition of an endectocide, such as ivermectin, resulted in lower simulated parasite prevalence and warrants further investigation. Simulation results indicate a high proportion of low density infections were missed by rapid diagnostic tests that would be treated and cleared with MDA. The optimal implementation strategy for MTAT/MDA will vary by background level of prevalence and rate of infections imported to the area. Success of the campaign depends on continued coverage of vector control interventions to ensure sustained gains in reduction of disease burden.

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Planning malaria elimination interventions and outbreak responses at the community level represents an operational challenge in most malaria endemic countries. This happens primarily because most health systems report surveillance data at the regional and country levels only, despite data collection at the community level. Therefore, when planning interventions and responses at the community level, decision makers often need to perform their own data analysis, which is time-consuming and resource-intensive. In an attempt to address this problem, we have developed the “Free Surveillance App” (FREESAPP), an online application that converts time-series data from national surveillance systems into interactive graphs similar to those produced by the Gapminder Foundation. The applet can output burden distribution and decision-making trees for cost-effectiveness assessments as well as utilize satellite maps for background illustration. The overall goal of this application is to allow public health officers to efficiently and accurately use the surveillance data to guide their decision-making practice at each of the levels of the local health system hierarchy. To achieve this goal, FREESAPP allows users to plot the trends in malaria burden using a variety of interactive displays (i.e., bubble, bar, and line charts). FREESAPP also potentially be used for other reportable diseases, therefore facilitating improved public health decision-making.

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Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many low transmission settings worldwide as a critical part of malaria elimination programs. ACD strategies include determining the origin of infection, case investigation, and responding to locally acquired cases of malaria, known as reactive case detection (RACD). Effectively implementing RACD requires substantial programmatic and human resources. Thailand is pursuing a spatially-progressive approach to eliminate malaria from 80 percent of the country by 2020. Between April and June 2014, the Thai Bureau of Vector-Borne Diseases (BVBD) conducted an evaluation in Ranong Province to identify best practices and inform RACD efforts in the province and across Thailand. Using a standardized monitoring and evaluation (M&E) tool, five districts within the province were evaluated, and included a mix of high, medium and low transmission settings. Case investigation and RACD rates and reporting timeliness were analyzed through secondary data extraction from the national malaria information system and district-level malaria clinics and measured against defined indicators. Questionnaires were administered to 60 malaria clinic staff regarding RACD operations and procedures. A financial analysis of RACD-related expenditures was also collected and analyzed to determine the primary cost drivers and operating costs for RACD. Findings from the evaluation will inform the BVBD on program efficiency within Ranong Province, identify best practices and gaps in RACD activities, and will assist the BVBD in optimizing RACD program effectiveness.
LAMP FOR THE DETECTION OF SUB-MICROSCOPIC MALARIA INFECTIONS IN THE CONTEXT OF MALARIA ELIMINATION IN CAMBODIA

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In the context of government’s commitment to eliminate malaria in Cambodia in 2025, additional efforts and new methodologies to detect malaria parasites in asymptomatic individuals are needed. Indeed, in very low transmission settings such as Cambodia, asymptomatic infections remain the major reservoir of malaria parasites contributing to maintain disease transmission. As a consequence, the detection and treatment of the asymptomatic carriers is a crucial step in progress towards malaria elimination. This represents a new challenge as the proportion of asymptomatic parasite carriers is unknown. To date, although PCR methods show lower detection threshold compared to microscopy, Nested or Real time PCR assays required fully equipped laboratory and trained technicians. These approaches are not suitable as point-of-care in the field, contrarily to LAMP which can be done on a simple bench top in a clinic, with basic reagents and equipment, by personnel with only a few days’ training in the technique. To assess the performance of this promising tool, we have conducted a retrospective study on 516 samples from asymptomatic individuals collected in Ratanakiri province, eastern Cambodia. Ten microliters of DNA extracted by Instagene matrix from dried blood spots were used for both LAMP (Pan detection) and Real time PCR. Positive specimens with Pan LAMP were screened for falciparum species (\textit{P. falciparum} LAMP reaction). The results between the two techniques were compared to calculate the diagnostic accuracy. Based on the LAMP detection, the prevalence of malaria infection was 19.8%. Compared to the Real time PCR, the specificity and the sensitivity of the malaria LAMP kit was 93.3% (95% CI: 90.5% - 95.4%) and 96.4% (95% CI: 76.6% - 92.7%), respectively. We concluded that LAMP detection has similar performances with the Real time PCR. In addition, LAMP results are available just one hour after sample processing begins for 14 samples. We suggest that high throughput LAMP assay for a large-scale screening would be developed for a step forward to malaria eradication.

STAGE 1 TRIALS OF THE CLINICAL DEVELOPMENT PLAN FOR PFSPZ VACCINE FOR GEOGRAPHICALLY FOCUSED MALARIA ELIMINATION CAMPAIGNS

Stephen L. Hoffman

International PFSPZ Vaccine Clinical Consortium; VRC, Laboratory of Malaria Immunology and Vaccinology, DMD of National Institute of Allergy and Infectious Diseases, UMB CVD, Bamako Malaria Research and Training Center, IHI Bagamoyo, Swiss Tropical and Public Health Institute, Naval Medical Research Center, Walter Reed Army Institute of Research, University of Tubingen, MoH Equatorial Guinea, CRESIB, Centers for Disease Control and Prevention, Sanaria, Rockville, MD, United States

A vaccine for geographically focused malaria elimination campaigns must provide sustained sterile protection against infection. The Sanaria\textsuperscript{2} PFSPZ Vaccine was developed to address this need. It is composed of aseptic, purified, cryopreserved, vialed PFSPZ. In a recent clinical trial at the Vaccine Research Center (VRC), NIAID, NIH, PFSPZ Vaccine protected 6/6 (100%) subjects against controlled human malaria infection at the highest dosage regimen administered (5 IV doses of 1.35x105 PFSPZ). There was a dose response in regard to antibody and T cell responses, and the vaccine was safe and well tolerated. An international consortium was established to facilitate development of PFSPZ Vaccine for use in geographically focused PF malaria elimination campaigns, and a 4-stage clinical development plan (CDP) delineated. In Stage 1, which is in progress, trials at 3 sites in the US, and in Mali, Tanzania, Equatorial Guinea, and Germany are assessing the reproducibility of the VRC 312 trial, and optimizing durability, heterologous protection, and dosage regimens. These trials are intended to establish: (1) the reproducibility of the findings from the study conducted at VRC; (2) protection against heterologous PF, including naturally acquired PF; (3) durability of protection; (4) protective efficacy of different dosage regimens of PFSPZ Vaccine - regimens of 5, 4, 3, 2 or 1 doses of 1.35x105 to 2.2x106 PFSPZ/dose by direct venous inoculation (DVI) or IM routes; (5) an assay/biomarker that predicts protection; and (6) optimal approaches for DVI administration. >400 doses of 2.7x105 to 2.2x106 PFSPZ/dose have been administered in the US and Africa, and the vaccine has been safe and exceptionally well tolerated. Stage 2 will include age de-escalation and escalation and regimen optimization trials; Stage 3 will be pivotal phase 3 trials; and Stage 4 will include mass administration campaigns to halt transmission and eliminate PF malaria from populations of > 200,000 individuals. Progress and plans will be explained.
SPECTRUM OF INFECTION DISEASES IN BURUNDIAN RURAL HOSPITAL IN GASURA IN DRY VERSUS RAINY SEASON - 2012/2013

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Seasonal cycles of infectious diseases have been variously attributed to changes in atmospheric conditions, the prevalence or virulence of the pathogen, or the behaviour of the host. Also malaria has been observed during last 10 years with increasing frequency in areas above sea level (highlands malaria). The aim of this research was to assess if rainy/dry seasons are related with seasonal variation of infectious diseases similar to Kenya or Uganda highlands (western Kenya and south-western Uganda).

Incident cases of malaria versus respiratory infections in June to November (rainy seasons) versus January to April (dry seasons) have been compared in 2012/2013 in community hospital in Gasura located in 1283 m.as.l. (Burundi). This hospital had about 50 beds and outpatients department. Hospital staffs was composed of 2 doctors, 8 nurses, 2 lab technicians and pharmacist, with a patient flow of 40-120 patients daily in the outpatients department and 2-10 inpatients daily. Malaria diagnosis was made microscopically (according to WHO guidelines) and was confirmed with rapid diagnostic test (RDT, according to manufacturer’s instructions). Malaria was responsible for approximately 42.5 - 48.8% of all admission or consultations in rainy seasons but only 12.5% -29.7% in dry seasons. In dry season, proportion of respiratory tract infections increased from 19% in June to 42% in November and replaced malaria. Both malaria and pneumonia showed significant seasonal variations in occurrence despite of attitude of community health care centre in Gasura (1283 m.a.s.). In Burundi highlands health care centre in Gasura seasonal variations despite of high attitude (1283 m.a.s.) was observed with increasing proportion of malaria from 29.7 to 42% during rainy season replaced respiratory tract infection increased from 19 to 42% in dry season vice versa. Malaria in the Burundi highlands represented growing problem with variations in prevalence in rainy versus dry seasons.

SEVERE MALARIA AMONG 3,707 ADMISSIONS IN SOUTH SUDANESE HOSPITAL FOR INTERNALLY DISPLACED POPULATION

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Severe malaria is responsible for 1.2 million deaths worldwide, 90% of them in Sub-Saharan Africa, mainly in children below 5 years of age. The aim of this study was to assess proportion of severe malaria among all admissions hospital in area with internally displaced population in south Sudan. Data on infectious diseases were analyzed at admission within 12 months 1.1. 2013 - 31.12.2013 at St. Frances of D’Assisi mission Hospital located in Marial Lou, South Sudan, built for internally displaced refugees coming from north to South Sudan due to civil war (1982 - 2005) and Darfour Conflict (2002 - 2012)and for about 50 000 Dinka population. Diagnosis of severe malaria was done clinically, plus microscopically, plus rapid diagnostic testing (RDT) has been used since 2013. Seasonality of severe malaria was observed among majority of cases in period from April to November, with 113 to 221 cases per month with up to 9 deaths on severe malaria (monthly among 3707 admissions, in 2013). Altogether, 1438 patients (38.8 %) had severe malaria clinically confirmed as fever plus severe anemia, or respiratory distress syndrome, or cerebral malaria, or liver, or kidney failure, or severe hypoglycemia with acidosis. Of 1438 severe malaria cases, 76 died (5.3 %). Relatively low mortality may be explained with: (i) good access to the hospital, (ii) pre-referral administration of antimalarial drugs due to education campaign with the in 2010 - 2013, and (iii) use of artemisinin-based combination therapy (ACT) since 2010 in Marial Lou. Severe malaria in travelers returning to Europe is associated with up to 20 % mortality. But it can by less successfully treated on site in tropics due to semi-immune population, early pre-referral administration of antimalarial drugs and early empiric intramuscular and venous administration of antimalarial drugs; resulting to 5 % mortality even in more severe cases.

GENETIC DIVERSITY AND POPULATION STRUCTURE OF PLASMODIUM VIVAX INFECTIONS AFTER RADICAL TREATMENT IN A RURAL COMMUNITY OF CENTRAL VIETNAM

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In Vietnam the malaria burden has been drastically reduced over the past two decades but Plasmodium vivax is becoming increasingly important mainly due to its relapsing nature and the difficulty to radically cure dormant parasites from the liver. A two-year cohort study was conducted in Central Vietnam to assess the efficacy of the radical cure regimen based on a 10-day Primaquine (0.5mg/kg/d) in combination with the standard 3-day chloroquine (total 25mg/kg) regimen. We report the genetic diversity and population structure of P. vivax infections before and after radical treatment. All day 0 (n=247) and post treatment P. vivax infections (n=788) detected by microscopy and PCR during the 2-year monthly follow-up were genotyped using 16 previously described microsatellites. Genetic diversity, linkage disequilibrium, population structure and haplotype clustering were analyzed in post treatment samples and compared to Day 0 samples. All markers were highly polymorphic with 3 to 30 alleles per marker and heterozygosity (He) values ranging from 0.35 to 0.90. Overall He values were not significantly different between day 0 and post-treatment samples (He = 0.64 and 0.66 respectively). In addition, 71.0% of all infections were polyclonal (76.9% at day 0 vs. 69.2% post-treatment samples) and the average multiplicity of infection (MOI) was 1.9 parasites/person (MOI = 2.1 at D0, MOI =1.8 at recurrences). Genetic diversity of parasite population experiment significant changes when parasite population before treatment was compared with parasite population in the second year following (FST= 0.21), which may suggest a delayed effect of the intervention or may reflect the intense follow up (with treatment of all cases) study design. In order to estimate multilocus linkage disequilibrium (LD) changes between day 0 and post-treatment samples, we calculated the index of association IsA (which is zero for LD). We observed higher LD in post-treatment than day 0 parasite population (IsA = 0.093, P=0.0001 and IsA = 0.039, P=0.0001, respectively), suggesting inbreeding and a clonal population structure. Overall parasite population in the study is genetically diverse, and has a low effective recombination rate that contrasts with the high number of polyclonal infections.
Seasonality in malaria transmission is a key driver of malaria epidemiology, and has important implications for the effectiveness of interventions. One aspect that is not well understood is how seasonality affects the number of repeat malaria episodes that occur soon after a previous clinical attack, and which might be prevented if a long-acting artemisinin combination therapy (LACT) was used to manage the initial episode. We estimated the separate and combined effects of transmission intensity and seasonality on the timing and concentration of repeat malaria episodes, using data from six cohort studies in West Africa, and then used an individual-based model of malaria transmission across sub-Saharan Africa to extrapolate these results across a range of settings. Seasonality was quantified using the Markham seasonality index (MSI), taking account of areas with bimodal seasonality patterns, and the concentration of malaria episodes in time was quantified using a modified version of the Gini index. We explored 10% intervals of the MSI, and simulated transmission intensity that equates to prevalence in 2-10 year olds ranging from 5-60%. In settings where prevalence is less than 10%, repeat malaria episodes constitute a small fraction of the total burden, and few repeat episodes occur within the window of protection provided by currently available drugs. However, in higher transmission settings, and particularly in highly seasonal settings, repeat malaria becomes increasingly important, with up to 20% of the overall burden in children estimated to be due to repeat episodes within four weeks of a prior attack. At a given level of transmission intensity and annual incidence, the concentration of repeat malaria episodes in time, and consequently the protection from LACTs, is always highest in the most seasonal areas. As a result, the degree of seasonality, in addition to the overall intensity of transmission, should be considered by policy makers when deciding between ACTs that differ in terms of the duration of post-treatment prophylaxis they provide.

Utility of PCR-based surveillance methods relative to age in rainy and dry seasons in southern Malawi

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Routine malaria surveillance often relies on microscopic or rapid diagnostic test (RDT) parasite detection, which misses low-density infections. We have previously shown in cross-sectional surveys that school age children are at high risk of PCR-detectable asymptomatic infections in dry and rainy seasons. Using the same survey data, we now assess risk factors for low density infection to determine the utility of PCR by age and transmission setting. Submicroscopic infection was defined as Plasmodium falciparum detected by PCR but with a negative malaria smear read by quality-controlled microscopy. Sub-RDT infection was defined as PCR detection with microscopy negative or <200 parasites per microliter. Among all PCR-detected infections, infections were more likely to be submicroscopic in the dry (56%, 180/319) vs. rainy season (38%, 208/544, p<.0001). The proportion of infections that were submicroscopic among adults (≥16 years), school age (6-15 years), and young children (<5 years), was 72% (69/96), 55% (93/168), and 33% (18/54) in the dry season and 56% (99/178), 30% (81/274), and 30% (27/91) in the rainy season, respectively (p<.0001 both seasons). In mixed modeling, relationships between age and low density infections varied by season. In the rainy season, adults had 3.0 [95% CI: 1.7, 5.2] increased odds of submicroscopic infection, while school age children did not differ from young children. In the same communities in the dry season, both adults (OR 5.3 [2.6, 11.0]) and school age children (OR 2.5 [1.3, 5.0]) had increased odds of submicroscopic infection relative to young children. Associations were independent of district, net use, house materials, and gender. The relationship between age and sub-RDT infections followed a similar pattern. Microscopy and RDTs have inconsistent performance across age groups and seasons.
both microscopy and molecular detection have demonstrated the highest malaria burden among school age children, surveillance with microscopy or RDT alone fails to detect most infections among adults and half of the infections in school age children.

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ESTIMATING THE MALARIA ATTACK RATE OF A TANZANIAN MILITARY COHORT IN THE SEARCH FOR NON-IMMUNE POPULATIONS FOR MALARIA PROPHYLAXIS, VACCINE AND TREATMENT STUDIES

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In Tanzania, malaria is the leading cause of outpatient and inpatient health service attendance, and accounts for about 32% of hospital deaths. The disease is unevenly distributed in the country. Some areas of Tanzania have little to no malaria transmission as a result of anti-malarial interventions (e.g., use of treated bednets and ACT) as well as differences in climate and geography, while other areas are highly endemic for malaria. Exposure to the malaria parasite gives individuals the ability to develop immunity and asymptomatic infection. However, naive populations from low-risk areas have no protective immunity and are at high risk of developing the disease. This study aims at determining the malaria attack rate of proposed non-immune individuals from non-malarious areas when entering a training camp in a highly endemic area. 500 recruits from Tanzania People’s Defence Forces (TPDF) from non-endemic areas were selected by multistage random sampling; consenting, eligible participants were followed for six months. Malaria smears were collected every fortnight by active and passive detection of infection at the camp health facility. Blood samples for PCR and serological tests were collected. Malaria diagnosis was confirmed by malaria microscopy. There was a high rate of study subject follow-up; 98.1% (491/500) individuals participated in all activities, while 8 withdrew their participation for personal reasons.

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MALARIA INCIDENCE IN A DISTRICT WITH THREE ECOLOGICAL ZONES IN SOUTHERN GHANA

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Information on malaria incidence, age patterns of morbidity, mortality and serological exposure can be combined with entomological data to help target enhanced malaria control. Although the Dangme West District in Ghana hosts a research centre and has conducted many malaria interventions, the last malaria transmission measurement was undertaken in 1994. We have therefore undertaken a new detailed malaria transmission study stratified by ecological zone. We followed a cohort of 2145 participants of all ages (715 per zone, selected by multistage cluster sampling) once a month from April 2011 to March 2012. A history of fever within the previous 2 weeks was elicited at each visit and further information obtained by questionnaire from those with a positive history, after which a finger-prick blood sample was taken for the preparation of blood smears. Data was analyzed in STATA 12. We completed 77% of all planned visits; 8% of participants reported fever, 3% had used an artemisinin combination therapy (ACT) for treatment of fever, and 6% had used an insecticide treated bed-net (ITN) the night before visits. The incidence of slide confirmed malaria per 1000 person years was 85 in the Forest, 41 in the Coastal and 13 in the Lakeside zones. Verified ITN use the night before visits in each of the zones was 3%, 4% and 9% respectively.

The absence of a ceiling in a room was associated with an excess risk of malaria of 15%. Malaria incidence per 1000 person years was 119 in those aged 0-4 years, 136 in those aged 6-9, 50 in those aged 10-19, 9 in those aged 20-29, 18 in those aged 30-39 and 24 in those over 40 years of age. Overall rates had decreased by 40% from the 1994 levels. The Lakeside zone had the lowest incidence despite vast irrigated fields and the lowest access to ACTs. The Forest zone with the lowest verified ITN use and ownership (25%) and highest access to ACTs bore the brunt of morbidity. The data suggest that an area of declining malaria transmission, efficient surveillance is required to promptly determine levels and patterns of morbidity to identify remaining foci for targeted interventions.

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CONTRIBUTIONS OF WOMEN WITH CHILDREN AND YOUTH WORKERS TO SPATIAL MALARIA TRANSMISSION IN SUB-SAHARAN AFRICA

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Recent investment in global malaria control has led to malaria prevalence dropping in many parts of the world. As people play a dominant role in parasite dispersal, a quantitative understanding of human movement patterns is relevant to determining how best to maintain gains made through these efforts. We conducted a survey of human movement patterns in four countries throughout sub-Saharan Africa - Mali, Burkina Faso, Zambia and Tanzania - with additional questions on malaria risk factors and cell phone usage behavior, the latter to enable anonymous cell phone signal data to be better-correlated with movement patterns. A total of 4,352 individuals were interviewed and 6,141 trips recorded. A cluster analysis highlighted two distinct traveler groups of relevance to malaria transmission - women traveling with children (all four countries) and youth workers (Mali). Women with children were predominantly between the ages of 16 and 45 and were more likely to travel to areas of relatively high malaria prevalence in Mali (p < 0.001) and Zambia (p = 0.035) compared to other travelers. They were also more likely to own bed nets in Burkina Faso (p = 0.001) and Zambia (p = 0.001), to use bed nets in Zambia (p < 0.001) and Tanzania (p = 0.046), and to own a cell phone in Mali (p < 0.001), Burkina Faso (p < 0.001) and Zambia (p < 0.001). Taking into account that children are especially receptive to malaria parasites, women with children were estimated to account for the majority of spatial malaria transmission in Mali, Burkina Faso and Zambia. Malian youth workers were predominantly between the ages of 16 and 29 and were more likely to travel to areas of relatively high malaria prevalence (p < 0.001) and for longer durations (p < 0.001) compared to other travelers. They were estimated to make a
significant contribution to spatial malaria transmission in Mali. Knowledge of the spatial patterns of malaria transmission and the contributions of key traveler groups to this spread will assist in the design of control and surveillance programs targeting "hot spots" of malaria transmission.

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EPIDEMIOLOGY OF MALARIA INFECTION AMONG SCHOOL-AGED CHILDREN IN KINTAMPO NORTH DISTRICT, GHANA: AN EVALUATION OF BEHAVIOR, NUTRITIONAL STATUS, HOOKWORM CO-INFECTION AND ANTIBODY RESPONSES

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A cross-sectional study was conducted in June 2010 in the Kintampo North Municipality, Ghana. Children (n=286) from 16 schools were enrolled after screening (n=844) if they had HAZ Z-score below -1.80 or above >0.10, with no more than one child from each household. Serum, fecal samples, and household surveys were used to assess the associations between the presence of malaria parasites, malaria parasitemia, and individual and household risk factors including nutritional status, hookworm co-infection, household risk prevention behaviors, and serum measures of parasite-specific immunoglobulin G (IgG). The primary factors associated with reduced risk of malaria infection included spraying the house in the past year (OR=0.04, p<0.001), the child having a health care visit in the past year (OR=0.39, p<0.001), household malaria in the past year (OR=0.37, p=0.001), higher hookworm antibody levels, and geographic location, while greater household food insecurity was associated with reduced risk of high levels of parasitemia. Primary risk factors for elevated parasite density included the house being sprayed in the past year (OR=9.83, p<0.001), household bednet usage (higher proportion of use associated with greater parasitemia), household and child history of malaria in the past year (OR=2.80, p=0.039; OR=0.15, p<0.001, respectively), frequency of consumption of protein-rich food groups, and geographic location, while those with the highest hookworm antibody levels showed reduced parasite density. Hookworm infection was associated with increased risk of malaria infection (OR=2.65, p=0.10) and higher density of malaria parasites among those infected (OR=2.81, p=0.001). These risk factors highlight areas of programmatic interest, particularly the elevated risks of malaria infection and higher density of parasites among those infected with hookworm. Further research should elucidate the mechanism of this interaction, and treatment measures should focus on reducing the burden of hookworm in malaria endemic areas.

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PREVALENCE OF TWO NEWLY RECOGNIZED HUMAN MALARIA SPECIES IN MALI: PLASMODIUM OVALE CURTISI AND P. OVALE WALLIKERI

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Ovale malaria is caused by two sympatric Plasmodium ovale sub-species; their biology and morbidity need to be investigated. In the present study we determine the presence and the prevalence of these sub-species in Mali using molecular analysis of blood blotted onto filter papers. Between 2011 and 2013, 7044 volunteers were screened by light microscopy in the context of various clinical studies conducted in five sites of Mali with different malaria epidemiology (Bougoula-Hameau, Faladje, Koli, Pongonon and Sotuba). Thick smears were made and read onsite by experienced microscopists. Genomic DNA was extracted using Qia-gen kits. First, ssrRNA-based PCR methods detecting the P. ovale species were performed. Second, nested PCR of P. ovale tryptophan-rich antigen (potra) gene designed to distinguish the sub-species P. ovale curtisi and P. ovale wallikeri were run. Overall, 84/7044 (1.2%) of slides were positive for P. ovale. To date, 483 dried blood spots were analyzed by PCR. ssrRNA analysis revealed 12 (2.5%) cases of P. ovale. Potra analysis showed that 6/12 (50%) were P. ovale curtisi, 5/12 (41.7%) were P. ovale wallikeri and 1 sample was not sub-typable. We show the two recently described P. ovale sub-species were both present in Mali.

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USING DHIS2 FOR ROUTINE MONITORING OF QUALITY OF HEALTH SERVICES IN THE PRIVATE SECTOR

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District Health Information System (DHIS2) has increasingly become the preferred Health information system for effective management of health data in many countries, mostly for collection and management of routine service data at various levels of the health sector. However, DHIS2 capabilities can also be optimised for routine monitoring of quality of health services in the private sector: Population Services International (PSI) is implementing a multi-country project aiming to create a market for malaria Rapid Diagnostic Tests (mRDTs) to improve quality of care in the private health sector by enabling effective treatment based on diagnosis. This project is being implemented by PSI in Kenya, Madagascar and Tanzania. The use of DHIS2 for the purpose of tracking provider level case management will be presented, with specific focus the power of DHIS2 to convert routine data into decision-making. DHIS2-enabled tablets can be used to (i) undertake provider quality of care and service preparedness assessments, (ii) automatically score and benchmark the provider’s performance on site, and (iii) provide effective on the spot feedback for continuous improvement. The power of DHIS2 dashboards to manage and give feedback to providers will be highlighted, bridging information on provider quality of service, productivity based on caseloads, and behaviour change based on the adoption stairway. In addition, an innovative adaptation of DHIS2 to allow effective allocation of resources through automated supervision planning, taking into account provider quality of service benchmarks will be discussed (frequency and scheduling of provider assessments in particular). The presentation will further demonstrate how DHIS2 can enable program managers to effectively track service provision and make informed decisions leading to program quality improvement through the use of tailored dashboards. Potential future links with national systems will also be discussed, given the widespread use of DHIS2 by governments in the countries we work.

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USE OF SATELLITE IMAGERY TO ESTABLISH A SAMPLING FRAME AND MEASURE HOUSEHOLD MOVEMENT IN SOUTHERN ZAMBIA BETWEEN 2007 AND 2011

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High-resolution satellite imagery can be used to establish a sampling frame for epidemiologic research and to describe patterns of household
distribution and movement. Assessing the frequency and geographic distribution of household movement by comparing satellite images taken over time may suggest a time period for satellite image accuracy and utility for epidemiological research. All households in a 575 km2 region of southern Zambia were enumerated on satellite images taken in 2007 and in 2011. Movement of households in the study area was assessed by comparing the images to calculate the percentage of households that were built, removed or stayed the same. We created a spatial intensity map to identify geographic areas of household movement, and to describe the spatial variation in household movement. There were a total of 3,287 households enumerated in 2007 and 3,721 in 2011. 970 households were newly observed in 2011 and 536 were no longer present. Reporting a net change of 434 households occurring over the four year period does not adequately describe the population movement within this region. Spatial variation around key features, such as around the new sealed road, points to non-uniform dynamics in population movement. These population dynamics may have implications for field studies working in this area over this time period.

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REDEFINING THE URBAN-RURAL CONTINUUM FOR MALARIA RISK: NEW APPROACHES TO CHARACTERIZING PATTERNS IN MALAWI

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Urban and rural setting is often included in the analysis of infectious disease risk, as it may be an important determinant of pathogen transmission. This dichotomous designation, however, is politically defined, and masks the true biological and social causal determinants of risk. To improve understanding of disease patterns and targeting of interventions, transmission-specific characterization of the urban-rural continuum is needed. We developed and tested a composite measure for malaria in Malawi that included features such as health facilities, roads, rivers, lakes and electricity, along with census-derived population density. Analysis was based on a household-level survey of infection and various risk factors among 6-59 month old children. Geographic distances to features were estimated and a principal component analysis (PCA)-based composite measure for each household was produced for all points on a fine-scale grid throughout Malawi. Statistical relationships of all factors were tested against Plasmodium parasitemia status using multivariate regression based methods, including potential household-level confounding factors such as treated net use and material wealth. Urban-like and rural-like areas existed throughout Malawi, even within areas classified as “urban” and “rural” by the Malawi Government. Individual factors associated with urban and rural divides, including proximity to health services and roads, as well as population density, were associated with Plasmodium infection. Community-level factors associated with human settlements and urban development were predictive of decreased malaria risk, even in the presence of more traditional household-level prevention methods such as ITN use. Infection probability was similar for most “rural” areas, but declined linearly after a breakpoint with increasing urbanicity, as measured by the PCA based composite. Dichotomized measures of urban and rural spaces fail to adequately characterize environments which might be associated with risks for infectious disease transmission. Politically determined destinations may ignore “urbanized” pockets within traditionally “rural” areas, and rural-like spaces within areas classified as “urban.” A composite measure which analyzes many factors associated to varying degrees with spaces roughly defined as rural and urban presents an opportunity to refine places of disease risk.

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MALARIA ATTRIBUTABLE FRACTION TO FEVER IN A COHORT OF PAPUA NEW GUINEAN CHILDREN

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In areas of high malaria endemicity, a high proportion of individuals is asymptomatic, thus are infected with malaria parasites without developing clinical symptoms. In addition, those who develop symptoms in the presence of malaria parasites, may be ill due to non-malaria diseases. Therefore, a case definition able to distinguish between malaria and non-malaria morbidity is essential to correctly estimate the burden of disease, design adequate control strategies -including case management-, and measure the effect of malaria interventions. In a cohort of 500 Papua New Guinean children aged 1 to 5 years and followed up for 9 months, logistic regression methods were used to estimate the risk of fever by parasite density above specific cut-offs. Plasmodium falciparum and P. vivax attributable fraction (AF) and population attributable fraction (PAF) to fever were calculated from odds ratio estimates for each case definition. Sensitivity and specificity of case definitions were also evaluated. P. falciparum AF ranges from 79% to 94% when all parasite densities and densities higher than 5000 P/µL are used as cut-off values, respectively. Overall, the PAF of fever to P. falciparum was 16% when all parasite densities in the presence of fever were considered. On the other hand, P. vivax AF increased from 9% (all parasite densities) to 85% when parasite densities higher than 10000 P/µL were used. P. vivax PAF exhibit the highest values (5-7%) when a cut-off of >1500 P/µL was used. Estimates of the sensitivity and specificity of case definitions cut-off by parasite density show that a low P. falciparum cut-off (<2500 P/µL) achieves high sensitivity (80-100%), while when only using high parasite density cut-off values high specificity (90%) is obtained. 80% sensitivity and specificity is achieved with P. vivax cut-off value > 10000 P/µL. Approximately 80% to 94% of fevers with P. falciparum infections occurring in Papua New Guinean children aged 1 to 5 years are attributable to malaria regardless of parasite densities. On the other hand, only 9% of fevers occurring in the presence of P. vivax infections, at any parasite density, are attributable to malaria, suggesting that clinical tolerance against low density P. vivax infections is already acquired at this young age.

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PRIVATE SECTOR READINESS FOR MALARIA CASE MANAGEMENT AND MALARIA MARKET COMPOSITION BEFORE AND AFTER THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM): RESULTS FROM THREE PILOT COUNTRIES

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People living in malaria endemic countries often turn to the private sector for fever case management. However, private sector markets are typically characterized by low levels of readiness for appropriate malaria case management, particularly in comparison with public health facilities. Efforts to improve this have included the Affordable Medicines Facility - malaria (AMFM), which aimed to improve availability and affordability of quality assured Artemisinin Combination Therapy (ACT) in both public and private sectors. The pilot demonstrated favorable improvements in private sector readiness for malaria case management in most countries. The private sector comprises a diverse set of actors, including regulated pharmacies and health facilities, and unregulated drug shops and general

astmh.org
TRENDS IN AVAILABILITY OF MALARIA MEDICINES AND DIAGNOSTICS IN KINSHASA, DR CONGO FROM 2009-2013

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Malaria is a leading cause of illness and death in the Democratic Republic of the Congo (DRC) where the malaria disease burden is estimated to be the second highest in the world. Malaria is endemic throughout the country, including in the large urban agglomeration of Kinshasa. National policy has recommended diagnostic testing since 2006, and the national treatment protocol includes two artemisinin-based combination therapies (ACT) for first-line treatment. However, according to past surveys, access to and use of malaria diagnostics and ACT remains low. A 2009 survey conducted by ACTwatch found that 60% of antimalarial-stocking public and private sector outlets in Kinshasa had ACT in stock, but a 2010 ACTwatch household survey found that just 15% of children with fever had received a diagnostic test for malaria and only 4.5% of those with fever had received an ACT. In a late 2013 follow-up to the 2009 survey, ACTwatch conducted a representative cross-sectional survey of 3,654 public and private sector health facilities and retail outlets in Kinshasa to assess the availability, price and market share of antimalarials and malaria diagnostics. Findings from this survey and a trend analysis will be presented. Preliminary results show that availability of malaria diagnostics remains low and the antimalarial market is still dominated by quinine in the private sector. Results will be examined in the context of qualitative research findings from in-depth interviews conducted in 2014 in Kinshasa with private doctors, pharmacists, drug shop owners and clients. There are renewed efforts in DRC to increase the quality of fever case management following World Health Organization recommendations calling for universal parasitological confirmation before treatment. This survey of the landscape of availability of ACT and malaria diagnostics, as well as ACT market share will inform renewed case management efforts in Kinshasa that can then be scaled up throughout the country.

EFFECTS OF AGE AND CONTROL INTERVENTIONS ON PREVALENCE OF PLASMODIUM FALCIPARUM GAMETOCYTEMIA DURING PEAK MALARIA TRANSMISSION SEASON IN WESTERN KENYA

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Gametocytes are the sexual stage of Plasmodium parasites responsible for malaria transmission from human to mosquito host. However, risk factors for gametocytemia such as age and ameliorating effects of interventions (antimalarial and ITN use) are poorly understood. We measured the prevalence and density of P. falciparum gametocytes by pfs25 gametocyte mature stage V marker using real-time quantitative nucleic acid sequence-based amplification assay (QT-NASBA) and by pfg377 female gametocyte marker using qRT-PCR on samples from a cross sectional survey conducted at peak transmission season in 2012 in Siaya County of western Kenya. We also measured parasitemia using 18S QT-NASBA. Total of 446 samples (225 malaria smear-positive and 221 randomly selected smear-negative) from 832 individuals were used to determine gametocyte carriage. Overall, 18S-NASBA detected 354 positives, of which 129 infections were from smear-negative individuals. Pfs25 was detected in 78.7% of smear positive and 10.9% of smear negative samples while pfg377 was found in 55.1% of smear positive and 2.7% of smear negative samples. In multivariable analysis, children (<5 and 5-15 years old) were more likely positive with pfs25 and pfg377 than adults >15 years old (pfs25: OR 3.4, CI 2.0-6.0 and OR 4.0, CI 2.1-7.7; pfg377: OR 15.3, CI 6.3-37.1 and OR 7.6, CI 3.0-19.3, respectively). Children <5 years were more likely pfg377 positive than children 5-15 years old (OR 2.0, CI 1.2-3.4); however, gametocyte density detected by pfg377 or pfs25 did not differ between these two age groups of children. Anemia (Hb < 11 g/dl) was associated with higher 18S density (1.45x per log10, CI 1.18-1.80, p=0.0005). Importantly, anemia was also associated with pfs25 and pfg377 positive status (OR 2.1, CI 1.4-3.4 and OR 2.4, CI 1.4-3.9). The odds of being pfs25 positive were lower in individuals using ITNs (OR 0.41, CI 0.23-0.71). No differences were seen for pfs25 density between individuals with and without ITN use. Antimalarial use (90% arteether-lumefantrine) during the two weeks prior to the survey was associated with fewer pfs25 carriers (OR 0.32, CI 0.17-0.62), but not with pfg377 density. These results show that children provided the highest gametocyte reservoir compared to adults and anemia was associated with an increased risk of gametocytemia. ITN and antimalarial use decreased the gametocyte prevalence, but not the gametocyte density in the study population.

IDENTIFYING FACTORS ASSOCIATED WITH MALARIA PARASITEMIA IN MOZAMBIQUE USING A GEOSTATISTICAL MODEL

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Malaria is among the largest contributors to child mortality in Mozambique. Donors such as the Global Fund and the US President’s Malaria Initiative have been successful in controlling malaria by supporting national scale-up of interventions, though transmission remains high in many parts of the country. Malaria Indicator Surveys (MIS) and Demographic Health Surveys (DHS) are intermittent household surveys
aimed at collecting nationally representative demographic and health related data, including socio-economic status (SES), insecticide treated net (ITN) ownership and use, coverage with indoor residual spraying (IRS), and malaria parasitemia prevalence by rapid diagnostic test (RDT) for children 6-59 months of age. When examined independently, these surveys may not capture annual variation in malaria transmission. To overcome this, we combined both the 2007 M5S and the 2011 DHS data to capture the inherent spatial and temporal variation in environmental factors and malaria transmission. Using these data, we estimated the associations between relevant factors and interventions and cluster-level parasitemia prevalence using a geostatistical model. This model included a fixed factor for survey year, IRS coverage, ITN use, age, SES (wealth quintiles), environmental factors, and a spatial random effect. Our results suggested that higher SES (OR=0.79; 95% CI 0.73, 0.85) and coverage of IRS up to 40% (OR=0.93; 95% CI 0.88, 1.00) were associated with increased odds of parasitemia, and higher monthly average enhanced vegetation index (increased greenness; OR=5.10; 95% CI 1.83, 16.91) was associated with increased odds of parasitemia; ITN use was not associated with parasitemia, although this could be related to the low levels of ITN use in both survey years (cluster median=0% and 33%, respectively). These findings suggest that socioeconomic status and vegetation are important factors to consider for understanding malaria transmission. Additionally, the lack of evidence using this analytic method for additional protection by IRS coverage above 40% bears further investigation.

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ASSESSING THE IMPACT OF CLINICAL MENTORSHIP ON MALARIA DIAGNOSIS AND TREATMENT PRACTICES IN UGANDA

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To address problems associated with malaria misdiagnosis and inappropriate treatment, Uganda has implemented nationwide case management trainings, and procured Rapid Diagnostic Tests (RDTs) widely in the public sector since 2012. Despite these efforts, only 60% of suspected malaria cases were tested and 35% of negative tests were provided with anti-malarials in early 2014. In this study, a clinical mentorship for health workers, as a potential tool to overcome barriers of testing and adherence, and to improve overall case management is evaluated. This is a cluster randomized control trial, with one control and two intervention arms: one with district-selected peer mentors and one with facility-in-charges as mentors. Mentorships occur on a monthly basis over 6 months, starting in April 2014 in 150 public health facilities and district locations. At baseline, there were no significant differences in clinical diagnosis and adherence between any of study arms. Over 6 months, it is expected that clinical mentorship improves confirmatory diagnosis and adherence to test results in both intervention arms, and that differences of at least 10% are identified pre and post intervention and between each intervention arm and the control group. Clinical mentorship can work as an effective method to increase confirmatory diagnosis and adherence to test results, and improve overall fever case management, providing countries with a novel and effective tool to meet their national malaria case management targets.
MALARIA INFECTION IS ASSOCIATED WITH PREGNANCY LOSS IN OUELESSEBOUGOU, MALI

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Malaria infection in peripheral blood was detected by blood smear, and submicroscopic infection by PCR when the BS was negative. The proportion of women with submicroscopic malaria infection at delivery was 25.5% and pregnancy loss occurred in 5.8% of the cohort followed up to delivery. Malaria infection in peripheral blood was detected by blood smear, and submicroscopic infection by PCR when the BS was negative. The proportion of women with submicroscopic malaria infection at delivery was 25.5% and pregnancy loss occurred in 5.8% of the cohort (80/1,377). Submicroscopic infection at delivery was associated with increased odds of fetal loss (unadjusted OR = 3.26, 95% confidence interval CI) 1.35 - 7.89, and adjusted OR = 3.35, 1.37 - 8.16). A recent positive blood smear also increased the odds of fetal loss. In summary, preliminary analysis indicates that a submicroscopic malaria infection is associated with four times increase in odds of the pregnancy loss.

ASYMPTOMATIC INFECTIONS AND MALARIA TRANSMITTED BY BLOOD TRANSFUSION: AN INVISIBLE RISK

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Transfusion transmitted malaria presents a major challenge, essentially due to the occurrence of asymptomatic infections. The vector transmission in Brazil mainly occurs in the Amazon Region, where 166,864 cases were notified in 2013. Outside the endemic region sporadic cases of autochthonous malaria are reported, including asymptomatic carriers of Plasmodium. In the state of São Paulo, transfusional cases were detected, due to asymptomatic donors harboring P. malariae, one of them leading to the death of the recipient. The occurrence of parasitemia without clinical symptoms in addition to the fact that Plasmodium can survive in stored blood cells between 2 and 6°C for up to three weeks, increases the risk of transmission. In order to minimize the possibility of transfusional cases, the use of platforms including molecular and serological tests might point out donors suspected of harboring Plasmodium. We tested samples from 56 candidates for blood donation living in an area of São Paulo State where asymptomatic infections are reported. Thick blood smear, PCR, ELISA with recombinant P. vivax MSP119 antigen and SD Bioline Malaria PI/Pv immunochromatographic test were used. Two samples (3.5%) (0.98 -12.1) were positive by thick blood film for Plasmodium, in a very low parasitemia. One of them was also positive by PCR, indicating the presence of P. malariae. ELISA detected 53.6% (40.7 - 65.9) of samples reagent for P. vivax, with Reactivity Index ≥1.0. SD Bioline detected antibodies against P. vivax MSP and CSP recombinant antigens in 48.2% (35.6 - 60.9) of the samples. The frequency of positive samples in the serological tests pointed out to the risk of transfusional malaria, even in areas of low endemicity, since asymptomatic donors could be accepted based on clinical screening. Moreover, the lack of knowledge about this silent malaria outside the Amazon Region increases the risk of transmission. The use of platforms with different approaches could minimize this invisible risk.

SUB PATENT INFECTION OF PLASMODIUM FALCIPARUM IN NORTHEASTERN PERU

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The North-western region in Peru, is an area categorized as low endemicity for malaria after the El Niño Southern Oscillation (ENSO) phenomenon that increased the number of malaria cases to a peak with more than 200,000 cases in 1998 for both species, Plasmodium vivax and P. falciparum. The new treatment scheme implement in 2000 in this area decreased the number of P. falciparum cases in this area and since 2008, no cases have been reported by Ministry of Health. In Peru, microscopy is the diagnostic test used as routine by the Ministry of Health; but there are other technics as the Polymerase Chain Reaction (PCR), which is more sensitive to detect and identified correctly the species of Plasmodium, under the microscopy detection limit. In June of 2013, a total of 750 individuals from 3 urban areas were enrolled in a surveillance study in Piura, a malaria endemic region North-western of Peru: 350 from Bellavista, 329 from Obrero and 71 from Queerocotillo. From each individual, a blood sample was taken to prepare 2 slides for microscopy and a filter paper for PCR diagnostic. Microscopy diagnosis was performed twice, one a local level and the second one by an expert microscopist as quality control. The DNA extraction from the filter paper was done by the Chelex-100 method and the PCR was based in a Real time protocol using specific probes to detect P. falciparum and/or P. vivax. No malaria cases was detected by microscopy; but PCR detected two positive cases for P. falciparum only, one case was located in Obrero and the other one in Bellavista. The parasitaemia level in both cases was lower than 450 parasites/μL and no symptom was present at moment when the sample was taken. These results showed the presence of P. falciparum in the North-western region of Peru and stress out the need to implement more sensitive tools for malaria diagnostic in areas of low endemicity where microscopy cannot detect if the country aims to improve control measures looking into malaria elimination.

IMPLICATIONS AND EFFECTS OF DIVERSE PLASMODIUM VIVAX RELAPSE DISTRIBUTIONS IN SIMULATIONS OF VARYING TRANSMISSION SETTINGS

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Control and elimination of Plasmodium vivax is complicated by recurring relapses from hypnozoites in the liver of infected individuals. Different strains of vivax exhibit different patterns of relapse, ranging from the Chesson strain with an initial infection followed by early relapses, to strains with mixes of short and longer relapses, to North Korean strains that have infrequent early infections and longer latencies to relapse. Which strains predominate in a given geographic region depends on the local transmission setting, and earlier observational and modeling studies by various groups have allowed classification of different malaria zones. We present a new model for P. vivax transmission, host interactions, and relapse distributions and incorporate it into the EMOD model for malaria transmission. The broad diversity of relapse patterns is recreated with...
a simple set of biological and immunological mechanisms, providing a mechanistic mathematical framework for comparing different strains. The fitness and population-level effects of different relapse patterns are then explored for a variety of transmission settings, with reference to earlier work by others on classification of malaria zones. Finally, implications of different relapse patterns for control and elimination efforts scaling up in the Solomon Islands and other settings are explored in simulation. Interventions simulated include primaquine and chloroquine combinations up in the Solomon Islands and other settings are explored in simulation.

The objectives of this research are to develop and optimize noninvasive sampling methods for macaque malaria in wild populations. By using naturally infected macaques, we will compare blood and fecal samples to determine if noninvasive samples offer a logistical solution to widespread surveillance of macaque malaria. Results of this project will be applied to field collected specimens and inform experimental designs for surveillance of this pathogen in Borneo and elsewhere. Results from this work are essential for understanding malaria prevalence in macaque hosts and controlling emergence of the pathogen in new human populations.

### A COMPARATIVE CASE CONTROL STUDY OF THE DETERMINANTS OF CLINICAL MALARIA IN THE GAMBIA

**Kalifa A. Bojang**, Julia Mwesigwa, Eugene L. Kama, Serign J. Ceesay, Serign J. Ceesay, Fanta Njie

The fifth human malaria parasite, *Plasmodium knowlesi*, is a novel public health threat in Southeast Asia. The parasite is primarily found in macaques, but within the last decade it has been increasingly recorded in humans, particularly on the island of Borneo. Human malaria treatment is effective for the parasite in humans, but to prevent transmission in the first place a better understanding of prevalence in its natural hosts is necessary. The objectives of this research are to develop and optimize noninvasive sampling methods for macaque malaria in wild populations. By using naturally infected macaques, we will compare blood and fecal samples to determine if noninvasive samples offer a logistical solution to widespread surveillance of macaque malaria. Results of this project will be applied to field collected specimens and inform experimental designs for surveillance of this pathogen in Borneo and elsewhere. Results from this work are essential for understanding malaria prevalence in macaque hosts and controlling emergence of the pathogen in new human populations.

### NONINVASIVE SURVEILLANCE OF ZOONOTIC MALARIA

**Josephine Siregar**, Christina Faust, Lydia Murdiyarso, Andrew Dobson

The fifth human malaria parasite, *Plasmodium knowlesi*, is a novel public health threat in Southeast Asia. The parasite is primarily found in macaques, but within the last decade it has been increasingly recorded in humans, particularly on the island of Borneo. Human malaria treatment is effective for the parasite in humans, but to prevent transmission in the first place a better understanding of prevalence in its natural hosts is necessary. The objectives of this research are to develop and optimize noninvasive sampling methods for macaque malaria in wild populations. By using naturally infected macaques, we will compare blood and fecal samples to determine if noninvasive samples offer a logistical solution to widespread surveillance of macaque malaria. Results of this project will be applied to field collected specimens and inform experimental designs for surveillance of this pathogen in Borneo and elsewhere. Results from this work are essential for understanding malaria prevalence in macaque hosts and controlling emergence of the pathogen in new human populations.

### HOUSEHOLD-LEVEL SOCIAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH BED NET OWNERSHIP AND DIFFERING MALARIA PREVALENCE IN SOUTHERN MALAWI

**Lindsay R. Townes**, Dyson Mwandama, Don P. Mathanga, Mark L. Wilson

Since 2002, Malawi’s primary malaria prevention program has been a nationwide, health facility-based distribution of insecticide-treated nets (ITNs). Despite these efforts, ITN ownership in households with under-5 children remains sub-optimal. Knowing what characterizes such low-ownership households may improve targeting of ITN distribution. From June to August 2011, data on household ITN ownership, child malaria ownership households may improve targeting of ITN distribution. From June to August 2011, data on household ITN ownership, child malaria ownership households may improve targeting of ITN distribution. From June to August 2011, data on household ITN ownership, child malaria ownership households may improve targeting of ITN distribution.
recent or current maternal pregnancy, number of children, or number of household residents. Among those with a bed net, there was no difference in whether they had received their bed net at MDH or whether the net was insecticide-treated. Curiously, within the higher malaria prevalence context of Sitola, ITN ownership was not significantly associated with child malaria status, land use/land cover, quality of housing materials, nor recent maternal pregnancy; however, current maternal pregnancy was inversely associated with net ownership (OR 0.35, 95% CI 0.13-0.95). In contrast, current maternal pregnancy had no association with net ownership in Nsamala (OR 0.69, 95% CI 0.31-1.50), while houses built with higher quality materials were more likely to own at least one bed net (OR 3.31, 95% CI 1.20-9.12). These results suggest a geographical disparity in ITN distribution between Nsamala and Sitola, which may be reduced through improved targeting of pregnant women in Sitola.

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**MALARIA TRANSMISSION IN BOLENGE HEALTH ZONE, EQUATORIAL SETTING, DR CONGO**

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Malaria constitutes a public health major problem in Democratic Republic of Congo (DRC). For malaria control, one of adopted approach is Insecticide treated Net(ITN). In order to evaluate the malaria transmission, level in a area, different parameters are therefore used. One of them is the parasitic parameter which includes plasmodic index (PI), gametocytic index (GI), parasitemia density (PD) and the plasmodial species. Another parameter is the sporozoitic index (SI). This study aimed to assess the level of transmission in a stable Heath Zone where ITN have been partially distributed. A transversal study has been conducted from 11th of October to 17th of November 2011 in Bolenge Heath Zone where in, there are 3 Health Areas(Bolenge, Wendji Secli and Bongonde) separated between themselves by the distance of at least 10 kilometers, have been selected. Thick blood smear and thin blood smear have been done in all members of the households which remount to 185 in total, which include by the way 1066 subjects. Anopheles were captured in household for determining SI Results The global PI in Bolenge Health zone was of 41.8 %. The rate of mosquito bednet utilization was 95 %, 13 % and 23 %, respectively in Bolenge health area, Bongonde and Wendji-Secli and in the same way, the PI was of 32.7 %, 50.4 % and 42.2 %; p<0.01. The global average parasitemia of 3 Health areas was of 2213±354 trophozoites/μl (2326.±54; 3182±603 and 965.±144 respectively in Bolenge, Bongonde and Wendji-Secli health areas and in the same way, GI was of 3.7 %, 10.4 % and 4.4 % SI was respectively 5, 7 et 10 in Bolenge , Wendji secli and Bongonde and .Plasmodium falciparum was found at 99.9 %. All anopheles were An.gambiae s.s M molecular form. In conclusion, transmission was high in Bolenge Health Zone, it was very raised in Bongonde Health Area, where the rate of the ITN use was low.

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**DEVELOPING A PASSIVE MALARIA CASE DETECTION STRATEGY IN TANZANIAN MILITARY HEALTH FACILITIES AND MALARIA EPIDEMIOLOGY DATA COLLECTED TO DATE**

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The capability to accurately track malaria incidence is essential to measuring the true impact and efficacy of malaria control interventions or field evaluations of malaria therapeutics, vaccines, etc. However, gathering reliable malaria epidemiology data in rural and resource-challenged settings is often a daunting and difficult endeavor. In Tanzania, malaria persists as a major cause of morbidity and mortality. The US Army has partnered with the Tanzania People’s Defence Forces (TPDF) and the Tanzania National Service Program (JKT) to support the TPDF’s efforts to improve malaria management in a number of TPDF and JKT camps. The TPDF is an important provider of health services for both military and civilian populations, especially in remote areas where TPDF camps are based. Our initial efforts to perform passive malaria case detection relied on collecting microscopy slides from sites for cross-checking. These efforts were resource intensive and resulted in dubious success and questionable data. This led us to transition our focus to the use of malaria rapid diagnostic tests (RDTs) with RDT readers. In our approach, we deployed the Deki Reader, a rugged, mobile in vitro diagnostic device which interprets commercially available RDTs. Several advantages provided by the Deki system include real-time quality control measures, the ability for remote quality assurance (QA), and the automatic organization of...
the data in a centralized web-based portal. After deploying devices to each site, training is provided to site staff regarding use of RDTs and Deki Readers and sites are allowed a period of practice. After the sites become active, QA monitors review the mRDT database and conduct QA quarterly visits for trouble-shooting, to cross-check mRDT results against laboratory, physician, and pharmacy records, and technician compliance and accuracy for data transmission. In our first year of implementation, our approach has undergone several adjustments to adapt to a number of challenges, however we have made great strides improving the reliability of the data. We will present the challenges and successes experienced with implementation of our approach and the data collected to date.

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PRECISE GENOTYPING TOOLS FOR INVESTIGATING TRANSMISSION DYNAMICS OF PLASMODIUM FALCIPARUM GAMETOCYTES

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Differentiation between gametocyte-producing Plasmodium falciparum clones depends on high stage-specific expression and high genetic diversity of a genotyping marker in the study area. High-resolution typing methods are crucial for longitudinal tracking of gametocyte producing clones in multiple infections. Pfs230 and pfg377 are classical length-polymorphic markers for differentiation of gametocytes. We have evaluated capillary electrophoresis-based differentiation of 6 length-polymorphic gametocyte genes. These assays were applied to asexual parasites by targeting genomic DNA from field samples and in parallel to gametocytes from the same blood samples by targeting gametocyte-specific RNA. Highest diversity was found for pfs230 with 18 alleles and for pfg377 with 15 alleles in 111 samples from PNG. When assays were performed in parallel on RNA and DNA from 46 samples from Burkina Faso, 85.7% of all pfs230 samples and 59.5% of all pfg377 samples contained at least one matching genotype in DNA and RNA. Out of the 93 PCR fragments amplified from DNA of all samples by pfs230, 41 (44.1%) were not observed in the corresponding RNA sample. Vice versa we found that 42.9% (39/91) of pfs230 fragments detected in RNA failed to be amplified from the corresponding DNA samples. The imperfect detection in both, DNA and RNA, was identified as major limitation for investigating transmission dynamics, owing primarily to the volume of blood processed and the incomplete representation of all clones in the sample tested. This finding emphasizes the importance of expression levels of gametocyte-specific markers as well as optimal sampling and preservation of DNA and RNA. Larger volumes may improve clone detectability of abundant low-density gametocyte carriers and of initially sequestered gametocyte clones in follow-up samples. Application of these methods to samples from cohort studies will help to explain additional factors influencing detectability of gametocyte clones, e.g. the dynamics of gametocytogenesis of a specific parasite clone over the duration of its infection.

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CAN HEALTH MANAGEMENT INFORMATION SYSTEM (HMIS) DATA BE USED TO MAKE INDOOR RESIDUAL SPRAY POLICY DECISIONS IN BENIN?

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The President’s Malaria Initiative (PMI) has supported Indoor Residual Spraying (IRS) in Benin as part of its malaria control strategy. The PMI/USAID-funded Africa Indoor Residual Spraying (AIRS) Project assessed the feasibility of using health facility data to inform targeted spray decision-making, and assess IRS’s effect on malaria caseseloads. AIRS accessed routinely HMIS data from nine IRS intervention districts and five neighboring comparison districts, covering the period 2006 - 2013, including the period of IRS implementation from 2011-2013. We then assessed the association between IRS and the number of malaria cases reported by health facilities for both the total population and children under five years of age. Two models were developed for this assessment. In the first model, we first assessed, for a subset of areas with available data, the association of IRS on the Entomological Inoculation Rate (EIR), we then analyzed the association between EIR and the number of malaria cases as reported in the HMIS data. Finally, we combined these two steps to evaluate the association of IRS on reported malaria cases through the EIR. The second model measured the association of IRS directly on reported malaria cases in the HMIS data. The sample size for the first model was small, yet it serves to assess the validity of the second model. For the analyses, we used a difference-in-difference approach that controls for rainfall, other malaria and health interventions, and time trends. We assessed the internal validity of the data assessing the association of other malaria interventions on health facility utilization and putting the treatment on a year before IRS took place to measure the association of a “false” treatment in the second model. We assessed the association separately for two classes of insecticides, carbamates and organophosphates. As estimated against the comparison group, initial results show a 20 to 30 percent decrease in the number of confirmed cases per person per month associated with carbamates and a 5 to 20 percent decrease per person per month associated with organophosphates. The sensitivity tests and the falsification tests make us question the internal validity of the second model; the falsification test showed a statistically significant effect in a year that IRS did not take place. Based on this research, we cannot recommend using HMIS data to make IRS targeting policy decisions in Benin without further data validation.
INVESTIGATING THE SPECIFICITY AND KINETICS OF PLASMODIUM FALCIPARUM-SPECIFIC IGG RESPONSES THAT ASSOCIATE WITH PROTECTION FROM MALARIA: A LONGITUDINAL STUDY IN MALI

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Antibodies play a key role in malaria immunity, but antibody-mediated protection is only acquired after years of Plasmodium falciparum exposure, leaving children vulnerable to repeated bouts of symptomatic malaria. Our previous work in Mali suggests P. falciparum protein microarrays can be used in population-based studies to investigate the antigen specificity of protective antibodies and the kinetics of their acquisition; however, our prior work was limited by small samples size, a lack of active surveillance for clinical malaria and P. falciparum infection, and few time points. To address these limitations we conducted an independent two year cohort study in the rural village of Kalifabougou, Mali where intense malaria transmission occurs from June to December. Active surveillance for clinical malaria and P. falciparum infection was done weekly and bi-weekly, respectively. Of the 695 enrollees in the cohort study (aged 2 months to 25 years), we focused the present analysis on the 268 subjects who were P. falciparum PCR negative at enrollment before the malaria season. A microarray with 1024 P. falciparum proteins was probed with plasma collected from these 268 subjects at four time points: before the six-month malaria season, during the first episode of febrile malaria of the ensuing malaria season (if it did occur), after the malaria season, and after the subsequent 6-month dry season. In ongoing analyses that we expect to complete by mid 2014, we are comparing antibody profiles of children who were prospectively classified as clinically immune (documented infection not followed by fever) or susceptible to malaria, as well as individuals who showed evidence of sterile protection. We are also modeling the breadth, magnitude and kinetics of P. falciparum-specific antibody responses from 2 months to 25 years—the age range over which clinical immunity to malaria is acquired in this population. This rich dataset is shedding light on fundamental properties of the human antibody response to malaria and may help identify novel malaria vaccine targets.

CIRCULATING IMMUNOGLOBULIN (IGG) AGAINST MSP1-42 AND PFEMP-1 ARE NOT ASSOCIATED WITH PEDIATRIC MALARIA SEVERITY IN WESTERN KENYA

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Malaria remains a major cause of morbidity and mortality among naïve immune children and pregnant women of Africa. The majority of cases are caused by Plasmodium falciparum. In holoendemic regions such as western Kenya, severe malaria cases in children under the age of five years manifests as severe malarial anemia [Hemoglobin (Hb) <6.0 g/dL; any density parasitemia]. High levels of Immunoglobulin (Ig)-G to a number of surface proteins and invasion ligands have been associated with protection from malaria. More recently studies suggest that MSP1-42 interacts with heparin-like molecules on the RBC. Adhesion is mediated by the P. falciparum erythrocyte membrane protein 1 (PFEMP-1), expressed at the surface of infected erythrocytes and is linked to both antigenic variation and cytoadherence. The role of antibodies against these antigens in the pathogenesis of SMA remains largely unknown. We therefore sought to elucidate the role of these antibodies by measuring circulating IgG levels against these antigens in children (n=117) presenting with acute malaria at Siaya County Hospital, western Kenya. Complete hematological measures were obtained with a Beckman Coulter Counter®, and Giemsa-stained slides were used to determine parasitemia. Participants were stratified based on Hb status as non-SMA (n=91), Hb26.0 g/dL and SMA (n=26), Hb<6.0 g/dL. Results presented here show that circulating IgG against MSP1-42 were comparable between non-SMA and SMA groups (Median Interquartile range) non-SMA 128.83 (295) and SMA 151.19 (321); P=0.963. In addition, the IgG levels against MSP1-42 did not correlate with parasite density (p=0.065; P=0.487). Similarly, circulating IgG against PFEMP-1 was comparable between the groups (Median Interquartile range) non-SMA 420.0 (590) and SMA 377.97 (710); P=0.632. Protection against severe malaria in p.232Thr/Thr (TT) Kenyan children may result from a missense mutation (p.Ile232Thr) that reduces inhibition is associated with critical inhibition of host immune responses. Homozygosity for FcγRIIB-RRIIb in modulating phagocytosis, further elucidating its role in malaria-infected erythrocytes (iRBC), which may select for this mutation. This hypothesis is supported by observations that TT frequency is high in malaria endemic areas (5-11%) and low elsewhere (1-3%). In Papua New Guinea, the frequency of I232T phenotypes did not significantly differ between groups living in different malaria endemic versus non-endemic areas (p = 0.3794), however preliminary analysis suggests that individuals with TT phenotype were less likely to be infected with P. vivax malaria (p = 0.0319, N = 544). Studies are underway to correlate TT phenotype with risk of malaria disease. To understand how the TT phenotype may mediate protection from malaria infection and disease, we examined malaria-specific antibody (Ab) levels among different FCGR2B genotypes since TT polymorphism reduces repression of B cell activating pathways. Unexpectedly, TT Papuans had smaller repertoires and lower levels of P. falciparum-and P. vivax-specific Ab than II Papuans (i.e. p = 0.0045 for Duffy Binding Protein). Studies are underway to determine the role of FcγRIIB in modulating phagocytosis, further elucidating its role in regulating host immune responses and susceptibility to malaria.
Unraveling the Human Immune Response to Symptomatic and Asymptomatic Plasmodium Vivax Infections Through Systems Immunology

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Approximately 2-3 billion people are at risk of Plasmodium vivax infection worldwide. The nature of the immune response during symptomatic and asymptomatic P. vivax infection remains elusive. We are applying systems biology tools such as genome-wide expression profiling by RNA-seq and multi-parameter flow cytometry to gain insight into host immune responses that associate with protection from disease during P. vivax infection. In the Brazilian Amazon we enrolled three groups: 1) P. vivax-infected adults with fever (n=19), 2) P. vivax-infected adults with no fever or symptoms for 30 days despite persistent parasitemia (n=17), and 3) age-matched uninfected controls (n=17). Blood samples were collected at enrollment from the first and third groups, whereas blood was collected from the second group after the 30-day period without fever or symptoms. Standard hematology and chemistry labs were done, and plasma, peripheral blood mononuclear cells (PBMCs) and RNA were isolated from whole blood. Analysis of the hematologic data showed that symptomatic subjects had a higher percentage of neutrophils (median 74.1%, p=0.0002) and a lower percentage of lymphocytes (median 21.0%, p=0.0007) compared to asymptomatic subjects. Symptomatic subjects also had higher levels of total bilirubin, creatinine and glucose (p<0.0001 for each comparison) versus asymptomatic subjects. Purified RNA was converted to cDNA and sequenced by next generation sequencing. In ongoing analysis of the RNA-seq data, differentially expressed pathways and gene sets in immune and susceptible individuals will be confirmed at the protein level and functionally using contemporary PBMCs and plasma samples. Molecular and cellular signatures that correlate with protection from malaria fever may yield new hypotheses regarding the biological mechanisms by which malaria immunity is induced by P. vivax infection. The resulting datasets may be of considerable value in the urgent effort to develop a malaria vaccine.

Evaluating Controlled Human Malaria Infection in Kenyan Adults with Varying Degrees of Prior Exposure to Plasmodium Falciparum Using Sporozoites Administered by Intramuscular Injection

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Controlled human malaria infection (CHMI) studies, where healthy volunteers are infected with Plasmodium falciparum have become a vital tool to accelerate vaccine and drug development. As CHMI trials are carried out in a controlled environment, they allow unprecedented, detailed evaluation of parasite growth dynamics and immunological responses to infection. However, to date CHMI studies have not been used to investigate mechanisms of naturally-acquired immunity (NAI) to P. falciparum infection. We conducted an open label, randomized CHMI study using aseptic, cryopreserved P. falciparum sporozoites (PSPz Challenge) administered intramuscularly to evaluate infectivity and parasite growth dynamics in healthy Kenyan adults (n=28) with varying degrees of prior exposure to P. falciparum. All participants developed blood-stage infection, however, one volunteer remained asymptomatic and blood film negative until day 21 post injection of PSPz Challenge, despite developing confirmed blood-stage infection by quantitative polymerase chain reaction (qPCR). A significant correlation was seen between parasite multiplication rate (PMR) and anti-schizont ELISA OD at screening (p=0.044; r=-0.384). Our study has shown that CHMI using PSPz Challenge is safe in African adults who have varying degrees of prior exposure to malaria and that NAI can impact on PMR post-CHMI, providing a novel method to investigate the dynamics and mechanisms of blood-stage immunity.
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Malaria is endemic in Malawi, with climatic factors supporting transmission in most of the country except at high elevation. Individuals at risk of malaria infection are mostly young children and pregnant women. The risk of severe malarial disease and clinical symptoms declines in children residing in endemic regions when they experience continuing exposure to infectious mosquito bites. Thus older children sustain only mild symptoms or are asymptomatic. To understand host responses that develop with repeated exposures and are associated with clinical disease immunity, we undertook a longitudinal cohort study at Mfera Health Centre, Chikhwawa district in Malawi. 120 subjects with uncomplicated malaria are being followed for two years to capture host responses during repeated infections. We will also examine host responses in three age groups (1-5 years, 6-12 years and 13-50 years), as age serves as a proxy for clinical immunity. We will present data by age group to include clinical symptoms, rate of recurrence, host response profiling and parasite genotypes. We will also analyse these features in individuals who have repeated infections to identify changes over time using repeated measures design. A primary focus of the host response studies is the role of type I IFN in the development of clinical immunity. The role of type I IFN to confer protection or susceptibility to clinical malaria remains controversial and thus we will focus on type I IFN responses (cytokine, interferon responsive genes, type I IFN receptor genetic variants, immunophenotyping of relevant cells) in association with disease markers and age. The data generated will provide an unprecedented opportunity to understand how residents of malaria endemic areas develop lesser severe clinical disease with repeated parasite exposures and these data may inform vaccine strategies.

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PD1 EXPRESSION ON NEONATAL VΔ2 T CELLS MODULATES FUNCTIONAL RESPONSES TO MICROBIAL ANTIGENS

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In utero exposure to microbial antigens primes the fetal immune system, often with negative consequences for infant immunity and responses to pediatric vaccines. VΔ2 T cells, a subset of γδ T cells, play important roles in antimicrobial immunity and participate in responses to Bacille Calmette-Guérin (BCG) vaccination. We observed that prenatal exposure to plasmodium antigens primes fetal VΔ2 cells, potentially causing dysregulation of infant VΔ2 cells. We are defining the impact of prenatal Plasmodium falciparum exposure on neonatal and infant immunity by measuring changes in VΔ2 cells. In this context, we want to identify inhibitory and activating receptors that modulate VΔ2 responses and differ in expression on fetal versus adult cells. These differences in regulation are important for normal immune function and may be altered by maternal infections. PD1 is a key negative regulator of immune responses and a marker of T cell functional exhaustion during chronic viral infections and malaria. We compared PD1 expression on healthy North American adult and neonatal VΔ2 cells after stimulation. For adult VΔ2 cells, PD1 expression peaked by day 4 and in most individuals returned to baseline by day 14. For neonatal (cord blood) VΔ2 cells, PD1 expression peaked between days 4 and 7, and in most subjects was still elevated at day 14, yielding a PD1+ fraction significantly larger than in adults (43.6% versus 8.5%, p<0.0001). PD1 expression on neonatal VΔ2 cells remained stable up to day 35. The ability of neonatal VΔ2 cells to produce the pro-inflammatory cytokine TNFα and mobilize cytotoxic granules in response to immobilized anti-T Cell Receptor antibody was inhibited by immobilized PD1-ligand in a dose-dependent manner. Our results suggest that VΔ2 cell function in the fetus is regulated by PD1 in order to limit inflammatory responses. Prenatal VΔ2 cell stimulation caused by maternal infection may induce long-term PD1 up-regulation, and hinder VΔ2 cell responses to pathogens and to BCG vaccination, affecting infant immunity.

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STATISTICAL MODELING METHODS REVEAL VARIABLE IMPORTANCE OF ANTI-MALARIAL ANTIBODIES IN KENYAN CHILDREN

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Naturally acquired immunity (NAI) to Plasmodium falciparum is characterized by age-related control of parasitemia and protection from clinical malaria. With the goal of advancing knowledge of how the magnitude and breadth of anti-malaria IgG antibodies contribute to NAI, we used plasma from 97 children (1-14 years) who participated in a treatment trial to infection study in western Kenya. IgG antibodies to 24 recombinant merozoite and pre-erythrocytic proteins were measured by multiplex microsphere assay. A global test had a p value of 0.0601 indicating that there is evidence that antibodies against at least one of the antigens is associated with delayed time to infection. Traditionally we have used only Kaplan-Meier analysis to examine the relationship between antibody responses and time to infection. Here we developed and compared 6 prediction models: 1) Kaplan-Meier, 2) Univariate Screening, 3) Backward Elimination, 4) Penalized Regression Model–Least Absolute Shrinkage and Selection Operator (lasso) 5) Penalized Regression Model–Elastic net, and 6) Random Survival Forests. Each method has benefits and limitations. By comparing the results of all analyses and evaluating the performance of each using the time-dependent Brier score, several promising antigen targets that appear to contribute to protection from infection such as AMA1(PVO), MSP3, MSP1(3D7), and EBA181 would have been missed if we only used Kaplan-Meier analysis. We conclude that high level statistical models offer insights into targets of NAI and identify potential candidates for inclusion in multi-antigen malaria vaccines.

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TRACKING THE DEVELOPMENT OF IMMUNITY TO PLASMODIUM FALCIPARUM AFTER IMMUNIZATION WITH IRRADIATED SPOROZOITES IN A MALARIA ENDEMIC SETTING

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Vaccination with irradiated Plasmodium falciparum (Pl) sporozoites (SPZ) has been shown to induce sterilizing protection against malaria infection in naïve volunteers, but as yet this has not been tested in subjects living in malaria endemic regions who would have pre-existing immunity. In collaboration with Sanaria Inc. and the Malaria Research Training Centre (MRTC), the first double-blinded randomized phase 1b trial of the Sanaria®

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PfSPZ Vaccine (radiation attenuated, aseptic, purified PfSPZ) in a malaria endemic region is being conducted in Mali. Ninety-three volunteers were randomized to receive five vaccinations of 2.7x10^1 PfSPZ or normal saline placebo. In addition, twelve volunteers received two vaccinations to ascertain safety of the vaccine, and nine of these volunteers will receive 5 immunizations. Vaccinations began in January 2014, and will be completed in July 2014. CD8 T cells may be the key mediators of protection against liver stages of P. falciparum, but other cellular subsets may also play a significant role. In earlier studies, CD38 and CD11a on CD8 T cells have been used to measure the development of immunity against malaria antigens after vaccination in mice and humans. Whole blood samples are being collected at baseline, 3, 7 and 27 days after each vaccination, and used to measure the percentages of CD8, CD4, T and NK cells expressing CD38 and CD11a using flow cytometry. The results after each of the 5 vaccine doses will be reported, and may be useful in discriminating the roles of various immune subsets in conferring protection after PfSPZ vaccination.

### 1562

**ANTIBODY PROFILING BY PROTEIN MICROARRAY IN NAIVE AND SEMI-IMMUNE INDIVIDUALS IN COLOMBIA AFTER EXPERIMENTAL CHALLENGE WITH PLASMODIUM VIVAX**

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Acquisition of naturally acquired immunity to malaria in low transmission intensity regions is often accomplished from relatively few exposures and can occur at any age. This stands in contrast to high transmission areas where protection is acquired from repeated exposures during the first two decades of life. In this study we have evaluated serological differences in response to live Plasmodium vivax challenge in two groups of individuals from Colombia. These comprised 7 individuals with no history of malaria (“naïve”), and 9 individuals naturally exposed to P. vivax previously (“semi-immune”). Each individual was infected with P. vivax sporozoites via mosquito bites. As a result, 6 naïve (86%) and 6 semi-immune (67%) individuals developed clinical symptoms in the second week post-challenge. Fever and headache were significantly more frequent or severe in naïve compared to semi-immune individuals, while blood alanine aminotransferase, aspartate aminotransferase (markers of liver function) and C-reactive protein were also significantly higher in naïve. Overall, the clinical data indicated previous exposure to P. vivax is associated with protection against clinical symptoms in response to P. vivax challenge. To test whether protection might also be associated with IgG profiles, serum from d0, d5, d11, 3 weeks, and 4 months post-challenge were probed on a protein microarray displaying 500 P. vivax and 500 P. falciparum sero-reactive exon products. The array did not reveal strong serological differences between naïve and semi-immune individuals at the time of challenge. However, a difference was observed in the response to challenge with P. vivax sporozoites. In both groups, the response peaked at week 3 and declined thereafter, with the response by the naïve group being noticeably stronger and broader in comparison with the semi-immune group. Interestingly, the bulk of the serological response seen in the semi-immune group was also associated with those individuals with fever or headache, while those that were asymptomatic had an attenuated response. Thus the association between previous exposure and protection against clinical malaria is also associated with lower serological reactivity as measured by protein array, possibly reflecting activity of the memory pool in previously-exposed individuals.

### 1563

**KIR3DS1 HOST GENOTYPE, IL17 SERUM CONCENTRATION AND PLASMODIUM VIVAX CSP GENOTYPES MODULATION OF VIVAX MALARIA PARASITEMIA**

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Malaria is the most important arthropod borne disease in Brazil, occurring mainly in North region, being a serious Public Health concern, in terms of its high morbidity and mortality rates. Both host and parasite genetic features, as well as host immune profile, modulate resistance to infection and heterogeneity of clinical/laboratorial manifestations. This study approached: i) the Plasmodium vivax genotypes in Circumsporozoite Protein (CSP) region; ii) host KIR genes polymorphisms and; iii) immune profile during the infection (concentration of six circulating cytokines: IL-17, INF-g, TNF-a, IL-10, IL-6, IL-4, IL-2). Furthermore, we evaluated how these factors modulate the parasitemia and how host KIR polymorphism influences the number of CSP genotypes in infected individuals. Fourteen KIR genes and their ligands were genotyped by PCR-SSP on 62 P. vivax-infected individuals living in the town of Goianesia do Pará (Pará, Brazil). Cytokine levels were quantified using a Becton Dickinson cytometric bead array. CSP genotypes (Vkh210, Vkh247 and P vivax-like) were determined by PCR-RFLP. Among the 14 KIR genes only KIR3DS1 presence was associated with higher parasitemia (Mann-Whitney test; p=0.01). Moreover, KIR3DS1 presence associates with P. vivax CSP multiple genotypes (Fisher Exact test; 0.0084). Interestingly, individuals presenting multiple genotypes of P. vivax CSP showed also higher parasitemia (Mann-Whitney test; p=0.028). Fulfilling this scenario IL-17 concentration correlated negatively with parasitemia (r=0.6702; p=0.024), suggesting a protective role in the parasitemia control. Noteworthy, KIR3DS1 is a key stimulatory receptor of Natural Killer cells that produces many cytokines related to immune response to P vivax infection being the results suggestive of a role of this gene in control of the number of different circulating CSP genotypes as well as parasitemia and highlights KIR3DS1 role’s in malaria immune response in an Amazonian population.

### 1564

**INDUCTION OF HOST CELL AUTOPHAGY PROMOTES THE DEVELOPMENT OF MALARIA LIVER STAGE**

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Host cell autophagy has been reported to be involved in the restriction of the growth of a variety of microorganisms, but its role in the development of malaria pre-erythrocytic stage is still unknown. Here, we found that sporozoite infection induced LC3 containing vesicle to surround the exo-erythrocytic forms in hepatocyte, indicating the hepatocyte autophagy of the malaria parasite. Rapamycin treatment significantly increased the number of the autophagy of exo-erythrocytic form by hepatocyte, and promoted the fusion of autophagy containing malaria parasite with lysosome. However, host cell autophagy induced by rapamycin could significantly promote the development of exo-erythrocytic form in vitro. Further study showed that parasites inside the autophagosome could still survive and replicate normally as same as those in the parasitophorous vacuole, and the acidification of autolysosome was greatly inhibited. Therefore, we firstly provide evidence that sporozoite infection could induce host cell autophagy of malaria parasite, and the
induction of hepatocyte autophagy promoted the development of pre-erythrocytic stage, which might be associated with its ability to suppress the acidification of autolysosome. This data indicated the induction of autophagy as a novel escape strategy of exo-erythrocytic stage, and shed new light on the prophylactic therapy against liver stage.

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TRANSCRIPTOME ANALYSIS OF ATYPICAL MEMORY B CELLS IN THE SETTING OF NATURAL PLASMODIUM FALCIPARUM INFECTION

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Higher frequencies of an “atypical” phenotype of memory B cells have been associated with chronic exposure to Plasmodium falciparum and other pathogens, but the function of these cells in malaria pathogenesis and the development of immunity is not understood. Atypical memory B cells have been hypothesized to be dysfunctional based on their poor production of antibodies in vitro, while others have hypothesized that these cells actively produce antibodies. However, antibody secretion is not the sole function of B cells. To better understand the functional nature of atypical memory B cells, we performed a systematic evaluation of the differences between atypical and classical memory B cells using transcriptome analysis of both subsets in the presence of naturally occurring P. falciparum infection. B cells subsets were isolated from 6 parasitemic but asymptomatic children aged 8-10 years old and analyzed on whole genome microarrays. Expression of select genes was confirmed by qPCR and/or flow cytometry. Consistent with previously hypothesized atypical memory B cell dysfunction, we found a number of inhibitory genes elevated compared to classical memory B cells. However, atypical memory B cells were not dormant, but clearly metabolically active. In addition to elevated inhibitory genes, atypical memory B cells also upregulated multiple genes associated with activation, migration, and secretion pathways. Upregulation of genes in these pathways suggests a more complex function for atypical memory B cells beyond antibody secretion. Indeed, we performed functional assays confirming that these cells did not spontaneously secrete IgG ex vivo. We are currently performing additional functional assays to help define other roles atypical memory B cells have in modulating cellular responses. In summary, the transcriptome data suggest a functional role for atypical B cells, which, while as yet unknown, may be independent of antibody production.

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AGE-SPECIFIC MALARIA SERO-CONVERSION RATES IN HAITI: AN ANALYSIS OF MALARIA TRANSMISSION IN THE OUEST AND SUD-EST DEPARTMENTS OF HAITI

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Malaria transmission continues to occur in Haiti, with 32,000 confirmed cases of Plasmodium falciparum reported in 2011. As rates of malaria decrease, passive surveillance measures become less sensitive for capturing transmission intensity. By implementing highly sensitive antibody detection methods we aimed to quantifying malaria transmission intensity over time. A total of 770 serum samples were screened for malaria antibodies using indirect enzyme-linked immunosorbant assay (ELISA) coated with vaccine candidates, apical membrane antigen (AMA-1) and merozoite surface protein-11-19 (MSP-1). The “exposed” cut off value was established based on three standard deviations above the normal distribution of our negative serum absorbances (OD of 0.37 and 0.48 for AMA-1 and MSP-1 respectively). Between February and May 2013, sample collection occurred at four different sites; a rural community, two schools and a clinic in the Ouest and Sud-Est departments of Haiti. Of the 770 samples screened, 170 (22.1%) had been exposed to malaria at one point in their life time. Age was highly associated with the likelihood of having been exposed (p-value <0.001). After adjusting for age, the sero-conversion rate calculations indicated that the annual malaria transmission in the Ouest and Sud-Est department is roughly 1.03%. This data suggests that despite the absence of sustained malaria control efforts in Haiti, transmission has remained relatively low over multiple decades. Our results are further supported by passive hospital based surveillance conducted by the Haiti Health Surveillance system, which found low country-wide transmission (<1%). These findings provide valuable information that can be used to make a case for the elimination of malaria on the Island of Hispaniola.

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TOWARDS FULL PLASMODIUM FALCIPARUM PROTEOME AND REACTOME ANTIBODY SCREENING ASSAYS USING REPRODUCIBLE HIGH-THROUGHPUT PROTEIN ARRAYS

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High throughput (HT) technologies have rapidly advanced discovery of novel targets for disease diagnostic tests and vaccines and is especially useful for pathogens with large, complex genomes, such as Plasmodium spp. We have developed a full proteome antibody screening assay for P. falciparum proteins expressed via an HT in vitro transcription and translation (IVTT) system and printed onto nitrocellulose-coated microarray chips. Here, we demonstrate the reproducibility of this protein array platform, the design and reagentogenicity of the full proteome array, and the development of a down-selected P. falciparum “reactome” array. Comparisons included variation in assay dates, sample position on microarray chips and print batches. Agreement between replicate protein array assays was good. Mean difference and limits of agreement, demonstrated graphically with Bland-Altman dotplots, was 0.03 +/- 0.6 normalized intensity (range -6 to 8 on log scale). Correlation between replicate measurements of antibody breadth and magnitude were 0.91 and 0.95, respectively. Classification of antigen reactivity showed high concordance (Cohen’s kappa: 0.95, p<0.01). The final down-selection and fabrication of a 1,000-feature “PF1000 reactome” chip is underway and expected completion is in summer of 2014. We are down-selecting based on a tiered antigen reactivity scoring algorithm using antibody responses from serum/plasma samples of highly exposed individuals from sub-Saharan Africa and Papua New Guinea and samples from experimental models of pre-erythrocytic immunity. Reactive antigens are cross-referenced for confirmation with previously published data from other regions, including South America and Southeast Asia. The latest generation of down-selected chips will be a useful tool for seroepidemiological studies and screening for antibody immune correlates of protection, and full proteome chips can be used for identifying novel targets of monoclonal therapeutic antibodies or potential vaccine target antigens.
IMMUNE MECHANISMS OF CROSS-STAGE PROTECTION BY VACCINATION WITH A LATE LIVER STAGE-ARRESTING GENETICALLY ATTENUATED PARASITE

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Immunizations targeting the pre-erythrocytic stages of Plasmodium have demonstrated sterile protection against malaria by blocking the sporozoite stages and/or eliminating liver stage parasites. Strategies such as irradiated sporozoite immunizations however face a formidable challenge in that they only confer stage-specific protection, and in consequence, less than complete protection can result in full-blown blood stage infection. However, we have demonstrated that immunization with sporozoites of P. yoelii genetically attenuated parasites (GAP), which arrest late in the liver stage development and do not progress to blood stage infection, engenders protective cross-stage immunity against a direct lethal blood stage challenge. Here, we demonstrate that immune mechanisms conferring cross-stage immunity are diverse. In C57BL/6 mice, antibodies are both sufficient and necessary for this protection as GAP-immunized mice depleted of T cells completely control blood stage parasitemia whereas immunized mice lacking antibodies succumb to uncontrolled blood stage infection. Conversely, BALB/c mice depend on T cells for cross-stage protection. C57BL/6 antibodies recognize antigens in late liver stages as well as on the surface of blood-stage merozoites but are not specific for the well-characterized merozoite surface protein (MSP)-1. In contrast, immunization of BALB/c mice engenders anti-blood stage antibodies but they are lower in quantity and do not recognize the surface of merozoites. Cross-stage protection is unique to late-liver stage arresting GAP as animals immunized with an early liver stage-arresting GAP are not protected from a lethal BS challenge and fail to generate antibodies which recognize late-liver stage/blood stage antigens. Therefore, immunization with a late liver stage-arresting GAP induces T and B cell responses capable of protecting against multiple stages of Plasmodium infection and thus constitutes the most potent among vaccination strategies. This unique system also offers an opportunity to identify novel protective antigens, which are shared with between both the liver stage and blood stage parasites.

PERIPHERAL BLOOD FOXP3+ REGULATORY CD4 T CELLS DECLINE WITH INCREASING MALARIA EPISODES IN YOUNG CHILDREN

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Plasmodium falciparum infection has been reported to induce immunoregulatory cell populations such as FoxP3+ CD4 regulatory T cells (Tregs). Studies of malaria-naïve adults and low exposure regions have indicated that Tregs expand during acute malaria infection and can be induced by malaria in vitro, however in children the relationship is less clear. Tregs expansion may be associated with the slow acquisition of immunity seen in children from malaria endemic areas. Indeed, other chronic diseases have been shown to induce Tregs which contributes to ongoing infection. We investigated the frequency and function of Tregs in well characterized cohorts of two-year-old (n=79) and four-year-old (n=72) children in Tororo, Uganda. All prior malaria episodes from age 6 months were documented and children were followed for 1 year following sampling. In both 2 and 4 year old children, we found that higher prior malaria incidence was strongly associated with lower frequencies of Tregs (2 yo cohort rho = -0.28, p=0.011; 4yo cohort rho = -0.35, p=0.005) However, there was no difference in frequencies of Tregs seen in children with or without current or recent parasitemia. Functional differences between Tregs from children with high or low prior incidences were investigated in ex vivo microarray analysis, specific and global suppression assays, and during the time course of acute infection. Our data suggests that repeated infection results in a loss of functionally suppressive Tregs from the peripheral blood. These data may indicate that although Tregs are induced following malaria infection in naïve individuals, this process becomes blunted after chronic repeated malaria exposure, which may have implications for the development of effective immune responses.

INTERLEUKIN 8 AND TRANSFORMING GROWTH FACTOR-BETA (TGFB) AMONG MALARIA PATIENTS IN LAGOS, NIGERIA

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The interaction between pro- and anti-inflammatory cytokines such as interleukin-8 (IL-8) and transforming growth factor beta (TGFB) plays an important role in malaria pathogenesis and outcome. TGFB, produced by a wide range of cells, has a pivotal role in the control of the transition between pro-inflammatory (Th1-type) and anti-inflammatory (Th2-type) response during the acute and resolving phases of malaria infection. The role of IL-8 in Plasmodium falciparum malaria is unknown although studies indicate it’s likely use as a biomarker of intensity of malaria. The aim of this study was to measure the plasma levels of IL-8 and TGFB-1 in 136 individuals with malaria and correlate the production of these cytokines with the severity of the disease. IL-8 and TGFB-1 levels were determined using enzyme-linked immunosorbent assay. The severity of malaria was established by parasitemia, clinical symptoms and haematological parameters. The level of IL-8 was found to be substantially elevated (508.8±755.1pg/ml) in malaria infected individuals and its value was significant in parasitemia levels (43200.0, p<0.05). In contrast TGFB-1 levels were found to be lower in malaria patients (23,672±30,703.8pg/ml) compared to non-malaria patients, the mean difference in levels of IL-8 between malaria positive and malaria negative individuals was statistically significant (p<0.05). The relationship between TGFB-1 levels and packed cell volume was negatively correlated (r = -0.27). These findings suggest that fine mechanisms regulate the interaction between TGFB-1 and IL-8 in the immune response to Plasmodium falciparum infection, seemingly directing in vivo modulations in red cell population, and indicating a likely role in susceptibility to malaria.

IMPACT OF ACUTE MALARIA ON PRE-EXISTING ANTIBODY LEVELS TO COMMON CHILDHOOD VACCINES

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Vaccine-induced protective immunity against many pathogens relies on long-lived plasma cells that maintain basal levels of antibody. Recent evidence from animal models suggests that Plasmodium infection mediates a transient drop in antibody titers induced by prior influenza virus infection, raising the possible public health concern that malaria may be detrimental to previously generated vaccine responses. Prior studies in humans have assessed the impact of concurrent P. falciparum infection on responses to standard childhood vaccines, but to our knowledge the impact of acute P. falciparum infection on previously induced vaccine responses is unknown. To address this question we conducted a
longitudinal analysis of IgG titers specific for common viral (measles, polio, Hepatitis B) and bacterial (Haemophilus influenzae type b, meningococcus, tetanus) vaccine antigens in 54 children living in an area of Mali where the 6-month malaria and dry seasons are sharply demarcated. Vaccine-specific IgG titers were measured for each subject at five time points over an 18 month period: before and after the first dry season, during and 10 days after the first episode of febrile malaria of the ensuing malaria season, and at the end of the second dry season. Preliminary analyses suggest that average IgG decay rates are not significantly accelerated by acute \( \text{P. falciparum} \) infection; however, at the individual level a minority of children exhibited accelerated IgG decay rates following \( \text{P. falciparum} \) infection compared to decay rates over the dry season. In addition, preliminary comparisons with studies of non-malaria exposed populations suggest that overall decay rates of vaccine-induced IgG responses may be higher in the population of children who experienced febrile malaria. These data highlight the need for additional studies to understand the factors underlying variability in vaccine-specific antibody decay rates in individuals residing in malaria endemic areas.

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THE IMPACT OF SCHISTOSOMA HAEMATOBIUM INFECTION ON \( \text{PLASMODIUM FALCIPARUM} \)-INDUCED IMMUNE RESPONSES

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\( \text{Plasmodium falciparum} \) and \( \text{Schistosoma haematobium} \) often overlap in tropical and subtropical countries and impose tremendous disease burdens. Evidence suggests that \( \text{S. haematobium} \) modifies the risk of febrile malaria; however, the nature of this putative immunomodulation remains unclear. To investigate this question we analyzed \( \text{S. haematobium} \)-infected individuals (n=15) and uninfected controls (n=24) within a longitudinal cohort study in Mali. Before the malaria season, peripheral blood mononuclear cells (PBMCs) were collected from \( \text{S. haematobium} \)-infected and uninfected individuals, all of whom were negative for \( \text{P. falciparum} \) by PCR. PBMCs were analyzed before and after in vitro stimulation with lysate of \( \text{P. falciparum} \) (3D7) infected red blood cells (iRBCs) and analyzed by flow cytometry with intracellular cytokine staining; and supernatants of stimulated PBMCs were analyzed by a multiplex cytokine assay. Compared to uninfected controls, \( \text{S. haematobium} \)-infected individuals had a higher baseline percentage of dendritic cells, but no differences were observed in the proportion of B cells, T cells, NK cells or their respective subsets. Stimulation with iRBCs showed a modest increase in IFN-\( \gamma \) producing CD4\(^+ \) T cells in \( \text{S. haematobium} \)-infected individuals. These preliminary data suggest that \( \text{S. haematobium} \) modulates the innate and adaptive immune response to \( \text{P. falciparum} \) infection. In an ongoing longitudinal study we are studying the impact of concurrent or recent \( \text{S. haematobium} \) infection on the host immune response to subsequent \( \text{P. falciparum} \) infection.

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AVIDITY OF NATURALLY ACQUIRED ANTI-\( \text{PLASMODIUM VIVAX} \) MSP1-19 ANTIBODIES IN INDIVIDUALS PRESENTING DIFFERENT CLINICAL EXPRESSIONS OF MALARIA

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\( \text{Plasmodium vivax} \) is the most prevalent species in Brazil accounting for around 85% of clinical cases. \( \text{P. vivax} \) infections cause non-severe malaria in most cases, but may also be asymptomatic or cause severe disease. C-terminal region of merozoite surface protein 1 of \( \text{P. vivax} \) (PvMSP1-19) is highly immunogenic conserved region that plays a major role in the protective immunity against asexual blood stages of malaria parasites. Since this protective immunity has been shown to correlate with levels of anti-MSP1-19 antibodies, this study aimed to evaluate the humoral immune response against PvMSP1-19 of individuals naturally exposed to malaria, from endemic areas of Brazil, in order to assess the IgG and IgM profile, the avidity of IgG antibody (functional affinity) and their association with different malaria clinical presentations. Serum samples from four groups of individuals were studied: severe malaria (N=18), asymptomatic infection (N=17), non-severe symptomatic malaria undergoing their first malaria episode (N=104) and non-severe symptomatic malaria with previous malaria episodes (N=102). All were positive for \( \text{P. vivax} \) by thick blood smear and/or PCR and for IgG and/or IgM antibodies by ELISA-PvMSP1-19. High avidity (>50%) IgG antibodies were observed in 92.9% of patients who had previous malaria episodes (median reactivity index: IgG=8.7 and IgM=1.1) and in 88.0% of asymptomatic individuals (median reactivity index: IgG=2.4 and IgM=0.3). Low/moderate avidity (≤50%) was seen in 89.1% of patients undergoing their first malaria episode (median reactivity index: IgG=6.7 and IgM=3.0) and in 94.0% of severe malaria patients (median reactivity index: IgG=7.7 and IgM=6.7). Predominance of high-avidity antibody in individuals with non-severe malaria that had multiple episodes of malaria and in asymptomatic infections corroborates the protective role of humoral immunity. It likely reduces the risk to develop mild and severe malaria. Our results show that protective immunity not only correlate with levels of anti-MSP1-19 antibodies but also with the quality of these antibodies.

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GENERATION OF “FULLY HUMAN” MONOCLONAL ANTIBODIES AGAINST THE CIRCUMSPOROZOITE PROTEIN (CSP) OF \( \text{PLASMODIUM FALCIPARUM} \) USING HUMANIZED HLA MICE

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“Fully human” monoclonal antibodies (hmAb) are a novel approach to treat infectious diseases. Fully human mAbs are devoid of the complications associated to the use of mAbs derived from mouse or humanized antibodies. We have generated humanized HLA mice in NOD. RagKO.I12RgKO background that develop a functional human immune system and respond to vaccination. Using human B cells from humanized HLA mice immunized with irradiation-attenuated \( \text{Plasmodium falciparum} \) sporozoites we have generated a panel of hmAbs against \( \text{P. falciparum} \) CSP. Herein we present data on anti-CSP hmAb immunocaracterization and in vitro and in vivo anti-parasitic activity.

1575

MONITORING AND EVALUATION OF EFFECTIVENESS OF IFAKARA HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEMS (HDSS) IN QUANTIFYING IMPACT OF MALARIA CONTROL STRATEGIES AND INTERVENTIONS

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Health and Demographic Surveillance Systems (HDSS) have been set up in various sites in Africa and Asia. In the absence of effective vital registration and information on mortality, these health and demographic surveillance systems have well developed structures and standard operating procedures established to periodically monitor vital events like births, deaths and migrations in well defined areas to produce population health information. In Tanzania Ifakara DSS remain as an instrumental infrastructure of
producing key demographic indicators that are useful for planning and resource allocation to the community. The aim of my study is to determine the effectiveness of Ifakara HDSS in quantifying the impact of malaria control strategies and interventions. A cross sectional study design will be used to assess HDSS documentation mechanism on the impact of different malaria control strategies and interventions performed in the area, and to determine if the project effectively quantifies the impact of those interventions and strategies. Systematic sampling will be used to obtain number and types of documents that will be reviewed to fulfill the aim of our study. Collection and analysis of data will be both qualitative and quantities approach. Findings will be explained based on: 1) extent the knowledge and skills of the project staff in content of the HDSS malaria forms and data quality checking has increased from the pre to the post intervention periods of the project, 2) extent to which the malaria information management has improved, 3) ways in which the project was able to promote quality malaria data generation, 4) extents to which costs were able to reach project goal of documenting impact of malaria control strategies and interventions, and 4) how the HDSS understood and cared about the importance of resources at work and if the managers supported them with training, supervision and needed resources. The conclusion will be based upon findings that will be obtained during the data collection.

1576
BUILDING A CRITICAL MASS OF HEALTH WORKFORCE TO FIGHT MALARIA IN NIGERIA
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The Presidential Malaria Initiative (PMI) through the Malaria Action Program for States (MAPS) in Nigeria currently supports seven states to implement effective malaria control, prevention, diagnosis and treatment activities. Nigeria health workforces are diverse and cut across administrative, programmatic and clinical areas including community health services. Effective and sustainable malaria program will need to build capacity in all these areas. This study documents the PMI achievement in Nigeria. In-service training using adult learning principles were conducted for all cadres of staff relevant to malaria prevention and control. Administrative and programmatic staffs were trained on malaria program management; clinical staff were trained on prevention of malaria in pregnancy (MIP); malaria diagnosis using rapid test and microscopy; malaria case management including severe malaria. Other trainings conducted were focused on strengthening health information management; behavior change communication and community acceptance of interventions. From October 2011 to September 2013, 2770 health workers had been trained on MIP; over 9400 health workers and Community Care Givers had been trained on case management and 3380 trained on malaria diagnosis. About 2498 health managers were also trained on malaria program management. Over 4504 health workers were trained on health information management; 3344 health educators and journalists trained on malaria BCC. Programmatic results recorded include increase in fever cases tested from 45.9% in April 2013 to 70.2% in March 2014; and referral of over 40,000 pregnant women and children under 5 for ANC and treatment of fever. The critical mass of health workers trained will contribute to the implementation of a sustainable, efficient, integrated malaria program at state and local government levels because many of these workers offer informal services in their communities. Pre-service training is needed to sustain malaria control program.

1577
QUALITY OF CARE FOR CHILDREN UNDER FIVE YEARS WITH UNCOMPACTED MALARIA AT PRIVATE CLINICS IN MAKINDYE DIVISION, KAMPALA DISTRICT
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Over 60% of the population in developing countries seeks for care from private health facilities. In Uganda over 80% of the Population access health care from small drug shops, private clinics and private-not-for profit providers because they are closer to the communities and are perceived to be affordable. However, health providers in private clinics more frequently violate accepted medical standards and guideline because there primary goal is making profit. Quality of care for children with uncomplicated malaria in such situations is generally sub-optimal with low adherence to treatment guidelines. We are conducting a study to assess the quality of care provided to children under five years with uncomplicated malaria at private clinics in Makindye division, Kampala district, Uganda. The study is a cross-sectional cluster survey conducted in 30 private clinics. Data was collected using patient exit interviews, questionnaires for health workers, observation of health workers during consultation, health facility audits and Key Informant Intervies. A total of 180 exit interviews were conducted with caretakers of children and 30 healthcare workers were observed while treating patients and were interviewed thereafter. A total of 8 Key informant interviews were conducted with heads of private clinics. Data is being analyzed to determine predictors of quality of care for children with uncomplicated malaria at private clinics. A composite index will be utilized to assess overall quality of care.

1578
FACTORS AFFECTING TREATMENT SEEKING FOR FEVER BEFORE AND AFTER INTERVENTIONS TO IMPROVE ACCESS AND TARGETING OF ARTEMISININ COMBINATION THERAPIES
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Artesinin combination therapies (ACTs) were introduced as the first line antimalarial in Tanzania in 2006, but access and targeting were poor. The main sources of treatment for fever included public and private health facilities, drug stores, pharmacies and general shops, but the probability of receiving both parasitological diagnosis and an ACT varied considerably across these outlet types. It is therefore essential to understand factors that determine choice of provider. We assessed this using data from large scale household surveys conducted before (2010) and after (2012) national rollout of rapid diagnostic tests in public facilities, and ACT subsidies under the Affordable Medicines Facility-malaria. We visited a representative random sample of households in each of 3 regions (Mwanza, Mbeya, Mtwara) with varying malaria transmission and access to health care. 5,423 households at baseline and 5,511 at endline were sampled using a multi-stage design. All household members reporting fever were asked about treatment sought. There was no significant change in the percentage of people with fever seeking care between baseline and endline (69.5% and 73.6%, p=0.07). However, there were changes in treatment source, with an increase in the percentage using drug stores (41.3% to 54.1%, p<0.001), and a fall in use of public facilities (25.3% to 16.8% p<0.001). Overall, children <5 years old were more likely to be taken to a public facility at baseline and endline. We will present results
of multivariable analysis assessing the adjusted odds of seeking care, and of seeking care at specific provider types at baseline and endpoint. Relevant exposures include age, sex, region, urban/rural location, education of the household head, time to the nearest treatment source, enrolment in health insurance scheme and local ACT stockout levels in public facilities. Key factors affecting treatment seeking decisions will be discussed, including how these have changed following implementation of two key interventions affecting malaria treatment provision, and the implications for future policy to enhance access and targeting of ACTs.

1579

AN ASSESSMENT OF THE MALARIA-RELATED KNOWLEDGE AND PRACTICES OF TANZANIA’S DRUG RETAILERS: EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

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Since 2005 Tanzania has been upgrading its approximately 7,000 drug stores to Accredited Drug Dispensing Outlets (ADDOs), involving dispenser training, introduction of record keeping and enhanced regulation. ADDOs are permitted to stock 49 prescription-only medicines, including artemisinin combination therapies. Prior to accreditation drug stores could officially stock over-the-counter medicines only, though many stocked prescription-only antimalarials. Oral artemisinin monotherapies and injectables were not allowed in any drug stores. By late 2011 ADDO conversion was complete in 14 of 21 regions. We explored variation in malaria-related knowledge and practices of drug retailers in ADDO and non-ADDO regions. We excluded Dar es Salaam where market conditions were not comparable to other regions. Data were collected as part of the Affordable Medicines Facility-malaria Independent Evaluation, involving a nationally representative survey of antimalarial retailer in October-December 2011. We randomly selected 49 wards, and interviewed all drug stores stocking antimalarials. Interviews were conducted in 148 drug stores in ADDO regions and 127 in non-ADDO regions. Drug stores in ADDO and non-ADDO regions were similar in terms of employing staff with health-related qualifications (96.1% and 96.2%, p=0.99); stocking the first line antimalarial (59.5% and 60.7%, p=0.89); and stocking artemisinin monotherapy (0.9% and 0.0%, p=0.43). Drug stores in ADDO regions performed better on knowledge of the first line antimalarial (99.5% and 91.5%, p=0.001). There was weak evidence of a lower price and higher market share of the first line antimalarial in ADDO regions. However, drug stores in non-ADDO regions were less likely to stock injectables (21.5% and 3.6%, p=0.003). ADDO conversion is frequently cited as a model for improving retail sector drug provision. Drug stores in ADDO performed better on some but not all indicators, possibly indicating weaknesses in ADDO regulation and high staff turnover. More evidence is needed on the value-added and value for money of ADDO roll out to inform retail policy in Tanzania and elsewhere.

1580

AUDITING VILLAGE HEALTH TEAMS’ CAPACITY FOR MANAGEMENT OF MALARIA: RESULTS OF THE 2013 ACTWATCH UGANDA OUTLET SURVEY

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Malaria is endemic across 95% of Uganda. The Ugandan government aims at 85% of malaria cases receiving prompt and recommended treatment by the end of 2014. To achieve this goal, integrated Community Case Management (iCCM) initiatives have been implemented in 34 districts by building capacity of Village Health Teams (VHTs) to manage febrile children. Within these initiatives, all suspected malaria cases are tested and confirmed cases are treated with Artemether-Lumefantrine (AL), and severe cases are referred for rectal Artesunate treatment. To enumerate VHT malaria capacity under iCCM, a census of VHTs was conducted in 16 iCCM sub-counties using ACTwatch methodology. A questionnaire was administered to assess knowledge, availability of antimalarials and rapid diagnostic tests (RDTs). We present descriptive results on availability and knowledge. We surveyed a total of 1,862 VHTs. Of these, 33.7% (628) stocked AL, 10.0% (187) rectal artesunate and 21.6% (403) stocked malaria RDTs during the survey visit. Notably, when AL was available, 56.5% (355/628) of VHTs stocked malaria RDTs. Of those with antimalarials, 91.6% (606/660) correctly stated recommended first-line medicine for uncomplicated malaria (AL) and 83.3% knew its dosing regimen. Among RDT stockists, 93% (375/403) stated they would never dispense antimalarial following a negative RDT result. The iCCM presents an important channel for increasing access to integrated case-management in Uganda. According to these results, VHTs are highly knowledgeable but lack antimalarial and RDT stocks, which may undermine iCCM goals. Enhancing stable and reliable supply of first-line medicines and RDTs may sustain increased access to prompt and correct malaria-case management.

1581

ASSESSING THE COST OF PRIVATE SECTOR ACT SUBSIDIES - THE FINANCIAL AND ECONOMIC COSTS OF THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM) IN THREE AFRICAN COUNTRIES

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AMFm was designed to improve uptake of quality-assured artemisinin combination therapies (ACTs). Hosted by the Global Fund to Fight HIV/AIDS, Tuberculosis and Malaria, it operated in eight national scale pilots, involving: (i) price negotiations with ACT manufacturers; (ii) a subsidy for ACTs at the top of the global supply chain; and (iii) supporting interventions, such as communications campaigns and provider training. An Independent Evaluation found that in most but not all settings AMFm improved the availability, affordability and market share of quality-assured ACTs. However, no data were available on AMFm costs. This study aimed to address this gap. We collected data on the costs of implementing AMFm in the private for-profit sector only in Nigeria, Kenya and Madagascar, representing a range of performance on AMFm indicators. Costs were included for all AMFm-related activities at both country level and the Global Fund headquarters. We adopted a “funders perspective”, covering all resources contributed by external funding agencies, NGOs and national governments, but excluding costs to commercial actors and households. Results are presented in terms of financial costs (actual expenditure) and economic costs (which include an annualised component
of start-up costs based on their expected useful life). All costs were converted to 2012 USD. The number of subsidised ACTs delivered for the private for-profit sector by the end of 2012 was 91.4 million (mn) in Nigeria and 28.3mn in Kenya, but only 2.1mn in Madagascar, where imports were much lower reflecting the very limited communications campaign, the predominance in the market of outlets not permitted to sell ACT, and political and economic disorder. Total financial costs for 2009-12 were $110.2mn in Nigeria, $39.9mn in Kenya and $4.9mn in Madagascar. Annual economic costs for 2011 were $57.2mn in Nigeria, $17.7mn in Kenya and $2.2mn in Madagascar, implying respectively an economic cost per capita of $0.35, $0.42 and $0.10, and per ACT dose delivered of $1.30, $1.51 and $1.91. The ACT subsidy itself accounted for 89% of economic costs in Nigeria, 80% in Kenya, and 48% in Madagascar. Sensitivity analysis will be presented to explore the impact of varying key assumptions and to estimate the costs of replication in other settings. The implications for the potential value for money of strategies to expand ACT use through the private sector will be discussed.

1582

INVESTIGATING THE DETERMINANTS OF DEMAND FOR ANTIMALARIAL MEDICINES IN BENIN, NIGERIA, UGANDA AND ZAMBIA

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Artemisinin-based combination therapy (ACT) is an essential health systems input in malaria endemic countries. By 2008, all of these countries had adopted ACT as the recommended first-line treatment for falciparum malaria. Although ACT may be available free of charge through public facilities, use of non-ACTs is common. This is particularly true for children under 5 years of age who may be treated presumptively where diagnostic capacity is limited. Much of these non-ACTs are purchased easily from private sector retailers who may also sell ACTs, but at much higher prices. Therefore, ensuring that children receive appropriate and quality treatment for malaria requires an understanding of how responsive household demand is to antimalarial prices and other determinants. We estimated econometric demand models for antimalarials to examine the determinants of antimalarial choice in Benin, Nigeria, Uganda and Zambia. In each country, data were collected through nationally representative surveys of households that experienced a recent paediatric febrile episode. This was complemented by survey data from all possible public and private sources of antimalarial medicines in the vicinity of these households. Treatment choices included ACTs, oral artemisinin monotherapies, non-artemisinin therapies, and no treatment. The range of determinants studied included various treatment price components (e.g. antimalarials, diagnostics, travel), and characteristics of the provider, household and caregiver. Our findings will focus on the most significant determinants of which antimalarial households obtain and from where, and examine how responsive antimalarial demand is to changes in antimalarial prices and household income. Given the considerable resources directed toward improving access to appropriate malaria treatment, we will also discuss how these findings may be applied to optimise the equitable impact of these investments in pluralistic health systems settings.

1583

DETERMINANTS OF STOCKING AND PRICING OF SUBSIDIZED ANTIMALARIAL TREATMENTS BY RETAILERS IN THE PRIVATE FOR-PROFIT SECTOR: EVIDENCE FROM NATIONAL SUBSIDY PROGRAMS IN KENYA, NIGERIA AND UGANDA

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There is increased interest in improving quality of care in the private for-profit sector in low and middle income countries, as private providers are an important source of treatment for many illnesses. In the case of malaria, a large proportion of patients purchase antimalarial treatments from for-profit providers. For example, data from 2009-2010 show that in Nigeria, Kenya and Uganda, 97%, 67% and 40% of total antimalarial volumes were distributed by for-profit providers, respectively. However, medicines obtained are often inappropriate, because non-recommended treatments are widely and cheaply available. Subsidies for artemisinin combination therapies (ACTs), the first-line treatment in most malaria-endemic countries, have been implemented in a number of settings in order to improve coverage of ACTs and discourage use of other treatments. The largest initiative of this type was the Affordable Medicines Facility-malaria (AMFm), which was implemented at a national scale in eight pilots from 2010-2013. By the end of 2013, over 310 million treatments were ordered for the private for-profit sector through the initiative, and an Independent Evaluation reported large improvements in ACT availability, price and market share in six of the eight pilots. However, little is known about the causes of inter- and intra-country variations in performance. The success of private sector ACT subsidy programmes is determined by providers’ decisions on whether to stock subsidized medicines and decisions on the pricing of subsidized medicines. This study used nationally-representative outlet-survey data from Kenya, Nigeria and Uganda to model provider decisions to stock ACTs subsidized through AMFm, and set markups for the drugs. These three countries were selected, because they have diverse contexts and AMFm had differing effects. For each country, multiple regression analysis was used to examine the determinants of markups and stock of subsidized ACTs. The determinants investigated were a set of product, provider and market characteristics, including measures of competition. The analysis addressed the endogenous and hierarchical nature of the data. The evidence presented will help identify settings suitable for ACT subsidies and the types of supporting interventions and their targeting that are most appropriate.

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OPTIMAL PRICE SUBSIDIES FOR APPROPRIATE MALARIA TESTING AND TREATMENT BEHAVIOR

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Malaria continues to be a serious public health problem particularly in Africa. Limited access to antimalarials and over-treatment with antimalarials are two seemingly contradicting phenomena that co-exist. A global subsidy on selected antimalarial drugs has been suggested to increase access to the most effective treatment both in the public and private sectors. In order to also reduce over-treatment we propose a combined price subsidy on malaria rapid diagnostic tests and antimalarial drugs. Focusing on the private sector, we analyse the optimal subsidy combination that incentivises individuals suspecting themselves to have malaria to purchase a parasitological test before buying the recommended treatment using an expected utility model describing the health-seeking behaviour of a representative individual. Solving our model numerically for individuals with a range of different health-seeking behaviours shows that the optimal policy of the health planner is to redirect some of the subsidy money from antimalarial drugs to parasitological tests.
IDENTIFYING MINIMAL EPITOPES ON THE SURFACE OF PLASMODIUM VIVAX DUFFY BINDING PROTEIN REACTIVE WITH NEUTRALIZING MONOCLONAL ANTIBODIES

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Plasmodium vivax Duffy Binding protein region II (PvDBPII) is an essential ligand for reticulocyte invasion, thereby making this molecule an attractive vaccine candidate to protect against asexual blood-stage P. vivax. Similar to other blood-stage vaccine candidates, DBP allelic variation elicits a strain-specific immunity that may be a major challenge for development of a broadly effective vaccine against vivax malaria. This study aims to identify conserved epitopes of neutralizing anti-DBP monoclonal antibodies (mAbs) using immunochemical and structural approaches to help design a strain-transcending vaccine. The crystal structure of PvDBPII consists of 2 α-helical bundles with an antiparallel β-hairpin near the N-terminus and may be assigned into three subdomains delineated by six disulphide bonds. The various subdomains and combination of subdomains were expressed in their correctly refolded and disulphide bonded conformation on the surface of the M13 filamentous phage. Additionally, a PvDBPII gene fragment library was used for biopanning to screen phage clones reactive with anti-DBP mAbs. Comparative analysis of specific targets of non-inhibitory anti-DBP mAbs with neutralizing anti-DBP mAbs will help determine essential regions of PvDBP for a subunit vaccine designed to protect against blood-stage Plasmodium vivax malaria.

ENHANCING PRE-ERYTHROCYTIC STAGE VACCINE EFFICACY WITH THE DEVELOPMENT OF A HIGHLY IMMUNOGENIC VIRUS-LIKE PARTICLE VACCINE AND A MULTI-COMPONENT VACCINE STRATEGY

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CSP-based subunit vaccines have been shown to protect against malaria in a range of models, but to date, none have been able to elicit high levels of durable sterilising efficacy in human field trials. Our aim is to enhance pre-erythrocytic stage vaccine efficacy by two methods. Firstly by increasing the immunogenicity of a CSP-based virus-like particle (VLP) vaccine, and secondly by combining the VLP vaccine with a liver-stage viral vector vaccine regimen. To enhance the immunogenicity of the CSP-based particle we have developed an improved RTS,S-like vaccine called R21. RTS,S is the leading CSP-based vaccine and consists of particles formed from a mixture of two proteins, with only ~20% of the total protein content being a CSP-HBsAg fusion protein. In the R21 particles, 100% of the total protein content is a CSP-HBsAg fusion protein and hence R21 will contain a much higher percentage of CSP than RTS,S. This could result in enhanced immunogenicity and efficacy and is currently under evaluation. The immunogenicity and efficacy of R21 + adjuvant was compared to non-particulate recombinant CSP + adjuvant in BALB/c mice. R21 was found to be more immunogenic and induced 10 fold greater levels of anti-CS antibodies as well as higher frequencies of CS specific T cells than CSP. These serum antibody titres were also durable and were maintained at high titres when measured 3 months after vaccination. R21 was also significantly more protective than non-particulate CSP in a BALB/c model against P. berghei transgenic for P. falciparum CSP. Vaccination with R21 + Matrix M sterilely protected 82.5% of mice compared to only 42.5% with CSP + Matrix M (p = 0.014). R21 was also assessed in combination with the ChAd63 ME.TRAP - MVA ME.TRAP vaccine regimen and there was no interference with the induction of vaccine specific immune responses when the vaccines were mixed and administered together. In addition, sterile efficacy against sporozoite challenge was significantly enhanced in the mixed vaccine group. R21 is now being taken forward for evaluation in Phase III clinical trials.

PROJECTED COST-EFFECTIVENESS OF RTS,S VACCINATION IN 43 SUB-SAHARAN AFRICAN COUNTRIES

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Phase III trials of the RTS,S malaria vaccine are generating new estimates of its efficacy and decay rate. To assess the appropriateness of introducing RTS,S into the EPI in endemic African countries cost-effectiveness estimates need to be updated with the most recent clinical efficacy data. We used methods for rapidly updating projections of country specific public health impact of the vaccine, and combined these with costing models for malaria case management and immunization to assess the cost-effectiveness of RTS,S introduction. We consider deployment with a 3 dose schedule targeting infants 6, 10 and 14 weeks of age, an older cohort vaccinated at 6, 7.5 and 9 months, and a 4 dose schedule as above, which includes a booster at 18 months after the third dose. Allowing for differences in epidemiological context and health systems we generate
predictions tailored to countries examined and apt to inform malaria control policy. An ensemble of individual-based stochastic simulation models of *Plasmodium falciparum* dynamics, with varied assumptions about immune decay, transmission heterogeneity, and access to treatment fit to an extensive library of field data were used to predict the impact of RTS,S. For each country average and incremental cost-effectiveness ratios were calculated relative to the routine case management and alternate vaccine deployment strategies. We show that RTS,S is likely to be a highly cost-effective intervention with a significant impact on malaria burden in endemic settings and cost per DALY averted generally comparable to routine malaria control interventions. Depending on vaccine properties, coverage, and age-related disease burden vaccinating younger cohorts may avert more disease and at a lower cost compared to older age groups, even if initial efficacy is lower. As the vaccine targets only a small fraction of the population susceptible to malaria and provides limited protection it does not eliminate the need for other control programs. Access to effective treatment is particularly important to sustain health gains achieved with the RTS,S.

### 1589

**METHODOLOGY TO ESTIMATE THE COST OF INTRODUCING RTS,S VACCINE INTO A NATIONAL IMMUNIZATION PROGRAM IN SUB-SAHARAN AFRICAN COUNTRIES**

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Having demonstrated moderate levels of efficacy in Phase II and III trials in Africa, the RTS,S vaccine against *P. falciparum* malaria is currently considered for use within the Expanded Programme on Immunization in a large number of endemic settings. Introduction of a new vaccines demands extensive resources from the health system. Aside from the vaccine and related immunization supplies, resources are required for storage and supply chain, training, development of educational materials, social mobilization, supervision, monitoring, vaccine delivery, and waste management. We propose a generalizable methodology to estimate these costs related to vaccine introduction in African countries. Costs are evaluated from a broad provider perspective using the ingredients approach; these reflect the economic value of resources, and take into account overheads and cost of inputs shared with other health interventions. To address the uncertainty about the level of existing capacity in the health system we consider several states of the EPI: no spare capacity, estimated current capacity, and sufficient spare capacity to accommodate the new vaccine. At each capacity level we develop a series of normative scenarios for service delivery and capacity scale-up in accordance with the current operational guidelines. Scenarios are adapted to a given country setting to take into account among other the structure of the EPI program, distribution model, geography, and population dynamics. Resource lists and quantity assumptions defined for each immunization scenario are matched with price and unit cost data via cost functions to assess the overall cost of the program. The methodology takes advantage of country data on prices of key inputs using routinely collected data from the cMYP, UNICEF, and WHO-CHOICE. The methodology is applied to assess cost of RTS,S introduction in 6 endemic countries. We test the robustness of estimates generated by varying core assumptions and prices of key inputs and validate against the literature on cost of EPI program and introduction of other new vaccines.

### 1590

**EFFICACY, SAFETY AND IMMUNOGENICITY OF HETEROLOGOUS PRIME-BOOST IMMUNIZATION WITH THE CANDIDATE MALARIA VACCINES CHAd3 ME-TRAP AND MVA ME-TRAP IN 5-17 MONTHS OLD BURKINABE INFANTS AND CHILDREN**

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The development of an effective vaccine against malaria is a high priority and of great importance in the context of coordinated efforts to reduce the burden of malaria. The protective efficacy of the candidate malaria vaccines ChAd63 ME-TRAP and MVA ME-TRAP is being evaluated in an ongoing phase IIbb double-blind randomized trial. This immunization regime has shown significant partial efficacy in controlled human malaria infection trials and an initial time-to-infection trial in adults in Kenya. Initial efficacy results from this first efficacy trial in African infants will be available in May 2014. The primary objective is to evaluate the protective efficacy against clinical malaria, for a period of 6 months after the last vaccination. Clinical malaria is defined as fever (axillary temperature ≥37.5°C) together with *Plasmodium falciparum* count > 5,000 p/µl. Seven hundred infants and children aged 5-17 months were randomized in 1:1 ratio to receive either ChAd63 ME-TRAP / MVA ME-TRAP in prime-boost immunization or rabies vaccine. Immunization schedule was 0, 8 weeks. Clinical malaria episodes were captured through a health-facility based passive case surveillance method. Venous blood samples for cellular and humoral immunogenicity were collected at various timepoints. Vaccine efficacy will be assessed using Cox regression models. For analysis of first or only episodes of *P. falciparum* malaria, the incidence of episodes for each group will be presented. Secondary analysis will examine multiple episodes, using the robust clustering method by individual. Analysis of vaccination immunogenicity will describe the arithmetic and geometric means and median spots per million PBMC by vaccine group and timepoints. The safety analysis will include all solicited and unsolicited local and systemic adverse events including clinically significant laboratory abnormalities, and serious adverse events. These results will help define the potential role of these recombinant viral vectors, either used alone or as part of a multi-component vaccine, in malaria control in Africa.

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**ANTIBODIES AGAINST A PLASMODIUM FALCIPARUM RHOPTRY NECK PROTEIN PROFRON12 INHIBIT MEROZOITE INVASION INTO ERYTHROCYTES**

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Proteins coating *Plasmodium* merozoite surface and secreted from its apical organelles are considered as promising vaccine candidates for the blood-stage malaria. The rhoptry neck protein 12 of *Plasmodium falciparum* (PROFRON12) was recently reported as a protein specifically
expressed in schizont and localized to the rhoptry neck of merozoite by immunoelectron microscopy (IEM). The characteristics of the PIRON12 suggest that it is a potential blood-stage vaccine candidate, and here we assessed its potential in this regard. We expressed a recombinant PIRON12 protein by the wheat germ cell-free system to obtain anti-PIRON12 antibody. Immunoblot analysis of schizont lysate detected a single band at approximately 40 kDa under reducing condition, consistent with the predicted molecular weight. In contrast, the anti-PIRON12 antibody recognized a single band at approximately 80 kDa under non-reducing condition, consistent with two-fold molecular weight of its reduced form, suggesting the native PIRON12 forms a disulfide-bond-mediated homodimer. Immunofluorescence assay and IEM revealed that PIRON12 localized to the rhoptry neck of merozoite in schizonts and to the surface of free merozoites. The biological activity of anti-PIRON12 antibody was tested by an in vitro growth inhibition assay, and the antibody significantly inhibits the merozoite invasion of erythrocytes. Since anti-PIRON12 antibody inhibited the merozoite invasion in vitro, we decided to investigate whether PIRON12 is exposed to the human immune system in P. falciparum-infected individuals. The sera from P. falciparum infected individuals in Thailand and Mali reacted with the recombinant PIRON12, indicating PIRON12 is immunogenic in humans. Our findings suggest that PIRON12 plays an important role in the merozoite invasion process, and that it merits an additional evaluation as a P. falciparum blood-stage vaccine candidate.

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ADMINISTRATION OF PFSPZ VACCINE BY DIRECT VENOUS INOCULATION IN AFRICA
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Malaria is a major cause of morbidity and mortality despite considerable investment in existing anti-malarial measures, highlighting the need for a vaccine. The vaccine candidate, Sanaria® PFSPZ Vaccine, contains radiation-attenuated, aseptic, purified, cryopreserved Plasmodium falciparum (PF) sporozoites (SPZ). Healthy 18-35 year Malians were enrolled in a randomized, double-blind, placebo-controlled trial to assess safety, tolerability, immunogenicity, ease of administration, and protective efficacy against naturally occurring malaria of PFSPZ Vaccine administered by direct venous inoculation (DVI). PFSPZ Vaccine was thawed, diluted to a 0.5 mL volume in a 1 mL syringe with a 25-gauge needle, and then passed to local physicians for administration. Local and systemic reactogenicity were solicited through 7 days after each vaccination. A survey on subject perception of vaccination procedure will be administered at the end of the vaccination phase in July 2014. In total, 105 volunteers have been vaccinated, 12 were enrolled in a pilot safety group and 93 at the end of the vaccination phase in July 2014. In total, 105 volunteers were enrolled in a randomized, double-blind, placebo-controlled trial to assess safety, tolerability, and administration after 5 doses of PFSPZ Vaccine will be presented. DVI administration of PFSPZ has been rapid, efficient and extremely well-tolerated. This is a 1st step toward establishing the conditions for operational implementation and logistics for mass administration of PFSPZ Vaccine in Africa.

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CHLOROQUINE NEITHER ELIMINATES NOR DELAYS LIVER STAGE DEVELOPMENT OF PLASMODIUM DURING CHEMOPROPHYLAXIS VACCINATION
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Chloroquine (CQ) has been used in Chemoprophylaxis Vaccination (CVac) against malaria, whereby sporozoite inoculations under the umbrella of CQ prophylaxis induce liver stage-specific immunity and long-lasting sterile protection against homologous parasite strain. CQ is known to kill blood stage parasites, but its effect on liver stages is poorly studied, and the mechanism by which CVac-CQ induces strong protective immunity is not understood. We used a luciferase expressing rodent parasite, Plasmodium yoelii-Luc (Py-Luc), to monitor the effect of CQ on Plasmodium liver stage development. Balb/c mice with or without CQ prophylaxis were infected with Py-Luc sporozoites. Primaquine (PQ), a liver stage-specific anti-parasitic drug, was included as a positive control. We followed parasite development by intra-vital imaging at 44h, 54h and 60h post-infection. Parasite burden in liver was measured by quantifying bioluminescence of whole body and isolated livers, as well as by quantifying liver stage parasite transcripts by qRT-PCR. Delay in appearance of parasites in the blood was monitored by microscopic observation of Giemsa-stained thin blood smears. The parasite load in livers of CQ treated and untreated mice did not differ (p=0.714), and this was consistent at all three timepoints. PQ treated mice had a significant reduction in parasite burden as compared to both CQ treated and untreated groups (p=0.008), and were similar to the non-infected control mice. Parasites appeared in the blood stream of both CQ treated and untreated mice at the 54h time point. Taken together, our findings indicate that CQ neither eliminates liver stage parasites nor delays their development. Further investigations into the mechanisms by which CVac-CQ induces protective immunity are required, and may give insights relevant to drug and vaccine development.

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DEVELOPMENT OF A METABOLICALLY ACTIVE, NON-REPLICATING, ASEPTIC, PURIFIED, CRYOPRESERVED, GENETICALLY ATTENUATED PLASMODIUM FALCIPARUM SPOROZITE VACCINE-PFSPZ (ΔSLARPΔB9) VACCINE
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Sanaria® PFSPZ Vaccine, composed of radiation attenuated, aseptic, purified, cryopreserved Plasmodium falciparum (PF) sporozoites (SPZ) protected 6/6 (100%) of volunteers, who received the highest dose. By mid 2014 PFSPZ Vaccine will be in clinical trials in the U.S. and 5 countries in Africa and Europe. It is intended for elimination campaigns and prevention of malaria in travelers. There would be potential manufacturing, potency and regulatory advantages if radiation-attenuated parasites were replaced with genetically attenuated parasites (GAPs). We recently reported on a new target gene, b9 and its deletion in combination with the slarp gene. In rodent malarials ΔslarpΔb9 SPZs elicit excellent protective immunity and do not lead to blood stage infection.
Elimination of Pf slarp and \( b9 \) genes leads to attenuation similar to radiation. All prior Pf GAPs showed leaky attenuation and breakthrough liver stage development in vivo or in vitro. After characterization in Nijmegen and Leiden, the parasites were transferred to Sanaria where master and working cell banks (MCB and WCB) were made and an engineering production run performed to demonstrate that Pf\( \Delta \)slarp\( \Delta b9 \) GAP was suitable for producing aseptic, purified, cryopreserved PfSPZ. The parasites demonstrated all growth characteristics necessary for cGMP production. The Pf\( \Delta \)slarp\( \Delta b9 \) SPZ were assayed in Sanaria's 6-day hepatocyte attenuation, 3-day hepatocyte potency, and sporozoite membrane integrity (viability) assays. For the 6-day assay we used Pf wild type and Pf\( \Delta p52 \Delta p36 \) SPZ as positive controls; the wild type Pf, Pf\( \Delta p52 \Delta p36 \), and Pf\( \Delta \)slarp\( \Delta b9 \) SPZ produced 21\( \pm \)1, 1.5\( \pm \)1.25 and 0, 6-day liver stage schizonts respectively. Pf\( \Delta \)slarp\( \Delta b9 \) SPZ were potent and viable. We will next use this genetically attenuated double-mutant parasite to manufacture, characterize and release a corresponding PfSPZ Vaccine, PfSPZ (\( \Delta \)slarp\( \Delta b9 \)) Vaccine (also known as PfSPZ-GA1 Vaccine) in compliance with cGMPs, conduct pre-clinical studies, submit to the appropriate U.S. and Dutch regulatory agencies, and conduct a clinical trial.

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FINE MAPPING OF ANTIBODY ISOTYPES AND IMMUNODOMINANT B CELL EPITOPES INDUCED BY MALARIA VACCINE, PFCELTOS/GLA-SE

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The secreted malarial protein Cell-Traversing protein for Ookinetes and Sporozoites (CelTOS) is highly conserved among Plasmodium species, essential to host hepatocyte invasion, and critical to malaria pathogenesis. We previously reported that immunization of mice with full-length recombinant CelTOS from \( P. falciparum \) (PFCelTOS) adjuvanted in Montanide ISA-720 achieved 60% heterologous protection against a \( P. berghei \) sporozoite challenge. The immune mechanisms leading to this cross-species protection are based on both cellular and humoral effector mechanisms. Formulating PFCelTOS with the clinically relevant adjuvant GLA-SE resulted in similar levels of protection in mice against challenge as seen with ISA-720, and these studies provided evidence to support its evaluation in a Phase 1 safety, immunogenicity with Controlled Human Malaria Infection (CHMI) clinical trial. Since protection is in part mediated by antibodies, we sought to identify protective B-cell epitopes and, thereby, we determined the antibody fine specificity of preclinical and clinical samples for PFCelTOS. To this end, various protein fragments were generated in \( E. coli \) or as synthesized peptides, and their reactivity with sera from PFCelTOS/GLA-SE immune mice, rats, non-human primates and human subjects was determined. We are currently implementing in \textit{vivo} functional assays such as inhibition of sporozoite gliding motility and the inhibition of sporozoite invasion and development within hepatocytes (ILSDA) to identify whether this vaccine formulation induces responses to functional B-cell epitopes within the identified immunodominant regions of CelTOS. Such characterizations will determine the role of these epitopes in mediating antibody-mediated protection from preclinical and clinical studies of the CelTOS antigen.

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GENETIC DIVERSITY OF THE PLACENTAL MALARIA VACCINE CANDIDATE VAR2CSA IN TWO MALARIA ENDEMIC SETTINGS IN AFRICA USING PACBio SEQUENCING

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Pregnancy-associated malaria, a leading cause of maternal anemia and low birth weight, is characterized by the sequestration of \( P. falciparum \)-infected erythrocytes in the placental microvasculature due to expression of VAR2CSA. Epidemiological and serological studies indicate that VAR2CSA is a target of naturally acquired immunity, suggesting that VAR2CSA could be a potential target for a pregnancy-associated malaria vaccine. However, based on limited data, there appears to be extensive genetic diversity within the var2csa gene that must be taken into account in the design of an effective vaccine. Genetic diversity has prevented the successful sequencing of var2csa from field isolates using standard sequencing platforms. Due to the low sequence complexity and high variant diversity of var2csa, applying a traditional Sanger sequencing strategy on field samples is inefficient and costly. To overcome this obstacle, we are characterizing var2csa genetic diversity in malaria parasite isolates from two different endemic regions in Africa using a combination of long range PCR amplification and the Pacific Bioscience next generation sequencing platform. We performed a multiple alignment of the publicly available 20 coding sequences and 12 upstream promoter region sequences of var2csa, and designed primers based on three of the 40 identified conserved regions with a minimum length of 25 nucleotides. A first set of primers targets a 5 kilobase (kb) region spanning the upstream promoter region to the DBLepam4 domain, and a second primer set targets a 5 kb region spanning the DBLepam3 domain to the intracellular acidic terminal segment region. We have successfully amplified the two fragments for the reference strains 3D7 and NF54 and the first fragment from the Dd2 and HB3 strains. We are employing this approach to determine the var2csa sequence of clinical isolates and characterize the extent of natural diversity in var2csa in Mali and Malawi.

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SIMULATION OF B CELL AFFINITY MATURATION EXPLAINS ENHANCED ANTIBODY CROSS-REACTIVITY INDUCED BY THE POLYVALENT MALARIAL VACCINE AMA1

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Polyvalent vaccines use a mixture of antigens representing distinct pathogen strains to induce an immune response that is cross-reactive and protective. However, such approaches often have mixed results, and it is unclear how polyvalency alters the fine specificity of the antibody response and what those consequences might be for protection. Here, we present a coarse-grain theoretical model of B cell affinity maturation during monovalent and polyvalent vaccinations that predicts the fine specificity and cross-reactivity of the antibody response. We stochastically simulate affinity maturation using a population dynamics approach where the host B cell repertoire is represented explicitly, and individual B cell sub-populations undergo rounds of selection, mutation, and differentiation. Antigens contain multiple epitopes and are present in subpopulations of distinct pathogen strains, each with varying degrees of cross-reactivity at the epitope level. This epitope and strain-specific model of affinity maturation enables us to study the composition of the polyclonal response in granular detail and identify the mechanisms driving serum specificity and cross-reactivity. We applied this approach to predict the antibody
response to a polyvalent vaccine based on the highly polymorphic malarial antigen AMA1. Our simulations show that polyvalent AMA1 vaccination induces an enhanced cross-reactive antibody response primarily through a shift in affinity maturation that favors B cells specific to shared and cross-reactive epitopes, and demonstrates how a polyvalent vaccine with a small number of strains and only moderate allelic coverage may be broadly neutralizing. These results present broad implications for general polyvalent vaccine design.

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HEPATITIS B, HEPATITIS C AND HIV INFECTION FREQUENCIES AMONG VOLUNTEERS SCREENED FOR MALARIA VACCINE CLINICAL TRIALS IN MALI

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Two clinical trials of malaria vaccine candidates (transmission blocking vaccine Pf925-EPA/Alhydrogel® in collaboration with LMIV-NIAID) and whole organism vaccine PfSPZ (in collaboration with LMIV-NIAID and Sanaria) have begun in Bancoumana and Doneguebougou, Mali. Both villages are located within 70km of Bamako, but are different in terms of ethnicity, surrounding terrain, and primary employment. We have screened 509 men and women volunteers aged 18-45 years in Bancoumana and 18-35 years in Doneguebougou. Screening has been conducted to exclude those with human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV), by hepatitis B surface antigen (HBsAg) and hepatitis C (HCV; by anti-HCV core antibodies). For HCV and HBV testing, AccuDiag ELISA was used in Doneguebougou and standard rapid diagnostic tests (RDT) in Bancoumana. Determine HIV1/2 Alere® RDT with positivity confirmation by ELISA GENSCREEN ULTRA Ag-Ab were used at both sites for HIV 1/2. Ten of 204 (4.9%; 95% CI [2.37-8.82]), and ten of 218 (4.6%; 95% CI [2.22-8.27]) volunteers were positive by HIV RDT respectively in Doneguebougou and Bancoumana. Four (2.0%; 95% CI [0.54-4.94])and five (2.3%; 95% CI [0.75-5.27]) were confirmed positive by HIV ELISA GENSCREEN ULTRA Ag-Ab. HIV prevalence rates were comparable (p > 0.05) at the two sites. Forty-six volunteers (22.5%; 95% CI [17.01-28.90]) and 21 volunteers (9.6%; 95% CI [6.06-14.35]) were HBSAg positive respectively in Doneguebougou and Bancoumana, while six (2.9%; 95% CI [1.08-6.29])and four (1.8%; 95% CI [0.5-4.63]) were anti-HCV positive at the sites respectively. The HBSAg positive frequency in Doneguebougou is higher than in Bancoumana, and higher than in previous studies, indicating that the prevalence of HBV infections may be increasing in this area. Together these viral diseases are an important consideration in the screening process for malaria vaccine trials.

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A GENOMIC EPIDEMIOLOGICAL APPROACH TO ASSESSING AND IMPROVING STRAIN-SPECIFIC WHOLE ORGANISM VACCINE EFFICACY

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Genetic diversity in Plasmodium falciparum is an obstacle for broadly efficacious malaria vaccines. A subunit blood stage malaria vaccine provides allele-specific efficacy, preventing only clinical malaria caused by parasites identical to the vaccine antigen at key polymorphic loci. A similar phenomenon may apply to whole-organism vaccines, albeit potentially at many, currently unknown, loci throughout the parasite genome. The attenuated whole-organism malaria vaccine PISPZ Vaccine, which protects against homologous challenge, is based on the African strain NF54, the parent stock of the reference P. falciparum strain, 3D7. Controlled human
malaria infection trials to assess heterologous protection will initially use the South American strain 7G8. To determine how NF54 and 7G8 relate to genetic variation in natural populations, whole genome sequence data from NF54, 7G8 and from twelve mono- or polyclonal clinical samples of *P. falciparum* from a Malian village were analyzed. SNPs for all 12 Malian strains, NF54 and 7G8 were called against the 3D7 genome using GATK. Using the most reliable of the SNP filters tested, an average of 37,000 SNPs were called for Malian strains. In contrast, 383 and 17,667 SNPs were called for NF54 and 7G8, respectively. As expected, NF54 was nearly identical to 3D7, and the Malian strains were on average considerably more dissimilar genetically from 3D7, and by proxy from NF54, than is 7G8. Principal coordinate analysis was used to compare genetic diversity between strains, both at the genome-wide level and in a subset of 26 single-copy antigenic genes. This analysis placed NF54 centrally among the 12 Malian samples, suggesting a fairly representative genetic composition. NF54 and 7G8 clustered tightly with one another and with a subset of the monoclonal strains, possibly representing an artifact of polyclonality. Principal coordinate analysis of single-copy antigens showed some of the field isolates to be more distant from NF54 than 7G8, suggesting that additional suitable challenge strains can be easily identified.

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**HUMANIZED DRAG MICE SUSTAIN THE VERTEBRATE LIFE CYCLE OF *PLASMODIUM FALCIPARUM* AND ELICIT PARASITE-SPECIFIC IMMUNE RESPONSES**

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*Plasmodium falciparum* is one of the deadliest protozoan parasites among the five species of human malaria, which accounts for the highest morbidity and mortality in tropical and sub-tropical countries. In addition of having multiple life cycle stages in the human host, *P. falciparum* parasites shows high antigenic diversity and cytoadherence properties, which increases the severity and complexity of the disease. Numerous efforts have been conducted over decades to address disease pathogenesis, immunity, and vaccine development, mostly in in-vitro or in rodent or non-human primate models. However, none of these models completely represent the disease, as it is in the natural human host, thus demanding the necessity of developing an accurate animal model. We generated a HLA- class II expressing humanized DRAG mice, which develop a functional human immune system, following human hematopoietic stem cell infusion. DRAG mice develop human hepatocytes, kupffer cells, liver endothelial cells and erythrocytes and sustain the complete life cycle of *P. falciparum* malaria parasite. Our data also demonstrate that the infected DRAG mice self-cure blood-stage infection following intravenous inoculation of live *P. falciparum* sporozoites and elicit humoral responses characterized by IgM and IgG antibodies against ring, trophozoite and schizont stage parasites. The infected DRAG mice also elicit cellular responses mediated by TNF-alpha against *P. falciparum* blood-stage parasites. Thus the DRAGs mice represent the first small animal model, which has the ability to sustain the complete *P. falciparum* life cycle and to elicit parasite-specific immune responses.
CD8 T CELLS MEDIATE STERILE PROTECTION OF OUTBREED MICE FROM *PLASMODIUM YOELII* CHALLENGE FOLLOWING RECOMBINANT DNA-PRIME/ADS-BOOST IMMUNIZATION EXPRESSING TWO CANDIDATE VACCINE ANTIGENS

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Previously, we identified two antigens, UIS3 and Falstatin, which conferred sterile protection of outbred mice from *P. yoelii* sporozoite challenge following recombinant DNA-prime/Adenovirus serotype 5 (Ad5)-boost immunization. In the present study, we aimed to identify the immune component(s) mediating this protection. CD1 mice were primed with Py UIS3 and Py Falstatin DNA vectors followed by a boost with recombinant Ad5 vectors. Protection ranged from 7% to 57% in multiple experiments, with the highest level of protection observed for Ad5 boost of 10^10 pu. Sporozoite IFA titers ranged from 1:40 to 1:640, while blood-stage titers ranged from 1:2560 to 1:20480. A wide range of ELISA titers was observed to UIS3 protein (range 1.25 to 1.78125) while those to Falstatin were more uniformly high (range 1.34576 to 1.14517). High frequencies of CD8 T cells producing IFN-γ following Falstatin stimulation were observed in the spleens of immunized mice (range 9.6% to 18.9% of CD8 T cells). A fraction of the responding T cells also produced TNF and/or IL-2 in addition to IFN-γ. Interestingly, the frequencies of Falstatin-specific CD8 T cells producing IFN-γ were significantly increased among mice immunized with both UIS3 and Falstatin (above) compared to those immunized with Falstatin alone (range 4.7% to 7.4% of CD8 T cells, p<0.01). Similarly, endpoint serum ELISA titers targeting Falstatin were also increased among mice immunized against both antigens (above) compared to those immunized against Falstatin alone (range 1.15663.4 to 1.108539, p<0.05). In vivo depletion of CD8 T cells prior to challenge resulted in complete loss of the protection. These data indicate that UIS3 and Falstatin are promising candidate malaria vaccine antigens. Further studies is required to fully understand the antigenic contribution to protection and its duration, as well as to identify an optimal platform eliciting high level durable T cell immunity.

D/HUADS-PFCSLAM, A *PLASMODIUM FALCIPARUM* MULTI-ANTIGEN MULTI-STAGE ADENOVIRUS VECTORED VACCINE CANDIDATE, IS IMMUNOGENIC IN BALB/C MICE

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We have demonstrated that a malaria DNA/human adenovirus serotype 5-vecorted vaccine platform (Ad) prime/boost vaccine expressing *Pc* *CSP* and *PFAMA1* antigens (*Pc* *CSP* is well tolerated, immunogenic, and efficacious in a Phase 1 clinical study (27%, 4/15 volunteers sterilely protected against controlled human malaria infection administered by mosquito bite) (Chuang et al., 2013). We are now evaluating strategies to increase efficacy. Here, we report the addition of antigens to broaden vaccine immunogenicity, with a focus on inducing a multi-valent, multi-stage, and multi-immune (cellular and humoral) response against both pre-erythrocytic and erythrocytic parasite life cycle stages. *Pc* *CSLAM* is a cocktail of *Pc* *CSP*, *PFSSP2TRAP*, *PfLSA1*, *PFAMA1*, and *PmMS1* DNA and Ad vectors; the first four antigens are designed to induce T cell responses targeting sporozoite and liver stages, while those encoding *AMA1* and *MSP1* are designed to induce antibody responses targeting asexual blood stages. We have previously demonstrated that Ad and DNA/Ad prime/boost vaccines are immunogenic in murine, swine, and nonhuman primate models, and that a *Pc* *CSP* DNA/Ad vaccine is protective in humans. Here, BALB/c mice were immunized i.m. with either the individual components, the 5 antigen *PCSLAM* mixture, or a 4-antigen *PCSLAM* mixture, administered as a single Ad dose (1x10^9 pu) on study day (SD) 28, two Ad doses on SD1 and SD28, or pDNA (50 μg) on SD1 and Ad (1x10^9 pu) on SD28. Animals were bled pre-, 2 and 6 weeks post-boost for antibody assays, and spleens were harvested 2 and 6 weeks post-boost for T cell assays. Results established that the 5- and 4-antigen mixtures, CSLAM mixture ± SSP2, induced antigen-specific T cell and antibody responses to each antigen comparable to those induced by the individual components, as assessed by IFN-γ ELISPOT or ELISA. These data support that the addition of antigens to the *Pc* *CSLAM* mixture which is protective in humans can broaden the vaccine specificity. Future plans include GMP manufacture and clinical testing of the CSLAM or CLAM DNA prime/Ad boost vaccine.

PLASMODIUM FALCIPARUM AMA1-BASED SUBUNIT VACCINE FMP2.1/AS02A ELICITS A DIVERSE AND STRONG YET UNPROTECTIVE IMMUNE RESPONSE IN A PEDIATRIC COHORT IN BANDIAGARA, MALI

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FMP2.1/AS02 is a blood stage malaria subunit vaccine candidate based on the ectodomain of apical membrane antigen 1 (AMA1) of the 3D7 strain of *Plasmodium falciparum*. In a Phase 2 vaccine trial in 400 children aged 1-6 years in Bandiagara, Mali, West Africa, the vaccine had only a statistically insignificant 17% efficacy against all clinical malaria episodes when compared to the rabies control vaccine. However, it had 64% efficacy against clinical malaria caused by homologous strains with respect to eight pre-specified polymorphic amino acid positions. To assess anti-AMA1 antibody specificity in AMA1 vaccine trials, we developed a protein microarray for measuring seroreactivity to 263 unique AMA1 ectodomain variants detected by sequencing the ama1 gene in field samples. We evaluated AMA1 seroreactivity in a random sample of 40 children (aged 1-6 years) and 20 adults (aged 18-55 years) pre- and post-vaccination with FMP2.1/AS02, or rabies control vaccine. Both children and adults immunized with the AMA1 vaccine had broad and strong immune responses to diverse AMA1 variants compared to controls 90 days after the first immunization. Due to the broad cross-reactivity of antibodies generated by the vaccine, we were unable to pinpoint specific AMA1 variants or polymorphic epitopes associated with protection from clinical malaria. Further analysis using multivariable logistic regression, as well as principle components analysis, Random Forest, and receiver-operating characteristic curves, suggest that antibodies stimulated by the FMP2.1/AS02 vaccine may be biased, binding preferentially to immunodominant but unprotective AMA1 epitopes. This form of deceptive imprinting has been described in HIV and influenza as a tool for immune escape and also has been characterized in malaria as the ‘smokescreen effect’. These
A MONOCLONAL ANTIBODY AGAINST THE N-TERMINAL REGION OF THE PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN STRONGLY INHIBITS SPOROZOITE INVASION OF HEPATOCYTES

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Studies in animals and human volunteers have demonstrated that antibodies against the Circumsporozoite Protein (CSP) can protect against infection by Plasmodium sporozoites. Due to its repetitive nature and tandem disposition, the epitopes at CSPs repeat region are the most prominent target of protective antibody responses. However, it has been long hypothesized that antibodies raised against epitopes outside the repeat domain can also confer significant protection against sporozoite invasion. Using a newly developed chimeric Plasmodium berghei strain bearing the N-terminal region of the P. falciparum CSP, we report the characterization of a monoclonal antibody (MAb) recognizing the P. falciparum CSP. We mapped the fine epitope specificity of this MAb (SDS) and established that it recognizes an amino acid sequence immediately adjacent to Region I of the P. falciparum CSP. Using both the novel P. berghei-P. falciparum chimera and P. falciparum parasites, we further characterized the SDS MAb epitope specificity and show that it can bind both live and air-dried fixed sporozoites. Most importantly, we demonstrate that SDS can strongly inhibit parasite infection in vivo and can inhibit cleavage of CSP, and so provide additional evidence that antibodies targeting epitopes other than those at CSPs repeat region can be highly protective. Furthermore, we propose that this MAb could be utilized as an antibody-based, therapeutic prophylaxis, which may be a critical tool in the face of growing malaria drug resistance.
LONGITUDINAL ANALYSIS OF HUMORAL AND CELLULAR IMMUNITY FOLLOWING VACCINATION WITH AN ATTENUATED PLASMODIUM FALCIPARUM SPOROZITE VACCINE

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A highly effective and durable vaccine for preventing Plasmodium falciparum (PF) malaria infection is a critical need for preventing the substantial morbidity and mortality incurred by this infection. PF sporozoites (PfSPZ) administered by mosquito bites are the only immunogens shown to induce high-level sterilizing protection (>80%) in humans. We previously reported that attenuated, aseptic, purified, cryopreserved PfSPZ (PfSPZ Vaccine) administered 4 or 5 times intravenously (IV) conferred high-level protection in humans in a dose-dependent manner. Moreover, initial analysis of humoral and cellular immunity showed that there was a dose-dependent increase in CSP antibody titer, functional inhibition of sporozoite invasion in vitro and the frequency of sporozoite specific IFN-g producing CD4+ and CD8+ T cell responses. Here, we substantially expanded this analysis and performed a longitudinal assessment of antibody and cellular responses throughout the course of vaccination and after controlled human malaria – 3 weeks after the final immunization and ~ 5 months later. For T cell assessment, multi-parameter flow cytometry with two recently developed 16-color panels as used to assess the magnitude, phenotype and quality of sporozoite specific T cell responses. Together, these data provide insights into the durability of immunity and protection after vaccination with the PfSPZ Vaccine and will guide ongoing and future studies to define the mechanistic correlates of protection.

CLINICAL DEVELOPMENT OF THE PFSpz VACCINE TO PROTECT THE WARFIGHTER FROM MALARIA

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A vaccine to prevent malaria for use by the Department of Defense (DoD) must provide sustained sterile protection against infection. The Sanaria® PfSPZ Vaccine has been developed to address this need. It is composed of aseptic, purified, cryopreserved, vialized PfSPZ manufactured in compliance with all regulatory standards. In a recent clinical trial, PfSPZ Vaccine protected 6/6 (100%) subjects against controlled human malaria infection (CHMI) at the highest dosage regimen administered (5 intravenous doses of 1.35x10^5 PfSPZ), and there was a dose response in regard to antibody and T cell responses. The protective regimen was safe and well tolerated, indicating that further dose escalation could be undertaken. Since completion of that study, 6 additional PfSPZ Vaccine trials have been or will be initiated, designed to optimize dose size, dose interval, number of doses and duration of protection. This study, conducted by the DoD, addresses the following questions: (1) Is PfSPZ Vaccine safe and tolerable administered by direct venous inoculation? (2) Can 5 doses of 2.7x10^5 PfSPZ provide protection 3 weeks (short term) after immunization against CHMI carrying a heterologous Pf strain? (3) Can 5 doses provide protection when subjects undergo a second CHMI at 24 weeks (long term) with homologous and heterologous Pf parasites? 4) Can the number of doses required to provide short term and long term homologous protection be reduced (to 3 doses of 4.5x10^4 PfSPZ)? We will present safety and immunogenicity results for both the 3 and 5 dose regimens. Additionally, we will present our plans to conduct late Phase 2 and Phase 3 trials supporting a BLA for licensure in adults. The potential for worldwide benefit results from the fact that DoD requirements - excellent safety and tolerability, efficient administration, and sterile protection lasting for at least six months - are characteristics that equally support deployment to malaria endemic areas to prevent disease and death and to promote campaigns aimed at halting malaria transmission and eliminate the disease from defined geographical areas.

DURABILITY OF POLYESTER-BASED LONG-LASTING INSECTICIDAL NETS IN THREE GEOGRAPHICAL ZONES OF NIGERIA - A THREE YEAR FOLLOW-UP OF NETS DISTRIBUTED THROUGH CAMPAIGNS

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The average survival of Long-lasting Insecticidal Nets (LLIN) has increasingly become of interest to malaria program managers and the international community as this measure of durability will determine the frequency of LLIN replacements at household level and the cost of sustaining universal coverage. With the recent publication of a WHO-recommended method
to estimate net survival, comparative analyses from different areas or for different brands have now become possible. Following the mass distribution campaigns of 2010/11 in Nigeria annual follow-up surveys to measure attrition and physical integrity of campaign LLIN (polyester, 100 Denier) were undertaken in three locations of Nigeria representing different eco-geographic and climatic zones: Shinkafi district in Zamfara State in the northern dry-savannah, Toto district in Nasarawa State in the central guinea-savannah and Abi district in the rain-forest area of Cross River State. In each district a population representative sample was drawn using a 20 cluster sampling design and in each selected community 15 households that had received nets from the campaign were included in the interview and net assessment. In the questionnaire reasons for any loss of campaign nets were explored as well as attitudes and practices towards net care and repair. The assessment of physical integrity of the nets was done according to WHO recommendations and the proportionate Hole Index used to evaluate the outcome for each net. In the first round of surveys a total of 900 households were sampled and 1,571 campaign nets assessed while for the second round the figures were 896 and 1,367 respectively. Two years after the campaign the survival of LLIN varied considerably between locations with 69.6% (95% CI 62.7-75.7) still in serviceable condition in Nasarawa State, 81.4% (75.0-86.5) in Zamfara and 89.4% (84.2-93.5) in Cross River State. The final round of data collections is currently ongoing and will be presented together with estimates of median LLIN survival. Reasons for differences by location will be explored and discussed.

MODELING THE EFFECTS OF TRANSNATIONAL MIGRATION ON PUBLIC HEALTH POLICIES IN SOUTHEAST ASIA: AN INVESTIGATION INTO THE IMPACT ON ELIMINATION STRATEGIES

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Human migration plays an important role in the spread of infectious diseases. In order to determine the impact of human movement between areas of unequal malaria endemicities on malaria transmission and the resulting control and elimination strategies, we need to improve our understanding of: the migration patterns present in the study areas of interest, the malaria acquisition risk borne by the migrants, and the impact of migration on malaria transmission. We hypothesized that persistent and unmonitored flow of people between porous borders between areas of unequal transmission, presents a significant challenge to the elimination of malaria in the area of low disease transmission. The present study aims to provide a quantitative assessment of malaria risks due to transnational migration and to evaluate subsequent intervention strategies for malaria control and elimination. Within the EMOD framework developed by the Institute for Disease Modeling, we have constructed simulations with two geographically-connected populations in order to model the impact of varying degrees of human migration rates and intervention methods on malaria transmission. Our preliminary simulation results suggest that the proportion of humans infected was markedly different between the various rates of human migration. We will continue to develop increasingly complex simulations that explore both human and vector-based interventions such as the administration of primaquine and the application of long-lasting microbial larvicides. The simulations results can be used to consider various feasible pathways to sustained local elimination as a function of cross-border migration rates. This approach has the ability to make a timely and significant contribution to public health by filling a critical gap in our knowledge of human migration patterns, especially in Southeast Asia. The utility of these data and this novel modeling tool is multidisciplinary and has the potential to inform sophisticated models in other research fields.

ANALYSIS OF DETERMINANTS OF INSECTICIDE TREATED NETS (ITNS) USE AMONG CHILDREN UNDER FIVE YEARS USING LOGISTIC REGRESSION

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Malaria causes between three hundred and fifty (350) and five hundred (500) million clinical episodes and over one million deaths annually. Children and pregnant women are the most vulnerable group and most endangered by the disease. Insecticide-Treated bednets (ITNs) range among the most effective measures of malaria prophylaxis, yet its implementation level in sub-Saharan Africa is still low. The goal of this study was to assess factors influencing the use of ITNs by children under five years in Ghana. A cross-sectional study was conducted using pre-tested, interviewer-administered questionnaires. Possible factors were measured using five hundred (500) mothers or guardians of children under five years, in twenty three (23) communities in the Asamankese sub-municipality in the West Akim District in the Eastern Region of Ghana. Logistic regression was used to assess the influence of five possible factors on ITNs use. In order of importance in determining one’s use of LLIN, ‘Sleeping area allowing for the use of Long Lasting Insecticides Net (LLIN)’ was the most important factor influencing the use of ITNs by children under five years in Ghana, followed by ‘Household’s expected monthly income’, then ‘Mother’s SHS level of education’ compared to ‘none’, while the least was ‘Number of children under five years’. The study also revealed that 86.2% of the participants owned ITNs out of which 92.81% used it the night before the study. The study revealed that there is a relationship between influencing factors and use of LLINs. To improve ITNs usage, there should be continuous distribution of LLINs and Insecticide Treated Materials (ITMs), such as insecticide treated curtains for doors and windows. This should be heavily supported by education and Behavior Change Communication (BCC) via radio, TV, and other media especially on the hanging techniques and need to provide adequate space for sleeping area.

EVALUATING DIFFERENCES IN HOUSEHOLD INSECTICIDE-TREATED BEDNETS (ITN) OWNERSHIP BETWEEN UGANDA AND ZIMBABWE DURING 2005-2011: AN ECONOMETRIC APPROACH

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The rapid scale-up of insecticide-treated bednets (ITNs) in African countries have received heightened attention with the availability of significantly greater resources for national malaria control efforts, particularly after 2005. In this study, we investigate the mean differences in household ITN ownership in Uganda and Zimbabwe for the years 2005 and 2011, using DHS and GPS data and MARA malaria endemicity maps and econometric methods. The probability of owning an ITN in Uganda was 12.8% higher compared to Zimbabwe in 2005. Although ITN ownership increased steadily in both countries, the difference in the probability of owning an ITN widened significantly and became 33.5% over this period. The Blinder-Oaxaca technique can be used to study the mean outcome differences between two groups (in our case two countries). Using this technique, we divide the ITN ownership differential between two countries into a part that is “explained part” by group differences in ITN ownership determinants, such as household characteristics and malaria risk, and a residual part that cannot be accounted for by such differences in ITN ownership determinants. This “unexplained” part captures the effects of group differences in unobserved predictors of ITN ownership. Our preliminary results showed that the larger fraction of the increase in the ITN ownership differential in this period was due to the unexplained part. To understand
this result we investigated what happened in these countries in terms of malaria control efforts between 2005 and 2011. Published literature points to a significant difference in malaria financing in these countries, yet the earmarked proportion of funding for increasing the ownership and use of ITNs is usually high in all countries. Next we will study to what extent malaria control efforts in these two countries affected ITN usage and all-cause child mortality rates and potentially extend our analysis to include other African countries.

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UNDERSTANDING INTRA-HOUSEHOLD DECISION MAKING FOR INSECTICIDE-TREATED MOSQUITO NET ALLOCATION IN UGANDA

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Intra-household allocation of insecticide-treated mosquito nets (ITNs) is an important concern for malaria prevention programs. Behavior change communication campaigns stress the need to prioritize children under five and pregnant women for net distribution and use, as these two groups are the most vulnerable to malaria infection. This study utilized a pile sort activity to help understand net allocation decision making in Luwero and Nebbi, two Ugandan districts. Sixty-four respondents, half men and half women, were asked to assign 10 individuals ranging in age from infant to older adult and including adults and children of both sexes as well as one visibly pregnant woman to one of four beds. One bed had a new net, one a slightly used net with a few holes, one an old net with large holes, and the last with no net. After sorting, respondents were asked why they allocated each individual as they had. The number of times a household member was placed under each net was evaluated, and a hierarchical cluster analysis was completed to determine which household members were typically grouped together. Responses were compared between male and female, rural/urban, and district of residence. Results demonstrate that net allocation differs by gender, rural/urban, and district, however the pregnant woman and baby were always clustered together and were given the best net greater than 50 and 60 percent of the time, respectively. Children ages 5-14 were also typically clustered together and given the best or second-best net. Young adults (around age 25), older adults, and elders were given the worst net or no net a majority of the time. Reasons given for these placements varied, but common themes include the baby and pregnant women being the most vulnerable to malaria, the older children being able to help repair nets, and the heads of household and young adults being robust enough to not succumb to malaria infection and having sufficient resources to purchase additional nets. The general consensus was that this household did not have enough nets, and that the family should strive to provide nets for all of their household members. This exercise supports current behavior change campaign messaging, demonstrating that, at least in their reported behavior, people are likely to prioritize vulnerable populations when it comes to net use.

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REDUCTION IN DISPARITY OF INSECTICIDE-TREATED NETS OWNERSHIP AND USE AMONG SOCIOECONOMIC GROUPS AFTER SCALE UP IN UGANDA

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The increase in funding for malaria control in the past decade resulted in an increase in Insecticide-Treated Nets (ITNs) ownership and use in many countries in sub-Saharan Africa, particularly in Uganda. However, with the shift in programmatic focus from target groups to universal coverage there is a need to ensure equal access and use of ITNs for all sub-populations regardless of their socioeconomic status. This study assessed change in disparity in ITN ownership and use among different socioeconomic groups in Uganda between 2006 and 2011. The authors used Lorenz Concentration Curve and Index (C-Index) to assess equity in household ITN ownership and use among children under five between wealth quintiles separately in 2006 (Demographic and Health Survey data) and 2011 (Malaria Indicator Survey data). C-Index values range between -1 to 1, a value of 0 suggests no difference in ownership and use among different socioeconomic groups. Household ownership of at least one ITN rose significantly from 16% (2006) to 60% (2011). Similarly, ITN use among children under five was very low (10%) in 2006 and increased substantially to 47% in 2011. The increase in ITN ownership was associated with significant reduction in inequity among wealth quintiles (C-Index 0.11, 95% CI: 0.08;0.34) in 2006 versus 0.02, 95% CI: 0.01;0.04 in 2011). Similarly the disparity in use of ITN use among children under five from different wealth quintiles greatly reduced from 2006 (C-Index: 0.04, 95% CI:-0.10;0.19) to 2011 (C-Index: 0.01, 95% CI:-0.04;0.06). This achievement is probably due to the shift to universal coverage in 2009 which led to free mass distribution campaigns of Long-lasting Insecticidal Nets (LLINs), with 7.2 million LLINs distributed by 2010. This achievement in parity between wealth quintiles should be sustained; however, efforts are needed to further increase overall ITN ownership coverage and use in Uganda. This is achievable through additional free mass campaign distribution combined with traditional distribution channels.

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ASSESSING THE CONTRIBUTION OF MALARIA CONTROL INTERVENTIONS ON REDUCTIONS IN ALL-CAUSE UNDER-5 MORTALITY IN ZAMBIA

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Between 2000 and 2010, Zambia rapidly scaled-up malaria control interventions such as insecticide-treated nets and indoor residual spraying. At the same time, Zambia recorded substantial declines in under-five mortality. Zambia’s experience is heralded as a malaria-control success story, but it is unclear whether the expansion of malaria control contributed to improved childhood survival beyond the impacts of other child health interventions. To quantify the impact of malaria control efforts in Zambia, we estimated trends for all-cause under-five mortality and a range of child and maternal health interventions at the subnational level. We quantified the reduction in child mortality associated with malaria control while taking into account trends in other key interventions as well as socio-demographic, health system, and environmental factors across districts. Our estimation methods included generalized linear models and functional data analysis and were validated with cross-validation and simulation techniques. We found that the bivariate relationship between malaria control and child mortality was strong and significant, but this relationship was attenuated when other factors were considered. Several other child health interventions also scaled up dramatically during the same time period, including pentavalent immunization, prevention of mother-to-child-transmission of HIV/AIDS, exclusive breastfeeding, and nutrition programs. Because of this simultaneous expansion, it was statistically infeasible to isolate the effects of malaria control efforts. In the absence of the combined scale-up of these interventions between 2000 and 2010, we estimated that child mortality would have been 11% higher in 2010. The scale-up of these interventions accelerated declines in mortality by 1% each year. Our findings emphasize the importance of constructing a comprehensive landscape of the drivers of progress in child mortality. A greater quantity of high-quality and localized data is critical for evaluating the independent impact of each intervention on childhood survival.
INCREASING ROLE OF ANOPHELES FUNESTUS AND AN. ARABIENSIS IN MALARIA TRANSMISSION IN THE KIŁOMBO VALLEY, TANZANIA

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This longitudinal study demonstrates the trends in malaria vector dynamics and their relative contribution to malaria transmission in hyper-endemic transmission settings in Tanzania. The study was conducted in two villages within the Kilombero valley, in rural Tanzania for five consecutive years (2008 - 2012). 72 houses were selected per village and each house was sampled for mosquitoes monthly using a CDC light trap. Collected mosquitoes were assessed for species identity and sporozoite infection status using PCR and ELISA respectively. Anopheles funestus susceptibility to insecticides was assessed using WHO guidelines. A total of 100,810 malaria vectors were collected, of which 76% were An. gambiae s. l. and 24% were An. funestus. Of all An. funestus samples that amplified with An. gambiae s.s. (n = 8,117), 93% were An. gambiae and 1% An. leesoni. Whereas for An. rivorulum and An. funestus s.s. identified by PCR (2,924) declined from 0.2% in the year 2008 to 0.1% in year 2012 and An. arabiensis dominated the wet season whereas An. funestus dominated the dry season. Malaria transmission intensity significantly decreased from an EIR of 78.14 infectious bites/person/year in 2008 to 25 ib/p/yr in 2011 but rebounded to 226 ib/p/yr in 2012 coinciding with an increased role of An. funestus in malaria transmission. Insecticide susceptibility tests indicated full susceptibility of An. funestus to deltamethrin (100% mortality), reduced susceptibility to diethyl (95%), permethrin (93%), and confirmed resistance to DDT (86%). Similar findings were also recorded for An. arabiensis, in separate study in same area. The results indicate the continuing role of An. arabiensis and the increasing importance of An. funestus in malaria transmission. These findings call for complementary vector control and surveillance tools that target these specific vector species, their behaviour and their ecology and an insecticide resistance management plan to preserve the efficacy of LLNs.

DEVELOPMENT OF AN IN VITRO TRANSMISSION BLOCKING (ITB) ASSAY AGAINST PLASMODIUM FALCIPARUM WITHOUT THE USE OF MOSQUITOES

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Major efforts are underway aimed at developing malaria vaccines that can be used for elimination of Plasmodium falciparum by preventing the development of the parasite within the mosquito and thereby preventing transmission of the parasite to humans. To assess candidate vaccines, antibodies are induced by immunization of animals and humans, and assessed for their transmission blocking activity against sexual erythrocytic and mosquito stage parasites in a standard membrane-feeding assay (SMFA). These SMFAs are biologically relevant, but highly consumptive and expensive to assess large numbers of candidate anti-sera in SMFAs. Sanaria’s technology platform generates live, aseptic, purified, cryopreserved P. falciparum sporozoites (PfSPZ) that can be administered as a highly protective malaria vaccine. PfSPZ are produced using Anopheles stephensi mosquitoes as bioreactors. Sanaria, in its quest for developing PfSPZ-based products without the use of mosquitoes, developed technology for the in vitro production of Pf oocysts. We have optimized gametocyte culture conditions that are optimal for the in vitro production of ookinetes and oocysts, established culture conditions to reproducibly produce and quantify 3 and 7 to 8 day oocysts, and demonstrated that in vitro produced oocysts are similar in size and morphology to mosquito produced oocysts. These developments have made it possible to assess the transmission blocking activities of candidate vaccines in an in vitro transmission blocking (ITB) assay without the need for mosquitoes. Preliminary data indicate correspondence between the results of SMFA and ITB assays. The transmission blocking activity of antibodies against PfS25 and PfS48/4S in SMFA and ITB assay will be presented.

IDENTIFICATION OF A NOVEL PLASMODIUM FALCIPARUM HEAT SHOCK PROTEIN 70 (HSP70Z), ALSO IDENTIFIED AS CG4, AS A TRANSMISSION BLOCKING VACCINE CANDIDATE

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A malaria transmission blocking vaccine (TBV) is critical to achieve the goal of malaria elimination in some areas. To date, only one sexual stage protein, PfS25, has been evaluated in humans and a second sexual stage protein, PfS230, will begin human testing soon. Other sexual stage proteins have failed to reach the clinic due to an inability to produce them at pilot-scale following cGMP. In order to expand the number of TBV candidates, a panel of monoclonal antibodies (mAbs) was produced against Plasmodium falciparum macrogametes to identify novel surface proteins that, when targeted by antibodies could interfere with parasite development in the mosquito midgut. Here we report on the evaluation of one mAb, identified as 1C7, which recognized a heat shock protein (HSP), identified as HSP70z or Cg4, by Western blot of a sexual stage parasite lysate and by pull-down studies using LC/MS/MS techniques. HSP70z is expressed in asexual and sexual stage parasites with a molecular mass of approximately 100 kDa. In macrogametes, HSP70z may be localized on the macrogamete cell surface by a live immunofluorescence assay. Most importantly, 1C7 blocked P. falciparum transmission in mosquitoes, with similar activity to that of a PfS230 domain 1 specific mAb using an ex vivo transmission blocking assay. A recombinant form of HSP70z (named rHSP70z) was produced in Pichia pastoris that was comprised of approximately 20% of the native protein which was recognized by 1C7 in Western blots. rHSP70z specific IgG purified from sera of immunized rabbits failed to block parasite transmission. In a competition ELISA, rHSP70z specific rabbit antibodies failed to compete for 1C7 binding to rHSP70z, likely explaining the lack of transmission blocking activity. Currently, the 1C7 epitope is being mapped to evaluate whether a synthetic peptide mimicking the 1C7 epitope will induce transmission blocking antibodies.
Impact of Indoor Residual Spraying on Entomological Indices of Malaria Transmission in the Bunkpurugu-Yunyoo District in the Northern Savannah Zone of Ghana

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Malaria remains a major public health problem in Ghana, especially in the northern savannah zone. This study was conducted to evaluate the impact of U.S. President’s Malaria Initiative (PMI) and the Ghana Health Services Indoor Residual Spraying (IRS) program on malaria transmission in the Bunkpurugu-Yunyoo District in northern Ghana. In 2011 and 2012, the district was sprayed with a pyrethroid, alphacypermethrin, at an application rate of 25mg/m². In 2013, an organophosphate, pirimiphos-methyl at a rate of 1g/m², was used based on declining susceptibility of local vectors to pyrethroids. Indoor resting densities (IRD), parity, sporozoite rate and entomological inoculation rates (EIR) of the local vector species were monitored through pre- and post-IRS monthly human landing and pyrethrum spray collections. The IRD of Anopheles gambiae s.l. (the predominant vector species, 99.2% of all Anopheles collected) was reduced from a mean of 2.91 mosquitoes/room recorded from the baseline surveys to 2.10 mosquitoes/room (27.7% reduction) in 2012 after spraying with alphacypermethrin. In 2013, the mean IRD of An. gambiae s.l. was further reduced to 0.22 mosquitoes/room, representing 89.2% decline compared to 2012. In 2012 there was a non-significant reduction (p=0.289) in the mean parity rate for An. gambiae s.l. from 75% to 43% (57% reduction). Spraying with Actellic in 2013, resulted in 67% reduction in parity rate from 43% to 32% (p = 0.130). A comparison of the pre and post-IRS EIRs also revealed a significant (p<0.05) reduction, from 0.35 infective bites/man/night (ib/m/n) in 2011 to 0.021 ib/m/n in 2012. In 2013, there was slight decrease in EIR to 0.018 ib/m/n after spraying with Actellic 300CS that year. The results show that the IRS operations resulted in reduction in key entomologic indicators. IRS with pirimiphos-methyl had the greatest impact on indoor resting densities and parity rates of An. gambiae s.l. but not EIRs. The PMI funded IRS program after three years contributed to 94.8% reduction in malaria transmission in the study area.

Scaling up of insecticide-treated nets (ITN) ownership and use in Mozambique: has the scale-up been equitable?

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Insecticide-treated nets (ITNs) are effective tools for malaria prevention and can significantly reduce morbidity and mortality due to malaria, especially among children under five in endemic areas. ITN ownership and use have rapidly increased in Mozambique, due to scaling up of ITN distribution efforts within the country over the past seven years, which have aimed to achieve universal coverage. The percent of households that owned at least one ITN rose by more than three-fold from 15.7% in 2007 to 51.4% in 2011. ITN access, defined as one ITN for every two people in the household also rose significantly, from 9.4% in 2007 to 37.0% in 2011. Similarly during this time, ITN use among children under five and pregnant women increased significantly, from 6.7% to 35.7% and from 7.3% to 34.3%, respectively. With the aim of achieving universal coverage, it is important to also assess distribution across subgroups, particularly groups from different socio-economic status. This analysis used Lorenz concentration curves and indices to assess the equity of ITN household ownership, access and use in Mozambique, using data from the 2007 Malaria Indicator Survey and 2011 Demographic Health Survey. Concentration Index (C-index) values range between -1 and 1, with a value of 0 representing perfect equality. From 2007 to 2011, equity in ITN household ownership (C-Index: 0.06 in 2007 and 0.04 in 2011), ITN access (C-Index: 0.12 in 2007 and 0.09 in 2011), and ITN use among children under five (C-Index: -0.04 in 2007 and 0.03 in 2011) showed slight improvements, while equity in ITN use among pregnant women remained the same (C-Index: .06 for both years). It is important to highlight that while improvements where shown, equity in ITN household ownership, access and ITN use among children under five, all were fairly equitable in 2007. The results demonstrate that ITN scale-up efforts have been successful as well as equitable across the population, however further improvements in access and ITN coverage are still needed to reach targets.

Differences in Deet and Picaridin Sensitivity Between Southeast Asian Vectors of Malaria and Arboviruses, Results of A Field Evaluation of Topical Repellents

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Scaling up of insecticide treated nets has contributed significantly to a substantial malaria decline. However, some malaria vectors, and most vectors of arboviruses, bite outdoors and in the early evening. Therefore, topically applied insect repellents may provide a crucial additional protection against mosquito-borne pathogens. Among topical repellents, DEET is the most commonly used, followed by others such as PDM or picaridin. A study was carried out in Cambodia to determine the entomological efficacy of DEET and picaridin repellents on wild populations of several mosquito genera, including vectors of arboviruses (Aedes aegypti and Aedes albopictus) and malaria (Anopheles dirus, An. minimus, An. maculatus and An. barbirostris). During 230 survey days in two consecutive years, the lower limbs of 5 persons were treated with repellents (“DEET 20%”, “picaridin 20%”, or “picaridin 10%”) or ethanol (2 negative controls), followed by mosquito collections on the treated limbs during 5 consecutive hours. The treatments were grouped following a 5x5x5 Graeco-latin square to equalize the effects of treatment days, collection sites, and test persons. Protection rates were high (91-99.2%), with significant differences between treatments, genera, and species. For malaria vectors, “DEET 20%” performed better than “picaridin 20%” or “picaridin 10%”. The protection rate against An. barbirostris was significantly lower as compared to the other vectors, especially for the picaridin repellents. As malaria endemic areas often differ in their vector species composition, this heterogeneity in repellent sensitivity between vector species might result in a geographically heterogeneous epidemiological impact of repellent use for malaria control.

Molecular and Environmental Interactions in Anopheles Gambiae Influence Both Reproductive Capacity and Plasmodium Development

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Reproduction in the major malaria vector Anopheles gambiae is influenced by a number of molecular and environmental factors. Male-female
molecular interactions following mating are important determinants of fertility and fecundity. At the same time, an increasing amount of evidence points to reproductive processes playing an important role in *Plasmodium* parasite development. Male transfer of the steroid hormone 20-hydroxy-ecdysone (20E) during mating activates the transcription of a Mating-Induced Stimulator of Oogenesis (MISO) gene that transduces the mating signal into an increase in egg development. Silencing MISO by RNA interference in laboratory colonies reduces egg development to levels observed in virgin females. This phenotype is caused by reduced expression of yolk protein precursors (YPPs) after MISO silencing, impairing lipid accumulation in the oocyte. Previous research shows that the same YPPs essential for lipid accumulation in the developing mosquito egg help parasites escape the immune system. We have found evidence that MISO depletion decreases *Plasmodium* infection in *A. gambiae*, further reconstructing the molecular pathways linking egg development and *Plasmodium* infection. Functional studies performed in field *A. gambiae* show that in natural populations MISO is essential for egg development, as its silencing abolishes oogenesis after blood feeding. These results suggest that in natural mosquito populations MISO is a key switch that directs resources derived from the blood meal towards oogenesis only in mated females. Besides molecular factors, environmental factors also affect *A. gambiae* reproductive biology in natural populations. Sequencing analysis of the microbiota from reproductive tissues of wild *A. gambiae* demonstrates enrichment of bacteria in specific villages and mating swarms, which may impact reproductive success and isolation. In addition we have identified bacteria in reproductive tissues that have been previously shown to impact *Plasmodium* survival. We are currently determining the effects of these bacteria on reproductive biology and mosquito fitness, two factors relevant for malaria transmission. By elucidating the different molecular and environmental interactions regulating reproduction and parasite development in *A. gambiae*, we hope to develop novel targets for vector control.

**Heterogeneity of Human and Mosquito Behavior in Relation to Outdoor and Early Malaria Transmission in Southeast Asia**

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Outdoor transmission is one of the key factors for malaria control and elimination in the Greater Mekong Region, including Cambodia, where malaria is now mainly reduced to forested regions inhabited by ethnic minorities and mobile migrants. Tackling outdoor transmission requires a better understanding of the heterogeneity in human and vector behavior during times when people are still active outdoors. Results from a mixed-methods social science study in Ratanakiri, Cambodia, indicate that local ethnic minorities have different socio-cultural characteristics than the majority society targeted by malaria control programs. (i) Mobility caused by a multiple residence system increases exposure to the sylvatic vector *An. dirus*, as during the malaria peak season people usually reside on their farms in the forest. (ii) Open housing blurs the boundary between indoor and outdoor biting. (iii) Differences in sleeping times between villages, farms and during forest activities creates diverse evening biting opportunities, if assumed that people sleep under insecticide-treated nets at night. However, (iv) evening resting is frequently done without nets, and (v) even night sleeping often occurs under non-impregnated bought nets, which are often torn, making it hard to establish to what extent transmission actually occurs early or outdoors. Results seem to suggest that the evolving interplay of vector and human behavioral heterogeneity maintains malaria hotspots/pop of mainly asymptomatic carriers. Targeting malaria transmission in low transmission settings requires a better understanding of this heterogeneity.

**No Product? No Program: Treatment Uptake and Availability of Antimalarial Drugs for Intermittent Preventive Treatment of Malaria in Malawi**

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Throughout Africa, 30 million pregnant women are exposed to malaria each year. Several interventions are central to malaria control efforts, including dispensing sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy (IPTp). While preventing malaria in pregnancy is a key focus of global interventions, trends in using SP to prevent malaria during pregnancy have largely stagnated. Twenty years after adopting IPTp as an official policy, the rates of pregnant women in Malawi who receive at least two doses of SP is only 55 percent. Research has identified factors impeding adherence to IPTp policies: antenatal clinic (ANC) client behavior/attitudes, provider actions/attitudes, and SP availability at facilities. Qualitative data indicate that stockouts significantly impact IPTp coverage. However, only a quantitative analysis will show if there is a relationship between IPTp use and stockouts. For four years, the USAID | DELIVER PROJECT examined trends for three complementary data sources: SP availability at health facilities using the country's logistics management information system; SP uptake at health facilities using national ANC service statistics; and IPTp coverage in households reported in the Malawi Demographic Health Surveys. Preliminary results show a general decline in SP stockouts between 2010 and 2013. SP stockouts peaked in late 2011 and the first quarter of 2012 (80 percent); low stockout rates were reported during the last half of 2012 and all of 2013 (6 percent). High stockout rates in early 2012 correlate with a sharp drop in SP uptake during the same time (62 to 31 percent). As SP availability improved, beginning in May 2012, the IPT coverage rates also improved. Women attending their first ANC visit after 12 weeks, who received any SP, increased to almost 100 percent by mid-2013. These results show a close relationship between SP availability and the uptake of preventive treatment during pregnancy. Using a mixed-method case study approach, this analysis will explore the impact of SP stockouts on MIP programming efforts.

**An Extended Molecular Barcode for Tracking Plasmodium Falciparum Parasite Populations**

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The lack of a generalizable approach to malaria control requires a real-time assessment of both epidemiological and parasite population genetics at the local level. The major epidemiological shifts associated with the transition into a malaria-eliminating country are mirrored by changes in the genetic diversity profile of malaria parasite populations. These genetic signatures can be monitored using population genetic-based tools to determine efficacy of malaria intervention efforts prior to measurable changes in disease prevalence. For these reasons, and to further understand the basic biological processes of parasite transmission, we have improved upon an interim panel of neutral, unlinked, single nucleotide polymorphisms (SNPs) that is specifically designed for resolving
individual parasites in highly related parasite populations and allows for quantification of allele balance at polymorphic loci to infer changes in transmission dynamics. The extended molecular barcode includes SNPs exhibiting high minor allele frequency and low divergence (e.g. FST) filtered from screening over 500 whole genome sequenced samples representing populations from Africa, South East Asia and South America. The accuracy and sensitivity of Sequenom’s Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry genotyping technologies allow for quantitative output that reflects proportions of each allele in multiplexed reactions. The increased number of loci and low dynamic range of the minor allele improve our ability to predict the number of distinct parasite genomes in samples with higher complexity of infection (COI). In silico testing of the extended molecular barcode demonstrates its utility for detecting recent common ancestry among parasites in a sample on a much more cost-effective basis than using variants called with whole genome sequence data. We are exploring the use of these approaches to identify shared regions of the genome that are identical by descent among highly related Senegalese populations and assess COI in high transmission settings.

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RECEPTIVITY OF ANOPHELINE MOSQUITOES IN SOUTHERN ZAMBIA: TWO YEARS OF BIONOMIC DATA FROM AN AREA TARGETED FOR MALARIA ELIMINATION

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Malaria prevalence in Macha, southern Zambia has reduced significantly over the past decade, with community parasite prevalence currently below 1%. The province is now targeted for elimination strategies. It is important, however, to establish the potential of the local mosquito population to transmit malaria to estimate the risk of resurgence if the parasite were re-introduced. To address this, light trap collections of mosquitoes were carried out monthly in randomly selected geo-referenced households in the catchment area of Macha Hospital from February 2012 to February 2014. Peak times of host-seeking were studied using collection bottle rotators. Approximately 1200 female anophelines were collected from 856 traps across 336 different households. 60% were identified as the vector An. arabiensis to give a mean catch of 0.88 An. arabiensis per trap-night over the study period. Catches ranged from 0 to 148 An. arabiensis in a single trap, with highest catch recorded in February 2013 (monthly mean 7.2 per trap-night). Households with anophelines appeared to be clustered. Spatial analyses are ongoing to overlay mosquito distribution on malaria risk maps. 10.8% of An. arabiensis were blood fed and the human blood index was calculated as 0.93. At least 9 other anopheline species were identified, some of which were highly anthropophilic. One specimen was found to be positive for Plasmodium falciparum sporozoites by ELISA. Preliminary analysis of host-seeking times indicated peaks between 22:30 and 02:30, but vector activity was recorded as early as 20:30. Despite substantial reduction in malaria cases in Macha, large numbers of the vector An. arabiensis exist at certain times of the year and in certain localities. Here An. arabiensis demonstrates high endophily and anthropophagy. There is the potential for human exposure outside the times of net use. Whilst drug-based elimination strategies are encouraged, vector control methods should be maintained and entomological surveillance continued to monitor any increase in dominant or secondary vectors.

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OPTIMAL COVERAGE AT MINIMAL COST: A DYNAMIC MODELING APPROACH TO SIMULTANEOUS ALLOCATION OF MULTIPLE ANTI-MALARIA INTERVENTIONS

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Malaria control efforts often involve a multipronged approach, including insecticide-treated nets (ITNs), indoor residual spraying (IRS), and pharmaceutical-based treatment and prevention. Public health agencies (government, private, NGOs) are constrained by conflicting demands on limited budgets. Determining the appropriate combination and magnitude of interventions is both complicated and context specific. Using a dynamic modeling approach, we analyzed the system dynamics of multiple malaria interventions functioning in concert, evaluated the cost-effectiveness of treatment and prevention strategies, and developed a resource allocation model to apportion resources across intervention types to maximize overall cost-effectiveness. This compartmental mathematical model (using MATLAB) includes age, human immunity, and seasonality in the Plasmodium transmission cycle, with interventions of IRS, ITNs, intermittent preventive treatment for pregnant women (IPTp), and mass screening and treatment (MSAT). We assessed individual and combined effects on various transmission parameters such as biting rate, force of infection, and mosquito death. For a set of parameters considered typical of high transmission malaria endemic settings, results show that after five years into the model run and compared to a baseline with no interventions, with a combination of 65% coverage of IRS and ITNs, infection prevalence in children under five years of age and pregnant women would decrease by about 18%. Those above five years of age exhibited decrease in prevalence by about 16% with 70% combined IRS and ITNs. The addition of IPTp and MSAT decreased long-term community prevalence in all age groups by an overall additional 12%. This modeling approach allows a careful assessment and optimization of costs and benefits to particular combinations of malaria interventions and should assist public health groups in maximizing benefit under constrained budgets.

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HOUSEHOLD EXPENDITURES FOR MALARIA TREATMENT FROM A POPULATION-BASED SURVEY

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Effective malaria control technologies not only improve health, but also reduce health expenditures by government and households. In Muheza, Tanzania, a randomized trial is assessing the effectiveness and cost-effectiveness of a non-pyrethroid insecticide-treated wall liner (IFWL) and indoor residual spraying (IRS). To project savings, the investigators are determining current expenditures for malaria treatment in conjunction with a cross-sectional epidemiological household survey. Following mapping and household enumeration, from December 2013 through February 2014 epidemiological teams visited 4200 randomly chosen households...
residents aged 6 months across 60 village clusters. Respondents who reported a malarial episode within the past 30 days were selected for a follow-up economic interview. Its questions included household income, treatment received, time required for travel and treatment, and expenses incurred. Overall, 18% of sampled residents reported a malaria illness episode within the last 30 days. Preliminary data are currently available for 467 representative malarial cases. Of these, 6% received inpatient hospital treatment, 91% received ambulatory treatment outside the home, and 3% received only in-home or no treatment. Overall combined travel and treatment time averaged 5 hours. Household expenditures per case averaged US$5.24 (TZS 8,545), but the median value of US$2.33 (TZS 3,800) was below the mean. This arose because expenditures on malaria treatment were highly skewed with a standard deviation equal to 213% of the mean. Analysis of household expenditures found 54% was for direct medical expense (consultations, beds, tests, and medications), 27% for transportation, and 19% for other expenses. Average expenditures of hospitalized patients US$32.82 (TZS53,491) were an order of magnitude above those of non-hospitalized patients of US$2.15 (TZS5,787).

By comparison, household monthly revenue was under US$46.01 (TZS75,000) for 71% of respondents. Average inpatient expenses represent about a month's median income. Given low rural incomes, even routine medical expenses can strain household budgets. Adjustment of censored expenditures for episodes in progress and valuing time lost would increase estimated costs. If ITWL and IRS prove efficacious in this district, households will save on treatment expenses.

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IMPACT OF MALARIA INTERVENTIONS ON REDUCTIONS IN NEONATAL MORTALITY IN MALAWI, RWANDA AND MAINLAND TANZANIA

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Between 2000 and 2010 sub-Saharan Africa’s impressive gains in under-five mortality have been accompanied by a more modest reduction in neonatal mortality. As a result, neonatal mortality now accounts for about a third of all under-five deaths in the region. This study selected three countries in SSA with significant reductions in NMR during this period: Malawi, Rwanda, and mainland Tanzania, in order to identify factors that contributed to the observed reductions, with special interest in the relative importance of intervention scale-up to protect women against malaria during pregnancy. In Malawi, the neonatal mortality rate among women’s most recent children born fell from 26 deaths per 1,000 live births in the five years preceding the 2000 DHS to 20 deaths per 1,000 live births in the five years preceding the 2010 DHS. In Rwanda, the NMR declined from 29 to 14 deaths per 1,000 live births, and in mainland Tanzania from 32 to 18 deaths per 1,000 live births between the 1999 and 2010 surveys. Multivariate decomposition procedures were used to examine the extent to which the scale-up of malaria interventions contributed to these observed reductions. Results show that in all three countries the rapid increase in mosquito net ownership was associated with the observed reductions in neonatal mortality, even after adjusting for changes in the distribution and effects of sociodemographic characteristics and key maternal and delivery services. In Malawi - where information on mothers’ use of IPTp was available - the study did not find evidence that the scale-up of IPTp was associated with the reduction in NMR. In conclusion, the findings reinforce the importance of consistent and universal mosquito net use in areas with high prevalence of malaria. While malaria interventions are most often geared towards saving the lives of children at older ages (6 months to 5 years), the study findings contribute to a growing body of evidence pointing to the importance of malaria interventions to neonatal survival.

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FIGHT AGAINST MALARIA BY STUDENTS AND SCHOOLCHILDREN IN KINSHASA, DR CONGO

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Malaria is a real public health problem in Africa. Despite the efforts made by countries, 655,000 deaths were recorded worldwide in 2010. And the Democratic Republic of the Congo accounts for nearly 10% of the mortality. And the fight against this disease should be of interest to all risk groups in the community. And new and innovative approaches should be considered to reduce disease. Thus, students of the Protestant University of Congo have been trained in the fight against malaria with the aim of training schoolchildren. Students’ knowledge was initially evaluated. And training was organized to provide knowledge about the transmission, prevention, particularly on ITN and finally what to do in case of fever. Method: 823 students in 9 schools in the city of Kinshasa sensitized by 20 university students who were trained by the managers of the National Program of Academic Medicine and University Kinshasa Parasitology Department. The age of children was between 9 to 11 years old. This awareness was marked by a strong interaction between children and sensitizers. At the end of awareness, the children were evaluated to assess their understanding of the subject. And the best schoolchildren were awarded. It was noted that over 70% of children could answer basic questions on malaria. In conclusion, at the end of this work, over 800 schoolchildren in the city of Kinshasa and 20 students have contributed to the fight against malaria, which is a small proportion of the population of said city. It is therefore important to continue these actions as awareness through children, future actors and relay in the transmission of knowledge from their families. This could be, an innovative approach to malaria control.

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INSECTICIDE-TREATED NET (ITN) UTILIZATION AND MAINTENANCE IN KINSHASA, DR CONGO

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Malaria remains at the beginning of the first century killer in DRC with more than 60,000 deaths, especially among children under 5 and pregnant women. Among the strategies, the LLIN is a tool of choice but its availability and handling are operational problems. Furthermore, its efficiency and sustainability are elements to evaluate. The present study was conducted with the aim to determine the proportion of households with at least one LLIN; the proportion screens installed (used); the proportion of pregnant women who slept under LLINs; the proportion of children who slept under LLINs; and determine the average age of the net and the number of washing on average per month. A cross-sectional study on the use and sustainability of ITN Long (LLINs) in the city of Kinshasa. We formed a cluster sampling and multistage. The sample size was 104 households per municipality. And 24 selected communes we got 2,512 households. The use of the net in Kinshasa was 59.4%. Pregnant women and children under 5 were using respectively 70% and 60%. Through the city's most important use is in the center where the mosquito nuisance with the Culex is the greatest with 72.7%. Whereas the periphery of the city use is low (44 to 55%) where anopheles populations are most abundant. The proportion of pregnant women and children under 5 years under nets was 70% and 60% respectively. In 2,512 households visited.
nearly 4,812 LLINs were counted. The average duration of the net in the household was in the range 19 to 24 months with 1.5 washes by month. More than 50% of household use detergent for washing ITN. The majority of nets found in the households have probably lost their effectiveness before 18 months of utilization. In conclusion, the use of LLINs was still low in Kinshasa. The inhabitants do not respect manufacturer’s recommendations in term of washing. Study on bio efficacy and durability in field use condition must be conducted to make evidence of efficacy.

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EFFECT OF MASS DRUG ADMINISTRATION OF IVERMECTIN TO HUMANS ON MALARIA TRANSMISSION AND EPIDEMIOLOGY IN WEST AFRICA

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Mass drug administration (MDA) of ivermectin to control filariasis and onchocerciasis has been shown to reduce malaria vector vectorv villagership and the proportion of mosquitoes that are able to transmit *Plasmodium falciparum* in the same communities. Therefore, we have proposed that ivermectin MDA be considered in malaria control and elimination strategies. In our ongoing efforts to fully characterize the effect of ivermectin MDA on malaria transmission, we compared its effect on *Anopheles gambiae* and *Plasmodium falciparum* natural populations in three different West African countries (Senegal, Liberia and Burkina Faso) across different seasons. Blood fed mosquitoes were collected indoors before and after MDA in treated villages by health authorities for either lymphatic filariasis (ivermectin + albendazole) or for onchocerciasis control (ivermectin alone), and concomitant mosquito sampling was performed in untreated control villages. We compared the blood fed mosquito survivorship, sporozoite rate and parasite genetic diversity in mosquitoes, taking account for temperature, humidity, mosquito species and type of treatment. The mosquitoecidal effect was consistent in all field sites and seasons, and did not vary with the addition of albendazole. The reduction in sporozoite rates were significant when compared to control villages but the observed reductions vary across field sites. In Burkina Faso in the treated village, sporozoite rate was significantly reduced by 79% following MDA (from 8% to 1.7%) and increased to pre-treatment levels after two weeks. Additionally, MDA completely eliminated sporozoite transmission from outdoor host-seeking mosquitoes for a period of two weeks. The potential impact of ivermectin on *Plasmodium* genetic diversity in mosquitoes and its consequence on malaria epidemiology and transmission will be discussed.

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IMPROVED ENVIRONMENTAL COMPLIANCE AND OPERATIONAL EFFICIENCY USING MOBILE SOAK PITS

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The President’s Malaria Initiative (PMI) employs thousands of spray operators and annually sprays millions of homes in Africa with a residual pesticide to kill malaria-bearing mosquitoes. At the end of the work day, the spray operators must clean their spray tank and their personal protective equipment (helmets, face shields, gloves, and boots), resulting in wash water that is contaminated with pesticide residue. This contaminated water is treated before release in soak pits, which are pits 1 meter wide x 2 meters length x 1 meter deep, filled with a bed of commonly available cooking charcoal and other materials to filter out and break down the pesticide. These soak pits are centrally located within targeted spray areas so that many operators can travel to and use these facilities for clean-up at the end of the day. PMI has built and/or refurbished hundreds of soak pits over the past several years of IRS project implementation. Ensuring that these soak pits are built properly so as to minimize negative environmental impacts from IRS is a major environmental compliance responsibility of the implementing partner. However, in sparsely populated spray areas, teams may travel hours to reach targeted communities, and they may not be able to return to a centralized location for clean-up at the end of the day. AIRS has developed a mobile soak pit (MSP) that can be transported from site to site with the spray team, can be installed in less than one half hour, and can remove pesticide contamination from wash waters. Advantages of this mobile soak pit include a marked reduction in the time that spray operators spend traveling from site to wash area, better adsorption of the pesticide due to the characteristics of the filter material, better control of soak pit materials, and better protection of the community because the pesticide contamination is taken away in the filter.

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BASELINE STUDIES ON ANGLOGOLD ASHANTIS’ INDOOR RESIDUAL SPRAYING PROGRAM (IRS) FOR MALARIA CONTROL IN GHANA

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Malaria is still high in many parts of Africa including Ghana. Private -public partnership are essential in significantly reducing the burden of malaria in high risk countries. In Ghana, there is rapid scale up of indoor residual spraying in various parts of the country with funding from the Global Fund and the United States President’s Malaria Initiative (PMI). AngloGold Ashanti (a mining company) is implementing an IRS programme in 25 districts in Ghana. The results of baseline studies (sensitivity of insecticides, prevalence of childhood malaria parasitemia and anemia) conducted prior to the IRS programme are presented here. Malathion was most effective with 100% An. gambiae mortalities in seven districts. Fenitrothion was effective in three districts while Propoxur worked in one district. Few (14) kdr susceptible strains were detected in samples analyzed with majority being homoygous kdrRR(120) resistant species compared to 32 Heterozygous kdrRS. Preliminary data shows high prevalence of malaria parasitemia (range: 30 - 50%) and anemia (range 40% - 60%). An organophosphate class of insecticide is considered most appropriate for IRS in eleven districts currently earmarked in Ghana. Rotation of different classes of insecticides over time is however recommended as it offers a practical solution for resistance management in light of rapid resistance development. Monitoring of malaria parasitemia and anemia during the IRS programme is required.

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TWO VARIANTS OF MULTIDRUG RESISTANT VIBRIO CHOLERAE O1 BIOTYPE EL TOR INVOLVED IN TWO CONSECUTIVE OUTBREAKS OF CHOLERA IN CAMEROON (2004 - 2005 AND 2010 - 2012)

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Outbreaks of cholera due to toxigenic *Vibrio cholerae* are always dangerous and need a quick taking care of patient. From may 2010 to February 2012, an outbreak of cholera raged in Cameroon. The epidemic started in the North and reached the south of Cameroon 4 months later, in September 2010. The isolated strains, *V. cholerae* O1 serotype Ogawa, were multidrug resistant with additional resistance to nalidixic acid. isolated from 2 subsequent outbreaks of cholera in Cameroon, 2004 - 2005 and 2010 - 2011. Geographically, the 2004-2005 outbreak was localized in the south of Cameroon, while the current outbreak (2010-2011) covered the whole area of Cameroon. A total of 200 V. Cholerae O1
isolated during the last outbreak of cholera in Cameroon were used in this study; the strains belonged to biotype El Tor, serotype Ogawa. The strains were resistant to multiple antimicrobials especially nalidixic acid, which was the newest character. Molecular detection of their virulence factors revealed that tcpA gene which encodes the toxin coregulated pilus was characteristic to El Tor biotype, while nucleotide sequence of ctxB which encode the sub-unit B of the cholera toxin, was closer to the classical biotype. A total of 3 mutations was observed on the ctxB nucleotide sequence of which 2 were PFGE fingerprinting types showed different patterns. This study reveals that V. cholerae strains isolated in 2010-2011 were different from strains isolated in 2004-2005, using antimicrobial susceptibility phenotypes, characterization of antimicrobial resistance, cholera toxin genotyping, PFGE. PFGE analysis revealed two different unrelated profiles. All the strains harboured tcpA El Tor allele, which was a supplemental argument ranging these strains in El Tor biotype.

PREVALENCE OF ENTEROAGGREGATIVE ESCHERICHIA COLI VIRULENCE GENES IN YOUNG CHILDREN FROM RURAL SOUTH AFRICA AND PATHOGENESIS OF POTENTIAL VIRULENCE MARKERS IN A MOUSE MODEL: THE MAL-ED COHORT

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Enteroaggregative Escherichia coli (EAEC) is recognized as a cause of growth shortfalls with or without diarrhea worldwide. A number of EAEC virulence-related genes (VRGs) have been described but their role in the clinical outcome of infection is not completely defined and may vary from one geographical location to another. Recently, we have characterized Aar (AggR activated regulator), a new negative regulator in EAEC. Deletion of Aar upregulated the expression of the EAEC master regulator AggR. Accordingly, we have found that an EAEC strain lacking Aar causes growth failure without diarrhea in our zinc deficient mouse model. We extracted DNA from 207 strains of EAEC isolated from the stool of 109 children followed from birth to 12 months of age from the Dzimuali community in the Limpopo Province of South Africa. We investigated the prevalence of EAEC VRGs using multiplex polymerase chain reaction. Samples were analyzed for identification of 18 VRGs. Plasmid encoded haemolysin (aar) was the most frequently detected (86.5%), followed by aggregative adherence regulator (aggR; 53.6%) and EAEC HIA homologue (eIA, 47.3%). Secreted autotransporter toxin gene (sat) was observed at lowest frequency (0%). Although only (5%) of participants had diarrhea in their first 12 months, children with EAEC had greater growth shortfalls (P< 0.03) and one child had an aar(-) EAEC with striking growth failure. These data confirm a high prevalence, endemicity and heterogeneity of EAEC strains in the Limpopo Province of South Africa and their association with growth failure. However, investigations are on-going to determine the impact of potential virulence determinants of the EAEC strains (aar(-) and aggR(+) in a murine model and their impact on the host on diarrhea and growth impairment.

SENDING PEACEKEEPING TROOPS INTO AN ONGOING CHOLERA OUTBREAK - THE CHILEAN EXPERIENCE DURING MINUSTAH (MISSION DES NATIONS UNIES POUR LA STABILISATION EN HAITI)

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Since 2004, Chile participates in MINUSTAH with a permanent contingent of 500 soldiers, who change every 6 months. The Preventive Medicine Service prepares troops and medical units for potential threats. After the 2010 earthquake, a cholera outbreak rapidly spread through Haiti, which was probably related to soldiers participating in the same UN mission. This work describes the experience with pre- and post-deployment measures of the Chilean Forces to reduce the cholera risk in Haiti as well as importation into Chile by asymptomatic carriers. Those actions included sanitary and hygiene education of troops, training for medical officers, pre-deployment immunizations with whole-cell oral cholera vaccine (WC/rBS), and post-deployment microbiological surveillance. In 2011, vaccine side effects were monitored using a standardized questionnaire. After deployment, all troops were screened for Vibrio cholerae carriage. Data on WC/rBS vaccination were available for 569 soldiers of whom 9.7% reported systemic and 10.5% gastrointestinal (GI) side effects. A shorter interdose interval (7 vs 30 days) was associated with more GI disturbances (17.4% vs 7.7%, p<0.001). In the subgroup receiving WC/rBS 30 days apart, systemic side effects were more common if peacekeepers simultaneously received other pre-deployment vaccines (11.9% vs 4.3%, p<0.05). All surveillance stool cultures were negative, except for 2 (in 2012) which both grew strains of non-pathogenic V. cholerae. Cholera is still a threat for underdeveloped countries and military operations within these countries need to take measures to prevent infection and further spread the disease by deployed troops. Our experience showed an acceptable safety profile of WC/rBS, especially if doses are separated by 30 days. This vaccine provides a fairly high protection rate for the first 6 months, but its influence on the rate of asymptomatic carriers is uncertain. Therefore, additional post-deployment stool cultures seem an appropriate surveillance tool for returnees to non-industrialized countries such as Chile.

NOVEL IMMUNOLOGICAL SIGNALS FOR DIAGNOSING ACUTE TYPHOID FEVER

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Salmonella Typhi is the causative agent of typhoid. There are a variety of methods for diagnosing typhoid; all lack sensitivity and specificity. Serological assays targeting LPS and/or flagella are commonly used settings to diagnose typhoid. However, cross-reactivity makes these antigens unsuitable for assessing disease burden and diagnosing acute illness. There have been few studies focusing on studying the immunological response to specific Salmonella antigens. Using an S Typhi antigen array we identified a number of antigens that elicited a significant IgM or IgG response greater than that of uninfected controls. We selected the best twelve (6 IgM and 6 IgG) antigens that gave a differential response between the groups for further investigation. Firstly, we expressed and purified these antigens and immunized mice to study the ability of the mouse serum to stimulate bacterial killing. All of the serum samples from immunized mice were able to stimulate a bactericidal response against S. Typhi, inhibiting >80% of the bacterial growth over three hours.
Additionally, eight of the serum samples had a bactericidal effect on S. Paratyphi A. None of the serum from immunized mice demonstrated any bactericidal activity against S. Typhimurium. We further investigated this bactericidal response by repeating the experiments with gene knockout strains. There was a marked reduction in bactericidal activity with the immunized mouse serum on the strains of S.Typhi strains harboring the respective specific antigen encoding gene knockout. Specifically, serum from mice immunized with CdtB (subunit of typhoid toxin) demonstrated a significant reduction bactericidal activity against a cdtB S. Typhi mutant in comparison to wild-type S.Typhi. These preliminary data show that S.Typhi 12 antigens appear to stimulate specific and strong immunological responses in patients with acute typhoid. Furthermore, we suggest these antigens may be candidate diagnostics or subunit vaccines, and provide a novel insight for further understanding of host immune responses induced during acute typhoid.

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THE SPATIOTEMPORAL DYNAMICS AND PHYLOGENETICS OF SALMONELLA PARATYPHI A IN KATHMANDU, NEPAL
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Enteric fever is a life threatening systemic disease caused by the bacteria Salmonella Typhi and Salmonella Paratyphi A, B, and C. Typhi is the major agent of enteric fever, yet Paratyphi A is emerging at an unprecedented rate. In our location in Patan Hospital, Kathmandu, standardised blood culture surveillance over the last ten years has shown annual increases in the proportions of individuals with Paratyphi A. More than two thirds of the culture confirmed enteric fever cases are now caused by this serovar. Both Typhi and Paratyphi A are systemic pathogens that induce indistinguishable syndromes. However, they exhibit contrary epidemiologies, different geographical distributions, and different propensities to develop resistance to antimicrobials. Additionally, they are genetically and phenotypically distinct, having gone through a lengthy process of convergent evolution to cause an identical disease. To understand the emergence and the molecular epidemiology of Paratyphi A in our setting we genome sequenced 182 organisms isolated from patients with acute or relapsed enteric fever, and a number isolated from the gallbladder of asymptomatic carriers. Performing phylogenetic analysis and evolutionary reconstruction we find that Paratyphi A is isolated and genetically distinct from Typhi. Our data show that Paratyphi A has been through a major clonal expansion in Kathmandu, apparently driven by resistance to fluoroquinolones and increased virulence through multi-copy effector proteins. Contemporary isolates of Paratyphi A have been introduced from other parts of Asia and induced a clonal replacement of the native strain(s). We surmise that Typhi and Paratyphi A have a dissimilar epidemiology in Nepal with Paratyphi A associated with spatiotemporal outbreaks and person-to-person transmission. Our study is the first to tackle the local phylogenetics and spatiotemporal dynamics of Paratyphi A. Our work outlines new perspectives on enteric fever and will pave the way for future genomic epidemiology investigations of this important emergent pathogen.

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INCIDENCE AND ETIOLOGY OF INFECTIOUS DIARRHEA FROM A MULTIYEAR FACILITY-BASED SURVEILLANCE SYSTEM IN GUATEMALA, 2008-2012
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Diarrheal diseases are a major cause of morbidity and mortality worldwide, yet data on etiology and population-level incidence in developing countries are limited. Diarrhea surveillance was conducted at two hospitals and 10 ambulatory clinics in the departments of Santa Rosa and Quetzaltenango in Guatemala. A case was defined as a person of any age having ≥3 loose stools in a 24-hour period and was admitted or presented to the surveillance facilities. Epidemiologic and clinical data were collected. Stool specimens were tested for bacterial, parasitic, and viral enteric pathogens. Estimated incidence rates were calculated by adjusting for healthcare seeking behaviors, based on results from a survey in the surveillance area assessing the proportion of those with reported diarrhea who visited a surveillance facility during their illness. From November 2008 to December 2012 there were 5,331 diarrhea cases. The weighted estimated community incidence averaged 659 diarrhea cases per 10,000 persons per year during the four year period. The estimated incidence was highest among children aged <5 years, averaging 1,584 cases per 10,000 children per year, while among those aged ≥5 years the estimated incidence averaged 311 cases per 10,000 persons. From 2008-2009 samples from 1,401 (26%) cases were tested for all the pathogens of interest. Among these, 846 (60%) specimens were from children aged <5 years in whom a virus was identified in 211 (25%) patients; of which, 178 (84%) tested positive for norovirus and 101 (48%) for rotavirus, including co-infections. Among the 555 patients aged ≥5 years the most frequently identified etiology was bacterial with 134 (24%) cases. Diarrheagenic Escherichia coli was detected in 94 (70%) cases, Shigella spp in 31 (23%), Campylobacter spp in 5 (4%), and Salmonella spp in 4 (3%) cases. Identification of parasites was low (24 cases, 9%), and most cases were among those aged 5-19 years. These data demonstrate a substantial burden of viral and bacterial diarrheal diseases in Guatemala, which may help guide public health policies aimed at reducing the burden of illness and death due to diarrhea.

1644

SALMONELLA ENTERICA SEROVAR ENTERITIDIS OUTBREAK AT A LODGE IN MOKOPANE, LIMPOPO PROVINCE, JANUARY 2014
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Salmonella enterica serovar Enteritidis is a leading cause of foodborne disease worldwide, but there is little data available in South Africa. Asymptomatic food-handlers have also been associated as a source of infection. We investigated the aetiology of an acute gastroenteritis outbreak among persons staying at a lodge in Limpopo Province, South Africa in January 2014. A retrospective cohort analysis was used to determine the risks of illness associated with consuming foods and/or beverages at the lodge. The at-risk population were contacted to complete a standard questionnaire related to food and beverages consumed at the lodge, symptoms of illness, visits to healthcare facilities and specimen submission for testing. Food and water samples were tested, as well as completion of an environmental assessment questionnaire by staff and external caterers. The data was categorised and STATA version 12 was used for multivariate analyses. A total of 73 ill persons, including
3 laboratory-confirmed infections, were identified: 69/109 (63%) of the selected cohort were seen at health facilities. Of the at-risk population 87% (109/124) completed the standard questionnaire: 66 cases of gastrointestinal illness and 43 healthy individuals were identified, with a corresponding attack rate of 61%. Most of the cases were females (86%, n=57) with a mean age of 33 years (S.D=7.1), and 36% (n=24) of the cases were hospitalised. Epidemiological data suggested a point source outbreak with no further transmission. Statistical analysis of survey data indicated consumption of diluted fruit juice (from concentrate) adjusted by other food and beverage items, presented a risk ratio of 1.5 (95% CI, 1.1-1.8, p=0.032). Environmental analysis indicated increased risks for cross-contamination of food/ beverages prepared in the lodge kitchen, and fruit juice consumption was the main exposure associated with ill cases. Feedback on food safety and hygiene practices to prevent cross-contamination at the kitchen lodge were provided.

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FECAL SHEDDING OF ROTAVIRUS FOLLOWING ROTARIX VACCINATION IN A COHORT OF BOLIVIAN INFANTS

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Worldwide children under the age of five suffer from an estimated 138 million cases of rotavirus (RV) diarrhea per year. Currently available oral RV vaccines, such as the human attenuated monovalent vaccine Rotarix, increase RV-specific immunity and prevent severe diarrhea. Following vaccination, RV vaccine strains are expected to replicate in the intestinal tract and be excreted in feces. Fecal shedding of vaccine strains could lead to horizontal transmission of such strains and theoretically afford protection to unvaccinated contacts, which would be of substantial benefit in impoverished countries such as Bolivia where rates of immunization are suboptimal or RV associated morbidity is high. With the ongoing rollout of RV vaccines in low-middle income countries, it is imperative to evaluate and describe RV vaccine-virus shedding in vaccine recipients. Between June 2013 and April 2014 a birth cohort of 462 Bolivian infants were enrolled and followed through receipt of Rotarix to quantify the prevalence of fecal shedding within 7 days of the initial vaccine dose (at approximately 2 months of age). Shedding was assessed using enzyme immunoassay (EIA) and in a subset of infants real-time reverse transcription-polymerase chain reaction (RT-PCR) was used for confirmation. Bivariate logistic regression was used to identify potential predictors of shedding. The median age at the initial (pre-vaccine) visit was 35 days (SD 8 days), and 55% of the infants were male. Baseline prevalence of stunting was 20%, prevalence of preterm birth was 19%, and prevalence of low birth weight was 7%. The mean maternal age was 26 years (SD 6 years), and 61% of mothers had completed secondary school. Shedding was identified in 6 infants out of 305 tested (2%). All before-mentioned predictors were tested, but no significant associations with shedding were detected (likely due to lack of power). Initial hypotheses for the low shedding rate include the high prevalence of exclusive breastfeeding, role of maternal antibodies, and circulating RV in the community; these and other potential explanations will be addressed in future investigation.

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INTESTINAL INFLAMMATION AND ALTERED BONE METABOLISM IN PERUVIAN INFANTS

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Low-grade inflammation resulting from frequent enteric episodes and resulting enteropathy is believed to be a cause of growth faltering among children in the developing world. In order to clarify these associations, markers of bone metabolism may be beneficial, as they are more dynamic over short intervals than anthropometry. In order to test this hypothesis, urine samples from 139 6-month olds and 145 15-month old Peruvian Amazonian infants, and serum samples from the same children at 7 and 15 months, were tested for markers of bone collagen formation (plasma osteocalcin (OC)) and resorption (urinary deoxypyridinoline (DPD)/creatinine (Cr)), as well as the acute phase protein plasma alpha-1-acid glycoprotein (AGP) and the plasma cytokines TNF-α, interleukin-6 (IL-6), and IL-1β, and fecal alpha-1-antitrypsin (AAT), a marker of intestinal inflammation. The mean plasma osteocalcin at seven months was 41.2µg/mL in boys and 36.4µg/mL in girls, by 15 month this had fallen to 34.1 µg/mL and 25.2 µg/L, respectively. The mean DPD/Cr at 6 months was 58.9 nmol/mmol Cr and 65.1 nmol/mmol Cr for boys and girls, respectively, and at 15 months, 68.3 nmol/mmol Cr and 52.1 nmol/mmol Cr for boys and girls, respectively. The mean length-for-age Z score (LAZ) at 6 months was -1.3 and 19.4% were stunted (LAZ < -2). By 15 months of age the mean LAZ was -2.0 and 53.1% were stunted. The mean weight-for-length Z-score (WLZ) was 0.9 at 6 months , 0.8 at 7 months, and 0.4 at 15 months. 76.4% of children at 6mo, and 69.1% at 15 month were classified as having subclinical inflammation, defined by AGP> 1g/L. Bone collagen metabolism was altered by nutritional status, as OC was inversely associated with both length-for-age and weight-for-length, and DPD/Cr was positively associated with length-for-age. Correspondingly, the ratio of OC/DPD was highest among shorter and leaner children. After adjusting for anthropometric status, age, and gender, OC and the ratio of OC/DPD were both inversely associated with fecal AAT, but not with plasma AGP, tnf-alpha, IL-6, or IL-1beta. Our findings suggest that bone metabolism is suppressed among children with chronic intestinal inflammation.

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EPIDEMIOLOGY OF SELF-REPORTED HEALTH EVENTS AMONG DEPLOYED U.S. MILITARY PERSONNEL

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Deployed military personnel are at risk of experiencing numerous adverse health events to include diarrhea, vomiting, fever and muscle aches while on humanitarian and war-fighting operations. These symptoms can negatively impact servicemember effectiveness and epidemiologic studies are needed to describe their frequency and associated risk factors; however, extensive prospective epidemiologic studies in operational environments are challenging. We utilized self-reported data collected through the Post-Deployment Health Assessment from individuals following operational deployments and compared prevalence estimates across region/country of deployment, multiple demographic characteristics as well as pre-existing medical conditions. Univariate and multivariate logistic regression methods were also used to identify unique risk factors while controlling for important covariates. Of 21,982 subjects, the top five self-reported symptoms included back pain (15.9%), feeling tired/problems sleeping (15.7%), swollen, stiff or painful joints (13.6%), diarrhea (12.7%) and muscle aches (11.1%). Among those reporting diarrhea/vomiting, a high proportion were assigned to limited duty/bed rest (36% and 54%, respectively). Further data will be presented on the estimated level of care required for reported adverse health events, and potential risk factors for increased self-report of diarrhea and vomiting. While these results are limited to self-report, the data support prior studies highlighting diarrhea

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and vomiting as significant causes of morbidity and troop down-time during operational deployment. Furthermore, recent studies highlighting the link between acute gastroenteritis and long-term adverse health outcomes raise the importance of these common, deployment-related health events. Continued evaluation of primary prevention strategies is needed.

### 1648

**REGULATION OF SMALL RHO GTPASES IN REDUCING INTESTINAL CELLS MIGRATION INDUCED BY STRAINS OF ENTEROPATHOGENIC ESCHERICHIA COLI**

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Intestinal epithelial migration provides an important early response when intestinal pathogens damage the host intestinal barrier. Small Rho GTPases Rac1, RhoA and Cdc42 are important regulators of this migratory response. We sought to test the hypothesis that EPEC impairs intestinal epithelial migration in vitro using small Rho GTPase-dependent mechanisms.

Methods: We investigated the effects of EPEC strain E2348/69, EPEC strain LD1001 (isolated from a malnourished child), and commensal E. coli HS on IEC-6 cell migration; as well as on the regulation of transcription and gene expression of small Rho GTPases by qPCR and confocal immunofluorescence microscopy, respectively. Results: We observed a significant reduction in IEC-6 cell migration for all E. coli strains tested. However, pathogenic EPEC strains reduced migration to a greater degree than E. coli HS. Only EPEC E2348/69 induced significant cellular necrosis. Gene analyses of small Rho GTPases revealed an increase in rac1 transcription in EPEC LD1001 infected cells and upregulation of rhoA transcription following infection with all strains. Confocal imaging showed an increase in Rac1 expression and decrease in RhoA in response EPEC LD1001 infection. We further observed increased expression of Cdc42 in all infected groups. Conclusions: The results suggest differential suppression of migration and co-regulation of small Rho GTPases in response to infection with enteropathogenic vs. commensal E. coli strains. These in vitro data corroborate an emerging in vivo and clinical understanding of the pathobiology of this infection and its associations with malnutrition and intestinal barrier injury.

### 1649

**INTEGRATING HERD PROTECTION INTO A COST-BENEFIT ANALYSIS OF TYPHOID FEVER VACCINE INTERVENTIONS**

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Recent studies have shed light on the costs and savings of typhoid fever vaccination campaigns, but no studies have been able to quantify the potential economic savings attributable to realistic estimates of herd protection, the protection conferred to individuals who did not receive the vaccine. In light of numerous budget constraints in resource-poor settings, it is necessary to more accurately estimate the indirect effects of vaccine campaigns as well as the additional potential financing mechanisms that may lower the costs of vaccination per DALY saved. Field studies are now available to assess the burden of typhoid fever and the possible impact of Vi-polsaccharide and Vi-conjugate vaccines. Using mathematical models for typhoid transmission, we can quantify the indirect protection of vaccines under different vaccine strategies. Moreover, surveys on the private demand for these vaccines in South Asian contexts, where the disease is endemic, inform calculations on the optimal vaccine subsidies necessary to achieve a desired level of vaccine coverage while recuperating some of the programmatic costs through user fees. With that in mind, we will show that past estimates of the costs of vaccination per life year saved have been overestimated when compared to an analysis that takes into account accurate estimates of indirect vaccine protection at different pricing levels.

### 1650

**CHARACTERIZING THE REGIONAL AND GLOBAL DISTRIBUTION AND BURDEN OF CHOLERA**

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There are an estimated 2.8 million cholera cases per year globally, but the majority of these cases are not detected or reported. Because of inadequate surveillance and reporting the global distribution of cholera risk and its public health burden are poorly known. Previous attempts to determine the global burden of cholera have relied on a limited number of case studies that do not capture the broad range of settings and environmental conditions where cholera occurs. Here we assemble a database of cholera surveillance and incidence reports from a variety of government, scientific, and non-governmental agency sources, with a particular focus on sub-Saharan Africa where a majority of cholera cases have been reported in the past several decades but where the distribution of risk and burden is still poorly understood. We will then use a formal modelling framework to associate cholera transmission with environmental and socioeconomic variables and map the global distribution of cholera risk and incidence. As a preliminary analysis we developed cholera incidence maps for the West African country of Guinea-Bissau using a hierarchical Bayesian framework with cholera data at spatial scales ranging from neighborhood-level incidence in the capital city of Bissau to country-level reports. Cholera incidence from 1986-2009 was highest in the island and coastal districts (including Bissau city). Incidence did not change significantly between the 1990s (which included outbreaks in 1994 and 1996-1997) and the 2000s (which included outbreaks in 2005 and 2008), except for an increase of 26-280% in the islands of the Bijagos Archipelago. Improving our understanding of the spatial distribution of cholera in Guinea-Bissau and associating incidence with climate, environmental and socioeconomic factors will provide a basis for planning public health prevention to reduce cholera transmission in this region.

### 1651

**BIOMARKERS OF ENVIRONMENTAL ENTEROPATHY FOR POSITIVELY ASSOCIATED WITH TOXIN-SPECIFIC B AND T CELL RESPONSES TO AN ORAL CHOLERA VACCINE IN BANGLADESI CHILDREN**

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Environmental enteropathy (EE) is a poorly understood condition that refers to chronic alterations in intestinal permeability, absorption, and inflammation that affects young children in resource limited settings. While EE has been linked to suboptimal oral vaccine performance in children, the causative immunological mechanisms are poorly defined. The objective of this study was to determine how markers of enteropathy are associated with immune responses to oral cholera vaccine (OCV). We collected blood and stool from 40 Bangladeshi children who received two doses of an OCV given 14 days apart. We measured five EE markers, including stool myeloperoxidase (MPO), a marker of intestinal inflammation, stool alpha anti-trypsin protein (iFABP), a marker of intestinal absorption, as well as plasma anti-endotoxin core antibody (EndoCab), plasma intestinal fatty acid binding protein (iFABP), and plasma soluble CD14 (sCD14), all markers of microbial translocation. We measured cholera toxin (CT) and lipopolysaccharide
LEPROSY IN NIGERIA (2008-2012): AN EVALUATION OF THE NATIONAL SURVEILLANCE SYSTEM

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Leprosy is a chronic infectious disease caused by Mycobacterium leprae an acid fast rod, shaped bacillus. It was historically associated with social isolation and psychological consequences. Although eradicated in most countries, ongoing transmission in African and Asian countries requires enhanced surveillance and monitoring. In 2012 Nigeria reported 3805 cases and no deaths. We evaluated the national Leprosy surveillance system to assess its usefulness and attributes. Centers for Disease Control (CDC) guidelines for evaluating surveillance systems were used. Ten Stakeholders from partner agencies, national and state level were interviewed using structured questionnaires. Secondary data (2008-2012) was abstracted from surveillance data submitted to the National Tuberculosis and Leprosy Control Program. Laboratory diagnostic capacity was also assessed. Out of 20,623 cases reported, 3805 (18.45%) were received in 2012, 3623 (17.57%) in 2011, 3913 (18.97%) in 2010, 4383 (21.25%) in 2009 and 4899 (23.76%) in 2008. The prevalence reduced from 4.3x103/10,000 population in 2008 to 1.2x10-4/1,000 population in 2011. Child proportion was 10.7% in 2008 and 8.0% in 2011. Grade 2 disability rate ranged between 11.7% and 13.4%. Being an adult (OR=1.97; 95% CI=1.37-2.82) and male (OR=1.25; 95% CI=1.05-1.48) was found associated with Multibacillary Leprosy. Residents of the Northwest region (OR=0.73; 95% CI=0.59-0.90) were less likely to have Multibacillary Leprosy. The system is active and rated simple by 17(85%) of respondents. Review of weekly, quarterly and monthly reporting forms and records at the national level showed timeliness. All suspected cases (100%) were laboratory tested within 24 hours of presentation. There is a high laboratory turnover of staff and low numbers of personnel trained in laboratory diagnosis. The system is fully integrated with surveillance of Tuberculosis at all levels. Leprosy transmission is still ongoing and the WHO elimination target (<1 case/10,000 population) has been achieved at the national level. Pockets of leprosy exist in the northwest region. We recommend intensification of surveillance activities in all zones, improvement of laboratory diagnostic capacity and recruitment of additional personnel. The system is acceptable, flexible, simple and timely. The system is meeting its purpose and promotes the achievement of the global elimination target for leprosy.

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OPTIMIZING THE CHEMOTHERAPEUTIC APPROACH FOR THE TREATMENT OF BURULI ULCER: POSSIBLE OPTIONS AND RESEARCH NEEDS

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Buruli ulcer (BU) is a serious necrotising skin infection caused by the environmental pathogen Mycobacterium ulcerans, and represents the third most common mycobacterial infection. Current WHO treatment has simplified the delivery of care for BU by recommending that early, limited lesions be treated with antibiotics alone. However, these recommendations still present significant disadvantages including the use of injectable agents. There is an urgent need to identify alternative oral regimens that are effective, short-course, have few drug-drug interactions with antiretrovirals and can be used in children. We performed a systematic literature review for publications focused on chemotherapy for M. ulcerans infection. We searched PubMed, EMBASE, Scopus, WHO Global Index Medicus, CAB Abstracts and Cochrane Library with standardized search terms, screened abstracts submitted to international conferences and assessed the ClinicalTrials.gov registry. While there were no restrictions by publication date or type, only articles in English, French and Italian as of December 31, 2012 were included. We included in vitro and clinical studies, with the primary outcomes of clinical resolution of the ulcer without surgery (clinical studies) and assessment of in vitro activity (pre-clinical data). We excluded all studies without microbiological confirmation of M. ulcerans infection. 49 clinical studies including 6 RCTs, 14 observational cohorts, 11 case series and 18 case reports were identified. Various drugs and drug combinations were identified as having clinical efficacy against BU disease in resource-poor settings. In particular, the combinations of clarithromycin + rifampin and clarithromycin + fluoroquinolones demonstrated good efficacy and safety. In vitro data reveal a number of promising compounds. Although recent studies indicate that a fully oral regimen for BU may be as equally effective as regimens containing aminoglycosides, further research is needed to identify and evaluate new treatments. The anti-tuberculosis research & development (R&D) pipeline represents a potentially rich source of novel compounds for BU treatment. We propose an R&D agenda aimed at delivering new, more efficacious and readily implementable treatments against Buruli ulcer in resource-limited settings.

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PERSISTENT GUT MICROBIOTA IMMATURITY IN MALNOURISHED BANGLADESHI CHILDREN

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Therapeutic food interventions have reduced mortality in children with severe acute malnutrition (SAM) but incomplete restoration of healthy growth remains a major problem. The relationships between the type of

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nutritional intervention, the gut microbiota, and therapeutic responses are unclear. In the current study, bacterial species whose proportional representation define a healthy gut microbiota as it assembles during the first two postnatal years were identified by applying a machine-learning-based approach to 16S rRNA datasets generated from monthly fecal samples obtained from a birth-cohort of children, living in an urban slum of Dhaka, Bangladesh, who exhibited consistently healthy growth. These age-discriminatory bacterial species were incorporated into a model that computes a ‘relative microbiota maturity index’ and ‘microbiota-for-age Z-score’ that compare development (defined here as maturation) of a child’s fecal microbiota relative to healthy children of similar chronologic age. The model was applied to twins and triplets (to test for associations of these indices with genetic and environmental factors including diarrhea), children with SAM enrolled in a randomized trial of two food interventions, and children with moderate acute malnutrition. Our results indicate that SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following two widely used nutritional interventions. Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements. Microbiota maturity indices provide a microbial measure of human postnatal development, a way of classifying malnourished states, and a parameter for judging therapeutic efficacy. More prolonged interventions with existing or new therapeutic foods and/or addition of gut microbes may be needed to achieve enduring repair of gut microbiota immaturity in childhood malnutrition and improve clinical outcomes.

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CONTRIBUTION OF THE COMMUNITY HEALTH VOLUNTEERS IN THE CONTROL OF BURULI ULCER IN BENIN

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Buruli ulcer (BU) is a neglected tropical disease caused by Mycobacterium ulcerans. Usually BU begins as a painless nodule, plaque or edema, ultimately developing into an ulcer. Striking is the high number of patients presenting with ulcers in an advanced stage. Such late presentation will complicate treatment and have long term disabilities as a consequence. The disease is mainly endemic in West Africa. Strategy for control this disease is early detection using community village volunteers. This study aims to understand the contribution of different actors in the current referral system in Benin, the role of the different referral systems on the stage of disease at presentation in the hospital and the diagnostic precision of Buruli ulcer. Patient information of Buruli ulcer patients that reported to one of the four BU centers in Benin between January 2008 and December 2010 was collected using the WHO/BU01 forms. Information traced from these forms were general characteristics of the patient, the results of diagnostic tests, the presence of functional limitations at start of treatment, lesion size, patient delay and the referral system. The role of the different referral systems on the stage of disease at presentation in the hospital was analyzed by a logistic regression analysis. About a quarter of the patients (26.5%) were referred to the hospital by the community health volunteers. In our data, community health volunteers seemed to refer patients more frequently in an earlier stage of disease but after adjustment for the health center, this effect could not be seen anymore. The Polymerase Chain Reaction (PCR) for IS2404 positivity rate among patients referred by the community health volunteers was not systematically lower than in patients referred by other systems. This study clarifies the role played by community health volunteers. It highlights that in Bénin, the community health volunteers are an important link in the control of BU.

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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING FLORAL EXTRACT OF GOMPHRENA GLOBOSA AND ITS ANTIMICROBIAL ACTIVITY AGAINST MULTI DRUG RESISTANT BACTERIA

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Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today. Silver nanoparticles (AgNPs) are well known biocidal substances that can be incorporated as antimicrobial agents in phar-macology, veterinary medicine, implants, wound dressings, and topical ointments. AgNPs were also found to exhibit antimicrobial activities. The present work reports one step ecofriendly method for the synthesis of AgNPs using Gomphrena globosa and its antibacterial effects against drug resistant bacteria. In the present results, AgNPs was characterized by ultraviolet-visible spectroscopy, X-ray diffraction spectroscopy, Transmission electron microscopy and particle size analyzer. The synthesized particles were found to be spherical in shape and sizes ranged between 55-60 nm. Further energy-dispersive X-ray spectroscopy confirmed the presence of silver. Furthermore these green synthesized AgNPs were found to show significant antimicrobial effect against the drug resistant Methicillin resistant Staphylococcus aureus, ciproflaxin resistant Escherichia coli, and carbapenem resistant Acetobacter baumanii. This outcome may pave a way for using floral extract of the AgNPs a drug carrier system to cure bacterial diseases.

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FREQUENCY OF VIRULENCE GENOTYPES IN ESCHERICHIA COLI STRAINS ISOLATED FROM URINARY TRACT INFECTIONS OF MEXICAN PATIENTS

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Uropathogenic Escherichia coli (UPEC) is responsible for a high percentage of urinary tract infections (UTIs) worldwide, among them cystitis and pyelonephritis. The purpose of this work was to determine the frequency of the genotypes: pap (pilus associated with pyelonephritis), papGI and papGII (pilus associated with pyelonephritis GI and GII), hlyA (haemolysin), afa (afimbrial adhesin), sfa (S fimbriae), iron (iron), iuc (aerobactin), cnf (cytotoxic necrotizing factor), astA (enteroaggregative toxin), sap (she pathogenicity island marker) and set (Shigella enterotoxin 1) in a group of E. coli strains isolated from Mexican patients suffering UTIs. E. coli strains were identified by biochemical tests and by PCR amplification of 16S rRNA. Genes pap, papGII, papGIII, hlyA, afa, sfa, iron, iuc, cnf, astA, sap and set were detected by multiplex PCR and by end-point PCR. Urine samples of 100 urinary tract infected patients were microbiologically analyzed. E. coli was identified in 60% of the samples (n=60). Of the E. coli strains, 48.3% (n=29) carried the set gene; 41.6% (n=25) carried papGII; 26.6% (n=16) carried hlyA; 23.3% (n=14) carried afa; 21.6% (n=13) carried pap; 20% (n=12) carried papGII; 18.3% (n=11) carried sfa; 16.6% (n=10) carried iron; 13.3% (n=8) carried iuc; 6.6% (n=4) carried cnfII; 10% (n=6) carried...
astA and 5\% (n=3) carried sap. The high frequency of the identified genes in the UPEC strains suggests that they are virulent and able to produce cystitis and/or pyelonephritis.

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COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS INTRODUCED INTO A BRAZILIAN PUBLIC PEDIATRIC CLINIC
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Low-income communities, health care professionals, and adults in frequent contact with children are all populations known to be at higher risk for methicillin-resistant Staphylococcus aureus (MRSA) colonization. The objective of our investigation was to determine risk factors for colonization with MRSA as well as staphylococcal cassette chromosome mec (SCCmec) genotypes among pediatric health care workers from a public hospital in Rio de Janeiro, Brazil. We collected nasal swabs and data on potential risk factors from 178 health care workers from all pediatric sectors from January to December 2012. Swab cultures were evaluated for antimicrobial resistance against Cefoxitin and Oxacillin, and resistance was confirmed by identifying the mecA gene by PCR. MRSA colonization was 5.1\% (n=9/178). Logistic regression analysis showed being a nurse and working in an inpatient unit as potential risk factors for MRSA colonization (adjusted OR= 11.6, 95\% CI 1.2 - 110.7 and 2.7, 95\% CI 0.3 - 23.7, respectively). No non-work related risk factors were identified. Four of the nine isolates were found to be SCCmec type IV, the genotype most commonly associated with community-acquired MRSA (CA-MRSA). Five isolates were observed to be SCCmec type III, with four collected from nurses of various pediatric sectors in August/September alone. We then compared these isolates with nasal MRSA isolates sampled from children within 48 hours of being admitted to the pediatric ward from December 2011 to July 2012. Of 11 CA-MRSA isolates from 92 children sampled (12.0\%), ten were found to be SCCmec type IV, including two which preceded (12.0\%), ten were found to be SCCmec type IV, including two which preceded.

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EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING ENTEROBACTERIAEAE ISOLATED FROM HEALTH CARE WORKERS’ CELL PHONES IN FIVE PERUVIAN INTENSIVE CARE UNITS: ANTIBIOTIC RESISTANCE PATTERNS AND MOLECULAR CHARACTERIZATION

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Technological advances allow rapid and efficient communication through cell phones; their unsupervised use in hospital environments however, is common. Outbreaks associated with ESBL-producing (Extended-spectrum beta-lactamase) Enterobacteriaceae have been widely described worldwide in intensive care units (ICU), we therefore hypothesized that cell phones might represent a source of these infections in Peru. We conducted a 5-month passive surveillance in 3 Pediatric ICUs and 2 Neonatology ICUs from 3 hospitals during February to June 2012. Swabs were collected from ICU health care workers’ cell phones twice monthly. Microbiological identification and resistance patterns were determined by standard methods. Suspicious ESBL-producing bacteria in the antibiotic were confirmed by the phenotypic CLSI ESBL confirmatory test. We then performed PCR for detection of blaTEM, blaSHV and blaCTX-M genes to characterize the ESBL. A total of 114 employees were enrolled, 114 devices were tested, resulting in 491 samples. Twenty-two percent (25/114) of providers phones were colonized with nosocomial pathogens. Among 105 Enterobacteriaceae isolated, 33.3\% (35/105) produced ESBLs, including 18.8\% (9/48) of Enterobacter spp., 55.9\% (19/34) of Escherichia coli, 26.1\% (6/15) of Klebsiella pneumoniae and 12.5\% (1/8) of Klebsiella oxytoca. blaCTX-M was the most prevalent ESBL. ESBLs resulted in a phenotype of Multidrug resistance: Tobramycin resistance represented 74.3\% of isolates, both Ciprofloxacin and Sulfamethoxazole/Trimethoprim 68.6\%, Gentamicin 62.9\%, Amikacin 17.1\%, and Cefoxitin 5.7\%. No carbapenem resistance was detected and Metallo-beta-lactamases (MBLs), Carbapenemases and AmpC beta-lactamases were not identified in isolated Enterobacteriaceae. Our data suggest cell phones can be an important source of ESBL spread in developing world ICUs. Methods to prevent outbreaks and transmission of these bacteria from commonly used fomites, such as cell phones, are needed.

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THE ROLE OF YERSINIA PESTIS PRESENSIBILIZATION AND GENETIC BACKGROUND IN RESISTANCE OF BLACK RATS AGAINST PLAGUE

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In Madagascar, plague remained endemic in the rural areas of the central highlands with the black rat, Rattus rattus, as the main reservoir. The occurrence of plague cases from one season to another in the same villages is questioning. One hypothesis is that part of black rats can acquire resistance to plague allowing survival of rat populations and maintenance of infected fleas and thus of the disease. We previously described resistance of field black rats living in endemic area against plague whereas those from non endemic ones remained sensitive. This study investigates whether this resistance is genetically driven or if pre immunization of rats with Yersinia pestis could increase survival during subsequent infections. F1 generation of black rats, originating either from plague endemic or plague free zones were obtained and challenged once or twice with Y. pestis. Rat survival, antibody production and gene expression were compared during the acute phase of the disease. First inoculation of a low dose of Y. pestis greatly increases survival of rats against a lethal dose of the bacteria. This protection of primed rats can likely be related to anti-F1 IgG. Transcription analysis of leukocytes five days after infection revealed that genes related to inflammation but also to apoptosis were more expressed in rats from non endemic than in those from endemic ones. In the other hand, anti-apoptotic Bcl2 pathway was highly expressed in resistant rats. This suggested that rat susceptibility to infection could be driven by apoptosis of activated leukocytes. Transmission of a resistance phenotype to the F1 generation for R. rattus from endemic plague foci is highlighted. These findings highlight the role of low transmission of bacteria in a resistance phenotype of R. rattus to plague. A genetic component of this resistance is also supported This study provides critical insights on the role of R. rattus in plague persistence in Madagascar.
CHARACTERIZATION OF ACREMONIUM AERUGINOSA ISOLATES POSITIVE FOR IMP CARBAPENAMASE FROM PERUVIAN HOSPITALS

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Carbapenem antibiotics constitute the last resort in treating multi-drug resistant Acinetobacter infections typically acquired during hospitalization. The effectiveness of carbapenems against Acinetobacter has been compromised in the last decade largely due to the emergence of carbapenem-hydrolyzing metallo-beta-lactamases (MBL). Metallo-beta-lactamases include the enzymes IMP, VIM, NDM, GIM, and SIM and account for the majority of carbapenem resistance in Gram negative bacteria. While extensively characterized in East Asia and Europe, currently there exist limited reports on MBL resistance in Acinetobacter infections from South America. Surveillance of hospital acquired Acinetobacter infections in Lima and Iquitos, Peru between March 2011 and February 2013 identified 32 suspected nosocomial isolates (11 from Lima and 21 from Iquitos) and 20 clinical and environmental isolates associated within ICU outbreaks in Lima. Four of the 52 Acinetobacter spp. isolates were positive for blaIMP, consisting of one A. baumannii, one A. haemolyticus, and two A. junii. Phenotypic carbapenem resistance as defined by minimum inhibition concentrations to imipenem indicates resistance in one A. junii and A. haemolyticus. Whole genome sequencing of the blaIMP-positive isolates identified multiple resistance genes and characterized the IMP-16 variant in the A. baumannii and A. junii isolates and IMP-18 in the A. haemolyticus isolate. Phylogenetic analysis indicates no relatedness between the A. junii isolates or any IMP-positive isolates with previously identified the IMP-positive Acinetobacter isolates referenced by the NCBI Whole Genome Shotgun Database.

CHARACTERIZATION OF CARBAPENEMASE-POSITIVE PSEUDOMONAS AERUGINOSA ISOLATES IN LIMA HOSPITALS

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Pseudomonas aeruginosa is an opportunistic pathogen that accounts for ten percent of all hospital-acquired infections. Therapeutic options for treatment of P. aeruginosa infections are increasingly limited due to inherent antimicrobial resistance an ability to acquire new mechanisms of resistance. Recently, carbapenemases have emerged as one of the major mechanisms of acquired resistance in P. aeruginosa and represent a significant clinical concern due to its ability to hydrolyze the majority of beta-lactam antibiotics. The most clinically relevant and widely disseminated carbapenemases include KPC, IMP, VIM and NDM. Currently, little information has been reported on the prevalence of carbapenemase genes present in P. aeruginosa isolates in Peru. To begin to define carbapenemase resistance in Peru, 124 carbapenem-resistant Pseudomonas aeruginosa isolates were collected from nosocomial and outbreak infections from three hospitals in Lima. Antibiotic resistance to beta-lactam was identified in 18 percent (23/124) of the P. aeruginosa, as defined by disk diffusion assay according to the CLSI guidelines. PCR was performed on all 124 isolates to detect the carbapenemase genes blaKPC, blaIMP, blaVIM and blaNDM. From the 124 isolates, 22 (18%) P. aeruginosa isolates were identified as the IMP-16 variant, one (1%) isolate positive for the VIM-2 variant, and none positive for KPC or NDM, being this 23 isolates extensively drug-resistant. Finally, in order to determine genomic-relatedness, the 23 isolates with carbapenemase genes were analyzed using rep-PCR on the Diversilab system. The 23 isolates clustered into four genomically distinct groups. Interestingly, several of IMP positive isolates were dispersed throughout different hospitals, suggesting possible clonal spread of IMP-16 positive P. aeruginosa between Lima hospitals. Also, the VIM-2 positive isolate demonstrate great than 95% homology with a reference to wild type strain reflecting the capacity of carbapenem-sensitive isolates to acquire to carbapenem resistant.

GENETIC CHARACTERIZATION OF RECOVERED BACILLUS ISOLATES FROM THE ENVIRONMENTAL SURVEILLANCE SWABS BY SEQUENCING OF GYRB GENE: A PUBLIC HEALTH APPROACH

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The primary mission of FDA is to enforce the Food, Drug and Cosmetic Act and regulate food, drug and cosmetic products. To assess adulteration in these commodities, FDA uses presence of pathogenic microorganisms in manufacturing and distribution areas as one of the regulatory action criteria and to ensure that the firm is following good manufacturing practices. Further, FDA provides guidance to achieve the said goal by establishing an environmental monitoring program in these facilities. This study was conducted to verify the effectiveness of pathogen control in a pharmaceutical compounding facility located in Southeast region of United States. A total of 28 environmental swabs were collected from several locations of a compounding company premises. The swab samples were initially examined by conventional microbiologic protocols. Of these, several swabs were found positive for the presence of rod-shaped, gram-positive bacteria, Bacillus. It is a diverse group of bacteria, and some of its species are human-pathogenic that can cause range of infections including ear infections, meningitis, urinary tract infections and septicemia. Species-identification of recovered Bacillus isolates were completed by our recently developed protocol based on nucleotide sequencing of PCR amplified gyrB gene products. Analysis of data confirmed four species of Bacillus (B. cereus, B. pumilus, B. subtilis, and B. thuringiensis) in the swabs examined. This newly developed gyrB-based molecular diagnostic protocol can be used as a suitable genetic marker for rapid detection of Bacillus in the environmental monitoring program of public health importance.

PRODUCTION AND EVALUATION OF A32 KDA FRAGMENT OF THE IMMUNOGLOBULIN-LIKE B PROTEIN FROM LEPTOSPIRA

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Leptospirosis is caused by spirochaetes of the genus Leptospira. It is considered to be the most widespread zoonotic disease in the world. Symptoms of leptospirosis are easily confused with a variety of other febrile illnesses (e.g., dengue and malaria) that require different treatment regimens. Currently, the Microscopic Agglutination Test (MAT) is the standard method for the diagnosis of leptospirosis. It is not only technically complex but also time-consuming. With the publication of the whole genome sequences of several pathogenic species of Leptospira, hundreds of genes encoding surface-exposed lipoproteins and outer membrane proteins have been identified as candidates for the development of rapid diagnostics of leptospirosis. An ELISA using recombinant antigens (rLipL32, rLipL41, and rLigA-Rep) for the detection of Leptospira-specific antibodies has been developed in our laboratory with sensitivity close to 90%. Here, we prepared a recombinant protein containing the coding region of amino acids 630-921 of LipB (LigB-Rep). The over-expressed rLigB-Rep, which contains a six-histidine tag at the N-terminus, was primarily bound in the inclusion body. The solubilized rLigB-Rep in 8 M urea was purified with a nickel column under denatured conditions. We achieved greater than 90% purity as demonstrated by SDS-PAGE. The purified rLigB-Rep was refolded by dialysis in buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) containing 6M, 4M, 2M, 1M, and no urea at 4°C. The refolded rLigB-Rep
has been shown that it was recognized by confirmed leptospirosis patient sera in western blot. These data suggest that rLigB-Rep antigen can be used to further improve the ELISA assay's sensitivity.

EPIDEMIOLOGICAL PROFILE OF LEPTOSPIROSIS CASES, GUATEMALA: 2008-2013

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Leptospirosis is a widespread zoonosis with a higher incidence in countries with humid subtropical or tropical climates. Leptospira are usually transmitted to humans through soil or water contaminated with infected urine from mammal hosts (mainly rodents). Outbreaks are associated with flooding, agriculture, as well as recreational, sporting and military activities. In Guatemala, there are reports of cases in humans and animals but the epidemiology of leptospirosis is unknown. Active surveillance for suspected leptospirosis cases was conducted during 2008-2013 among patients with acute febrile illness (AFI) attending the Culipa National Hospital (932 M) or Nueva Santa Rosa ambulatory facilities (1005 M) in the Department of Santa Rosa, in southern Guatemala. Leptospirosis is typically an undifferentiated AFI but suspected cases are seldom confirmed with laboratory diagnosis. In this study, AFI was defined as self-reported fever or measured temperature ≥38°C that began <7 days before presentation with no other diagnosis (e.g. pneumonia or diarrhea). Blood samples were taken and tested for IgM anti-Leptospira by enzyme-linked immunosorbent assay. Of 553 patients studied (396 hospitalized cases and 157 ambulatory cases), 25 (6%) hospitalized patients were positive while 8 (5%) ambulatory patients were positive. The median of age (IQR) of cases was older (22 years (16-34)) than the leptospirosis negative cases (16 (7-29)). Most cases (79%) were between 10-39 years and 53% were male. The majority of the patients presented with nonspecific signs, such as headache (91%), nausea (85%), myalgia (81%), vomiting (79%), arthralgia (67%), and hemorrhages (12%). During 2008-2013, 22 cases (67%) were detected in the rainy season (May-Oct). Cases were higher in 2010, the year with the most rain in a decade due to tropical storm Agatha, with 18 (55%) cases detected and 14 (78%) in the rainy season. Given endemic nature of leptospirosis in Guatemala and Central America, efforts on prevention and control should focus on these events and the greater risk among adults.

Extended Empirical Treatment Contributes to Changes in Bacterial Resistance Profile

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Extra intestinal infections acquired in the community with Escherichia coli (E. coli) showed in the United States, a rate of frequency of six million to 8 million cases of uncomplicated cystitis and 127,500 cases of sepsis per year. Empirical treatment has been associated with the emergence of bacterial resistance. although some countries are concerned to switch drugs used to treatment infectious, others keep the same treatment (for treatment empirical) over a long period. This study aims to assess the sensitivity and resistance profile of E. coli across the different drugs used for treatment. A cross-sectional and retrospective study (January 2008 to December 20013) at a public hospital, university, belonging at System of Health of Minas Gerais/Brazil (empirical treatment = fluoroquinolones), was considered only in patients ambulatory that presented symptoms (dysuria). The antibiotics tested were as follows (potency in µg/disc): ampicillin + sulbactam (10/10), cephalothin (30), ciprofloxacin (5), norfloxacin (10) e nitrofurantoin (300) (standard disc diffusion method as per CLSI) guidelines using discs of standard potency. Furthermore the costs of different classes of antibiotics were compared. Statistical analysis was performed using the program “Prism” from Graphpad. The results showed that all antibiotics tested here are effective (p<0.05). However there was an increase in the sensitivity of nitrofurantoin in relation to other antibiotics with a decrease of the resistance of the same antibiotic in compared with others (p<0.05), and a variation in the sensitivity and resistance among the fluoroquinolones with the ampicillin more inhibited beta-lactamase. There was also a significant difference in cost, and showed are more affordable the nitrofurantoin (p<0.05), followed by fluoroquinolones, beta-lactamase inhibitor and cefalotin. In conclusion, this work shows that should be considered an alternation of treatments for infection by E. coli, thus favoring the control of bacterial resistance and the cost effective to the population.

Significant Differences in Ultrasound Findings Between Malnourished and Non-Malnourished Schoolchildren in Madagascar

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Childhood malnutrition contributes to high mortality rates and decreased educational and adult capacity among survivors. Not just closely linked to an increasing burden of infectious diseases, it is also increasingly recognized as a cause of chronic morbidity in later life. We explored the utility of bedside ultrasound in identifying specific pathologic findings in malnourished children in Madagascar, with the aim of improving the
management of co-morbidities. 83 children with stunting and/or severe acute malnutrition underwent bedside abdominal sonography and compared with 76 non-malnourished children. Liver and spleen size, liver echogenicity, intraluminal bowel evidence of massive helminthic infections, enlargement of abdominal lymph nodes, thickening of the gallbladder wall, and other pathologic findings were assessed. Malnourished children had hepatosplenomegaly (36% vs 18%) and fatty liver (41% vs 18%) more frequently than non-malnourished children. Hepatosplenomegaly was more common in the Antaimoro area, where malaria and sickle-cell anemia are more prevalent. Evidence of intestinal helminth infections were common in both groups, but in non-malnourished children were mostly associated with fever and acute diarrhea. Other pathologic findings were present in 17 malnourished children (20%) compared with 8% of non-malnourished children. Preliminary results of this study suggest that ultrasound evaluation of malnourished children is feasible and can be aid in identifying co-morbidities. The high rates of fatty liver infiltration particularly deserves more attention, as a possible marker for the development of metabolic diseases and liver fibrosis in adulthood.

1669 LEPROSY, A MIMICKER OF OTHER DISEASES IN A DEVELOPED COUNTRY
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Leprosy (Hansen’s disease) is an uncommon disease in the U.S. and therefore unfamiliar to many health-care providers. This disease primarily occurs in immigrants from countries with higher endemicity such as India and Brazil. Initial misdiagnosis can lead to significant delay in therapy, morbidity and loss of function. Here we reviewed our database of 172 patients with leprosy, who were evaluated and treated in our Hansen’s disease public health satellite clinic from 1998 to 2014. There were 18 patients (10%) with prior misdiagnoses; eleven of them initially presented with skin rash, six with neuropathy and one with rheumatologic symptoms. Skin rash from Hansen’s disease can be difficult to distinguish from more common dermatologic conditions such as fungal skin infection, allergic dermatitis and cutaneous sarcoidosis. Idiopathic mono-neuropathies with wrist or foot drops, as well as mononeuritis multiplex, were among the common misdiagnoses for patients with lepromatous leprosy. Leprosy can also mimic rheumatoid arthritis with solely joint symptoms without initial involvement of skin or nerve. In our review, the diagnosis could be delayed for a significant amount of time, for even up to 10 years in 2 cases. This led to irreversible loss of neurologic function (foot/wrist drops), neuropathic ulcers and osteomyelitis. We will present 3 illustrative cases: one of lepromatous misdiagnosed as mononeuritis multiplex from sarcoidosis, a second of polyarthritis from leprosy mimicking rheumatoid arthritis, and lastly, a case of borderline tuberculoid leprosy with skin rash thought to be from cutaneous sarcoidosis based on initial evaluation of a skin biopsy. To summarize, it is of crucial importance to include leprosy in the differential diagnoses for patients with chronic rash, neuropathy or joint symptoms in the appropriate epidemiologic setting, because early recognition and treatment can prevent progression of the disease and its morbidity.

1670 TWO FATAL CASES OF MELIOIDOSIS ON THE THAI-MYANMAR BORDER
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Meliodosis, caused by the gram-negative environmental bacterium Burkholderia pseudomallei, is an infectious disease of clinical importance in endemic areas, and is associated with a high case-rate fatality in humans and animals. Once considered an esoteric tropical disease, research on B. pseudomallei has gained prominence due to its potential for epidemic spread, increasing numbers of case reports from non-endemic regions, and classification by the United States as a potential bioterrorism agent. Lack of awareness among physicians, along with a wide variability in disease manifestations, contributes to underdiagnosis and delayed treatment, and also confounds accurate assessment of global prevalence. Although melioidosis is endemic in Northern Australia and parts of Southeast Asia, there are no published reports from the Thai-Myanmar border. Here we report the first two documented cases of fatal melioidosis in this region.

1671 HISTOPLASmosis in Oregon EX Ecuador
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A 41 year old river raft guide returned from a 2 week long rafting trip in a jungle region of Ecuador on March 30, 2012. He had received appropriate travel vaccinations and had taken mefloquine for malaria prophylaxis. Shortly after his return he developed fever to 103 F range, chills, sweats, headache, myalgias, arthralgias and fatigue. He self-medicated with amoxicillin and subsequently ciprofloxacin was prescribed by a physician. On examination on April 9th he was afebrile, had some shotty cervical and inguinal lymphadenopathy but otherwise had a normal physical examination including that of the lungs. Initial laboratory investigations including malaria smears, blood cultures and stool studies were unrevealing. A chest x-ray had evidence of a right sided infiltrate. Ceftriaxone and doxycycline were given but fevers persisted. A CT scan of the chest was carried out and revealed extensive mediastinal and hilar lymphadenopathy and too numerous to count lung nodules. Fiberoptic bronchoscopy was nondiagnostic. On April 20th therapy with ketoconazole was initiated to treat possible paracoccidioidomycosis. On April 24th, histoplasmosis serologies were reported positive. He was started on liposomal amphotericin B. On April 25th he underwent a minithoracotomy for definite diagnosis as there was concern about possible coexisting malignancy. Pathology and intraoperative cultures were consistent with histoplasmosis. He subsequently completed a 3 month course of itraconazole and did well. We herein discuss briefly travel-associated histoplasmosis and the current recommendations for drug therapy of severe primary histoplasmosis infection.

1672 DESCRIPTION OF DENGUE-RELATED HOSPITALIZATION AND DISEASE SEVERITY FROM AN ENHANCED DENGUE SURVEILLANCE SYSTEM IN PUERTO RICO
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Dengue is an acute febrile illness (AFI) that is endemic in Puerto Rico. The clinical spectrum of dengue ranges from mild AFI to a life-threatening illness. Although timely identification of dengue patients can reduce medical complications and mortality, this complicated by clinical manifestations that overlap with other AFI. To identify early clinical features that can be used as predictors for severe dengue, we evaluated the clinical course of laboratory-positive (i.e., DENV nucleic acid detected by RT-PCR or anti-DENV IgM antibody detected by ELISA) dengue patients with dengue fever.
enrolled in the Sentinel Enhanced Dengue Surveillance System (SEDSS) site located in Ponce, Puerto Rico. Patients were those presenting with AFI during May 7, 2012 to May 6, 2013 that were hospitalized (n = 262) or were out-patients that returned for follow-up evaluation (n = 120). Of all 382 patients, there were no significant differences in age or sex between hospitalized and non-hospitalized patients. Admitted patients sought care later than non-hospitalized patients (mean day of presentation = 4 vs. 2 days), and had a mean hospital stay of 4 days. Clinical findings associated with hospitalization were anorexia (p = 0.002), diarrhea (p = 0.021) and dengue warning signs of persistent vomiting (p < 0.001), abdominal pain (p < 0.001) and bleeding (p = 0.013). Laboratory findings at presentation associated with hospitalization were leukopenia (p = 0.021) and thrombocytopenia (p < 0.001). Mean platelet count was significantly different between hospitalized and non-hospitalized patients (mean = 81,000 vs. 151,000) (p < 0.001). Patients that presented 4-7 days after illness onset had greater odds of having thrombocytopenia (OR = 2.18; CI: 1.24-3.83) or elevated liver transaminases (OR = 4.74; CI: 1.53-19.45). Enhanced dengue surveillance revealed that hospitalized dengue patients presenting late for clinical care were more likely to present with dengue warning signs, and were hospitalized more frequently. Further analysis will assess correlation between early presentation and ultimate disease severity.

**ACCESS MATTERS: AFRICAN-BORN U.S. MILITARY TRAVELERS UTILIZE TRAVEL CARE AT HIGH RATES**

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Immigrant travelers that visit friends and relatives (VFRs) are less likely to have a pre-travel health encounter and more likely to experience serious illness. Immigrants comprise more than 5% of active duty service members in the United States Department of Defense and have free access to travel medicine care, yet their travel health seeking behaviors are unknown. 351 African-born service members and 470 U.S.-born comparators completed an Internet-based survey to assess pre-travel health care utilization, perceived potential barriers, and health outcomes. While overall use of travel medicine services was equivalent if official duty was included (p=0.94), when traveling on leave status (not on official duty) African-born service members were more likely than U.S.-born to see a physician, 65% vs. 35% (p=0.001) prior to their most recent travel to a low, low-middle or upper-middle income country. This persisted when stratified by malaria risk at destination with African-born service member VFRs reporting pre-travel health care more than American-born comparators, 65% vs. 45% (p < 0.001) Both African-born and American-born service members reported easy access to medical care. African-born military service members perceive less risk of illness when traveling to Africa compared to American born travelers (p=0.001) yet, somewhat paradoxically, place more importance on pre-travel medical services (p = 0.007). African-born service members are more willing to self-diagnose and treat illnesses such as malaria (p<0.001) and rely on locally purchased medications (p<0.001). There was no difference in reported adherence to malaria chemoprophylaxis. This United States Military Health System study revealed data that opposes national health-care reform and community engagement programs. Disclaimer: The Views expressed are those of the author(s) and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences, the U.S. Army, or the Department of Defense.

**SURVEILLANCE FOR ARTEMISININ, QUININE, AND MALARONE RESISTANCE AMONG IMPORTED PLASMODIUM FALCIPARUM MALARIA - CALGARY, CANADA (2013-14)**

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Calgary has seen an increase in imported malaria cases in recent years with a large proportion coming from sub-Saharan Africa where Plasmodium falciparum is hyper-endemic. Surveillance for emerging resistance to antimalarial drugs such as Artemisinin, Quinine, and Malarone is essential in returning travelers. Artemisinin resistance has been reported in Cambodia and mutations within the K13-propeller gene associated with resistance to this drug. Mutations in the cytochrome B (cytB) are linked to Malarone resistance. In this study, we prospectively determined the susceptibility and resistance genotype of imported P. falciparum malaria to Calgary from April, 2013 to April, 2014. Travelers were mostly male (n=12, 67%) VFR (n=17, 94%) with mean age of 35.3 years destined for sub-Saharan Africa (n=17, 94%) predominantly West Africa (n=11, 61%). Malarone (oral) was the commonest treatment option (n=11, 61 %) followed by artesunate (n=3, 17%) and quinine (n=3, 17%). Positive malaria samples from patients (n=18) were tested with a standardized panel of antimalarials using the EUSA-based HRP2 ex vivo drug sensitivity protocol developed by WWARN. DNA was extracted from patient EDTA blood samples (n=18) and primers flanking the K-13 propeller gene and cyt B were used for PCR amplification of this gene. PCR products were bidirectionally sequenced and analyzed for mutations. Our ex-vivo results showed IC50 values of 17.36 ± 11.92 nM, 4.34 ± 2.34 nM, 4.06 ± 1.66 nM, 4.00 ± 1.39 nM for Artemisinin, Artesunate, Artemether and Dihydroartemisinin, respectively; mean IC50 of 39.04 ± 15.73 nM, 16.33 ± 4.36 nM, 80.44 ± 25.75 nM, 17.23 ± 3.65 nM and 127.38 ± 36.97 nM for Chloroquine, Mefloquine, Quinine, Amodiaquine and Piperaquine respectively, and mean IC50 = 27.2 ± 22.26 for Atovaquone. Analysis of the K-13 propeller and CytB gene showed that all imported malaria were wild type to date. Our results confirm that imported P. falciparum malaria to Calgary from sub-Saharan Africa remains wild-type at key resistant loci (K13 and cyt B) and susceptible to Artemisinin, Malarone and quinine the commonest treatment options when tested ex vivo.

**A SURVEY ON KNOWLEDGE, ATTITUDES AND PRACTICES AMONG INTERNATIONAL TRAVELERS IN UGANDA**

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In an increasingly international environment, global travel rates are increasing. However, significant risk exists amongst travelers. Counseling against preventable morbidity and mortality, such as transit related injuries, vaccine preventable diseases, violent crimes, and tropical illnesses, is essential for international travelers. The majority of data around traveler attitudes and counseling is based on pre-travel assessments, or in evaluation of returned ill travelers, but is biased towards those with health seeking behaviors. This study was designed to look at attitudes and practices amongst international travelers while actively travelling. The study took place Jinja, Uganda, a popular tourist destination near Kampala known for white-water rafting and the Nile River. Participants were recruited at tourist locales in Jinja, and a semi-structured questionnaire was administered to tourists after obtaining voluntary informed consent. A total of 153 travelers were surveyed. The majority was female, with age range 31 years, and predominantly from the USA, Australia, and the Netherlands. Most participants had received pre-travel advice through a travel clinic (64.0%) or a general practitioner (25.4%), and others cited the Internet, friends, and relatives as sources of information. Participants
endorsed malaria (94.7%), vaccines (92.1%), and diarrhea (59.5%) were the most important pre-travel counseling items, but few mentioned traffic accidents (15.0%) or sexually transmitted infections (13.7%). Malaria prophylaxis was prescribed to the majority (80.1%), but only three quarters of those people took prophylaxis, with adherence issues attributed to side effects or a lengthy duration of stay. Finally, when asked about health and safety issues experienced during the trip, nearly a third had encountered an issue, most of which either related to gastrointestinal, malaria, or traffic accidents. Although many travelers seek medical care prior to departure, counseling regarding non-infectious issues such as road traffic accidents, personal safety, and risk behaviors are lacking, despite these being a major cause of morbidity and mortality. Integration of this information into Internet resources and clinical practice may help to decrease mortality amongst global travelers.

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FACTORS ASSOCIATED WITH MORTALITY BY DENGUE

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Dengue is a public health priority in Colombia due to the significant increase in the number of cases from 26817 in 2008 to 57589 in 2013. In 2010 a 183% increase was noted in respect to 2009 and 9393 cases of severe dengue was reported and 217 confirmed deaths. A case-control study was conducted in the departments of Cundinamarca, Norte Santander, Santander, Cauca Valley and Meta to identify the clinical factors associated with mortality in patients with severe dengue in hospitals of level II and III attention between the periods 2009-2013. A case was considered to be death by dengue and a control pertained to survivors, all were confirmed through IgM or RT-PCR. The sample size was 50 cases and 150 controls. For the analysis logistic regression was utilized. 42% (63) of the patients originated from Cauca Valley, 24% (36) Meta, 18% (27) North of Santander, 10% (15) Santander and 6% (9) Cundinamarca. 60% (90) were less than 16 years and 52% were women. The factors associated independently with mortality were: comorbidities OR 3.18 (IC 95%: 1.33; 7.60), social risk OR 1.5 (IC 95%: 1.21; 9.17), tachycardia OR 8.94 (IC 95%: 2.57; 31.05), tachypnea OR 2.71 (IC 95%: 1.16; 6.28), altered state of consciousness OR 12.09 (IC 95%: 2.72; 57.30), respiratory difficulty OR 5.61 (IC 95%: 2.24; 14.06), pleural effusion OR 2.85 (IC 95%: 1.33; 6.11), main organ damage OR 3.14 (IC 95%: 1.29; 7.64), severe bleeding OR 2.66 (IC 95%: 1.19; 5.95), and previous consultation OR 2.68 (IC 95%: 1.16; 6.19). In the multivariate analysis the factors associated with increased death were: social risk social OR 9.88 (IC 95%: 1.26; 77.11), altered state of consciousness OR 11.48 (IC 95%: 1.34; 97.93), respiratory difficulty OR 9.84 (IC 95%: 1.96; 49.36) main organ damage OR 9.55 (IC 95%: 1.77; 51.41) and severe bleeding OR 8.08 (IC 95%: 1.95; 33.66).

Patients with these clinical characteristics should be hospitalized for extended observation and opportune treatment to avoid death.

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ATORVASTATIN FOR THE TREATMENT OF RHEUMATOID ARTHRITIS IN IRAQ

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Rheumatoid arthritis (RA) is a common chronic inflammatory and disabling disorder that is characterized by synovitis, articular destruction, and many systemic extra articular features. In addition, RA is associated with both morbidity and mortality due to accelerated atherosclerosis and risk of increased cardiovascular disease. Atorvastatin is well known anti- dyslipidemic agent that is considered to have potential anti-inflammatory and immune modulatory functions in patients with RA. To explore the possible anti-inflammatory effects of Atorvastin in patients with RA, we designed a study to evaluate the effect of atorvastatin compared to therapy with two more standards RA medications, methotrexate (MTX) and etanercept (EPT). The study group included Iraqi patients with moderate to highly active RA. A double blind, randomized, placebo - controlled clinical trial was conducted in which 100 RA male and female patients were enrolled from a group who were already on MTX or EPT for at least 1 month. This pool of subjects was divided into two groups, one to receive 20 mg atorvastatin tablet and the other to receive placebo capsules for three consecutive months. This study revealed first that only 49 patients completed the 3 months trial, 25 patients in atorvastatin and 24 patients in placebo group. All patients were clinically evaluated by measuring swollen joint count (SJC), tender joint count (TJC), visual analogue scale (VAS) and disease activity score (DAS28). Blood samples of all subjects patients were evaluated for erythrocyte sedimentation rate (ESR), C reactive protein (CRP) at baseline, monthly and at the end of the study. RA patients undergoing 20 mg atorvastatin treatment showed a significant (P < 0.05) reduction in CRP, SJC and TJC compared to those who received placebo. In addition, atorvastin treatment groups trended toward reduced ESR, VAS, and DAS28, but these differences did not achieve statistical significance (p > 0.05). In conclusion we believe that 20 mg atorvastatin is a safe and well-tolerated drug that has modest anti- inflammatory effect in patients with moderate to severe active RA.
Snakebite is an important medical emergency in rural Nepal. Although accurate figures are lacking, over 20,000 cases are recorded annually, with a case fatality rate close to 15%. Important variations exist between hospitals in the management and outcome of snakebite envenomation. In particular, striking disparities are observed in the dosage of antivenom, reflecting poor adherence to the complex national protocol. Although clinical studies have been conducted on viperid snake envenomation, well-designed dose-finding studies are almost non-existent for elapid envenomation. The purpose of this trial was to compare the efficacy and safety of two antivenom dosing schemes in the treatment of neurotoxic snake envenomation. The trial was conducted between May 2011 and March 2013 in 3 health facilities of southern Nepal. 157 patients presenting with signs of neurotoxic envenomation were randomized either to a high initial dose regimen (intervention) or to the low initial dose regimen as recommended by the Nepalese national protocol (control). The primary composite outcome included death, requirement for manual ventilation and worsening of neurotoxicity. Secondary outcomes included time to recovery, occurrence of adverse reactions, and cost. There was no statistically significant difference between arms in the proportion of patients reaching the primary endpoint (control 48.7% vs intervention 38.5%, p=0.264). No differences were observed in the analysis of safety outcomes. In 51 patients the snake species could be identified. 29 had Naja spp, 13 Bungarus spp. More patients bitten by cobras (Naja spp) and 22 by kraits (Bungarus spp.). Those bitten by kraits experienced more primary outcomes (68.2% versus 38.5%, p=0.264). No differences were observed in the analysis of safety outcomes. Of the 750 mothers interviewed, (36.6%) were fully immunized. Children whose mothers possessed primary or no formal education were less likely to be fully immunized compared to children of mothers with at least a secondary level education (OR=0.50, 95% CI=0.34-0.73). Children delivered at health facilities were more likely to be fully immunized (OR=1.81, 1.21-2.69). The major determinants of complete immunization status were knowledge level, maternal educational status and place of birth of the children. Raising the level of knowledge and increasing maternal literacy level as well as encouraging health facility births are essential to improve immunization coverage in these rural communities.

Factors associated with complete routine immunization status of children 12-23 months in rural areas of Osun State - Southwestern Nigeria

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Immunization is a cost-effective public health intervention to reduce morbidity and mortality associated with infectious disease. Incomplete immunization status especially in rural areas has led to a high burden of VPD in children. The Nigerian Demographic and Health Survey, 2008 showed that only 57.8% of children had received all recommended vaccines in Osun State far below WHO target of 80%. We conducted this study to identify the factors associated with complete immunization status of children in rural areas of Osun State. A total of 750 mothers of children aged 12-23 months were interviewed, using the WHO 30 cluster sampling technique. We collected data on socio-demographic characteristics, history of vaccination and factors associated with immunization status using semi-structured questionnaire, vaccination cards were also reviewed. We defined a completely immunized child as a child who had received one dose of BCG, three doses of oral polio vaccine, three doses of Diptheria-Pertussis-Tetanus vaccine and one dose of measles vaccine by 12 months of age. Bivariate and multivariate data analysis was performed using Epi-info software. Of the 750 mothers interviewed, (36.6%) were fully immunized. Children of mothers with poor knowledge on immunization were less likely to be fully immunized (Odds ratio (OR) =0.55, 95% CI=0.23-0.51). Children whose mothers possessed primary or no formal education were less likely to be fully immunized compared to children of mothers with at least a secondary level education (OR=0.50, 95% CI=0.34-0.73). Children delivered at health facilities were more likely to be fully immunized (OR=1.81, 1.21-2.69). The major determinants of complete immunization status were knowledge level, maternal educational status and place of birth of the children. Raising the level of knowledge and increasing maternal literacy level as well as encouraging health facility births are essential to improve immunization coverage in these rural communities.

Variations in presentation of Erythema nodosum lepromatous leprosy: report of three cases seen at a U.S. Hansen’s disease clinic

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Hansen’s disease (leprosy) remains the leading infectious cause of disability, with 250,000 new cases reported globally yearly. Erythema nodosum lepromatous leprosy (ENL) reactions, are humoral responses of the immune system that can cause systemic illness, including fever, skin lesions, joint pains and neuritis. ENL reactions occur in patients on the lepromatous end of the spectrum, classified by the Ridley-Jopling system. In non-endemic countries, Hansen’s disease (HD) remains rare and often underrecognized with the literature lacking in clinical descriptions of leprosy complications in the United States. To fill this gap, we report three patients with lepromatous leprosy who were seen at a HD clinic in Atlanta, GA with complicated ENL reactions within the last three years. The first patient was a 33-year-old Bangladeshi woman who presented with high fever, abdominal pain, and arthralgias. She lacked the distinctive skin lesions usually seen in ENL, but was incidentally found to have spenic lesions. She responded well to prednisone and was able to be weaned off after 6 months. Second, a 42-year-old Vietnamese man initially presented with classic ENL lesions, fevers, and lymphadenopathy that progressed in severity despite increasing doses of corticosteroids. He eventually was admitted to the intensive care unit with a severe systemic inflammatory response syndrome. He was subsequently started on thalidomide without recurrence. The last patient was a 68-year-old U.S.-born man, who displayed symptoms representative of both Type 1 and Type 2 reactions as his initial presentation of HD. These included joint pain, severe extremity swelling, skin nodules and a progressive neuropathy. He had been misdiagnosed with a seronegative arthritis prior to this presentation. While all three cases reported are ENL, the differences of clinical courses and presentations highlight the complexity of the disease and the need for increased awareness of unique manifestations of lepromatous leprosy.
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SEROLOGICAL STUDY OF ANTIBODIES ANTI-TOXOCARA CANIS EVALUATED BY ELISA AND WESTERN BLOT IN PEDIATRIC PATIENTS WITH CRYPTOGENIC EPILEPSY

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It has been suggested a correlation between infection by Toxocara canis and epilepsy and it is thought that epileptic seizures can be derived from the immune response generated by the presence of the parasite or direct lesions that can cause in the brain. The objective was to determine antibody anti-T. canis against antigen of the parasite through the technique of ELISA and Western blot in pediatric patients with cryptogenic epilepsy attending outpatient consultations in the area of Neurology of the Children Hospital of Mexico Federico Gomez (HIMFG). We analyzed 111 patients from 6 to 16 years of age with confirmed diagnosis of epilepsy with clinical and epidemiological background who attend the external consultation of Neurology of the HIMFG. We analyzed the presence of antibodies anti-T. canis by ELISA using excretion-secretion antigens obtained from larvae of L2 T. canis cultured in vitro and the children who tested positive by this technique were evaluated by the technique of Western blot to determine the molecular weight of excretion-secretion proteins recognized by sera from patients with antibodies anti-T. canis. It was found that 12.5% sera had antibodies against antigens of excretion-secretion for T. canis. Nine children were evaluated by the technique of Western blot and only 5 were positive for this technique, recognized two main antigens of 24 and 35 kDa. The analysis in sera from pediatric patients with epilepsy, showed a rate of 6.9% to antigens from T. canis, after analysis by ELISA and Western blot.

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MAPPING THE POTENTIAL RISK OF MYCETOMA IN SUDAN USING MAXIMUM ENTROPY ECOCLOGICAL NICH MODELLING

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WHO recognized mycetoma as one of 17 neglected tropical diseases (NTDs) worldwide. Studies revealed a soil-borne mediated or thorn prick-mediated origin of mycetoma, but no studies are available to investigate the effect of soil type and Acacia distribution on mycetoma in Sudan. Here, we report efforts to investigate risk factors associated with mycetoma risk in Sudan using ecological niche modeling. Records of mycetoma cases were obtained from the scientific literature, PubMed, and GIDEON. Acacia records were obtained from the Global Biodiversity Information Facility. We developed ecological niche models (ENMs) based on digital GIS data layers summarizing soil, land-surface temperature, and greenness, summarizing environmental variation across Sudan. ENMs calibrated in endemic districts were transferred across all of Sudan, and suggested that greatest risk was in a belt across central and southern Sudan. We visualized mycetoma in environmental dimensions, and the results revealed that mycetoma in ecologically diverse landscapes under wide ranges of environmental conditions. We tested niche similarity between Acacia and mycetoma, and found significant niche similarity. These results revealed contributions of different environmental factors to mycetoma risk, identify suitable environments for disease emergence, raise the concerns for mycetoma-acacia association, and provide steps towards a robust, predictive risk map for the disease.

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THE EPIDEMIOLOGY OF ORAL HUMAN PAPILLOMAVIRUS INFECTION AMONG HEALTHY MEN AND WOMEN IN LIMA, PERU

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The incidence of head and neck cancers associated with human papillomavirus infection has been increasing in Peru. However, the burden of oral HPV infection in Peru has not been assessed in a healthy population. The objective of the study was to estimate the prevalence and correlates of oral HPV infection in healthy male and female residents from Las Pampas, a shantytown in Lima, Peru. A population-based sample of 1,500 healthy men and women between the ages of 8-85 in low to middle income areas of Lima, Peru was identified through random household sampling between January and August 2010. Adjusted odds Ratios (aOR with 95% CI) were used to assess the association of demographic factors, sexual practices, and oral hygiene on the prevalence of oral HPV infection. The prevalence of any HPV and any high-risk HPV (HR-HPV) was 6.8% and 2.0%, respectively. The three most common types were HPV 55 (3.4%), HPV 6 (1.46%), and HPV 16 (1.09%). Male sex (aOR, 2.32; 95% CI: 1.29, 4.18), age 19-27 (aOR, 2.77; 95% CI: 1.02, 7.56) and 46-55 years (aOR, 3.52; 95% CI: 1.07, 11.5) were significantly associated with prevalent HPV infection after adjustment. The prevalence of oral HPV in this population-based sample of healthy men and women from Peru was similar to estimates observed in the United States. Higher prevalence of oral infections in men were consistent with a male predominance of HPV-associated HNC and may signal a sex-specific etiology in the natural history of infection.

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DIAGNOSIS OF LIVER TUMORS USING IMAGE-BASED STATISTICAL FEATURES

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Tumors located in the liver can be detected using CT imaging and comparison of intensity histograms with the associated statistical parameters for characterizing different regions of the liver including normal and cancerous. Identifying whether the tumor is benign or malignant is an important step in image-based liver cancer diagnosis. In this paper we describe an automated system for image-based liver segmentation of CT imagery using a multi-stage process. Each CT liver image is pre-processed to remove noise and enhance image quality to recognize structures within the liver. A key challenge is related to separating the liver from the rest of the abdominal cavity in CT imagery. We used a statistical feature descriptor to characterize healthy tissue versus cancerous regions and then applied a modified K-means classifier to improve the accuracy of the tumor segmentation process.

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ASSOCIATION OF HEMOGLOBIN LEVELS AND SELECTED BIOCHEMICAL MARKERS WITH DIABETIC NEPHROPATHY DISEASE

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The present study was designed to investigate the relationship between hemoglobin levels (Hb), serum creatinine, lipid profile and urine albumin excretion in diabetic patients in Thi-Qar province, Iraq with various degrees
of nephropathy. The study cohort included 60 patients and 30 healthy subjects (control group) presented at the Al-Nasiriyah Endocrine Centre in Thi-Qar province, Iraq. The diabetic subjects were divided into three groups each with 20 subjects presenting diabetes mellitus (DM) disease for 1-5 years, 6-10 years and more than 10 years. The clinical results showed a significant decrease in the levels of Hb (p<0.01) in patients with DM compared to the control group. Also, there was a significant increase in blood sugar and urine albumin excretion in patients with DM compared with the control group (p<0.01). Serum creatinin increased significantly in patients with more than 10 years of DM compared with the control group. These results indicate a dyslipidemia in patients with DM compared with the control group.

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THE EFFECT OF METFORMIN ON GHRELIN SERUM LEVEL IN TYPE 2 DIABETES MELLITUS

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Ghrelin is an orexigenic peptide hormone. A great deal of evidence suggests that ghrelin is involved in the development of Type 2 diabetes mellitus (DM). The aim of this study was to investigate the effect of metformin on ghrelin serum level in Type 2 DM patients. This clinical control study was carried out at the Al-Wafa Medical Center for Diabetic and Endocrine Disorder patients in Mosul from October 2011 to March 2012. Fifty-five Type 2 diabetic patients and 20 control healthy subjects were enrolled. Patients and subjects were divided into 4 groups. Blood samples were collected from all subjects and the body mass index (BMI) was calculated for each person. Fasting blood sugar (FBS) level and ghrelin serum level were estimated for each patient. This study demonstrated a non-significant lower mean ghrelin serum level in the diabetic group compared to healthy controls. There were, however, significant differences in ghrelin serum levels between the diabetic group without metformin and the diabetic group treated with 1,000 mg metformin daily (p<0.05). In this study we found that ghrelin serum levels had a negative correlation with age of patients over 30 years and BMI in both healthy and diabetic individuals.

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PEDIATRIC MENINGITIS IN THE AL-ABBASEYA FEVER HOSPITAL OF CAIRO

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Meningitis in children can be a fatal disease. Early discovery and prompt treatment using effective antimicrobials greatly reduces the mortality and resulting complications of the disease. Since meningitis is caused by a number of different microorganisms, detection of the causative agent is essential for proper treatment and to establish any preferential pattern of prevalence in different age groups or geographical regions. We conducted our clinical study on 61 patients admitted to Al-Abbassaya Fever Hospital in Cairo during the period of December 2010 to June 2011. The patients ranged in age from one month up to 17 years. Diagnosis of these cases was based on history, clinical data, laboratory tests, cerebrospinal fluid (CSF) examination and other visual diagnostic tests. There were 61 cases with 35 males and 26 females. Based on the CSF culture, gram stain, cell count and cell type diagnostic information, the patients were grouped into two classes: Acute bacterial meningitis (Group 1) and acute non-bacterial meningitis (Group 2). Group 1 consisted of 31 cases (20 males and 11 females) while Group 2 had 29 cases (15 males and 14 females), and one case of recurrent meningitis. The yield of microbial isolation from Group 1 was only 32.2% with four cases of N. meningitides, two cases of S. pneumonia, three cases of H. influenza and one case of Gram negative rods. The clinical and laboratory information and antimicrobial treatment regimen we used will be described. Pediatric meningitis needs special attention and a high rate of clinical suspicion as the yield of microbial isolation is low primarily due to the use of antibiotics prior to hospital admission. The choice of empirical antimicrobial usage might need to be reviewed from a clinical and public health perspective.

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UTILIZING THE COMMUNITY HEALTH WORKER NETWORK FOR LYMPHATIC FILARIASIS (LF) MORBIDITY MONITORING: THE DEVELOPMENT OF AN SMS-BASED SURVEILLANCE TOOL

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Lymphatic filariasis (LF) is a parasitic infection that is responsible for over 15 million lymphoedema and 25 million hydrocele cases globally, resulting in LF morbidity being one of the leading causes of long-term disability. Currently, there are no standardised methods available for quantifying and mapping LF morbidity burden. The purpose of this study was therefore to pilot a novel method for collecting LF morbidity data in endemic areas of Malawi and Ghana. The premise of this method is that community health workers are able to quickly identify lymphoedema or hydrocele cases in their villages, but do not currently have a standardised method of collating this information. We have therefore developed an SMS-based tool which enables health workers to submit information on each identified case in their communities using a basic mobile phone. This tool was trialled under two scenarios: in March 2014 the tool was trialled by qualified, salaried health workers in southern Malawi; in May 2014 the study was repeated in Ghana using volunteer community health workers. In both scenarios, each health worker was asked to submit each identified case's village of residence, age, sex, condition and severity of condition (if lymphoedema) via SMS to a smartphone housed in-country. This information was then instantly compiled into a single database. A random sample of cases was visited by a medically qualified person to confirm the health workers' diagnoses, and GPS coordinates of their villages were recorded. The feasibility of the method was assessed in terms of the ease in which health workers were able to correctly identify cases (true positive rate), and the ease of use of the SMS-based tool (data entry error rates). A comparison between the performance of salaried health workers and volunteer health workers was also undertaken. Preliminary results for Malawi indicate that the true positive rate for reported lymphoedema and hydrocele cases using this method were 90% (95% CI [80%, 97%]), and 92% (95% CI [77%, 97%]) respectively.
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EPIDEMIOLOGY OF PODOCONIOSIS IN ETHIOPIA: RESULTS FROM A FIRST NATIONWIDE MAPPING

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Podococoniosis (endemic non-filarial elephantiasis) is a major cause of tropical lymphoedema and is endemic in Ethiopia. To guide targeting and implementation of the National Neglected Tropical Diseases control strategy, an integrated nationwide survey of lymphatic filariasis (LF) and podococoniosis was conducted between June and September 2013. Here we present a description of podococoniosis epidemiology in Ethiopia resulting from this survey. District health offices' reports of podococoniosis and LF were used to guide selection of survey sites. Data and cluster level GPS coordinates were collected via smartphones by trained local health workers. Individual level data were available for 129,959 randomly-sampled individuals from 1,315 communities in 659 districts. Blood samples were tested for Wuchereria bancrofti antigen using immunochromatographic card tests (ICT). A clinical algorithm was used to diagnose podococoniosis by excluding other potential causes of lymphoedema of the lower limb. Mixed-effects logistic regression was used to identify individual-level correlates, adjusting for dependence within district and municipality. Overall, 8,110 of 129,959 (6.2%, 95%CI: 6.1 to 6.4%) surveyed individuals were identified with lymphoedema with 5253 (4.0%, 95% CI; 3.9 to 4.1%) confirmed as podococoniosis cases. Prevalence among men and women was 3.4% (95%CI; 3.3 to 3.5%) and 4.7% (95% CI; 4.5 to 4.8%) (p<0.001), respectively. During the survey 85.2% (95%CI: 84.9 to 85.3%) of respondents were wearing protective shoes, but only 57.9% (95%CI: 57.6 to 58.2%) of them were wearing protective shoes. Female sex, older age, wearing shoes after 12 years of age, wearing feet less frequently than daily were significantly associated with increased odds of having podococoniosis. Attending formal education, living in a house with a covered floor were associated with decreased odds of having podococoniosis. The survey confirmed that podococoniosis remains a significant public health problem and is widely distributed in Ethiopia; it is endemic throughout 30% of the country’s landmass, where more than 40% of the population live. Results provide a current benchmark of the burden of the disease, against which future podococoniosis control programmes can be measured.

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EFFECTS OF ELIMINATION CAMPAIGN OF LYMPHATIC FILARIASIS SEEN IN NEPAL

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Effects of Elimination Campaign of lymphatic Filariasis seen in Nepal, 2014* Lymphatic filariasis (LF) is one of the five infectious diseases targeted by WHO for elimination as public health problem in Nepal. They are LF, Kala-azar, Leprosy, Yaws and Chagas disease. WHO launched GPELF with a goal to eliminate LF as public health problem by 2020 A.D. Two key strategies are: Interruption of transmission of LF infection in endemic countries by reducing microfilarial prevalence levels (below 1%) through Mass Drug Administration (MDA); Prevention and alleviation of disabilities and sufferings in individuals already affected by LF. Nepal: A total of 61 districts are considered LF endemic. Some districts with high prevalence are as high as 40%. The population at risk in Nepal are 25 millions. The causative agent are Wuchereria bancrofti, and transmission vector is Culex quinquefasciatus. The reported chronic conditions in 2012 are 28,835, majority were hydrocele. The 10 most morbid districts with hydrocele were Morang, Jhapa, Bardia, Banke, Saralahi, Dhading, Nuwakot,Kapilbastu, Bara, and Mahottari.LF Elimination Strategies: Interruption of transmission by Mass Drug Administration (MDA) using two drugs regimen, Diethylcarbamazine (DEC) and Albendazole, once yearly for six years. Morbidity management by self care and with support using intensive but simple, effective and local hygiene technique.MDA 2013: The number of MDA districts were 56. The total population in MDA districts was 25087450. The estimated eligible population for MDA: Phase I : 37 districts of eastern, central and western regions and Phase II : 19 districts of mid western and far western regions.

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REAL-TIME PCR AND MELT-CURVE ANALYSIS (QPCR-MCA) AS A REFERENCE LABORATORY TOOL FOR THE DETECTION OF ONCHOCERCA VOLVULUS AND ITS IMPORTANCE FOR MONITORING AND EVALUATION ACTIVITIES

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The efforts to eliminate onchocerciasis in most of Africa by 2025 necessitate re-examination of current monitoring and evaluation tools. In particular, assessment of hypoendemic zones, stop-treatment determinations, and post-intervention surveillance will require sufficiently sensitive tools to detect low-intensity infections. Mass drug administration with ivermectin (IVM) is expected to decrease microfiladermia and thereby decrease the usefulness of skin snip microscopy, currently the standard assessment tool. Using a pan-filarial qPCR-MCA, we assessed 1) the utility of this single-step assay for detecting evidence of microfilaria (MF) in residual skin snips and 2) the sensitivity of skin snip microscopy relative to our QPCR-based assay. Specificity of the qPCR-MCA to Onchocerca volvulus was verified using DNA from O. volvulus macrofilariae; MF of B. malayi and pahangi, L. loa, M. ozzardi, and W. bancrofti; and uninfected human controls. Utility of the qPCR-MCA assay and the relative sensitivity of microscopy were evaluated with residual skin biopsies (i.e., after 24-hour incubation in saline) collected from hyperendemic regions of Uganda and Ethiopia (n=500 each) which had received limited rounds of IVM. qPCR-MCA detected over 94% of known positive skin snips (139/147 total microscopy positive), identifiable by consistent, well-defined dissociation curves at 79.35°C (S.D. 0.22) with a minimum 1°C difference from other filarial species. Using qPCR-MCA as the reference test, the sensitivity of the skin snip microscopy was only 74.7% (121/162) and 28.1% (18/64) in Uganda and Ethiopia, respectively. Combined across countries, qPCR-MCA detected an additional 87 positive samples (38.5%), indicating a combined microscopy sensitivity of 61.5% (139/226). When evaluating low-intensity infections (≤2 MF/snip), the sensitivity of microscopy was only 46% (74/154). Thus, skin snip microscopy does not appear to be sufficiently sensitive to assess transmission in areas with low microfilaridaemia or to make stop-treatment decisions in the absence of other transmission assessments (e.g., vector data). qPCR-MCA can augment sensitivity and provide diagnostic confirmation of skin biopsies and will be useful for validating new monitoring tools that may be developed to support elimination efforts.
FIFTY-EIGHT YEARS OF MAN AGAINST THE WORM IN BUDONGO ONCHOCERCIASIS FOCUS OF UGANDA: INTERRUPTION OF TRANSMISSION IS FINALLY IN SIGHT

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The battle against onchocerciasis in hyperendemic Budongo focus (where the baseline microfilaria (mf) rate was 80% and fly infection rate 20%) has lasted over a half a century. Vector control of Simulium neavei using DDT commenced in 1955, and larviciding was halted when no larval/pupal stages of S. neavei were observed on crabs, and no adults flies were found in human landing captures. Six months after stopping larviciding, however, rapid repopulation of S. neavei was observed. In August 1956, a new series of DDT larviciding was initiated. In 1958, the early stages of the vector could not be found in the rivers and the adult fly had disappeared by 1962 indicating elimination of S. neavei from the focus. Repeated courses of DEC were provided to individuals in the communities who were infected. To avoid vector reinfestation, maintenance larviciding continued until about 1971. Political unrest in Uganda led to the collapse of this work, and by 1989 S. neavei had again recrudescence had occurred. Annual mass drug administration (MDA) with ivermectin alone for onchocerciasis or with ivermectin and albendazole for LF. Years of ivermectin distribution for onchocerciasis may have decreased or interrupted LF transmission in certain areas. The Kedougou region, Senegal, has been historically known to be co-endemic with LF (immunochromatographic test [ICT] prevalence ≥1%) and onchocerciasis (microfilaria prevalence 9.0-68.4%). MDA for onchocerciasis started in 1988 and as of 2014, albendazole had not been added to target LF. The objective was to assess in an integrated manner the status of LF and onchocerciasis in three districts of Kedougou after ≥15 years of ivermectin MDA. Sixteen villages close to rivers and breeding sites for onchocerciasis vector, Simulium spp, were selected. LF antigenemia testing (ICT) was added to skin snip microscopy for onchocerciasis evaluation. Convenience sampling of residents ≥5 years was performed. Dried blood spots were collected to test for antibodies against Wb123 (LF) and Ov16 (onchocerciasis) antigens (results pending). One village refused to participate and one was excluded because it was treated with ivermectin too recently. Forty percent (1154/2925) of residents participated; 50% were males and the median age was 15 years. In two districts, no participants were ICT or skin snip positive. In the third district, 3.4% (6/176) were ICT-positive (village range 1.9-6.4%) and 0.7% (1/150) were skin snip-positive. The mean age of ICT-positive participants was 49 years (range 25-79); the participant with a positive skin snip was 79 years old. After ≥15 years of ivermectin distribution, LF prevalence was still above treatment threshold in one of the three districts included in the evaluation. The integrated evaluation of LF and onchocerciasis provided important information on both diseases that should help program managers make decisions about treatment interventions.

EVALUATION OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS IN THREE SENEGALESE DISTRICTS TREATED FOR ONCHOCERCIASIS WITH IVERMECTIN FOR MORE THAN 15 YEARS

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In Africa, there is significant overlap between onchocerciasis and lymphatic filariasis (LF). Current efforts to eliminate these two diseases are through mass drug administration (MDA) with ivermectin alone for onchocerciasis or with ivermectin and albendazole for LF. Years of ivermectin distribution for onchocerciasis may have decreased or interrupted LF transmission in certain areas. The Kedougou region, Senegal, has been historically known to be co-endemic with LF (immunochromatographic test [ICT] prevalence ≥1%) and onchocerciasis (microfilaria prevalence 9.0-68.4%). MDA for onchocerciasis started in 1988 and as of 2014, albendazole had not been added to target LF. The objective was to assess in an integrated manner the status of LF and onchocerciasis in three districts of Kedougou after ≥15 years of ivermectin MDA. Sixteen villages close to rivers and breeding sites for onchocerciasis vector, Simulium spp, were selected. LF antigenemia testing (ICT) was added to skin snip microscopy for onchocerciasis evaluation. Convenience sampling of residents ≥5 years was performed. Dried blood spots were collected to test for antibodies against Wb123 (LF) and Ov16 (onchocerciasis) antigens (results pending). One village refused to participate and one was excluded because it was treated with ivermectin too recently. Forty percent (1154/2925) of residents participated; 50% were males and the median age was 15 years. In two districts, no participants were ICT or skin snip positive. In the third district, 3.4% (6/176) were ICT-positive (village range 1.9-6.4%) and 0.7% (1/150) were skin snip-positive. The mean age of ICT-positive participants was 49 years (range 25-79); the participant with a positive skin snip was 79 years old. After ≥15 years of ivermectin distribution, LF prevalence was still above treatment threshold in one of the three districts included in the evaluation. The integrated evaluation of LF and onchocerciasis provided important information on both diseases that should help program managers make decisions about treatment interventions.

THE USE OF HUMAN SWEAT METABOLITES AS BAIT FOR MONITORING VECTORS OF ONCHOCERCIASIS IN WEST AFRICA AND LATIN AMERICA

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Onchocerciasis, a.k.a. river blindness, is a parasitic disease caused by infection from the nematode Onchocerca volvulus. The parasite is transmitted to humans by the bite of infected black flies (genus Simulium spp,).
Simulium. The World Health Organization (WHO) estimates 18 million people suffer from onchocerciasis, the vast majority occurring in central Africa, and isolated foci in the American Tropics. Treatment of the disease relies on accurate epidemiological data, which is best achieved though real-time data of infection prevalence in the vectors. The need for a new monitoring method is crucial. To this end we identified key primary metabolites in human sweat, which putatively attract black flies to humans. Laboratory studies were then conducted in Southern Mexico and West Africa to test which compounds attracted these vectors of onchocerciasis, using electroantennography and y-tube olfactometry. The attractive compounds will be developed into baits to lure black flies to a novel trap for monitoring vector abundance and infection prevalence in both Latin America and Africa. In this study we describe the identification of key human sweat components via GC-MS, the identification of attractive metabolites to the two major species of Simulium as well as trap development and bait formulation for the continued monitoring of these important disease vector.

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LYMPHATIC FILARIASIS ELIMINATION: ASSESSMENT OF TWO VILLAGES WITH DIFFERENT ENDEMICITY LEVELS IN A PREVIOUSLY HIGHLY ENDEMIC REGION (SIKASSO) OF MALI

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Lymphatic filariasis (LF) is a disfiguring neglected tropical disease that is targeted for elimination by 2020 using annual mass drug administration (MDA) in endemic areas. We previously reported the Anopheles gambiae complex frequencies in 2 neighboring villages of Kolondiéba, one of the highly LF endemic district of Sikasso, Mali. To assess transmission interruption after 6 annual MDA in the district of Kolondiéba and determine the potential impact of vector density on MDA-induced transmission interruption. A cross sectional study with quantitative and qualitative data collection methods in 2 villages of Sikasso region. The village of Boundioba had a lower Anopheles density as compared to Bougoula (1,494 versus 251 specimen from July to December 2011). A total of 481 volunteers in Bougoula including 340 female (70.5%) and 332 in Boundioba including 221 Female (66.6%) were included in this study. The 6-7 years/15 years and above composition was 113/368 and 127/205 respectively in Bougoula and Boundioba. Microfilaremia was significantly more frequent in the 15 years and above in Boundioba(1.95%, 4/205) as compared toBougoula (0%, 0/368), (p=0.02, Fisher exact test). Additionally, the 2 villages showed comparable low prevalences in 6-7 years olds with respectively 1/127 and 0/113 for Boundioba and Bougoula. Anopheles vector density may be misleading because it is not necessarily associated with a higher endemicity in a village under MDA.
reasons for systematic non-compliance with MDA. A total of 486 subjects (170 men and 316 women) were questioned, of whom 16.1% (79/486) reported never swallowing MDA drugs. The most common reasons given were being unaware of MDA (24/486, 4.9%), being pregnant or breast-feeding (8/486, 1.6%) and not willing to take the drugs (6/486, 1.2%). Although systematic non-compliers were more likely to be younger [OR = 1.7 (1.006-2.921) for individuals 15-30 vs. >30 years of age], compliant and systematically non-compliant subjects were similar with respect to participants’ instruction level [OR = 1.2 (0.59-2.51)] and the presence of lymphoedema / hydrocele [OR = 0.5 (0.11-2.63)]. These data suggest that significant rates of systematic non-compliance can be present despite adequate overall coverage rates. Whether persistent infection in systematic non-compliers provided the reservoir for re-emergence of transmission in the 6 study villages requires further study.

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NON-MANSONELLA OZZARDI ATYPICAL MICROFILARIA IN THE PERUVIAN AMAZON BASIN
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Filariasis is a neglected tropical disease around the world. This disease is a vector-borne infection caused by nematodes (roundworms) of the family Onchocercidae. Since 1950s, filariasis caused by Mansonella ozzardi has been regularly reported in the Peruvian Amazon basin. Isolated cases have been diagnosed as M. perstans, Brugia spp, Onchocerca spp. and Dirofilaria spp. Since 2010, by examining de-identified blood samples (thick smear and Knott’s concentration techniques) we have characterized the prevalence of filarial infections and associated clinical symptoms in both rural (n=383) and urban (n=755) human populations located on tributaries of the Amazon River near and in Iquitos, Peru. In the rural communities, prevalence of microfilariae was 28.5% (109/383) overall; but the majority 99% (108/109) were M. ozzardi. Prevalence rates were not heterogeneous ranging from 0 to 72.5% in 11 communities. In contrast, of 755 samples from residents of Iquitos, 2% and 4% were infested with M. ozzardi and an atypical microfilaria, respectively. Interestingly, those infected with M. ozzardi tended to be male adults with occupations associated with rural areas, whereas those infested with the atypical parasite were rarely febrile and were often children or housewives. Surveillance in local hospitals identified at least one morphologically distinct atypical microfilariae and one co-infection with M. ozzardi in seven symptomatic patients; one had subacute skin lesions and the others fever. The atypical microfilariae were macroscopically distinct from M. ozzardi, measuring 600 x 8 µm, with no sheath and no nuclei in the tail. In a subset of samples tested by PCR. All M. ozzardi were confirmed, but five of the atypical microfilariae tested negative the internal transcribed spacer rDNA sequence of M. perstans and Onchocerca volvulus. In conclusion, atypical microfilariae with a distinct epidemiology from M. ozzardi, and not related to M. perstans, or O. volvulus, are sufficiently prevalent to warrant investigation of their health impact in the Peruvian Amazon.
interviewed did not know about the disease, what caused it, how it was transmitted or were aware of the national programme to eliminate LF. A follow-up microfilaria (MF) survey was conducted in the study site with the most ICT positive individuals, and included the majority of community members (n=300). Preliminary results indicate the average MF prevalence was 41.5%, and ranged from 10.5% in children under 10 years, to 54.6% in adults over 50 years of age. MF prevalence was found to be higher in males (46.4%) than females (34.9%), and among those living in houses made of bush material (45.8%) compared with other semi-permanent materials (23.1%). The field work is still in progress and expected to be finished with final results by November 2014. This research highlights that LF is endemic in remote areas of the country and the national LF programme has to scale up its efforts to control and eliminate the spread of infection with particular emphasis on LF advocacy and education to those most at risk.

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METHODS FOR ASSESSING LYMPHATIC FILARIASIS TRANSMISSION IN LOW ENDEMIC AREAS OF BANGLADESH: ONE STEP CLOSER TO THE ELIMINATION GOAL
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Bangladesh had one of the highest burdens of lymphatic filariasis (LF) at the beginning of the Global Programme to Eliminate LF (GPELF), with an estimated 80 million people at risk of infection. Baseline mapping in 2000 using the rapid diagnostic ICT cards, found that 34 of the 64 districts in the country were endemic, however, only 19 districts required mass drug administration (MDA) using the drugs albendazole and DEC to interrupt transmission. The Bangladesh LF Programme has successfully scaled up MDA in these districts, and is moving into the elimination phase and using the WHO recommended Transmission Assessment Survey (TAS) to assess their success in interrupting transmission - so far with excellent results. The outstanding important question for the LF Programme was how to assess the 15 endemic districts that were found to have low prevalence levels (<1%) and not be eligible for MDA. Follow-up night blood microfilaria (MF) and community clinical surveys undertaken in 2008 -2010 in selected areas of these districts found little or no evidence of infection and disease such as lymphedema and hydrocele. Currently, there is no recommended strategy for assessing low endemic districts, therefore, in order to address this issue and provide more rigorous evidence that LF is not a public health problem, the TAS method is being used as an assessment tool with additional systematic patient searching at household level. The assessments are planned for each month of 2014 and being carried out by trained field teams visiting schools for TAS (targeting children) and using local community clinic workers and volunteers to visit households (targeting individuals with clinical manifestations). To date five districts have been assessed with good results, and if the remaining districts are also found to have little or no LF infection or disease, the national LF programmes can ‘shrink the LF map’ by approximately 30 million people and move one step closer to their elimination goal, with an increased focus on the new priorities of surveillance and morbidity management.

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SYSTEMATIC REVIEW AND META-ANALYSIS OF DOXYCYCLINE IN CONTROL OF LYMPHATIC FILARIASIS
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Lymphatic Filariasis control programs rely on periodic population-wide administration of microfilaricidal agents repeated over several years to decrease transmission. Effective microfilaricidal agents could decrease the years necessary to achieve elimination. Doxycycline has emerged as a possible agent due to its effects on the Wolbachia endosymbiont. A systematic review identified 9 published randomized controlled trials evaluating the effects of various Doxycycline regimens on Wuchereria or Brugia microfilaria levels at 12 months post-treatment in 5 countries, with or without interim single-dose microfilaricide (ivermectin or DEC). For elimination of microfilariae at 12 months (compared to placebo), the pooled Risk Ratio was 3.22 (1.95, 5.32), with high heterogeneity (I²=68%). Subgroup analysis showed: Doxycycline 6-8 week regimens, RR= 3.96 (2.07, 7.59); Doxycycline 3-4 week regimens, RR= 2.14 (1.08, 4.26); Ivermectin or DEC 4 months after Doxycycline, RR= 2.29 (1.73, 3.04); no interim microfilaricide, RR= 5.80 (2.36, 14.24). Multi-day Doxycycline regimens effectively eliminate LF microfilariae at 1 year after treatment. Applicability of such multi-day regimens to population-wide control programs is limited. Further studies should evaluate shorter-term treatment.

1704
SIMULTANEOUS DETECTION OF ONCHOCERCA VOLVULUS AND O. OCHENGI IN INFECTED SIMULIUM FLIES USING A NEW MULTIPLEX REAL-TIME PCR
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Onchocerciasis is a parasitic worm infection estimated to affect 75 million people in sub-Saharan Africa and parts of Latin America, causing dermatitis, skin atrophy and visual impairment. In 27 endemic countries in Africa, 130 million people at risk of the disease. The January, 2014 report of the International Task Force for Disease Eradication has stated the continued need for improved diagnostics to assess when mass drug administration efforts can be halted and to monitor for recrudescence. Currently transmission is monitored by identifying larvae in dissected Simulium damnosum species, the vector of O. volvulus, which are also able to transmit O. ochengi, a parasitic worm of cattle that does not infect humans. We developed a multiplex real-time PCR based on the NDS gene of the Onchoeceridae genus with specific TaqMan probes to differentiate O. volvulus and O. ochengi from other Onchoeceridae. A blinded study with 217 flies from O. volvulus and O. ochengi endemic and O. volvulus/O. ochengi co-endemic areas in Cameroon (n=23) showed 100% specificity in all analyzed Simulium flies. Vector monitoring to assess transmission potential in endemic areas is reliable. Our multi-plex real-time PCR offers time and cost savings over species identification via microscopy.

1705
MODELING THE EFFECTS OF MASS DRUG TREATMENTS AND VECTOR CONTROL ON CO-INFECTION WITH MALARIA AND LYMPHATIC FILARIASIS
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Malaria and lymphatic filariasis (LF) are both transmitted by anopheles mosquitoes and are co-endemic in many regions of the tropics. For ongoing global campaigns to eliminate LF and malaria, it is important to understand interactions between both parasites and hosts where they co-exist. The use of mass drug administrations (MDA) to reduce prevalence and intensity of microfilariae may increase the lifespan of anopheles mosquitoes and thereby potentially increasing the transmission of malaria, while interactions at the host level may affect susceptibility, disease severity, and co-transmission of both diseases. Each parasite system alone exhibits complex dynamics where factors such as vector biting rates and threshold prevalence of human infection contributes to either extinction or stabilization to an endemic level. Knowledge of how interactions between both systems may affect co-infection endemicity and extinction dynamics is important for designing effective disease management programs such as MDA and vector control (VC). We extend a mathematical model of
malaria-LF co-infection to describe how the interplay between these two infections influence threshold behaviors in the system, and how MDA and VC interventions can influence elimination or resurgence of both diseases.

1706

IMPACT OF MEROWE DAM ON ONCHOCERCIASIS VECTORS OF ABU HAMED, NORTHERN SUDAN

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Abu Hamed, the northernmost onchocerciasis focus in the world, is located along the River Nile banks in the Nubian Desert. Hydroelectric dams can alter activity of black flies and may provide breeding sites for black fly. Merowe Dam, the largest hydropower project in Africa, was built west of Abu Hamed focus in 2009. The impact of the Dam on onchocerciasis and its black fly vectors in Abu Hamed focus was measured in this study. Entomological surveys for aquatic stages and adult Simulium hamedense were conducted before and after the inception of Merowe Dam in 2007/2008 and 2010/2011. There was no black fly breeding or adult activity in the previously known breeding sites upstream of the Merowe Dam with the western most breeding site found in Al Saraf village near the center of the focus. No adult or aquatic stages of black flies were found downstream of the Dam. The artificial lake of the Dam flooded all the breeding sites in the western region of the focus and no aquatic stages and/or adult black fly activity were established in the study area upstream of the Dam. The Dam seems to have positive impact on onchocerciasis and its black fly vectors in Abu Hamed focus. These outcomes of the Merowe Dam might have contributed to the recently declared interruption of onchocerciasis transmission in Abu Hamed focus. Continuous entomological surveys are needed to monitor presence of black fly vectors and its impact on the disease.

1707

THE CURRENT STATUS OF LYMPHATIC FILARIASIS IN COTE D’IVOIRE PRIOR TO IMPLEMENTATION OF A NATIONAL PROGRAM OF MASS DRUG ADMINISTRATION

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Côte d’Ivoire is planning to implement a coordinated national program of mass drug administration (MDA) for elimination of onchocerciasis (Oncho) and lymphatic filariasis (LF) in the near future. The country has complex patterns of endemicity for these infections with extensive areas of coendemity, areas that have received mass drug administration with ivermectin for variable periods, and extensive migration to and from neighboring countries (Liberia, Ghana, and Burkina Faso). For example, some areas in the northern part of the country have received many rounds of ivermectin for onchocerciasis control during OCP and APOC, and LF rates tend to be low in the North. In contrast, Oncho is uncommon in coastal areas (with some exceptions), and little ivermectin has been used in the South. Some 47 of the country’s 82 health districts (mainly in Central and Southern districts) are considered to be co-endemic for LF and Oncho. LF mapping circa 2001 was based on antigen testing (Binax Now Filariasis, card test) of 50-100 people in two villages per district. The current study was performed to obtain more current information on the distribution of LF in the country and to identify sentinel sites for monitoring and evaluation of the impact MDA on LF. More than 3,900 people were tested for filarial antigenemia in 40 villages in 6 districts in the central and Southeastern part of the country. Antigen rates ranged from 4-22% in Lakota, 4-21% in Tiebissou, 15-41% in Akoupe, 21-25% in Agboville, 9-14% in Bettie, and 6-35% in Abengourou districts. Microfilaria rates ranged from 1% in Lakota to 11% in Abengourou and Agboville. Prior ivermectin distribution in areas with coendemic onchocerciasis may partially explain the highly variable Mf rates in these areas. This study has helped to establish the current LF situation in Côte d’Ivoire, and this information will be used to plan and implement the national LF elimination program based on MDA.

1708

PROGRAMMATIC IMPLICATIONS OF EXTENSIVE VECTOR CONTROL ON THE ELIMINATION OF LYMPHATIC FILARIASIS IN ZAMBIA

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Lymphatic filariasis (LF) is widely endemic in Zambia, and the National LF Programme is planning the first nationwide mass drug administration (MDA) to interrupt transmission in 2014. Overall the LF prevalence is low (<10%) in most regions of the country and it is possible that the well established vector control programme for malaria has already impacted LF transmission over the past decade. To better understand the distribution of vector control that has occurred across the country and the potential implications for the LF Programme as it moves to scale up MDA, this study developed a spatially modelled vector control map and examined it in relation to the baseline LF prevalence data collected across 108 geo-referenced sites in 2003 and 2011, and the sentinel site data collected across 32 geo-referenced sites in 2014. Information on bed nets, including long-lasting/insecticide treated bed nets (LLIN/ITNs) and indoor residual spraying (IRS) distributions was obtained from the Ministry of Health, and public data sources such as the Demographic Health Survey (DHS) data, President’s Malaria Initiative (PMI) reports, and combined in a weighted sum to form a multiple vector intervention score, which was then used to produce district-level maps of vector control intensity. Each district was classified according to LF prevalence and the vector intervention score which included the following combinations i) low LF high vector control, ii) low LF/low vector control iii) high LF/low vector control and iv) high LF/low vector control. These groups will help the LF Programme as it scales up MDA to determine if a district has potential for elimination, in need of very high MDA coverage and will require standard or enhanced surveillance.

1709

MODELING IMPACTS OF INTEGRATED VECTOR CONTROL ON LYMPHATIC FILARIASIS TRANSMISSION DYNAMICS AND ELIMINATION PROCESS

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Lymphatic filariasis (LF) is a target for global eradication by 2020. Launched in 2000, the Global Programme to Eliminate Lymphatic Filariasis relies mainly on large-scale preventative chemotherapy programs via mass drug administration (MDA) to eliminate this major vector-borne parasitic disease from all endemic settings. Modeling studies of LF transmission and control have been crucial for designing national control programs by establishing and quantifying the presence of infection and vector biting thresholds below which the transmission is interrupted. Renewed malaria control efforts have witnessed large-scale applications of vector control
(VC) through the use of long lasting insecticidal nets (LLIN) alone or in some combination with indoor residual spray (IRS) across the majority of LF endemic regions. LLIN/IRS affects the transmission of mosquito-borne infections either by directly killing, or by preventing mosquitoes from coming into contact with infected hosts through several mechanisms. Recent community trials have shown the substantial impact that VC may have in enhancing LF transmission interruption particularly when infection prevalence has been depressed to low levels using MDA. Despite these observations, theoretical and quantitative modeling of the impact of VC on LF transmission dynamics that takes explicit account of the various effects the different VC options may have on mosquito populations is scarce. Such analysis is vital when chemical insecticides require repeated applications in the affected communities due to their variable durations of effectiveness. This need for frequent insecticide applications introduces a number of factors such as the effects of adherence to the recommended timeframe for the replenishment of LLIN/IRS and the maintenance of the required community coverage, which may contribute to different outcomes from VC between communities. We aim to extend our present Bayesian Melding LF modeling framework by incorporating the specific effects that the application of LLIN and IRS, used separately or in combination, may have on the effective mosquito biting rate to quantify and gain better insights on the role of VC in the MDA-based LF control programs.

1710
AXENICALLY-DERIVED CAENORHABDITIS ELEGANS ANTIGEN FOR THE TREATMENT OF AUTOIMMUNITY

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The potential use of helminth infections as a protective measure against inflammatory disease has been validated in both animal and clinical studies. However, several obstacles impede the use of parasitic helminths in clinical practice. These include difficulties in obtaining large quantities due to complex lifecycles, challenges in purification from lifecycle hosts, inter-batch variability in product, and the potential of live infections to cause clinical symptoms. To overcome these hurdles, we tested whether a homogenate of soluble antigens prepared from axenically grown Caenorhabditis elegans (aCeAg) would protect against autoimmunity. Using a TLR4 reporter cell line, we demonstrated that, in contrast to soluble antigen prepared from C. elegans grown on Escherichia coli lawns, aCeAg lacks LPS and does not activate TLR4. Twice weekly intraperitoneal injections of 100 mcg of aCeAg protected against the development of type 1 diabetes in non-obese Diabetic (NOD) mice (80% T1DM in PBS-injected controls, vs 10% in aCeAg group). Histological analysis demonstrated twice as many pancreatic islets in aCeAg-treated mice (p<0.001) as well as greater numbers of uninfiltrated islets. As observed in studies using antigens from parasitic helminths, aCeAg treatments increased the levels of basophils, eosinophils, and polyclonal and helminth-specific IgE immunoglobulins. Further, we observed increased production of the suppressive cytokine IL-10 (p<0.05), but not of the pro-inflammatory cytokine IFN-gamma, from splenocytes of aCeAg-treated animals. This study demonstrates proof-of-concept that antigens obtained from the non-parasitic nematode C. elegans can be used to obtain the same immune responses, and same immunoprotective effects, as parasitic helminths. Given that C. elegans can be grown axenically in controlled conditions without the need of any intermediate hosts, aCeAg may be able to overcome many of the current obstacles facing helminthic therapies for inflammatory diseases.

1711
DIFFERENCES IN OV-16 ELISA IMMUNE RESPONSES AMONG CHIMPANZES INOCULATED WITH ONCHOCERCA VOLVULUS

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Onchocerciasis, also known as river blindness, is a neglected tropical disease caused by the filarial parasite Onchocerca volvulus. Detection of immunoglobulin G4 (IgG4) antibodies to Ov16 recombinant antigen is the basis for serological tests for onchocerciasis. However, the dynamics of this immune response have yet to be thoroughly characterized. A non-human primate model was used to evaluate the temporal evolution of antibody responses under controlled infection conditions. Nine chimpanzees were inoculated with third-stage larvae of O. volvulus. Three chimpanzees were inoculated with approximately 100 stage three larva (L3) of Guatemalan origin, either one, three, or five times. Six chimpanzees each were inoculated once with 200, 300, or 400 L3 of Liberian origin. On a monthly basis, serum was collected and the presence of microfilariae (Mf) was determined via skin snip microscopy. Seven of nine chimpanzees developed patent infections, and six were used to evaluate the temporal responses over a median number of 1,660 days post-inoculation (PI). The seventh chimpanzee with patent infection was not evaluated due to health complications and was withdrawn at 535 days PI. Infections were categorized based on average microfilariaemia of three consecutive dates as: weak (<10 Mf/snip), mild (≥10 and <20) and strong (≥20 Mf/snip). One chimpanzee had a weak infection, two developed mild infections, and three had strong infections. No positive IgG4 responses to Ov16 were detected in the two inoculated but uninfected chimpanzees. The mean time to develop IgG4 responses and detection of Mf were 414 and 485 days PI. Four chimpanzees showed decreases in IgG4 values towards the end of the study. In three of these chimpanzees, decreased IgG4 responses were detected with decreasing microfilariaemia. These findings indicate that positive serology to Ov16 occurs only among chimpanzees that developed patent infections, and suggest that anti-Ov16 antibody responses may decrease over time after reductions in detectable Mf loads in skin snips.

1712
CHARACTERIZING REACTIVITY TO ONCHOCERCA VOLVULUS ANTIGENS IN MULTIPLEX BEAD ASSAYS

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Onchocerciasis is a neglected tropical disease targeted for elimination in Africa by 2025. Multiplex assays can provide a powerful platform for monitoring and evaluation, as well as integrated surveillance of onchocerciasis and other co-endemic diseases; however, the specificity and sensitivity of O. volvulus antigens have not been well-characterized within this context. A multiplex immunoassay was developed and used to evaluate three antigens (Ov16, Ov17 and Ov33) for onchocerciasis. The performance of each antigen was characterized using a panel of 499 specimens. One hundred ten samples were positive for onchocerciasis by skin snip microscopy and PCR, while the 389 controls were from people living in areas where onchocerciasis was not endemic and had infection with Wuchereria bancrofti, Brugia malayi, Loa loa, Mansonia spp, Strongyloides stercoralis, Hymenolepis nana, cysticercosis, schistosomiasis, or other human pathogenic parasites. All samples were analyzed in duplicate for IgG and IgG4 reactivity. Receiver Operator Characteristics (ROC) analyses were used to determine optimal cutoffs for all antigens. High sensitivity and specificity were detected for Ov16 and Ov33, while the Ov17 assay had specificities below 80%, identifying 75 false positives.
among controls with lymphatic filariasis (LF). The Ov16 cutoff values for IgG or IgG4 were 379 and 32 fluorescent units (MFI), with sensitivities of 96.3 and 96.3% and specificities of 98.7 and 99.7%, respectively. For Ov33, a cutoff of 5,216 MFI in IgG reactivity resulted in 90.8% sensitivity and 97.2% specificity. The IgG4 cutoff was 67 MFI with a higher sensitivity of 96.3% and specificity of 98.5%. The IgG4 assay for both Ov16 and Ov33 detected few false positives, although the Ov33 assay detected 5 additional false positives among onchocerciasis-negative samples that were positive for either LF (3) or schistosomiasis (2). While no statistical difference was detected between the IgG and IgG4 assays for Ov16 and Ov33 (p>0.3), assays with the highest specificity and lowest cutoff values will help to ensure the ability of programs to monitor their work towards reaching desired elimination endpoints. Overall, Ov16 and Ov33 are highly sensitive and specific antigens in the multiplex platform. Further analysis of these antigens, either alone or in combination, may be useful for monitoring and evaluating progress towards the elimination of onchocerciasis.

1713

EVALUATION OF HLA IMMUNOINFORMATICS FOR THE IDENTIFICATION OF BRUGIA MALAYI PUTATIVE T CELL EPITOPES CONSERVED WITH Wuchereria bancrofti AND Loa loa

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The availability of filarial genome sequences and improved immunoinformatics tools promises to accelerate the identification of highly conserved and immunogenic filarial vaccine components. The work done in this study has taken advantage of the functional similarities, rather than genetic diversity, of HLA binding residues in efforts to further identify putative T cell epitopes as potential vaccine antigens to combat lymphatic filariasis (LF). Predictions were previously made using the IVAX website containing both the EpiMatrix and ClustiMER immunoinformatics tools. 20-mer peptide sequences were selected for peptide synthesis from proteins within the Brugia malayi secretome. The 20 sequences were selected based on predictions to bind up to 8 of the most common HLA alleles represented within the software toolkit: DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0801, DRB1*1101, and DRB1*1501. In silico filarial conservation analyses were done using basic local alignment tools within BROAD Institute’s Filarial Worms Database. These analyses identified cross conservation for 6 out of the 20 sequences that shared sequence identity between B. malayi and Wuchereria bancrofti and/or Loa loa.

Hookworm infection in school-aged Kenyan children is associated with lower physical fitness

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Children living in parasite-endemic resource-limited areas are heavily burdened by infection and the comorbidities associated with these diseases. Reduced physical fitness can result from anemia, undernutrition and chronic parasitic infections including soil transmitted helminths (STH), Entamoeba histolytica, Giardia lamblia, malaria and schistosomiasis. Our goal was to determine the prevalence of parasitic infections and their association with physical fitness as measured by the validated multi-stage 20 meter shuttle run (20mSRT) method. From January to March 2014, a cohort of 101 children aged 4-7 years in coastal Kenya was evaluated. At the visit, blood, stool, and urine were collected and tested for presence of infection as follows: blood smear for malaria, Ritchie stool examination for STH, E. histolytica, and G. lamblia, and urine filtration for S. haematobium. 20mSRT were scored based on level achieved. Descriptive statistics were used to estimate infection rates. Wilcoxon scores determined the association between each type of infection and 20mSRT level achieved. The cohort included 101 children with a mean age of 5.8 years, 53% male. 43% reached 20mSRT level 1, 48% reached level 2, 5% reached level 3 and 4% reached level 4. Age, sex, and hemoglobin level (mean 10g/dL) were not significantly associated with the shuttle run level achieved. Trichuris was the most prevalent parasitic infection (13%), followed by hookworm (7%), E. histolytica (7%), giardia (5%), malaria (4%), Acanthamoeba (1%), and schistosomiasis (1%). Hookworm infection was associated with a lower level achieved in the 20mSRT (p=0.002, 95% CI 0.014-0.113). Parasitic infections are common in school-aged children in coastal Kenya and may impair physical fitness. Hookworm infection, in particular, is associated with decreased physical fitness as measured by the 20mSRT.

1715

INFEERENCE OF PARASITE BURDEN FROM INDIRECT INTENSITY DATA AND IMPLICATIONS FOR STUDY DESIGN

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A substantial proportion of data collected in monitoring and evaluation (M&E) of macro-parasites records secondary indicators of the infection, such as egg counts or microfilaria, as a measure of parasite burden. Arguably, however, the most useful information for diagnosis, estimation of morbidity and for understanding the transmission dynamics of disease are quantities such as mean adult parasite burden and levels of aggregation of this burden within the population. Since adult worm counts are often very difficult to obtain, and we have only a few studies from which to estimate the relationship between adult worms and transmission stages, we need robust statistical descriptions of the distribution of measured quantities, such as egg counts, in terms of basic parameters of parasite biology. These will enable reliable inference of quantities such as mean worm burden, parasite aggregation and density-dependent processes from standard M&E data. We have developed a method for doing this calculation using extensive egg count and worm count data to identify and parameterize models of the distribution of Acanthamoeba and microfilaria. These models allow us to make robust predictions for the measurement of the effect of interventions on worm burden. These results show that egg counts are best described by a negative binomial distribution with mean egg production from individual subject to an exponentially decreasing fecundity. Models of this kind will allow the optimal use of available data sources to extract reliable estimates of...
basic biological parameters and their associated confidence intervals. This has strong implications for the tailoring of study design and choice of diagnostic techniques to optimize information gained against cost incurred. As an example, we discuss the reappraisal of M&E as elimination is approached and egg intensities fall.

1716

EFFECT OF A SINGLE DOSE OF 8 MG MOXIDECTIN OR 150 \( \mu \)G/KG IVERMECTIN ON INTESTINAL HELMINTHS IN PARTICIPANTS OF A CLINICAL TRIAL CONDUCTED IN NORTHEAST DRC, LIBERIA AND GHANA

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In the Phase 3 study comparing the effects of a single dose of 8 mg moxidectin vs. ivermectin standard dose (150\( \mu \)g/kg) on \( O. \) volvulus skin microfilariae levels and participant well-being in 941 males and 531 females, including 79 children 12-17 years, participants were randomized in a 2:1 ratio to moxidectin : ivermectin. 1465 participants underwent a single sample Kato-Katz test at screening. 876/1465 (60\%) were infected with \( \geq 1 \) type of intestinal helmint: Ascaris lumbricoides 44 (3\%), Trichuris trichuria 22 (1.5\%), hookworm 783 (53.4\%), Schistosoma mansoni 233 (15.9\%), Strongyloides 4 (0.3\%). 835 with \( \geq 1 \) type of intestinal helmint were treated with either moxidectin or ivermectin and tested again 1 month later with a single sample Kato-Katz test. Results obtained are expressed as cure rate (CR) and egg reduction rate (ERRam) using arithmetic mean egg counts post treatment relative to arithmetic mean egg counts pretreatment (EPG) for each species. Results for ivermectin treated subjects: \( A. \) lumbricoides \( n = 10 \), EPG = 408, CR = 100\%, ERRam = 100\%; \( T. \) trichuria \( n = 6 \), EPG = 2856, CR = 83\%, ERRam = 76\%; hookworm \( n = 259 \), EPG = 842, CR = 29\%, ERRam = 52\%; \( S. \) mansoni \( n = 67 \), EPG = 236, CR = 54\%, ERRam = 73\%. Results for moxidectin treated subjects: Ascaris lumbricoides \( n = 26 \), EPG = 408, CR = 97\%, ERRam = 97\%; T. trichuria \( n = 11 \), EPG = 1409, CR = 91\%, ERRam = 99%; hookworm \( n = 491 \), EPG = 601, CR = 48\%, ERRam = 82%; \( S. \) mansoni \( n = 143 \), EPG = 168, CR = 64\%, ERRam = 66\%. Co-administration of either ivermectin or moxidectin with drugs like benzimidazoles and/or praziquantel may help achieve high efficacy in preventive chemotherapy programmes for soil-transmitted nematodes and schistosomiasis.

1717

EPIDEMIOLOGY OF ANTHELMINTIC TREATMENT FAILURE IN HOOKWORM (NECTAR AMERICANUS) INFECTIONS IN CHILDREN IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. II. BASELINE PARASITOLOGY DATA

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Previous studies in multiple communities in the Kintampo North Municipality (KNM), Ghana in 2007 revealed a high prevalence of hookworm infection (45\%) and albendazole (single dose, 400mg) treatment failure (39\%) school age children. Subsequent study in 2010 confirmed the high prevalence of hookworm (39\%) and an even higher rate of albendazole treatment failure (56\%) in this population. The overall goal of an NIH-funded longitudinal study is to fully characterize the epidemiology and molecular basis of benzimidazole treatment failure in KNM. Parasitological, anthropometric, socioeconomic, nutritional and immune status data were obtained from a cohort of consented 273 school-aged children (48.1\% females and 51.9\% males). Fecal samples, demographic data were also obtained from three consented household members of participants in two communities. We report here, the baseline parasitology results. Stool and blood samples were analyzed for intestinal helminths (Kato-Katz) and malaria (RDT and microscopy) infections, and PCR used to identify the hookworm species. All the participants gave fecal samples and 260 donated blood, and those found infected treated with albendazole. Fifty-eight (21.2\%) were infected and 16 of 46 (34.7\%) failed treatment and the overall cure rate was 61.7\%. The geometric mean of intensity infection was 376.1epg (± 890.5) at pre-treatment and was 57epg (± 46.3) post treatment. The fecal egg count reduction rate was 81.2\%. Sixty nine hookworm specimens were all identified as Necator americanus. 76.54\% (199/260) and 67.7\% (176/260) were positive by RDT and microscopy respectively. Geometric mean of Plasmodium falciparum intensity was 1377.3 (± 3623.9) parasites/ml of blood. 16.6\% (45/173) participants were co-infected with both parasites. Hookworm and malaria co-infection rate was 17.3\% (45/260). The cross sectional survey revealed hookworm prevalence of 33.3\% (46/138) among household members, and 56.9\% (29/51) of households, and the percent positive child with at least one household member positive was 73.3\% (11/15).

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EPIDEMIOLOGY OF HOOKWORM (NECTAR AMERICANUS) INFECTIONS IN CHILDREN AND ANTHELMINTHIC TREATMENT FAILURE IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. III. EVALUATION OF NUTRITIONAL RISK FACTORS AT BASELINE

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An ongoing NIH-funded longitudinal study of school-age children aims to fully characterize the epidemiology of hookworm in the Kintampo North Municipality (KNM) where previous studies have found high albendazole treatment (400mg) failures in children. This arm of the study is to investigate the influence of modifiable host factors including overall nutritional status,food diversity as risks factors of hookworm infestationat baseline. Structured questionnaires and food frequency questionnaires were used to collect data from 271 study participants. Haemoglobin concentration, weights and height z- scores were used to determine nutritional status of the study participants, WHO criteria was used to determine food security and food diversity. Fifty-eight participants (21.2\%) and sixteen out of 46 studied participants (34.8\%) were hookworm infected pre- and post-treatment respectively.
The overall mean Hb levels (anemia defined as <12.0g/dl) was 11.47g/dl (±1.28), 11.50g/dl (±1.25) and 11.35g/dl (±1.38) for negative and positive cases, respectively. In conclusion, most of the study participants were anemic, underweight, stunted and consumed less diverse food. Moreover, infected children were significantly underweight than their non-infected counterparts.

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COMPARING METHODS TO ASSESS SCHISTOSOMA RESPONSE TO PRAZIQUANTEL TREATMENT

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To diagnose Schistosoma infection, stool or urine samples are examined for worm eggs, but there is no definitive agreement as to how best express treatment efficacy. We analyzed data from 24 trials conducted in Africa, Asia and Latin America enrolling overall 4740 individuals infected with S. mansoni (Sm, n=1804; 38.1%), S. haematobium (Sh, n=2633; 55.5%), or S. japonicum (Sj, n=303; 55.5%), and treated with praziquantel at doses of 40 (n=3713), 60 (n=690) and 80 (n=337) mg/kg. Efficacy was measured using cure rate (CR) and egg reduction rate (ERR) calculated using either geometric or arithmetic means (ERRgm, ERRam). We compared efficacy outcome measures: 1) ERRam vs. ERRgm, 2) ERR vs CR, and 3) ERR and CR based on quadruplicate vs single Kato-Katz thick smear examination for Sm. We found that: 1) ERRam and ERRgm can be used interchangeably only if treatment efficacy is very high (>95%); as efficacy falls, estimates are higher with ERRgm than ERRam. Modeling data shows that consistency between means is better for Sm and Sj than for S; 2) poor correlation between ERRgm/am and CR except when ERRs are very high (>97%); 3) using a single rather than quadruplicate Kato-Katz thick smear excluded 19% of Sm-infected individuals; the effect on estimating ERR was negligible by individual studies; however, on aggregate ERRam and CR were 8-9% higher (no effect on ERRgm). A valid complement for drug efficacy monitoring is to study the distribution of individual responses to identify suboptimal responders. Of the 2358 Sm-infected individuals with complete data records 61.3% were negative post-treatment (cure rate, CR), 32.4% had reduced egg counts (rEC), 6.3% had no change/increased egg counts (nEC). For Sm (n=1699) individuals CR was 75.4%, rEC 20.5%, nEC 4.1%. For Sj (n=300) CR was 90%, nEC 8.3%, nEC 1.7%. The response achieved by the 5th centile (the 5% worse responders) was 79.1%, 77.9%, and 23.6% for Sj, Sh and Sm; for the 10th centile it was 100%, 88.2%, and 70.3%, and for the 25th centile 100%, 97.8%, and 100%, respectively.

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EPIDEMIOLOGY OF HOOKWORM (NECATOR AMERICANUS) INFECTIONS IN CHILDREN AND ANTHELMINTIC TREATMENT FAILURE IN THE KINTAMPO NORTH MUNICIPALITY, GHANA. I. BASELINE INDICATORS OF EPIDEMIOLOGY AND SOCIOECONOMIC RISK FACTORS

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In 2007 and 2010, high prevalence rates of hookworm infections (45%) and (39%) respectively in school age children was observed in the Kintampo North Municipality (KNM), Ghana. Also observed were high rates of albendazole (single dose 400mg) treatment failure; (39%) and (54%) respectively. As part of an NIH-funded study which aims to fully characterize the epidemiology and molecular basis of benzimidazole treatment failure in KNM, a census/enumeration survey was conducted in eight communities in KNM, then 271 children aged between 8 and 12 years were randomly selected for a longitudinal study. To define the epidemiology of hookworm infection at baseline and the specific host factors associated with albendazole treatment failure, demographic, socioeconomic and environmental information was collected using structured questionnaires. The cohort comprised of 138 males and 133 females. The cohort’s mean age was 9.49 (± 1.69). The overall hookworm prevalence was 21.4% (58/271), of which 67.2% of infected children were the group 10 years and above. Significant associations were found between hookworm infections and possession of cattle and dogs (P=0.049 and P=0.029), ownership of shoes (P=0.015) and wearing shoes daily (P=0.020). No significant associations were found with gender, age, access to agricultural land, and types of water sources and toilet facilities (P>0.05). No associations were also found between treatment response and any of the socio-economic parameters (P>0.05).

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RICE FORTIFIED WITH IRON AND OTHER MICRONUTRIENTS IMPACTS HOOKWORM INFECTION RISK IN SCHOOLCHILDREN

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Soil-transmitted helminth (STH) infections often co-exist with micronutrient deficiencies in the developing world. There is evidence that these major health issues can influence and exacerbate each other. Fortification of staple foods such as rice can be an effective tool to improve micronutrient status of vulnerable groups, but its impact on STH infection is currently unclear. In a cluster randomized, placebo-controlled, double-blinded trial, 3 different types of fortified rice were introduced through the World Food Program (WFP) School Meal program in Cambodia. The 3 types of fortified rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no school meal (control) for 7 months. Stool samples were collected and analyzed by Kato Katz method at baseline, 3 months and 7 months. After baseline, all children received a single dose of 400mg albendazole.
The effects of consumption of fortified rice on hookworm infection were analyzed by multiple logistic regression. Baseline prevalence of STH was 17.0%, which were mainly hookworm infections (16.6%) of light intensity. A risk factor for hookworm infection was being a boy (P=0.011). After 7 months (n= 1236 children), hookworm infection prevalence was between 17-24% in control, placebo and in the NutriRice fortified rice groups. In the children receiving 2 types of UltraRice fortified rice, hookworm prevalence was 33-34% (P<0.001). The new infection rate was highest in the UltraRice group with the highest iron content (24.6%), intermediate in the UltraRice group with less iron (21.8%), and lowest in the control and placebo and groups (12.5% and 11.9%, P=0.001 for difference among groups). Fortifying rice with micronutrients, especially iron, can increase risk of hookworm infection. Type of fortificant appears to be a major effect modifier. These findings have big implications for policies aiming to improve child health and nutritional status in tropical regions.

**How does the scale of deworming programs affect their cost-effectiveness?**

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The predominant control strategy for the soil-transmitted helminths (STHs) is regular periodic mass drug administration (MDA), targeting school-aged children. The World Health Organization (WHO) and London declaration on Neglected Tropical Diseases (NTDs) has set goals to scale up MDA, so that by 2020, 75% of the pre-school and school-aged children in need, will be treated regularly. It has been observed that increasing the number treated can reduce the per capita costs of MDA programmes (economies of scale). This is because a number of the costs associated with MDA delivery are fixed (i.e. do not depend on the number treated), and therefore increasing the number treated reduces the average fixed cost per treatment. However, the implications this has on the cost-effectiveness of scaling up control for STH infections, and the optimum treatment strategy have not been explored. We developed costing functions which account for the changes in the per capita costs of treatment with scale, and incorporated them into STH dynamic transmission models. We found that, due to these economies of scale, the cost effectiveness of STH control programmes markedly increased with the number treated. This has notable implications for programmes considering scaling up MDA, in line with the current goals set by the London declaration.

**The effect of seasonality on the prevalence of Ascaris lumbricoides and implications for the optimal timing of mass treatment programs**

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Previous studies have shown that changes in temperature affect maturation times, development proportions, and mortality rates of Ascaris lumbricoides and suum eggs, which suggests that climate could impact both overall prevalence and reinfection rates after treatment. Depending on the size of this effect, this could have implications for the optimal time of year for mass treatment in order to get the maximum impact out of the large number of donated drugs. However, there has been little quantitative investigation of the influence of seasonal timing of mass chemotherapy on treatment programmes. Using historical data from experimental studies on A. suum eggs – which have been shown to display equivalent behaviour to A. lumbricoides eggs – to characterise relationships between temperature and egg maturation and survival, models were developed which investigated the effect of temperature dependent development of eggs on mean worm burden in the human population. These reveal fast maturation and low egg mortality at high temperatures, but also a drop in proportion of eggs reaching maturity above 30°C. To demonstrate implications for the optimal timing of mass treatment campaigns, models were applied to districts with differing levels of A. lumbricoides transmission across Kenya. District-level prevalence estimates were generated using predictive risk maps developed by the Global Atlas of Helminth Infection and monthly temperature patterns were derived from MODIS. Results suggest timing of treatment campaigns could have important consequences for programme impact. Depending on region, changing the treatment date resulted in an estimated 18-55% comparative decrease in prevalence after four yearly treatment rounds. This highlights the potential importance of appropriate timing of the established Kenya National Deworming Programme. More generally, this approach provides insights into the epidemiology of A. lumbricoides infection, methods for testing and validating these predictions, and can help guide optimal long-term helminth control strategies in diverse settings.

**A novel next-generation sequencing approach to develop improved molecular diagnostics for the detection of soil transmitted helminth infections**

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The soil transmitted helminths (STHs) are a group of parasitic worms responsible for causing extensive morbidity in many of the world’s most economically depressed locations. With an estimated 880 million children infected with one or more species of STH parasite, accurate and cost-effective diagnostic measures are of premier importance to global control and elimination efforts. Accordingly, the use of molecular
diagnostic measures, such as real-time PCR, has shown great promise for the improved detection of STH infections. To date, such molecular assays have utilized ribosomal and mitochondrial target sequences, as previously reported. While effective, such sequences are frequently not the highest copy number target and will not yield the most sensitive assay possible. The most sensitive assay will utilize the most highly repetitive, unique, non-coding DNA sequences found within the genome of each species. Consequently, we have coupled next-generation sequencing technology with the Galaxy-based software RepeatExplorer, to identify the most numerous, non-coding DNA sequences within multiple species of STH parasites including Trichuris trichiura, Necator americanus, Ancylostoma duodenale, and Ascaris lumbricoides. Following the application of this approach to each STH species, we designed TaqMan-based primer-probe combinations for each candidate sequence. Species-specificity was verified for each assay and repeatable detection of genomic DNA isolated from each parasite was demonstrated at concentrations ranging from 1.0ng to 1.0fg. Through this novel approach to the identification of species-specific, high copy-number target sequences, we have developed a new strategy for the design of a PCR-based diagnostic assay with improved sensitivity.

**THE ROAD TOWARDS EFFICIENT 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 disease affecting approximately 200 million people worldwide. Since a long time, schistosomiasis has been known to be endemic in certain provinces of the Democratic Republic of Congo. However, the most recent national data available on schistosomiasis prevalence and distribution were published in the early sixties. Recently, the Ministry of Health adopted a national plan against schistosomiasis aiming at to distribute Praziquantel (PZQ), the treatment of choice for schistosomiasis, to all individuals infected. For effective introduction of control strategies, data on national prevalence and distribution of schistosomiasis in the DRC urgently need to be updated. The present study assessed the knowledge of health workers on schistosomiasis as well as the availability of the facilities needed for adequate diagnosis and management of the disease in the endemic provinces of Kinshasa and Bas-Congo in the DRC. This study was conducted in 9 health zones (HZ) of Kinshasa and 2 HZ in Bas-Congo. Health workers could name all symptoms of schistosomiasis. Kato-Katz, urine filtration or sedimentation were not available as diagnostic methods in any health facilities. Diagnosis therefore almost solely relied anamnesis. The knowledge on schistosomiasis did not differ between the rural Bas-Congo and urban Kinshasa. The fees for consultation, diagnostics and treatment were three times higher in Kinshasa than Bas-Congo. Health workers in Kinshasa and Bas-Congo are able to name the symptoms related to schistosomiasis. However there is a lack of availability of adequate diagnostic tools and treatment. The fees of diagnostics and treatment are high for a population often living in extreme poverty.

**GUT INSTINCTS: EVALUATING PARENTAL ATTITUDES TOWARD INTESTINAL WORM TREATMENT IN RURAL CHINA**

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Nearly forty percent of elementary schoolchildren in rural provinces of Southwest China are infected with soil-transmitted helminths. However, parasitic worm infection is neglected as a major public health problem in these villages and deworming treatment is rarely sought, despite its efficacy and low cost (one treatment dosage costs less than 3 cents USD). Surveys and interviews were conducted in six rural villages in Guizhou, China to evaluate what factors influence parental decisions to seek or not seek deworming among rural Chinese schoolchildren. It was found that knowledge about helmint infection and prevention was severely lacking and often influenced by deep-rooted myths, such as the local belief that deworming medicine can harm a child’s future fertility. The majority of household interviewees were highly skeptical of high worm prevalence in their children, despite the nearly universal practice of regularly deworming their pigs. A comprehensive deworming program involving biannual administration of deworming treatment, household health education, and village health system strengthening is necessary to effectively mitigate the disease burden of helminth infection in rural China.
Humans can be chronically infected with Strongyloides stercoralis for decades because of the mechanism of autoinfection. Although most infected individuals are asymptomatic, S. stercoralis is capable of transforming into a fatal illness in settings of HTLV-1 and steroids. Chronic infection with S. stercoralis presents both diagnostic and therapeutic challenges. Parasitologic diagnosis of chronic infection is difficult, because the larval output can be low and irregular. The diagnostic accuracy of serologic testing has a sensitivity and specificity of 93% and 95%, respectively. Ivermectin is the treatment of choice for this parasitic infection, although optimal dosing is yet to be determined. This study evaluated the serologic response to S. stercoralis infection after treatment with ivermectin 200 mcg/kg given 2 weeks apart based on the auto-infective cycle (Group A) vs. given on two consecutive days (Group B). Patients were referred from outpatient clinics or identified on the in-patient services and invited to participate in the study. Participants were randomized to either treatment arm and repeat serologies were performed at 3 months intervals for 9 months after treatment. Forty-seven cases were enrolled, mean age was 54.1 (SD 15.2), 61.7% male, 55.3% Hispanic, mean eosinophil count 0.55x10^3/μL (SD 0.53), IgE 608.0 mg/dL (SD 680.4) and HTLV-1 was negative in all cases. There were no significant differences in baseline demographic or clinical variables between the two groups. Of the 47, 51.0% had completed the 9 months follow-up. Mean eosinophil count (p=0.002) and IgE value (p=0.045) both decreased after treatment of cases in both treatment groups. Of the 47 patients, 9 cases remained sero-positive on follow-up; five (22.7%) in Group A and four (16.0%) in Group B (p=0.751). In this randomized controlled trial, there was no difference in serologic outcome in the two treatment arms, but treatment resulted in decreased eosinophil and IgE values.

**EMERGENCE OF A TROPICAL DISEASE IN U.S. DOGS: A PROSPECTIVE STUDY OF LEISHMANIA IN U.S. FOXHOUNDS**

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Leishmania is the causative agent of leishmaniasis, a deadly protozoan disease which affects approximately 12 million people worldwide. Generally associated with tropical and subtropical regions, Leishmania can be found as far north as the United States. Similarly, the vector for Leishmania transmission, the sand-fly, has been found within areas as far north as Missouri and Ohio. Leishmania infantum, infects canines as well as humans. In endemic areas dogs serve as the domestic reservoir. Within the United States, foxhound hunt populations have developed endemic disease. The first documented foxhound case of Leishmania in the US was reported in 1980. Leishmaniasis is a chronic disease with long latency, diagnosis can be difficult with gold standards limited to invasive techniques including bone marrow aspirate with culture. Serological testing has been used as a diagnostic technique but has cross reactivity with Trypanosoma cruzi also found in the southern U.S. To help with diagnosis, a highly sensitive and specific real time quantitative polymerase chain reaction (RT-qPCR) assay was developed. While this assay is capable of identifying parasite DNA within the peripheral blood it lacks the ability to determine whether the Leishmania is actively proliferating. Overall the course of the last 8 years, diagnostic testing using PCR increased. Cases of Leishmaniasis can be tracked throughout regions of the US within the foxhound hunt populations. This study is the first to report changes in Leishmania prevalence and incidence over a six-year span (2007-2012) within US foxhound hunts. Trends in infection over time and across regions.
were examined. *Leishmania* infection has stayed consistent over time with a point prevalence in 2007 of 3.54 per 1000 foxhounds and 3.23 per 1000 foxhounds in 2012. Incidence rates over the 5-year period began at 4.77 per 1000 foxhounds in 2007 and ended at 3.08 per 1000 foxhounds in 2012. The consistent prevalence and incidence of this infection stresses the need for appropriate risk management and disease prevention techniques in this community.

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**MATERNAL TRYPANOSOMA CRUZI INFECTION AND INFANT GROWTH**

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Maternal *Trypanosoma cruzi* infection and subsequent congenital transmission is a serious, yet neglected, global health issue. Infant growth assessment can provide an understanding of the health of children and an indirect evaluation of the quality of life for an entire population afflicted with Chagas disease. The objective of this study was to determine if maternal *T. cruzi* infection indirectly affects a newborn’s physical development. Infected mothers and their infants (*n=153*) were followed at birth, 4-8 weeks and 10 months post-partum in Tucuman, Argentina from April 2011 until April 2013. Age- and sex-specific estimates of infant weight, length, weight-for-length, and head circumference were compared to an international child growth standard. All mean z-scores were between ±1 standard deviations (SD) of the standard. However, the prevalence of infants falling below -2 SD of the WHO standard peaked at 16% for weight-for-age for females, 10.9% for length-for-age in males and 10.8% for weight-for-length in females, all at visit 1. Infants who experienced growth faltering were more likely to be female and weighed 0.8 kg less at birth, 0.9 kg less at 4-8 weeks and 0.5 kg less at 10 months of age. They also were 3 cm shorter and had a 2 cm reduction in head circumference. This analysis provides evidence of progressive stunting over the 10 month period and early failure to thrive with improvement by 10 months. Nutrition and health interventions, as well as socioeconomic changes, may be helpful in improving the growth and development of infants from Chagas affected populations.

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**COMPARISON BETWEEN PATIENTS WITH CLASSICAL AND ATYPICAL PRESENTATIONS OF CUTANEOUS LEISHMANIASIS, FROM AN AREA OF LEISHMANIA (VIANIA) BRAZILIENSIS TRANSMISSION IN NORTHEAST BRAZIL**

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The purpose of this study was to compare clinical, immunological and parasitological aspects between patients with atypical cutaneous leishmaniasis (ACL) and classical cutaneous leishmaniasis (CL) of a region with high endemcity for American tegumentary leishmaniasis (ATL) in northeast Brazil. Fifty one ACL and an equal number of CL patients were enrolled. ATL was confirmed and parasite species was determined by PCR of lesion biopsies. For each patient clinical data was annotated, peripheral blood was collected, and parasite isolation was attempted. Cultured parasites were genotyped according to the sequence of a locus in chromosome 28, previously shown to be polymorphic in this population of *L. (V.) braziliensis*. All cases had their living sites geographic coordinates acquired by GPS then distributions of ACL and CL cases were compared by the Cusick and Edward’s test (CE). Among ACL included there was no pregnant women or HIV positive subjects. ACL presented the same distribution as CL patients in the affected region (CE *p* = 0.26), but had a greater proportion of lesions above the waist line (94% in ACL x 33% in CL, *p* = 0.0001) and of failure to antimony treatment (41% in ACL x 0% in CL, *p* = 0.0006) than CL individuals. Immunologically, ACL showed lower production of TNFα (average 316.5 pg/ml in ACL x 1906.1 pg/ml in CL, *p* = 0.0001) and IFNγ (average 747.1 pg/ml in ACL x 4445.9 pg/ml in CL, *p* = 0.0002), but higher IL-10 (average 392.8 pg/ml in ACL x 171.9 pg/ml in CL, *p* = 0.0006) and IL-17 (average 218.4 pg/ml in ACL x 69.4 pg/ml in CL, *p* = 0.0008) after in vitro stimulation of peripheral blood mononuclear cells with leishmania antigen than CL patients. All subjects were infected with *L. (V.) braziliensis*, but parasites from ACL presented genotypes that were not found in isolates from CL individuals. Therefore, in the region studied, patients with ACL consist in a more homogeneous group of individuals than originally suspected, and are distinct from classical CL regarding treatment outcome, immune response and causative strain of *L. (V.) braziliensis*.

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**MODELING ECO BIO-SOCIAL DETERMINANTS FOR HOUSEHOLD INVASION OF SYLVATIC TRIATOMA DIMIDIATA IN NORTHERN BELIZE**

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Initial reports have confirmed presence of *Triatoma dimidiata*, an important Chagas disease vector throughout northern Belize. To date, *T. dimidiata* remains the sole vector species reported from this Central American country, yet much of the disease transmission dynamics remain unclear. Here, we report updated infection rates of the vector population as well as infestation rates for villages in north and central Belize. In order to further characterize the epidemiological risk of human-vector contact, Over 225 households have been surveyed and characterized with respect to 30 key determinants related to the probability of household infestation by *T. dimidiata*. These key variables included: presence of domestic animals, distance of household to village periphery, and proximity of community light sources. The infestation behavior of *T. dimidiata* in Belize is confirmed to be distinct from what would classically be designated a domiciliated vector population. Risk factors reported here can be used to guide integrated control efforts to reduce infestation and limit human-vector contact.
incidence will align more closely. This will facilitate understanding the epidemiology of this disease and result in more focused and successful control and prevention strategies.

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**TRYPANOSOMA CRUZI AND OTHER TRYPANOSOMATIDS IN COMMONLY HUNTED WILD MAMMALS FROM REMOTE LOCATION OF THE PERUVIAN AMAZON**

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Chagas disease is generally transmitted by contact with the feces of triatomine bugs infected with Trypanosoma cruzi, but oral transmission has also been documented. We evaluated the prevalence of T. cruzi and other trypanosomatids in four orders of mammals hunted for subsistence to better understand the risk of infection associated with human consumption and contact. Blood samples from wild mammal species were collected on filter papers by subsistence hunters from a remote small community in Loreto, Peru, bordering Brazil. DNA was isolated from filter papers and amplified using a nested-PCR targeting the 24S alpha subunit rRNA gene. Primers D75/D76 and D71/D72 were used to amplify specific regions of trypanosomatids and T. cruzi, respectively. Comparisons of prevalence between orders were performed using Chi-Square and Fisher’s test. A total of 142 mammalian blood samples from four orders (10 species) were tested: Carnivora (n=34), Edentata (n=24), Artiodactyla (n=28) and Rodentia (n=56). The prevalence of T. cruzi in Carnivora (18%) was significantly higher (p=0.008) compared to other orders (0% - 4%). The prevalence of trypanosomatids ranged from 7% in Carnivora to 27% in Rodentia with no significant differences (p=0.180), possibly due to the small sample size. Nasua nasua (ring-tailed coat), Dasypus novemcinctus (nine-banded armadillo), Agouti paca (spotted paca) and Tayassu tajacu (collared peccary) accounted for 89% of the samples and all positive animals. Among these four species the prevalence of T. cruzi was 19%, 4%, 2% and 0%, respectively (p=0.016); and the prevalence of trypanosomatids ranged from 9% to 31%. The high prevalence of T. cruzi in Nasua nasua, a type of racoon, suggests the importance of carnivores in sylvatic T. cruzi transmission. The multiple hunted species infected with trypanosomatids highlights the risk of human infection by consumption of improperly cooked meat.

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**PREVALENCE OF TRYPANOSOMATIDS AND TRYPANOSOMA CRUZI IN WILD AND CAPTIVE NON-HUMAN PRIMATES FROM PERÚ**

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Non-human primates (NHPs) can be infected with Trypanosoma cruzi, the etiological agent of Chagas disease, and other trypanosomatids. Primates may act as reservoirs and close contact between humans (traders, owners, hunters, zookeepers, etc.) and NHPs is a potential risk of accidental infection. We compared the prevalence of T. cruzi and other trypanosomatids in wild and captive Peruvian NHPs to assess this risk. Blood samples were obtained from captive NHPs (n=192) at zoos, wildlife rescue centers, wet markets and households in six Peruvian cities, and wild NHP hunted for subsistence (n=126) in two remote communities in the Peruvian Amazon. Blood smears from 88 captive NHPs were stained with Giemsa and examined by microscopy. Samples were collected from 318 NHPs on filter paper, FTA cards or EDTA tubes and tested with a nested PCR protocol using primers for the 24S alpha subunit rRNA gene. Primers D75/D76 target the conserved flanking sequences of the D7 alpha domain in trypanosomatids, while primers D71/D72 target a region in the same domain that is specific to T. cruzi. PCR was used as gold standard to calculate the sensitivity and specificity of microscopy. Trypanosomatid and T. cruzi prevalences were compared using Chi2 and Fisher’s exact tests. We studied captive NHPs from five families (14 species) and wild NHPs from three families (11 species). Wild NHPs had significantly higher prevalence of both trypanosomatids (56% vs 27%, p<0.001) and T. cruzi (9% vs 3%, p=0.034), compared to captive NHPs. Pitheciae had the highest trypanosomatid prevalence (18/20, 90%) and Cebidae had the highest T. cruzi prevalence (14/16, 12%). Captive NHPs from wet markets (n=38) had very high trypanosomatid (53%) and T. cruzi (13%) prevalence. Compared to PCR, microscopy was 83% sensitive and 98% specific. T. cruzi and trypanosomatids are common in Peruvian NHPs and pose a risk to human and animal health that has not been properly studied. Although microscopy is poorly sensitive compared to PCR, it may still be useful for screening in the field.

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**CHAGAS DISEASE, POVERTY AND BIODIVERSITY IN MEXICO**

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Since 1928 Chagas disease (ChD) has been studied in Mexico. Official data from the National Council for the Evaluation of the Politics for Social Development (Consejo Nacional de Evaluación de la Política de Desarrollo Social) [CONEVAL] in 2012 shows that poverty is 50% in Mexican population out of the 110 M people (2010 national census: National Institute of Statistic and Geographical Information) [INEGI], from this, 15% is under extreme poverty. In the Southern region, Mexico has high level of poverty and the highest biodiversity richness in the country, which is currently under serious ecological threatening. There is evidence that reduced biodiversity affects the transmission of infectious diseases in humans and other animals. This situation matches perfectly in maps with the high prevalence of ChD in that region. The importance of this work is related to the role of multinational control initiatives against ChD. In some way ChD is a neglected disease in Mexico. This situation is also relevant giving the immigrant phenomenon between Mexico and the US. In this work we present data and geographical evidence that even the great number of Mexican scientists working on ChD, this zoonotic parasitic disease is still under estimation. Back in 2006 we published information about ChD in Mexico form our data base “CHAGMEX”, now we are working on a new data base (2004-2014), and so far, the new bibliographic information shows that the number of human cases is increasing considering all clinical and epidemiological forms of the disease: vectorial transmission, blood transfusion, congenital transmission. From the 32 species of Triatominae identified in Mexico, more than 10 are reported with domestic habits. Also Trypanosoma cruzi is becoming quite common in domestic dogs, from urban and rural areas. We think that besides academic research, is urgent to implement vector control programs by each climatic region of Mexico, along with a wide epidemiological and socio-economical approaches of ChD in Mexico.
1740

MAPPING THE PREVALENCE AND CASE DETECTION RATES OF GAMBINESE HUMAN AFRICAN TRYPANOSOMIASIS

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Recorded human cases of gambiense human African Trypanosomiasis (HAT) have undergone a remarkable decline over the past decade, prompting plans for elimination of the disease. Effective planning for this elimination programme will require accurate and reliable spatial information on the contemporary distribution of the disease. The Atlas of HAT, recently established by WHO and FAO, aims to map the locations of all known HAT cases. These data provide an unparalleled resource for spatial risk assessment. However, under-reporting of cases and spatial variation in reporting rates complicate their interpretation and reduce their utility for continental-scale planning. To overcome these issues, a collaboration between the Spatial Ecology and Epidemiology Group (Oxford), WHO and FAO is developing a spatial modelling framework to simultaneously map the prevalence of gambiense HAT cases and the probability of detection of cases through the passive reporting system. In order to construct this framework, a novel Bayesian spatio-temporal joint model has been developed to integrate data from both active and passive case detection. The modelling framework will be outlined and preliminary results presented for the first time.

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THE HEALTH IMPACT OF VISCERAL LEISHMANIASIS AND HUMAN AFRICAN TRYPANOSOMIASIS WHEN REACHING THE 2020 WHO CONTROL AND ELIMINATION TARGETS

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Visceral leishmaniasis (VL) and human African trypanosomiasis (HAT) are neglected tropical diseases (NTDs). The London Declaration was established in 2012 to support the WHO control and elimination targets for ten NTDs by 2020. This initiative intends to globally generate a positive health and socioeconomic impact by decreasing and eliminating the disease burden caused by these NTDs. In our study the global health impact for VL and HAT is calculated for the ideal situation that the 2020 targets are met. The global burden of disease (GBD) study provides prevalence data for VL and HAT in 1990 and 2010. The 2020 targets, as formulated in the WHO Roadmap, provide the ideal situation: control for VL (100% detection and treatment at global level, and 1/10,000 new cases at subdistrict level per year on the Indian subcontinent) and elimination of HAT by 2030. Linear trends between 1990, 2010, 2020 and 2030 provide a simplification of the real situation, representing the number of remaining cases with disease, per country, age group and sex. Continuing the 1990 prevalence until 2030, corrected for demographic changes based on UNPOP data, serves as baseline situation without interventions. The difference between the baseline and the remaining cases results in the number of averted cases. The total number of averted years lived with disability (YLD) is calculated by multiplying the number of averted cases with the GBD disability weights. The total number of averted disability adjusted life years (DALYs) between 2010 and 2030 results in app. 140 and 100 million DALYs for VL and HAT, respectively. The DALYs are almost completely determined by the number of years of life lost (YLLs). The number of averted deaths over these two decades is 2.4 million and 1.7 million for VL and HAT, respectively. Although there have been many successful interventions for VL and HAT, it is important to emphasize the need for continuation and even increase of these efforts, especially when recognizing the sizeable health impact that can be gained when achieving the 2020 targets.

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ANTI-LEISHMANIA ANTIBODIES IN BLOOD DONORS FROM BRAZIL USING RECOMBINANT L. INFANTUM PROTEIN K39 ELISA

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In Brazil, visceral leishmaniasis (VL) caused by Leishmania infantum has a wide geographic distribution throughout the country with 3,894 reported cases in 2011, 47% in the Northeast region. Most of L. infantum-infected individuals (around 95%) are asymptomatic and may be undetected and accepted as blood donors in endemic areas. Since leishmaniasis can be transmitted through blood transfusion, this study aimed to investigate the presence of anti-L. infantum K39 antibodies in blood samples from Brazil endemic areas. We used ELISA-rK39 (rK39 antigen kindly provided by Infectious Disease Research Institute, USA) that yielded 95.45% sensitivity, assaying 44 sera from parasitologically confirmed symptomatic VL patients with positive Direct Agglutination Test (DAT) and 100.0% specificity assaying 44 healthy endemic DAT negative control samples. The present study was carried out with 916 blood samples from Brazilian Northeastern states, Bahia (N=604) and Ceará (N=312). Anti- rK39 antibodies were detected in 26 out of 916 samples (2.8%): Bahia (2.8%) and Ceará (3.0%). The reactivity index (RI = absorbance/cut-off) varied from 1.010 to 6.756. Immunochromatographic rK39 test (ICT) applied to the 26 reactive samples showed one positive (from Ceará). Using L. major-like promastigote antigen (Lm), ELISA-Lm and indirect immunofluorescence test (IIFT-Lm) detected respectively seven and one out of 26 positive samples. The sample showing RI of 6.756 in ELISA-rK39 was positive also in ELISA-Lm and ICT. Of note, the studied samples had been screened for Chagas’ disease using antigen that cross-reacts with Leishmania and has been approved for transfusion; however, the present results showed that this assay using cross-reactive antigen missed those 26 samples with anti-Leishmania antibodies that likely result from an asymptomatic L. infantum infection. As in endemic areas, it is not easy to differentiate transfusion- or vector-mediated transmission; the occurrence of transmission by transfusion is probably underestimated and raises concerns on blood transfusion safety.

1743

THE RELATIONSHIP BETWEEN CLIMATIC AND OTHER ENVIRONMENTAL FACTORS AND ANNUAL FLUCTUATIONS IN INCIDENCE OF VISCERAL LEISHMANIASIS IN GEDAREF STATE, EASTERN SUDAN

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Visceral leishmaniasis (VL, kala azar), in Gedaref State, eastern Sudan, is caused by Leishmania donovani, which is transmitted by Phlebotomus orientalis sand flies. The endemicity of the disease in this region is characterized by marked annual fluctuations and occasional severe epidemics that claim the lives of many people. Since no significant interventions are undertaken against the disease in this region, the fall and rise in the incidence of VL in Gedaref state may be due to climate...
variability, which characterizes the Sahelian region. Previous studies conducted by our groups and other research teams in Sudan and the Republic of South Sudan (RSS) showed marked variation in spatial distribution of kala azar that can be related to a number of environmental and socio-economic factors that may be acting together or independently to increase the vulnerability of specific populations to the disease. Our findings supported the previous notion that the vector and the disease are associated with Acacia seyal - Balanites woodland and chromic vertisol soils. We used this knowledge to produce a general kala azar risk map based on environmental prediction of the distribution of P. orientalis, the VL vector in Sudan and RSS. However, no attempt has yet been made to correlate annual incidence of kala azar with climatic factors and it is not known whether the flare up in disease incidence is associated with dry or wet years. In this study we analyzed VL records of MSF-Switzerland and MSF-Holland, form 1996-2004 and 2010-2012, in relation to a number of climatic and environmental variables, including temperature, humidity, rainfall and normalized difference vegetation index (NDVI). Our results indicated that the incidence of kala azar in this region is related to late onset of the rainy season. Results are discussed in relation to the epidemiology of the disease. Findings from the study may be used in the future to develop an Early Warning System and construct high resolution Geographical Information System (GIS) risk-maps for the disease.

**1744**

**CO-STIMULATORY MOLECULES ARE INVOLVED IN ANERGY IN SYMPTOMATIC VISCERAL LEISHMANIASIS**

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The proliferation and differentiation of naive T cells require signals provided by co-stimulatory molecules on antigen presenting cells (APCs), in addition to antigen-induced signals. In the absence of co-stimulatory signals, T cells become anergic. We hypothesized that co-stimulatory molecules might be involved in part in the reversible anergy observed during symptomatic visceral leishmaniasis. We analyzed the profile of co-stimulatory molecules in lymphocytes and in CD4+ monocytes in whole blood collected from subjects with symptomatic visceral leishmaniasis (sVL) and after their clinical recovery (rVL). An increase in CD8+ T cells expressing CTLA-4 after stimulation with soluble Leishmania antigens (SLA) was observed (p<0.01) in sVL. An increase in percentage of CTLA-4 in CD4+ and CD8+ in ex vivo condition was observed, when compared sVL versus rVL (p<0.05). No difference in lymphocytes expressing CD28 after SLA stimulation was observed in sVL or rVL, but rVL showed a high percentage of CD8+CD28+ in ex vivo condition when compared with sVL (p<0.05). An increase in the percentage of OX-40 in CD4+ T cells after SLA stimulation in sVL (p<0.05) was observed, as well as, an increase of ICOS in CD4+ and CD8+ T cells after SLA stimuli in sVL (p<0.01). Furthermore, a high percentage of ICOS in CD4+ in ex vivo condition was observed in sVL (p<0.01). There was no difference in CD40, CD86, CD80, ICOSL and HLA-DR in CD14+ monocytes after SLA stimulation in sVL or rVL. There wasn’t also difference in the median fluorescence intensity (MFI) of CD40, CD80, ICOSL or HLA-DR after SLA stimulation in sVL or rVL observed in CD14+ monocytes. But, CD86 showed a high expression in rVL after SLA stimulation (p<0.05). These data support the role of co-stimulatory molecules in the reversible anergy observed during symptomatic VL and might indicate pathways to be explored for immunotherapy against leishmaniasis.

**1745**

**DEFICIENCY OF PROLACTIN-INDUCIBLE PROTEIN LEADS TO IMPAIRED TH1 IMMUNE RESPONSE AND SUSCEPTIBILITY TO AN INTRACELLULAR PATHOGEN**

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The prolactin-inducible protein (PIP) is a secretory protein strategically located at several ports of pathogen entry into the body suggesting it might play a role in host defense. To date, no study has addressed the contributions of PIP in immunity against infectious agents. Here, we assessed the phenotype and responsiveness of immune cells from PIP KO mice to polyclonal T cell stimulators and model antigens in vitro and in vivo. We found comparable numbers of immune cells, (including T, B, natural killer and dendritic cells) in the primary and peripheral lymphoid organs of wild type and PIP KO mice. Further in-depth phenotypic analysis revealed that PIP KO mice had slightly but significantly lower numbers of CD4+ T cells in their spleens and lymph nodes. CD4+ T cells from PIP KO mice showed significantly decreased proliferation, IL-2 production and impaired Th1 differentiation in vitro. The impaired in vitro Th1 response was confirmed in vivo where CD4+ T cells from OVA-immunized PIP KO mice showed significantly impaired proliferation and IFN-γ production following in vitro restimulation. Furthermore, PIP KO mice were highly susceptible to Leishmania major infection as evidence by inability to control lesion progression and parasite proliferation. This impaired resistant was associated with dramatic impairment in IFN-γ and nitric oxide production by splenic and draining lymph node cells from infected mice. Collectively, our findings implicate PIP as an important regulator of CD4+ Th1 cell response, and play a critical role in resistance to intracellular pathogens.

**1746**

**T CELL ACCUMULATION IN THE SPLEEN DURING CHRONIC PROGRESSIVE VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis (VL), caused by the protozoan, Leishmania donovani, is a chronic systemic infection that contributes to half a million new cases each year. During progressive VL, there is profound expansion of immune cells in the spleen. Type 1 and type 2 cytokines are increased during VL in patients, but the T cell population in the spleen during VL has not been fully characterized. In the Syrian hamster model of progressive VL, which mimics human VL, we found a significant increase in expression of the T cell transcription factors Tbet, GATA3 and Foxp3 in spleen cells over the course of chronic infection. This suggests the presence of a mix of Th1, Th2 and Treg cells. In purified splenic CD4 T cells from 28 day infected hamsters we found an increase in Tbet (p<0.001) and GATA3 and Foxp3 in spleen cells over the course of chronic infection. This suggests the presence of a mix of Th1 and Th2 cells are present in the spleen during chronic infection, although one would expect Th2 cells to prominently express CCR4. Notably, we also found a significant population of CD4 T cells that expressed both Tbet and GATA3. The increase in Th1 and Th2 cells in the spleen during chronic infection could be due to local proliferation or splenic recruitment by T cell attracting chemokines. We found an array of chemokines (CCL2, CCL4, CCL5, CCL17, CCL22) increased in the spleen of hamsters over a course of disease. This suggests that T cell attracting chemokines may be playing a role in T cell accumulation at the site of infection.
EVALUATION OF THE EFFICACY OF ANTIGEN DELIVERY BY THE TRANSCUTANEOUS IMMUNIZATION ROUTE IN A MURINE MODEL OF CUTANEOUS LEISHMANIASIS

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Transcutaneous immunization (TCI) is a novel attractive vaccination method which offers advantages over traditional vaccination routes, exploiting the abundance of antigen presenting cells in the skin. We provide the first report of Transcutaneous immunization (TCI) is a novel attractive vaccination method which offers advantages over traditional vaccination routes, exploiting the abundance of antigen presenting cells in the skin. We provide the first report of TCI induced immune responses to Leishmania antigens. Leishmania major soluble antigens (SLA) and Phlebotomus papatasi salivary gland homogenates (SGH) were delivered transcutaneously with cholera toxin (CT), a potent adjuvant for developing mucosal immunity. Sixty inbred BALB/c mice were immunized three times at weeks 0, 3 and 6 with the vaccine formulations (different doses of SLA or SGH, SLA+SGH). TCI was well tolerated. Two weeks after the last vaccine boost, we assessed humoral IgG titer to antigens and CT) and cellular immune responses IFN-γ ELISPOT and cytokine levels from splenic cell culture. In contrast to SGH alone, we showed that transcutaneous immunization of mice with SLA resulted in high titers of anti-SLA IgG that increased when SLA was combined with SGH antigen. Immunization was also associated with high anti-CT IgG titers. A Th1-type immune response was demonstrated with high levels of IFN-γ production and lower levels of IL-10 resulting in a significantly higher IFN-γ production and lower levels of IL-10 ratio compared to the control groups. A high frequency of IFN-γ secreting cells was also seen in groups of mice immunized with SLA. Altogether, these data are consistent with reported protective immune responses and indicate the strong potential of our TCI strategy to protect against Leishmania major infection, with the combined antigen SLA and SGH showing the strongest responses. Experiments using the same regimen of immunizations followed by parasite challenge are in progress. Results of lesion evolution and parasite load along with immune responses pre and post challenge will be presented.

MECHANISMS OF DISEASE-PROMOTING MACROPHAGE PROLIFERATION IN VISCERAL LEISHMANIASIS

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Macrophages have classically been viewed as mature non-proliferative cells, which originate from bone marrow monocytes released to peripheral blood to infiltrate tissues in response to injury. However, recently it was discovered that macrophages can be locally self-maintained without major contribution of infiltrating monocytes, and that M2 macrophages are able to proliferate locally within a Th2 environment. The mechanisms(s) that drive this proliferation have not been defined. In a model of chronic progressive visceral leishmaniasis, we found huge expansion of disease promoting macrophages in the spleen, with signs of Th2-amplified M2 activation, increased STAT-6 activation, and pathological arginase expression. We discovered that growth factors IGF-I and FGF-2 were key contributors to both STAT-6 activation and arginase expression in L. donovani infected macrophages, and inhibition of growth factor signaling blocked arginase expression and parasite replication. Since that these growth factors also drive cellular proliferation and differentiation, and arginase contributes to the cell growth through polyamine production, we explored the possibility that these factors had a role in macrophage expansion in visceral leishmaniasis. L. donovani infection of bone marrow and splenic macrophages resulted in increased cell number, DNA synthesis (BrdU incorporation) and mitosis (ki-67 antigen expression). The combination of growth factor FGF-2 or IGF-I with IL-4 significantly increased macrophage proliferation, suggesting that they interact to control the cell cycle. Inhibition of FGF, IGF and PI3K significantly reduced mitosis indicating that growth factor signaling through PI3K was a major contributor to macrophage proliferation. The local amplification of macrophages in response to chronic infection through the expression of type 2 cytokines and growth factors may have broad significance to other chronic infectious diseases.

FACTORS CONTRIBUTING TO TISSUE DAMAGE IN HUMAN CUTANEOUS LEISHMANIASIS

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Human cutaneous leishmaniasis (CL), due to Leishmania braziliensis infection, is characterized by intense immune mediated tissue inflammation and skin ulceration. For many years immunological studies in CL patients have focused on the evaluation of host response mainly using peripheral blood cells assays. In this context, high levels of IFN-γ and TNF and low levels of regulatory cytokine, IL-10, is observed in cultures of peripheral blood mononuclear cells stimulated with soluble Leishmania antigen. Histopathology studies show mononuclear cells infiltration and low number of parasites. Our hypothesis is that the inflammatory environment helps to control parasitemia but mediates tissue destruction. In the present study we evaluated cytokine and chemokine profile at lesion site. Cells from CL lesion produced high levels of pro-inflammatory cytokines, TNF, IL-6 and IL-1b in absence of stimuli. To determine the contribution of skin epithelial cells to the production of these cytokines we cultured epidermis and dermis separately. TNF was only produced by cells composing the dermis, while IL-6 and IL-1b were produced by dermis and epithelial cells from epidermis. High levels of CCL2, a chemokine that recruits mononuclear phagocytes, and CXCL9 and CXCL10, involved in lymphocyte recruitment, were also observed. Metalloproteinase-9 (MMP-9) is a zinc-dependent enzyme that degrades collagen type 4 (present in basal membrane) and has been associated with tissue damage in skin inflammatory diseases. We found increased production of MMP-9 in CL lesion when compared to healthy skin. TNF is known to induce MMP-9 production. To determine the role of TNF in MMP-9 production in CL, we cultured CL cells in presence of monoclonal antibodies anti-TNF. Blockage of TNF decreased MMP-9 production in CL. Ou study contributes to the understanding of immunopathology in CL and reveals possible targets for immunotherapy.

IMMUNOGENICITY AND PROTECTIVE EFFICACY OF PVAX-NH36 AS A DNA VACCINE AGAINST CUTANEOUS LEISHMANIASIS IN A CANINE MODEL

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Leishmaniasis is a major tropical disease affecting 2 million people annually, against which there is no effective treatment. Vaccine development for humans requires preclinical testing in animal models including canines. One of the best vaccine candidates is NH36, a Leishmania donovani 36 kDa protein. As DNA vaccine NH36 provides better protection than the recombinant protein or purified fucose-manose
can confer some protection against qPCR. In conclusion, pVAX-NH36 is safe and immunogenic in dogs, and parasite burden as two vaccinated animals showed negative results by canines. In addition, only vaccinated animals were DTH positive against NH36 in comparison with the control group. High IFN and low IL-10 levels were produced by PBMC stimulated with NH36 from vaccinated against NH36 in comparison with the control group. High IFN and low IL-10 levels were produced by PBMC stimulated with NH36 from vaccinated canines. In addition, only vaccinated animals were DTH positive against recombinant NH36. Finally, some protection was observed based on skin parasite burden as two vaccinated animals showed negative results by qPCR. In conclusion, pVAX-NH36 is safe and immunogenic in dogs, and can confer some protection against L. mexicana.

**1751**

**LEISHMANIA SPECIFIC CD4 T CELLS RELEASE IFN-γ THAT LIMITS PARASITE REPLICATION IN PATIENTS WITH VISCERAL LEISHMANIASIS**

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Visceral leishmaniasis (VL) is associated with increased circulating levels of multiple pro-inflammatory cytokines and chemokines, including IL-12, IFN-γ, and TNF-alpha, and elevated expression of IFNγ mRNA in lesional tissue such as the spleen and bone marrow. However, an immunological feature of VL patients is that their peripheral blood mononuclear cells (PBMCs) typically fail to respond to stimulation with leishmanial antigen. Unexpectedly, it was recently shown that Leishmania specific IFNγ can readily be detected when a whole blood stimulation assay (WBDA) is used. We sought to define the conditions that permit whole blood cells to respond to antigen stimulation, and clarify the biological role of the IFNγ found to be released by cells from VL patients. CD4+ T cells were found to be crucial for and the main source of the IFNγ production in Leishmania stimulated whole blood (WB) cultures. Complement, antibodies and red blood cells present in whole blood do not play a significant role in the IFNγ response. The IFNγ production was reduced by blockade of human leukocyte antigen (HLA)-DR, indicating that the response to leishmanial antigens observed in WB of active VL patients is a classical HLA-T cell receptor (TCR) driven reaction. Most importantly, blockade of IFNγ in ex-vivo splenic aspirate cultures demonstrated that despite the progressive nature of their disease, the endogenous IFNγ produced in patients with active VL serves to limit parasite growth.

**1752**

**ISOLATION AND INITIAL CHARACTERIZATION OF TRYpanosoma cruzi ISOLATES FROM A POPULATION OF CYNOMOLGUS MACAQUES NATURALLY INFECTED IN THE UNITED STATES**

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The protozoan parasite Trypanosoma cruzi, causative agent of Chagas disease, is capable of infecting not only humans, but also virtually any mammalian species. Non-human primates housed in outdoor facilities in the southern United States are known to acquire T. cruzi infection, as is the case for a group of 64 cynomolgus macaques from Texas who are seropositive for T. cruzi infection. This group provided a unique opportunity to investigate natural T. cruzi infection in an entire population

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**IMMUNOREGULATORY NETWORKS AND IMMUNOPATHOGENIC PATHWAYS IN VISCERAL LEISHMANIASIS**

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Visceral Leishmaniasis (VL) caused by Leishmania donovani, is an important problem in tropical and subtropical areas of the world. VL is characterized by a progressive increase in visceral parasite burden, cachexia, splenomegaly, pancytopenia and ultimately death. We have studied the pathogenesis of VL in Syrian hamsters (Mesocricetus auratus) since it closely mimics active human disease. We demonstrated previously that the simultaneous expression of Th1 (IFN-γ) and Th2 (IL-4) cytokines, parasite activation of STAT6, and decreased production of nitric oxide are associated with susceptibility to the infection. To fill the gaps in our understanding of the pathogenesis of VL we examined global changes in gene expression in spleen tissue and splenic (adherent) macrophages. We used a novel approach of deep sequencing (RNAseq) coupled with de novo assembly of full-length transcripts because the Syrian hamster does not have an annotated reference genome. Differentially expressed transcripts in L. donovani infected (28 days) vs. uninfected hamsters were determined by alignment back to the de novo constructed transcriptome and cross-species BLAST, after the removal of contaminating parasite sequences. Transcriptome analysis confirmed that adherent cells were enriched for expression of macrophage (CD14, CD64 and Merk) but not T cells markers (CD4, Gata3 and Tbet). Differentially expressed genes analyzed with IPA software revealed a number of highly enriched canonical pathways in spleen and splenic macrophages, including hepatic fibrosis, pathogenesis of multiple sclerosis, atherosclerosis signaling, communication between innate and adaptive immune cells, and the glucocorticoid receptor signaling. Notably, within the differentially expressed transcripts we identified mixed expression of genes associated with classical (M1) and alternative (M2) activation of macrophages that was confirmed by qPCR. This approach provides a valuable tool to overcome the obstacles of working with a non-model organism without a reference genome. With it we can begin to understand the complex immunopathogenic mechanisms at the site of visceral infection.
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ENHANCEMENT OF MURINE VISCERAL LEISHMANNIASIS DUE TO CROSS REACTIVE LEISHMANIA MAJOR ANTIBODIES

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Leishmaniasis is a global disease found in regions with compatible temperatures for the phlebotomine sandfly vector to survive and lacking rigorous vector control programs. An estimated 1.3 million new cases and 20,000 - 30,000 deaths occur annually due to this parasitic protozoan. More than twenty different species of Leishmania infect humans with multiple species occurring in the same geographic areas. Symptoms range from a minor cutaneous lesion at the bite site due to dermotropic species such as L. major, to life threatening disseminated disease with multiple organ involvement, caused by viscerotropic species such as L. infantum. We have previously shown susceptible BALB/c mice infected with a low self-healing dose of cutaneous L. major and challenged with L. infantum, develop a markedly worsened disease with higher parasite burden, relative to naive mice. There was little notable difference in the cytokine profiles between L. major exposed and naive mice in response to L. infantum. Cross-reactive antibodies were seen in both groups of L. infantum infected mice regardless of their immune history. Opsonizing antibodies have been shown to lead to increased disease in visceral leishmaniasis. The present studies focus on exploring the role cross-reactive antibodies may play in exacerbation of visceral disease seen in mice previously exposed to L. major. Mice receiving passively transferred serum from L. major infected mice, 48 hours prior to challenge with L. infantum, developed equivalent organ parasitemia to age and L. major-infected matched control naive mice. Naïve mice inoculated with control serum did not suffer any disease enhancement with L. infantum. We speculate that cross-reactive antibodies are augmenting visceral disease in mice with immunological memory to L. major. While L. major is known to produce long lasting immunological memory and protect against recurrent cutaneous disease, antibody enhancement due to inter-Leishmania infection may enhance disease in regions with multiple circulating Leishmania-species and suggests leishmanization might be riskier than previously thought.

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HUMAN SEROPREVALENCE OF LEPTOSPIROSIS AND RICKETTSIOSIS IN FOUR REGIONS OF PERU

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Rickettsia and Leptospira are obligate intracellular bacteria with global distributions and a wide variety of animal hosts. Two disease-causing groups of Rickettsia are noted: the spotted fever group (SFGR) and the typhus fever group Rickettsia (TFGR). We explored the prevalence of exposure and risk factors for human Rickettsia infection in four ecologically distinct regions of Peru (Lima, Cusco, Puerto Maldonado and Tumbes) and for Leptospira in Puerto Maldonado and Tumbes. In January 2012 we randomly collected 2165 serum samples from participants in a surveillance cohort for respiratory disease in the aforementioned sites and tested them for IgG by ELISA (Rickettsia) and microscopic agglutination test (Leptospira). Overall antibody prevalence across the four sites was 10.6% for SFGR (ranging from 6.2-14.0%, with the highest prevalence in Tumbes) and 3.3% for TFGR (ranging from 2.6-6.4%, with highest prevalence in Puerto Maldonado). Factors associated with positive IgG for SFGR on multiple logistic regression analysis were male sex (OR 2.2, 95% CI 1.5-3.3), increasing age (OR 1.02, 95% CI 1.01-1.04 per year), contact with backyard birds (OR 2.1, 95% CI 1.4-3.0), and working in agriculture or with livestock (OR 4.3, 95% CI 1.7-10.8). However, exposure to any kind of animal within the household decreased the odds ratio by half (OR 0.50, 95% CI 0.31-0.81), perhaps indicating that arthropod vectors on birds preferred non-human hosts when they were present, thus diminishing exposure to humans. Age was the only variable associated with antibody positivity to TFGR (OR 1.03, 95% CI 1.02-1.05). The antibody prevalence to Leptospira was 11.3% in Puerto Maldonado and 5.8% in Tumbes, with a borderline association with keeping animals in the household (OR 2.5, 95% CI 1.0-6.2). Exposure to Rickettsia, especially SFGR, and Leptospira appears to be frequent in Peru. We plan now to perform testing in domestic animals in some of these sites to determine the specific reservoirs and vectors for these agents and to obtain pathogen isolates for identification of the specific species.

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PROXIMITY TO PIG POPULATIONS AS A KEY RISK FACTOR FOR JAPANESE ENCEPHALITIS DISEASE; RESULTS OF A FIVE-YEAR SURVEILLANCE STUDY FROM NORTHWESTERN BANGLADESH

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Japanese encephalitis (JE) is a mosquito-borne virus that causes severe disease in humans with 10-20% case fatality. It is the commonest cause of encephalitis in Bangladesh. Humans are dead-end hosts. Pigs, by contrast, are viral amplifying hosts, so the distribution of pig populations may be particularly important for human disease risk. Previous studies have shown that the highest incidence of human JE infections occur in northwestern Bangladesh, where JE is also endemic among pigs. The objective of this study was to explore proximity to pigs as a risk factor for human JE disease in Bangladesh. We first geocoded the locations of residence for all JE patients identified through hospital-based surveillance in Naogaon, Chapainawabganj, and Rajshahi districts in northwestern Bangladesh between 2007 and 2011. Next, we used data from a 2009 pig census in these areas to map all pig raising households. To explore the impact of proximity to pigs as a risk factor for JE disease we compared the odds of a human JE case living within a set distance of a pig-raising household to that of a randomly selected control population. We identified 81 human JE cases from throughout the region, with a mean age of 32 years (range: 0 - 75 years); 11% died. Disease patterns were highly seasonal with 90% of cases occurring between the months of August and November. Humans infected with JE were 2.7 times more likely to live within 500m of a pig-owning household compared to controls (95% confidence interval [CI] 1.3 - 4.6) and 1.7 times more likely to live within 5km (95% CI: 1.0 - 3.7). Results from this analysis suggest that proximity to pig populations is an important risk factor for human JE disease in northwestern Bangladesh. JE vaccination is not currently included in the Bangladesh immunization program; therefore, interventions to reduce infections among pigs could be an important strategy for reducing human risk in these areas and should be explored.

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Leptospira spp. infection was also reported in a wide range of animal hosts. 11 out of 100,000 based on surveillance studies of island populations. Leptospira febrile illness ranged from 2.3% (n=43) to 47.5% (n=59). Estimates of leptospirosis in hospital-based cohort studies of patients with non-malarial human and animal criteria, 89 (14.1%) were considered eligible. Eligible articles described databases using the terms ‘ OR ‘leptospirosis’ AND ‘Africa’ for infection in animals. We searched eight international and regional scientific review of acute haemorrhagic fever caused by Leptospira infection. However, relatively little is known for the geographic distribution of hematophagous bats. Such information would help define areas and populations at risk for rabies and inform effective prevention campaigns. We therefore modeled the potential epidemiological distribution of D. rotundus using the ecological niche modeling algorithm MaxEnt. Incorporating climatic, environmental and anthropogenic factors that may relate to the geographic distribution of D. rotundus, rabies-related bat deaths, and rabies-infected farm animals, we developed a risk map for bat-associated rabies transmission in Peru. D. rotundus occurrence was found to be associated with the colder, drier months of the year. In addition, land classification data show that bats prefer firmer, non-flooding low lands. Variables associated with occurrence of animal rabies included livestock population density, mean diurnal temperature range, and precipitation in the drier months of the year. This study offers a first glimpse of the environmental and bioclimatic factors associated with the distribution of hematophagous bats and animal rabies using novel techniques that extract the maximum information and offer robust results from the little data available.

**THE PROBLEM OF LEPTOSPIROSIS IN AFRICA: REVEALING A NEGLECTED ‘ONE HEALTH’ CHALLENGE THROUGH A SYSTEMATIC REVIEW**

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Recent evidence from Tanzania indicates that leptospirosis is an important cause of non-malarial febrile disease. However, relatively little is known about the incidence and geographic distribution of leptospirosis in Africa as well as the diversity of infecting Leptospira spp. in human and animal populations. To examine current knowledge, we performed a systematic review of acute human leptospirosis and confirmed Leptospira infection in animals. We searched eight international and regional scientific databases using the terms ‘Leptospira’ OR ‘leptospirosis’ AND ‘Africa’ for articles published between 1930 and June 2013. Of 630 unique articles identified and reviewed against predetermined inclusion and exclusion criteria, 89 (14.1%) were considered eligible. Eligible articles described human and animal Leptospira spp. infection in 26 (44.8%) of 58 countries included in the UN continental definition of Africa. Prevalence of acute leptospirosis in hospital-based cohort studies of patients with non-malarial febrile illness ranged from 2.3% (n=43) to 47.5% (n=59). Estimates of annual human leptospirosis incidence ranged from 4.1 to 101 cases per 100,000 based on surveillance studies of island populations. Leptospira spp. infection was also reported in a wide range of animal hosts. 11 out of 15 human-infecting Leptospira serogroups were isolated from one or more animal host species in Africa. For several important human-infecting serogroups, multiple animal host species were identified. L. borgpetersenii, L. interrogans and L. kirschneri were the predominant genetic species reported in human and animal populations across Africa, although some local variation was observed. In conclusion, this systematic review highlights the importance of acute leptospirosis in febrile patients in Africa and reveals many areas of uncertainty that remain in our understanding of this complex, multi-host disease. A ‘One Health’ approach is advocated to integrate human and animal studies in future work, and to explore local and regional variation in leptospirosis epidemiology in Africa.

**RISK FACTORS FOR HUMAN EXPOSURE TO WILDLIFE ZOOONES IN NIGERIAN HUNTING COMMUNITIES**

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Bushmeat hunting clearly increases the risk of zoonotic pathogen transmission. However, limited information exists on the nature and frequency of contact with wildlife in communities that practice bushmeat hunting, especially with respect to socioeconomic drivers of hunting behavior. We surveyed 188 hunters and 139 non-hunters in five rural communities in Cross River State, Nigeria. Responses were used to: 1) quantify contact rates with wildlife, 2) identify socioeconomic factors predisposing individuals to hunt, and 3) identify specific hunting behaviors that increase frequency of contact with wildlife. Among hunters, 95% hunted rodents, 91% ungulates, 90% carnivores, 78% primates and 35% bats. Hunters used traps (75%), guns (71%), machetes (71%), and dogs (18%) to hunt animals both day (78%) and night (69%). We constructed generalized linear mixed models to examine socioeconomic predictors of individual hunting behavior and frequency of contact with wildlife, especially primates. We found that lower education level (<0.01), having a father who hunts (<0.0001), and larger household sizes (<0.05) were all associated with becoming a hunter. Among hunters, high rates of wildlife contact were associated with high hunting frequency (<0.05), hunting...
both night and day (p<0.05) and with a gun (p<0.05); while sleeping in the forest (p<0.0001), hunting night and day (p<0.0001) and with a dog (p<0.05) were associated specifically with high rates of primate contact. Results demonstrate that hunters have risky contact with a diversity of wildlife, and that the decision to become a hunter is deeply rooted in family history and modified by economic necessity. Improved education, reduced family sizes, and alternative livelihoods may reduce the risk of zoonotic disease exposure in rural hunting communities in Nigeria. Public health programs aimed at reducing zoonotic transmission of wildlife pathogens in such settings will be most efficient when they target root socioeconomic drivers that lead to hunting behavior and risky wildlife contact.

ENVIRONMENTAL RESERVOIRS OF ANTIBiotic Resistance ASSOCIATED WITH SMALL SCALE POULTRY FARMING IN NORTHWESTERN ECUADOR

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Non-therapeutic use of antibiotics in agriculture poses a threat to human health by contributing to an environmental reservoir of antibiotic-resistant (AR) bacteria. Small-scale “backyard” broiler chicken production using antibiotics for feed promotion is becoming increasingly common in developing countries. We use E. coli antibiotic susceptibility profiles to assess the potential for transmission of AR from broiler chickens to the surrounding environment (water, soil, surface) in the context of small-scale poultry farming in Northwestern Ecuador. Field work spanned 190 households in 17 villages visited between 08/2010-08/2012. We collected a total of 529 samples from drinking water, soil and food preparation surfaces, and surveyed water, sanitation and antibiotic use practices. From a subset of 91 households involved in broiler production, we collected 131 cloacal samples from production chickens and 66 soil and surface samples from chicken coops. In addition, 153 non-production (“free-range”) birds were sampled across all villages, and 54 water samples from local rivers were collected. Up to five E. coli isolates from each sample were tested against 12 antibiotics using disc diffusion. Zones of inhibition and their categorical interpretations were compared using mixed-effect models. AR was more common in broilers than free-range chickens for every antibiotic tested (p<0.01), particularly tetracycline (76.8% vs 33.1%), sulfisoxazole (66.9% vs 19.2%) and streptomycin (61.1% vs 26.7%). A pattern of AR to gentamicin, fluoroquinolones and beta-lactams that was unique to broilers was also found in coop surfaces and soils, but not in household samples. The prevalence of this phenotype declined with bird age, implying importation form hatcheries outside the study system. AR was more common in coop than household samples. Farming and non-farming households showed no difference in AR profiles. These results suggest broiler chickens carry AR and pass it to their immediate environment. However, transmission to households may operate at different scales.

COMPARATIVE GENOMIC ANALYSIS OF CoCCIDIOides AND RELATED SPECIES

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Dimorphic fungi Coccidioides immitis and C. posadasii are primary pathogens of immunocompetent mammals, including humans. Coccidioides infection results from environmental exposure, which is believed to grow as a soil saprophyte in arid desert environments. To investigate hypotheses about the evolution of Coccidioides, the genomes of several Onygenales, a close, nonpathogenic relative, and a more diverged pathogenic fungus, were compared with those of 13 more distantly related Ascomycetes. This poster aims to identify shifts in gene family size associated with a host/substrate shift from plants to animals in the Onygenales. Comparison among Onygenales revealed distinct evolutionary changes in Coccidioides that may underlie its infectious phenotype, coccidioidomycosis. Phylogenetic analysis suggest that Coccidioides species are not soil saprophytes as previously hypothesized. Data indicate that they have evolved to remain associated with their dead animal hosts in soil. Using a bioinformatics workflow, we show that metabolic pathway genes, membrane-related proteins, and putatively antigenic compounds have evolved in response to interaction with an animal host.

FACTORS ASSOCIATED WITH GIARDIA IN HUMANS AND ANIMALS RESIDING IN RURAL AND URBAN AREAS OF COASTAL ODISHA, INDIA AND ENVIRONMENTAL LOADING ESTIMATES FROM ANIMALS

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Globally, Giardia lambia, is one of the most commonly detected intestinal parasites and is particularly relevant in developing countries such as India, where inadequate sanitary conditions, high population densities, and frequent contact with animals capable of carrying zoonotic pathogens can exist in both urban slums and rural areas. We present a study to estimate and compare the prevalence of Giardia infection in rural and urban settings for both human and animal populations residing in coastal Odisha, India, evaluate if gender or age is associated with Giardia infection in humans, and estimate fecal loading rates of Giardia cysts from animal host populations residing in the study area. From April to May of 2012, human fecal samples from 85 diarrhea patients presenting at three diarrhea wards and 111 pooled animal fecal samples across seven host species (cattle, buffalo, goat, sheep, chicken, cat, and dog) were collected across urban and rural residential areas served by the wards. Samples were screened and fluorescent microscopy used to enumerate Giardia cysts and a subset of dog and human samples analyzed by molecular methods to identify isolate genotypes. Giardia cysts were detected in 12% of tested diarrhea patients, while 32% of pooled animal samples were positive. No evidence for difference in the presence of Giardia cysts among humans was observed between urban and rural settings, gender, or age groups (<5 years, 5-59 years, >59 years). There was substantial support for a location effect on Giardia shedding among animals, with rural animals shedding higher numbers of parasites. Of the seven animal host groups screened, dogs and cattle, both reported to shed zoonotic genotypes of Giardia in India, shed decisively more Giardia cysts per gram of feces, as much as 2-3 orders of magnitude greater than other animal types (adjusting for location). Molecular characterization of isolates identified host specific Assemblages in dog samples and a possible zoonotic Assemblage in a human sample. Using current animal populations and observed Giardia shedding rates, cattle were estimated to contribute >99% of Giardia animal cysts into the study area environment, followed by dogs as the next largest source. This study shows Giardia prevalence is similar for humans living in rural and urban settings, but different for animals and that exposure from infected cattle and dogs may be an important public health concern in Coastal Odisha.
Zoonotic bacterial pathogens present significant threats to human health and compromise the economic well-being of pastoralist herding communities. The dynamics of transmission of pathogens such as *Brucella* spp., the causative agent of Brucellosis, however, are poorly understood. Prior studies have suggested that transmission of Brucellosis cannot be sustained in the small to medium size herds characteristic of most pastoralist systems. These analyses, however, did not consider the joint effects of animal trading between herds and inter-species mixing within herds. Using quantitative and ethnographic data collected in a pastoralist community in Laikipia, Kenya, we create a deterministic SIR model of *Brucella* sp. transmission to examine how these transmission heterogeneities may facilitate disease persistence. We explore dynamics of infection given various herd sizes and management strategies observed in Laikipia Kenya. Specifically, we model a community that engages in multispecies livestock raising, assuming different types and levels of interaction between herds. We find that transmission is unsustainable in small herds of all species when in isolation, confirming prior research. Multi-species herds which include a high proportion of goats compared with large stock such as cattle can sustain itself for a longer time, but not indefinitely. Links through sales and purchases or other between-herd contact, however, create conditions where *Brucella* can transmit indefinitely. Though it is likely that herding strategies historically accommodate potentially devastating risks to herd health, it is possible that herders are unaware of threats, both to health and economic wellbeing, presented by bacterial infections such as Brucellosis. In addition to education programs on specific diseases, herders should be encouraged to cull animals showing obvious signs of illness as soon as possible and should be discouraged from selling such animals to minimize risks to human health.

### MARKETS AS HUBS OF RISKY CONTACT BETWEEN HUMANS AND WILD/DOMESTIC ANIMALS: CASE STUDIES FROM REPUBLIC OF CONGO (ROC) AND DEMOCRATIC REPUBLIC OF CONGO (DRC)

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Traditionally, market studies have focused on elements of market dynamics such as the volume of wildlife and domestic animals being sold. We are examining markets through a different lens: to assess them as locations to education programs on specific diseases, herders should be encouraged to cull animals showing obvious signs of illness as soon as possible and should be discouraged from selling such animals to minimize risks to human health.

### MODELING BRUCELLA INFECTION DYNAMICS IN PASTORALIST COMMUNITIES: THE ROLE OF HERD MANAGEMENT AND SPECIES COMPOSITION IN SUSTAINING BRUCELLA TRANSMISSION

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USAID’s PREVENT Project is examining markets through a different lens: to assess them as locations to which the volume of wildlife and domestic animals being sold. We are conducting key informant interviews, consumer exit interviews and then a household survey in the market catchment areas (as determined by exit interviews) to learn where people shop, what they buy and why. In addition, since February 2014 we have carried out monthly one-week full-day observations of vendor stalls selling bush meat and/or poultry. This presentation will describe what we have learned to date about hygiene conditions, infrastructure, and biosecurity practices in these markets and about the importance, diversity and seasonal variation of the bush meat trade as well as how the forms of animals sold (live, freshly dead, large pieces, small pieces, smoked, raw) and change with the length of time they are in the market. We will discuss the implications of all of these factors for human exposure and risk.

### MASSTAG PCR DETECTION OF EV-D68, RSV-A AND B, AND MORE, IN CLUSTERS OF UNEXPLAINED ACUTE FEBrILE ILLNESS IN CAMBODIA

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Fever of unknown origin constitute a substantial disease burden on patients in Southeast Asia with the majority of the cases remaining undiagnosed. To expand the breadth of possible infectious pathogens, we used MassTag PCR to test for the presence of 20 bacterial and viral respiratory agents from 85 patients with unexplained respiratory illness representing six disease clusters that occurred in Cambodia between 2006 and 2012. We detected potential pathogens in 62 (73%) of 85 total cases, identifying a virus in 37 patients (44%) and a bacterium in 53 (62%) cases. In a cluster from Kandal province from August 2009, we detected a high frequency of enterovirus 68 and human rhinoviruses. Among 22 cases that occurred during October 2009 in Kampong Speu province, we detected human respiratory syncytial virus B. Finally, a cluster of children < five years of age from the Ratanakiri province previously diagnosed with pneumonia, revealed infection from human respiratory syncytial virus A. These findings provide insight into the etiologies of previously undiagnosed acute febrile illness in Cambodia and point to the utility of multiplexed diagnostics during disease outbreaks.

### DIAGNOSTIC VALUE OF CLINICAL FEATURES FOR DIAGNOSING PNEUMONIA IN CHILDREN UNDER FIVE YEARS OF AGE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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The aim of this review is to assess the diagnostic value of clinical signs and symptoms in identifying radiological pneumonia in children under the age of five and to review the accuracy of WHO criteria for diagnosing clinical pneumonia in developing countries. Electronic databases (Medline and Embase) and reference lists of relevant studies were searched to identify articles assessing clinical predictors of radiological pneumonia in children. 1697 potentially relevant studies were identified. Selection was based on: design (diagnostic accuracy studies), target disease (pneumonia), patients (children below 5 years), setting (ambulatory or hospital care), index test (clinical features), reference standard (chest radiography). Quality assessment was based on the 2011 Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria. For each index test, sensitivity and specificity were calculated. Meta-analyses with the Bivariate model and hierarchical SROC plots were done for index tests assessed in four or more studies. Eighteen articles were included in the analysis. Age-related fast breathing (six studies, pooled sensitivity: 0.62 [95%CI 0.26-0.89]; specificity: 0.59 [0.29-0.84]) and lower chest wall indrawing (four studies, 0.48 [0.16-0.82]; 0.72 [0.47-0.89]) showed poor diagnostic performance in the meta-analysis. Features with the highest pooled, positive likelihood ratios were: respiratory rate above 50/min (1.90 [1.45-2.48]), grunting (1.78 [1.10-2.88]), chest indrawing (1.76 [0.86-3.58]), and nasal flaring (1.75 [1.20-2.56]). Features with the lowest pooled negative likelihood.
ratio were: cough (0.30 [0.09-0.96]), history of fever (0.53 [0.41-0.69]), and respiratory rate above 40/min (0.43 [0.23-0.83]). No single clinical feature was sufficient for definitively diagnosing pneumonia. Combining clinical features in a decision tree may improve diagnostic performance, but the addition of new point-of-care tests for diagnosing bacterial pneumonia would help to reach an acceptable level of accuracy.

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COMMUNITY ACQUIRED PNEUMONIA IN ADULT HOSPITAL ADMISSIONS NORTHERN VIETNAM: CLINICAL FEATURES AND ETIOLOGY
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Community acquired pneumonia (CAP) causes an estimated 1 million deaths in Asia each year including 160 000 amongst those aged 15 - 60 years. Knowledge of local etiology is critical to formulating treatment guidelines and vaccination priorities. Despite this studies of the etiology of CAP in South East Asia have been limited. We conducted a study of the causes of CAP in adult patients admitted to 3 hospitals in Ha Noi, Vietnam. Adults with an infiltrate on chest radiography and one of: cough, dyspnea, fever (≥38.3C) or hypothermia (<36.0C), purulent respiratory secretions, bronchial breathing or rales on auscultation, leucocytosis or leucopenia that had not been residing in hospital or a long term care facility in the 14 days prior to onset were eligible for admission. Consenting patients were enrolled and recieved a standardized clinical evaluation. All patients had routine haematology and biochemistry test results recorded and received blood culture, sputum culture and PCR for the following bacterial pathogens: Streptococcus pneumoniae, Mycoplasma pneumoniae, M. amorphoforme, Chlamydocephila pneumoniae, C. psittaci, Legionella pneumophila and L. longbeachae. PCR was also performed for 14 respiratory viruses on nasal/throat swabs and/or sputum. Urine was tested for pneumococcal antigen and L pneumophila serogroup 1. In a selection of cases where acute and convalescent serum was available serology for C. pneumoniae, M. pneumoniae, Orientia tsutsugamushi, Rickettsia typhi and R. prowazekii was also performed. Preliminary results only are available at this time, full results should be available in time for presentation. Preliminary results show a case fatality rate (died in hospital or palliative discharge) of 12/116 (10.3%). The rate of positivity for blood culture was low (5/112, 4.5%). Sputum PCR rate (died in hospital or palliative discharge) of 12/116 (10.3%). The frequency of monoresistance 33.63 % (n = 37) , poliresistance 9.09 % (n = 10 ) , 45.45 % MDR - TB (n = 50) and 12.72% sensitive cases (n = 14 ) . The overall frequency of isoniazid resistance was 90.18 % (n = 92) , rifampcin 79.09 % (n = 87) , Streptomycin 18.18 % (n = 20) Etambutol 11.81 % (n = 13) and pyrazinamide 7.27% (n = 8).The initial cultures were bacsiloscopias negative and 44.6% (n = 50) and 32.1% (n = 36) respectively. Only 1.8% (n = 2) showed HIV positive reaction. In the present study we found that there is a high prevalence of resistant TB in patients admitted to retreat in the Madre de Dios Region where HIV positive reaction is not related to the presentation. MDR-TB were the most frequent type of resistance and isoniazid is the drug resistance most often generated in monoresistence patients.

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INFLUENZA VACCINE EFFECTIVENESS IN THE TROPICS: MODERATE PROTECTION IN A SURVEILLANCE POPULATION IN BANGKOK BETWEEN AUGUST 2009 AND JANUARY 2013
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Influenza in the tropics occurs year round with peaks that correspond variably to temperate regions. However, data on influenza vaccine effectiveness (VE) in the tropics is sparse. We report on the effectiveness of influenza vaccine to prevent medically attended laboratory confirmed influenza from a sentinel surveillance study conducted at a Thai military medical facility in Bangkok, Thailand from August 2009 to January 2013. Patients ≥6 months old presenting with influenza-like illness underwent nasal/throat swabs which were tested by influenza RT-PCR. A case test-negative study design was used to evaluate VE. Of 2992 samples available for analysis, 1058 (35.4%) were PCR-positive (cases) and 1934 (64.6%) were PCR-negative (test-negative controls). Five hundred and eight (16.9%) of these patients reported being vaccinated within the previous 12 months. Periods of high and low influenza activity were defined based on publicly available Thai Ministry of Public Health data. Overall adjusted VE was found to be 51.6% (95%CI: 36.8, 63.1%). Adjusted point estimate for VE was highest in the 18-49 year age group (77.0%) followed by 6-23 months (55.1%) and 2-17 years (44.6%). Adjusted estimates were not done for those ≥50 years of age due to small numbers. VE in patients with underlying disease was 75.5% compared to 48.6% in those without. VE appeared to be much higher during high versus low influenza activity periods. Among those who reported receiving vaccine 14 days-3 months prior to illness, VE was 55.8% (95% CI 26.6 to 74.1%), and tended to decrease as the interval between vaccination and illness increased (46.8% at ≥3 to 6 months; 48.9% at ≥6 to 9 months; 31.5% at ≥9 to 12 months). Our findings demonstrate moderate protection by influenza vaccination and support the utility of influenza vaccination in the tropics including in very young children and those with underlying disease. Our study also suggests that booster vaccination may be useful within several months even when the formulation does not change.
SEASONAL INFLUENZA'S ASSOCIATION WITH SPECIFIC HUMIDITY IN THREE TROPICAL CENTRAL AMERICAN COUNTRIES: HONDURAS, NICARAGUA AND COSTA RICA

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Studies have demonstrated the association between seasonal influenza & meteorological factors. We previously showed that seasonal influenza in Guatemala, El Salvador & Panama was associated with specific humidity. In this work, we investigated the association in 6 departments from three other Central America countries: Cortes & Francisco Morazan in Honduras; Managua in Nicaragua; Alajuela, Cartago & San Jose in Costa Rica. As an indicator for influenza activity, we used the weekly proportion of samples which tested positive for influenza in the period 2008-2013 from each country's National Influenza Centers. Respiratory samples were collected from case-patients presenting with influenza-like illness or severe acute respiratory infection. Meteorological factors - rainfall, temperature & specific humidity (SH) - were obtained from NASA's satellites & models. We used logistic regression and adjusted for previous influenza activity & co-circulating viruses (respiratory syncytial virus, adenovirus and parainfluenza virus). We found that SH was proportionally associated (p<.05) with influenza activity in all departments (Odds Ratio (OR)=1.2-1.6). Temperature was inversely associated with influenza activity in Alajuela of Costa Rica (OR and 95% Confidence Interval=0.7(0.6-0.8)) & Cortes of Honduras (OR=0.8(0.7-0.9)). There was no statistical association (p<.05) with rainfall in any locations. Among the meteorological factors, SH had the highest contribution (2-15%) to the model in all locations except in Cortes. The model estimated influenza activity accurately (R=0.6-0.9) for the final 6 months in all countries except Honduras. Time-frequency analysis using Hilbert-Huang Transform showed that seasonal components of influenza activity were positively correlated with SH (R=0.2-0.6, p<.05), further corroborated the SH findings from logistic regression. Our results highlighted influenza's proportional association with SH in these countries, which was consistent with other studies in the tropics. Understanding of climate role in influenza may help in estimating epidemic timing and intensity.

THE NEED FOR ENHANCED LABORATORY BASED DIAGNOSIS CAPACITY IN THE TESTING AND SURVEILLANCE FOR OTHER RESPIRATORY VIRUSES FROM PATIENTS REPORTING WITH INFLUENZA LIKE ILLNESSES

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The capacity of Laboratories to promptly identify particular strains or subtypes of organisms using modern diagnostic techniques has become essential for rapid and efficient response to disease outbreaks and preventing potential epidemic or pandemic spread. In Influenza surveillance, patients present with symptoms that match the case definition of Influenza Like Illnesses (ILI) and Severe Acute Respiratory Infection (SARI). Up to 24% of the samples collected and tested for influenza at the National Influenza Centre are positive for influenza while the biggest percentage are negative. There are various respiratory viruses and bacteria that affect humans which include adenovirus, human rhinovirus A, coronavirus OC43, parainfluenza virus 1, parainfluenza virus 3, respiratory syncytial virus B, human metapneumovirus, respiratory syncytial virus A, parainfluenza virus 2 and coronavirus 229E. Nasopharyngeal and oropharyngeal swab specimens collected from patients presenting with ILI and SARI in 8 sentinel sites between December 2011- April 2014 in Uganda were tested for Influenza by RT-PCR, subtyping and isolation. Of a total of 5931 samples tested, 645(10.9%) were positive for Influenza with 20.3% co-infections, 405(62.8%) Influenza A and 238(36.9%) Influenza B; though, 5286(89.1%) were negative yet the patients presented with symptoms that match the case definition for ILI and SARI. This raises a need to establish baseline information on the prevalence of other respiratory pathogens that cause upper and lower respiratory disease in populations through strengthening laboratory diagnostic capabilities for the identification and characterization of infectious agents likely to cause public health emergencies. There is need for more-simplified testing systems that enable researchers and clinicians to perform multiplexed molecular diagnostics quickly and easily. The results would be useful to guide future surveillance and case management strategies involving other respiratory infections in Uganda.

THE ECONOMIC BURDEN OF VALLEY FEVER IN CALIFORNIA

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Coccidioidomycosis or Valley Fever (VF) is a mycotic disease endemic to southwestern US. In California, where 33% of VF cases nationwide occur, VF incidence increased from 4.2-10.8/100,000 in the period 2002-2012 (157% increase). There is no complete study of the economic burden of VF to guide clinical care and public health planning. We estimated the total and per person lifetime direct and indirect cost of VF in 2012 US $. The 4,904 incident VF cases reported in 2012 in California were symptomatic VF infections, which we followed for lifetime costs. Unreported cases were assumed to be asymptomatic infections with no costs. We included early treatment costs of VF misdiagnosed as community acquired pneumonia. We costed VF by disease categories; (uncomplicated pneumonia [UP], chronic/diffuse pneumonia with [CD] and without dissemination [CN], chronic pulmonary nodule [PN], and chronic pulmonary cavity [PC]). Direct costs were VF diagnosis, treatment, and follow-up including physician visits, ER, hospitalization, tests, procedures, and medications. VF epidemiology data were from literature and expert interviews. Treatment and utilization were from published guidelines and 2 hour prepared interviews with 5 expert VF physicians. Hospitalizations were from the 2012 California Patient Discharge Dataset and HCUP prices, medication costs from average wholesale price minus 17% for contract pricing, physician visit were costed using CPT based Medicare estimates. Total lifetime costs of 2012 VF incident cases in CA was $212 million (M), $51,743/person. UP accounted for 85% of our population and 31% of direct lifetime costs ($65.4M), CD 2.5% and 42% costs ($89.2M), CN 2.5% and only 11% of costs, pulmonary nodules and cavities 10% and 16% of costs, respectively, primarily due to cost of differential diagnosis of cancer. Short term work loss costs were $6.4 M, and mortality another $126 M. VF causes a large cost burden, especially for disseminated cases. Variation exists by geographic regions, insurance status and practice patterns. Areas for cost control exist.
EPIDEMIOLOGY OF RESPIRATORY VIRAL PATHOGENS FROM SENTINEL SURVEILLANCE IN WESTERN CAMBODIA NEAR THE BORDER WITH THAILAND

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Little is known about influenza and other respiratory viruses in remote populations along the Thai-Cambodia border in Western Cambodia. Real-time PCR for influenza was performed on combined nasal and throat specimens from outpatients presenting with influenza-like illness (ILI) at 4 sentinel sites in Western Cambodia between May 2010 and December 2012; a subset was further characterized by antigenic analysis, antiviral susceptibility testing and full genome sequencing for phylogenetic analysis. PCR-negative ILI-specimens for influenza were cultured; cultured negative samples were then tested with RT-PCR for enteroviruses and rhinoviruses (EV/VR) and enterovirus EV71. Among 586 ILI-patients (median age 5 years, range 1-77 years), 168 (29%) tested positive for influenza by RT-PCR and at least 1 respiratory virus was detected in 258 (44%) patients. Influenza strains were highly related and matched circulating strains and although vaccination coverage was low, most strains matched the vaccine strains. No intrasubtype reassortment was detected. Our Western Cambodian H1N1(2009) isolates were more closely related (based on full genome analysis) to 10 earlier isolates from Cambodia (94.4% genome conservation) compared to 13 Thai isolates (75.9% genome conservation). Aside from adenovirus (5.74%) and parainfluenza virus (3.8%), detection of non-influenza viruses by viral culture was low (<10%), with no detection of coronavirus, human bocavirus, human metapneumovirus and respiratory syncytial virus. We detected 5.9% of non-polio enteroviruses among our culture-negative specimens: human Coxsackievirus types A4, A6, A8, A9, A12, B3, B4 and human echovirus types E6 and E9. We conclude that influenza epidemiology in this sample of isolates is following similar trends as observed elsewhere in Cambodia. Further research to clarify the burden of adenovirus and non-polio enteroviruses as etiologic agents for acute respiratory infections is needed in Cambodia.

CONCORDANCE BETWEEN SOLID AND LIQUID CULTURE FOR ANTITUBERCULOSIS DRUG SUSCEPTIBILITY TEST (DST) IN PERU

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Löwenstein Jensen (LJ) has been the most widely technique for MTB detection and DST. However, its long incubation and time to detection has led to develop alternative methods. Automated Mycobacteria Growth Indicator Tube (MGIT) constitutes a rapid alternative with comparable results, but manual MGIT (mMGIT) is favorable in low-resources areas because its lower cost. This study evaluates the concordance for DST of LJ and manual MGIT in a country with one of the highest prevalence of tuberculosis in the Americas. Sputum samples collected from respiratory TB suspects, enrolled in diagnostic trials during 2007-2011 in Lima (Capital of Peru), underwent LJ and MGIT. Only samples with positive Capilia test for Mycobacterium tuberculosis (MTB) were included in the DST analysis. Resistance to Isoniazid (INH), Rifampin (RIF), Streptomycin (SM) and Ethambutol (EMB), as well as resistance to INH plus RIF (MDR) were evaluated by Proportion Method (PM) in LJ medium and SIRE system in MGIT. Comparison between performance of MTB detection and susceptibility patterns were assessed by Kappa indices. DST in both solid and liquid mediums was performed in 319 samples. PM-LJ and SIRE-MGIT detected resistance to INH: 21.6% (69/319) and 20.7% (66/319) (Kappa=0.92, p<0.001); RIF: 12.0% (38/319) and 11.0% (35/319) (Kappa=0.94, p<0.001); SM: 26.3% (84/319) and 25.1 (80/319) (Kappa = 0.82, p<0.001); and EMB: 11.9% (38/319) and 9.4% (30/319) (Kappa = 0.77, p<0.001). Furthermore, PM-LJ and SIRE-MGIT found 10.0% (32/319) and 10.0% (32/319) and 8.8% (28/319) as MDR cases, respectively (Kappa = 0.89, p<0.001). Manual MGIT emerges as a faster DST alternative to LJ in low resources settings like Peru, with optimal concordance between LJ and mMGIT DST.

DIFFERENT FROM EQUATORIAL BRAZIL, SOUTHERN HEMISPHERE WHO VACCINATION RECOMMENDATIONS ARE ADEQUATE FOR MOST OF SOUTHERN PARTS OF BRAZIL

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Influenza vaccination is the most important public health measure to prevent severe cases and deaths due to influenza infection. However, influenza viruses are constantly evolving, forcing continuous vaccine reformulation. In this study we investigated the annual genetic matching between circulating influenza viruses and recommended A(H3N2) vaccine components for over a decade (1999-2012) in three regions of a southern hemisphere country with continental dimensions (Brazil). A total of 237 hemagglutinin sequences from Northeast (NE), Southeast (SE) and South (S) Brazilian regions were compared against the corresponding vaccine strains recommended annually by the WHO. We used MEGAv5.1 to infer nucleotide and amino acid distances between sequences and annual vaccine prototypes. PhyML was used for phylogenetic reconstructions by Maximum Likelihood (ML) to infer the antigenic relationship between viral samples and vaccine composition. We next compared the putative effectiveness of the influenza vaccination in the three regions using hypothetical vaccination scenarios where alternative vaccine delivery timing and vaccine compositions (either Southern or Northern Hemisphere WHO recommendations) were considered (comparison following method of Mello et al 2009). We found that, although influenza circulates in most (NE) or all (S,SE) months of year, the current Southern Hemisphere recommendation in Brazil is adequate for these regions. This was less expected in the NE region, but we attribute it to the fact that most of the samples of the NE actually came from the its southernmost part (Bahia, at approximately 13oS), where influenza seasonality differ from the equatorial pattern of circulation. Our results show that WHO hemisphere vaccine recommendation decisions must consider differences in influenza circulation patterns even within regions of a country.
Influenza-like illness (ILI) is a leading cause of illness globally. A substantial proportion of ILI is not detected by health services-based surveillance because not all persons with ILI seek healthcare; this may be exacerbated in settings where healthcare access or utilization is limited. Active surveillance through household visits can detect most ILI cases, but is costly and labor-intensive. We followed a cohort of pregnant women in rural Quetzaltenango, Guatemala for ILI (fever and cough or sore throat) by using either weekly phone calls or home visits. Women with <20 weeks gestation were randomized 1:1 to either a weekly phone call or home visit. Staff attempted up to 3 contacts per week to administer a study questionnaire for ILI symptoms. Probable ILI cases identified by the call or visit were then evaluated within 24 hours by a nurse during a home visit. Participants with ILI had a nasopharyngeal swab collected which was tested by polymerase chain reaction for respiratory syncytial virus (RSV), human metapneumovirus, influenza A/B, parainfluenza virus 1/2/3, and adenovirus. During May-November 2013, 167 women were enrolled, of whom 85 (51%) were randomized to weekly phone calls and 82 (49%) to home visits. Surveillance was completed for 864 (63%) of the 1,384 expected person-weeks of follow-up by phone calls versus 1,010 (73%) of the 1,381 expected person-weeks by home visits (p=0.01). Weekly follow-up identified 9 ILI cases in the phone call group versus 13 ILI cases in the home visit group (p=0.5 for ILI). We detected 3 infections in the phone call group (adenovirus, parainfluenza-2, RSV) versus 6 infections in the home visit group (RSV, flu-B, parainfluenza types 2 and 3) (p=0.8). Although more costly and time consuming, home visits were more likely to have successfully completed questionnaires than phone calls. The sample size was inadequate to determine the difference in detection of lab-confirmed ILI cases between visits and calls. The choice between phone calls and home visits to identify case-patients will depend on the surveillance objectives and available resources.

HAMSTER WEIGHT PATTERNS PREDICT THE INTENSITY AND COURSE OF SCHISTOSOMA HAEMATOBIUM INFECTION

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Although Syrian golden hamsters are a widely used host for experimental infection by Schistosoma haematobium, surprisingly little is known about the associated intensity and course of infection, making the use of these animals potentially unreliable. As such, we sought to define inexpensive, simple, noninvasive, and accurate methods for assessing and predicting the severity of disease in S. haematobium infected hamsters in order to prevent premature hamster sacrifice and unexpected morbidity and mortality. Through monitoring the weight and behavior of infected hamsters, we determined that the weight loss patterns of infected hamsters are highly correlated with commonly used measures of the severity of infection (i.e. numbers of eggs passed in the stool and worm burdens). In contrast, we found no significant correlation between hamster weight loss patterns and egg yields from liver and intestinal tissues.

Our findings suggest that a more complex relationship exists among weight loss patterns and egg yields from liver and intestinal tissues. Our data may be useful for workers seeking to optimize harvests of S. haematobium eggs and worm pairs from infected hamsters for downstream applications.

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Despite the increased use of prophylactic therapy and improved access to antiretroviral therapy, Pneumocystis jiroveci pneumonia (PCP) remains one of the most common life-threatening opportunistic infections in HIV-infected patients worldwide. Difficulty obtaining an adequate sputum sample is often a barrier to the diagnosis of PCP in sputum-scarce patients where bronchoalveolar lavage is not available. This study examined the rates of PCP in HIV patients with respiratory symptoms in Cochabamba, Bolivia, and evaluated detection of PCP in samples other than sputum in an attempt to overcome the challenge of sample collection for the diagnosis of PCP in resource-limited settings. Fifty-one HIV patients admitted to the hospital for respiratory symptoms in Cochabamba, Bolivia in 2010 were enrolled in the study. In addition to induced sputum, an oral rinse, stool, and gastric secretion sample obtained by the string test were collected from each subject. Presence of PCP was evaluated by real-time quantitative PCR in a laboratory in Lima, Peru after the completion of sample collection. Of the 51 induced sputum samples collected, seven (13.7%) were positive for PCP. The oral rinse and string test each detected PCP in one of the samples. PCP was not detected in any of the stool samples. The mortality rate of those diagnosed with PCP was 42.9% (3/7). Although other studies have reported the ability to diagnose PCP by the use of PCR on oral rinse samples, our study was not able to repeat this. Additionally, we were not able to detect PCP in either stool or gastric secretion samples obtained by the string test (as has been used for the detection of Mycobacterium tuberculosis in other studies). Our study does show that PCP is a cause of substantial morbidity in the HIV population in Bolivia, and it is possible that there were additional cases of PCP that went undiagnosed in our study because of we were limited to induced sputum. Thus, improved diagnostic modalities are needed for the detection of PCP in resource-limited settings (delete all in italics). Although the rates of PCP in our study, and in most resource-scarce settings, are significantly less than tuberculosis, our study shows that PCP is still a cause of substantial morbidity and mortality in the HIV population in Bolivia. We were not able to detect PCP in stool samples, and our yield was very low in oral rinse and gastric secretion samples obtained by the string test. It is possible that there were additional cases of PCP undiagnosed in our study because we were limited to samples of induced sputum. Our study highlights the fact that improved diagnostic modalities are needed for the detection of PCP in resource-limited settings.

COMPARATIVE PERFORMANCE OF WEEKLY TELEPHONE CALLS VERSUS WEEKLY HOME VISITS TO IDENTIFY CASES OF INFLUENZA-LIKE ILLNESS AMONG A COHORT OF PREGNANT WOMEN - GUATEMALA, 2013

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Influenza-like illness (ILI) is a leading cause of illness globally. A substantial proportion of ILI is not detected by health services-based surveillance because not all persons with ILI seek healthcare; this may be exacerbated in settings where healthcare access or utilization is limited. Active surveillance through household visits can detect most ILI cases, but is costly and labor-intensive. We followed a cohort of pregnant women in rural Quetzaltenango, Guatemala for ILI (fever and cough or sore throat) by using either weekly phone calls or home visits. Women with <20 weeks gestation were randomized 1:1 to either a weekly phone call or home visit. Staff attempted up to 3 contacts per week to administer a study questionnaire for ILI symptoms. Probable ILI cases identified by the call or visit were then evaluated within 24 hours by a nurse during a home visit. Participants with ILI had a nasopharyngeal swab collected which was tested by polymerase chain reaction for respiratory syncytial virus (RSV), human metapneumovirus, influenza A/B, parainfluenza virus 1/2/3, and adenovirus. During May-November 2013, 167 women were enrolled, of whom 85 (51%) were randomized to weekly phone calls and 82 (49%) to home visits. Surveillance was completed for 864 (63%) of the 1,384 expected person-weeks of follow-up by phone calls versus 1,010 (73%) of the 1,381 expected person-weeks by home visits (p=0.01). Weekly follow-up identified 9 ILI cases in the phone call group versus 13 ILI cases in the home visit group (p=0.5 for ILI). We detected 3 infections in the phone call group (adenovirus, parainfluenza-2, RSV) versus 6 infections in the home visit group (RSV, flu-B, parainfluenza types 2 and 3) (p=0.8). Although more costly and time consuming, home visits were more likely to have successfully completed questionnaires than phone calls. The sample size was inadequate to determine the difference in detection of lab-confirmed ILI cases between visits and calls. The choice between phone calls and home visits to identify case-patients will depend on the surveillance objectives and available resources.

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Although Syrian golden hamsters are a widely used host for experimental infection by Schistosoma haematobium, surprisingly little is known about the associated intensity and course of infection, making the use of these animals potentially unreliable. As such, we sought to define inexpensive, simple, noninvasive, and accurate methods for assessing and predicting the severity of disease in S. haematobium infected hamsters in order to prevent premature hamster sacrifice and unexpected morbidity and mortality. Through monitoring the weight and behavior of infected hamsters, we determined that the weight loss patterns of infected hamsters are highly correlated with commonly used measures of the severity of infection (i.e. numbers of eggs passed in the stool and worm burdens). In contrast, we found no significant correlation between hamster weight loss patterns and egg yields from liver and intestinal tissues.

Our findings suggest that a more complex relationship exists among weight loss patterns and egg yields from liver and intestinal tissues. Our data may be useful for workers seeking to optimize harvests of S. haematobium eggs and worm pairs from infected hamsters for downstream applications.
LONG DELAY IN DIAGNOSIS AND HIGH LITHIASIS PROPORTION IN SURGERY SUGGEST UNDERESTIMATION OF HUMAN FASCIOLIASIS IN ARGENTINA

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A retrospective overview of human fascioliasis in Argentina highlighted the long delay with which many patients were diagnosed. Calculated delay average of the time elapsed between the appearance of symptoms and confirmation of infection by appropriate diagnosis is very high, of 1262 days, nearly 3.5 years, and there are references about patients having suffered from symptoms for ten or more years without diagnosis. This suggests either infected subjects not looking for professional diagnosis due to mild symptoms of low fluke burdens and/or misdiagnosis of patients due to the non-pathognomonic clinical picture, easily confused with other diseases when the patient attends a health centre not used to dealing with fascioliasis. Moreover, the number of cases in which a surgical procedure contributed to the diagnosis when Fasciola hepatica specimens were unexpectedly found upon liver exploration appeared to be surprisingly high. In the majority, surgery was indicated due to abdominal pain and biliary obstruction suggestive of lithiasis. The importance of intraoperative cholangiography was highlighted in cases in which, even though gallstones were removed, evidence of obstruction observed during the cholangiography led to the finding of flukes. In most of these surgical cases with lithiasis suspicion, the patient inhabited a large city (Buenos Aires, Córdoba, Mendoza, Tucuman) as opposed to a rural area where attending a health care centre is less usual due to economic reasons or at least complicated due to the long journey that has to be made. This additionally suggests a far greater underestimation of the problem in rural areas. There were patients in whom fluke infection was detected only after a second surgical intervention. Both long delay in diagnosis and high lithiasis proportion suggest that many patients are frequently overlooked and pose a question mark about fascioliasis detection in the country.

RNAi OPTIMIZATION IN FASCIOLA HEPATICA NEWLY EXCYSTED JUVENILES: LONG DSRNA INDUCE MORE PERSISTENT SILENCING THAN siRNA

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The exponential growth of the genomic and transcriptomics knowledge of parasitic flatworms allows the identification of several novel putative genes of unknown function. In trematodes RNA interference emerges as almost the only available tool to analyze gene function since classical genetics or other reverse genetics approaches still remain unavailable. Whereas this approach has been tested in several parasites of this group it has been optimized only in schistosomes likely reflecting the difficulties in the establishment of the technology as a routine tool. In this report we present progress in the optimization of this technology in the liver fluke Fasciola hepatica, causative agent of fascioliasis. This disease is one of the most problematic infections affecting livestock worldwide, and the increasing appearance of human cases had lead the WHO to recognized this disease as a reemerging zoonosis. Using a single copy gene encoding leucine aminopeptidase (LAP) as the target, we refined delivery conditions, identifying electroporation (electroporation and subsequent incubation) as the most efficient method to introduce small RNAs into the fluke. We observed consistent knock down of LAP with low (2 µg/ml) dsRNA concentrations. While this low concentration may reduce or obviate off-target effects, it also compromise the tracking of the RNAi incorporation by fluorescent labeling. We also tested the effects of long dsRNA and short interfering RNA (siRNA) are equally effective at inducing a short-term knock down, dsRNA induced more persistent silencing up to 21 days after treatment, suggesting that mechanisms of amplification of the interfering signal can be present in this parasite. Persistent silencing from invasive stage for up to 3 weeks (close to what it takes for the parasite to reach the liver) opens the possibility of using RNAi for the validation of new putative therapeutic targets.

PREVALENCE OF HAPLOCHIRIS TAICHUI AMONG HUMANS AND FISH IN LUANG PRABANG PROVINCE, LAO PDR

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This study confirmed the prevalence of the intestinal fluke Haplorchis taichui (Trematoda: Heterophyidae) among people and fish in Luang Prabang Province, Lao PDR. Fecal specimens were collected from 559 riparian people (229 males and 330 females), residing in 4 Districts (Luang Prabang, Xieng Ngeun, Pak Ou, and Nam Bak) and were examined by the Kato-Katz fecal smear technique. The overall helminth egg positive rate was 64.9%. The positive rate for small trematode eggs (STE), which may include H. taichui and other heterophyids, Opisthorchis viverrini, and lecithodendriids, was 15.2%. For recovery of adult helminths, 10 STE-positive people were treated with 40 mg/kg praziquantel and 15 mg/kg pyrantel pamoate, and then purged. Mixed infections with 3 Haplorchis species (H. taichui, H. pumilio, and H. yokogawai), a species of cestode (Taenia saginata), and several species of nematodes including Enterobius vermicularis and hookworms were found. The worm load for trematodes was exclusively high for H. taichui with an average of 7691 specimens per infected person, followed by H. yokogawai (8.3 specimens) and H. pumilio (4.1 specimens). Out of 207 freshwater fish (17 species) purchased in a market in Luang Prabang District, 138 (67%) harboured H. yokogawai metacercariae (metacercarial burden per fish; 520). Lower prevalence of fish and lower metacercarial density were observed for H. yokogawai (52% and 50 per fish, respectively) and H. pumilio (18% and 3 per fish, respectively). STE found in the surveyed population of Luang Prabang Province were verified to be those of intestinal flukes, particularly H. taichui.

MOLECULAR MODELING OF ADENYLATE KINASE 1 AND 8 KDA CALCIUM-BINDING PROTEIN OF CLONORCHIS SINENSIS

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Allosteric proteins involved in signal transduction transforms their molecular structure by binding co-factors, and thus they have a potential
for new drug development. In this study, two allosteric proteins, adenylate kinase 1 (ADK1) and 8 kDa calcium-binding protein (CaBP) of the Chinese liver fluke _Clonorchis sinensis_ were cloned and modeled. _C. sinensis_ EST clone Cs63 and Cs296 were cloned and sequenced. To compare them with other proteins of parasites, multiple sequence alignment and phylogenetic analysis were performed. For molecular modeling, both sequences were subjected to SWISS-MODEL. Recombinant proteins generated bacterially were used for their functional analysis. By BLAST search, Cs63 and Cs296 were confirmed as ADK1 and 8 kDa calmodulin-like CaBP, respectively, and thus they were named as CsADK1 and CsCa8, respectively. Sequence and hydrophobicity of them were similar to those identified from parasitic helminthes. Molecular model of CsADK1 contained CORE, Lid and NMP domains and expected to transform its structure by binding co-factor-like APS. CsCa8 was predicted to have two distinctive EF-handed calcium-binding sites by molecular modeling. Calcium ion could bind to each of EF-hands of CsCa8 model. Both recombinant proteins were functionally active in biochemical assay. Results obtained from the study provide structural basis of _C. sinensis_ ADKs and CaBPs for the development of new anthelmintic drugs.

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**AN OPEN-LABEL, RANDOMIZED, MULTICENTRIC STUDY OF TRICLABENDAZOLE FOR FASCIOLIASIS IN CHILDREN FROM PERU**

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Human fascioliasis is an important public health problem in Latin America mainly affecting school-aged children in poor areas of the Andean Region (Peru and Bolivia). The optimal therapeutic scheme of triclabendazole (TCBZ), the recommended anthelmintic against the trematode _Fasciola hepatica_, has not been well defined (with a 10 mg/kg single dose being the most common regimen recommended), and clinical trials that assess effectiveness and tolerability in children are scarce. We aimed to evaluate the efficacy and tolerability of 2 therapeutic schemes of TCBZ in different areas of Peru. A total of 84 individuals (mean age ± SD: 9.27± 2.48 years) with _F. hepatica_ eggs in their stools (chronic infection) were enrolled in an open-label, phase II clinical trial from areas located along the Peruvian Andes. Individuals were randomly allocated into 2 groups: 44 received 2 dosages of TCBZ at 7.5 mg/kg each, with a 12 h interval post-prandially (group I-tested group), and 40 received a single dose of 10 mg/kg, post-prandially (group II-standard group). The efficacy (parasitological cure) was evaluated by the presence of eggs in stools at regular intervals and up to 60 days post-treatment. Tolerability was evaluated by the presence of clinical symptoms during the first week after TCBZ administration. A parasitological cure was obtained in 100% of individuals from the tested-group, and 95.0% in the standard-group (p>0.05). The most common adverse event was biliary colic, documented in 25.0% in group II (95% CI = [Ed.1] 11.9-38.9) on day 2, and in 20.5% in group I (95% CI = 7.8-32.7) on day 4, possibly related to the expelling of the adult worms through the biliary tract. In conclusion, the tested scheme was highly efficacious (100% cure rate) and tolerable, and it may be an optimal therapeutic scheme for the treatment of fascioliasis in children in Peru. This represents the largest series of children treated with TCBZ in a non-hospital setting and the largest in Peru.

**1785**

**IDENTIFICATION, CHARACTERIZATION AND EVALUATION OF RECOMBINANT ANTIGENS FOR THE DIAGNOSIS HUMAN PARAGONIMIASIS**

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Paragonimiasis is a foodborne trematode infection that affects 23 million people mainly in Asia. The parasite causes chronic cough with fever and hemoptysis, and lung fluke infection is often confused with tuberculosis. We used a systems biology approach to identify antigens that might lead to improved diagnostic tests for this infection. Antibodies from patients with _Paragonimus kellicotti_ were used to isolate antigens for proteomic analysis, and RNAseq data from adult worms were used for protein identification. Among the 22 most abundant identified proteins were a number of orthologues to known diagnostic antigen as well as novel candidates. Sequences for these proteins have 80-90% identity with amino acid sequences for orthologues in _P. westermani_. We expressed five _P. kellicotti_ proteins as his-fusion proteins in _E. coli_, and these were used to raise antibodies in mice. Immunohistology performed with sections of adult worms showed that four of them were localized to the tegument, at the parasite host interface. In contrast, a known egg antigen was absent from the tegument but present in developing and mature eggs. We evaluated the diagnostic potential of these antigens by Western blot with sera from patients with paragonimiasis (from Missouri and the Philippines), fascioliasis, and schisosoimiasis and with sera from healthy North American controls. Two recombinant proteins showed high sensitivity and specificity as diagnostic antigens. Antibodies to the egg protein seemed to be specific marker for patients with mature adult worm infections (with _Paragonimus ova_ in stool or sputum). In conclusion, this study has identified and characterized promising antigens for diagnosis of human paragonimiasis.

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**IMPACT OF SCHISTOSOMA MANSONI IN SCHOOL-AGED CHILDREN LIVING IN KASANSA, DEMOCRATIC REPUBLIC OF THE CONGO**

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_Schistosomiasis (SCH)_ is an important public health problem in developing countries and school aged children are the most affected. The aim of this study was to evaluate the impact of SCH on the population of school-aged children living in the high endemic area of Kasansa HZ in terms of malnutrition, anemia and low school performance. The overall health status of the children was poor with very high prevalence of _S. mansoni_ infection (99.3%), malaria infection (65.1%), anemia (61.4%) and stunting (61.0%). School performance was also negatively affected with 54.6% of the children having failed at least one class. Regular contact with river water was the most significant risk factor related to SCH infection. Anemia was influenced by SCH infection (p=0.003) and weak egg load was associated with stunting (p=0.04). However, due to poverty the causality between chronic malnutrition and anemia can be in either direction, potentially aggravated by SCH. Low school performance was mainly influenced by low income (<1 USD). Poverty exacerbated both health and school performance. Control measures are urgently needed to improve the health status of these children with in depth studies to demonstrate the causalities for each of the diseases in this population.
FIELD EVALUATION OF MINI-FLOTAC®, SPONTANEOUS SEDIMENTATION TECHNIQUE IN TUBE OF TELLO, RAPID SEDIMENTATION TECHNIQUE BY LUMBERNAS AND KATOKATZ FOR THE DIAGNOSIS OF ENTERIC PARASITES IN THE HIGHLANDS OF PUNO, PERU

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Enteric parasites show a peculiar distribution along the geography of Peru. Although a low-cost, reliable, local coproparasitological technique such as the Spontaneous Sedimentation Technique in Tube of Tello (SSTT) has been used over decades this has not been compared to standardized methods. Here, we compare the performance of SSTT with the mini-FLOTAC®, a coproparasitological technique under validation, in an area where intestinal multiparasitism and fascioliasis are common. Rapid Sedimentation Technique (RST) by Lumbreras, and Kato-Katz (KK) were included. We conducted a stool survey among school-aged children in Naupa Pampa, Calapampa, and Progreso, located in Azángaro (3859 m) (Puno, Peru). Four techniques, mini-FLOTAC®, SSTT, RST, and KK were performed. The sensitivity and negative predictive value (NPV) of each technique were compared using the combined results of all positive techniques as the “gold standard”. The inter-technique agreement (κ) was also evaluated. A p value 10%: Entamoeba coli (77.2%), Blastocystis hominis (66.5%), Endolimax nana (50.3%), Iodamoeba buetschlii (15.0%), Entamoeba histolytica/dispar (10.8%), and Chilomastix mesnili (10.2%). The area of study is hyperendemic for human fascioliasis with high prevalence in Naupa Pampa (25%). Mini-FLOTAC® showed a higher performance than SSTT and RST for H. nana (13 vs. 11 vs. 8) and F. hepatica (15 vs. 10 vs. 8), and higher sensitivities than SSTT, RST and KK for both helminths (p<0.05). The use of SSTT with the mini-FLOTAC® showed a higher performance than SSTT and RST

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INFLUENCE OF HISTONE MODIFYING ENZYMES AND MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY ON SCHISTOSOMA MANSONI SURVIVAL AND REPRODUCTIVE DEVELOPMENT

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Praziquantel is an efficacious drug against schistosomiasis, however, there is the risk of drug resistance development and therefore new drugs are necessary. Although, the roles of Histone Modifying Enzymes (HMEs) and Mitogen-activated protein kinases (MAPKs) are unclear in schistosomes, they are increasingly approved as targets for drug development with a rising number of inhibitors under development. In other organisms, HMEs and MAPKs influence a number of tissue-specific biological activities such as cell survival, differentiation and proliferation. Here, we employed RNA interference (RNAi) to elucidate the functional role of 16 HMEs and 6 genes involved in MAPK signaling pathway in S. mansoni. First, the HMEs and ePKs were identified in the predicted proteomes of Schistosoma mansoni, S. japonicum, and S. haematobium by HMM searches. Genes were annotated and selected regarding their putative function in the parasite. One histone deacetylases (HDAC8), 10 methyltransferases (HMTs), 5 demethylases (HDM), SmRas, SmERK1, SmERK2, SmJNK, SmCaMK2, and Sm3p8 were chosen for experimental validation. RNAi and pharmacological inhibition were used to elucidate the functional role of HDAC8 and Smp38 dsRNA treated worms exhibited tegumental damage. We also observed that Sm3p8 is involved in the activation of detoxification enzymes. Our results help characterize the importance of HMEs and MAPK pathway in the normal development and survival of the schistosome parasite and suggest some of these enzymes as useful drug targets to prevent schistosomiasis progression.
In order to better understand the effector mechanisms associated with parasitic encapsulation reactions, whole hemolymph containing hemocytes was extracted from susceptible (NMRI) and resistant (BS-90) Biomphalaria glabrata strains and incubated in the presence or absence of newly-transformed Schistosoma mansoni sporocysts (Sp). After 18 h of incubation at 26°C, hemocyte capsules were isolated, frozen en bloc, cryosectioned and subjected to laser capture microdissection to yield samples enriched for cells involved in hemocyte/Sp reactions for comparison to hemocyte capsules without Sp. Isolated cryosections were analyzed by nanoLC-EASI-MS/MS for peptide isolation and sequencing. Putative protein identification were made by BLAST analyses vs. the non-redundant NCBI protein database (db) and a 6-frame translated B. glabrata protein db in VectorBase. Preliminary analyses revealed at total of 358 putatively identified proteins of which 71 were from Schistosoma spp. (mainly S. mansoni) and 284 were non-Schistosoma sequences. Significantly more larval sequences were identified in NMRI/Sp capsules compared to BS-90/Sp samples, consistent with greater killing and larval rejection typically seen in R hemocyte reactions in vitro. After normalizing the dataset across samples using total unique peptide counts for actin and tubulin, other notable immune-related observations were made: (a) A greater reduction of HSP70 peptides in BS-90/Sp capsules compared to NMRI/Sp (67% BS-90 vs. 37% NMRI); (b) Frep2 was the only Frep identified in all samples, and only in the BS-90/Sp sample, implying an upregulation of Frep2 during parasite encounters; (c) An upregulation of extracellular matrix/adhesion proteins (dermatopontin2, HMG1, matrilin2 and α-integrin) only in BS-90/Sp or BS-90 control samples suggesting possible roles in immune-related cell-parasite or cell-cell adhesion reactions; and (d) Enrichment of MnSOD, a potential effector molecule, in the BS-90 hemocyte capsules compared to those of NMRI snails. It is anticipated that continued mining of this rich dataset will yield valuable insights into hemocyte immune function.

Comparative analysis of gene expression in Schistosoma mansoni-exposed Biomphalaria glabrata (BS90 stock) snails maintained at permissive and non-permissive temperatures

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We have shown previously that the refractory phenotype in the Biomphalaria glabrata BS-90 stock is a temperature-dependent trait. Thus, using mild non-lethal heat (32°C) to induce stress genes such as Hsp70 and Hsp90 prior to S. mansoni infection rendered these normally resistant snails susceptible. In order to determine differences between transcription profiles of these snails responding to early parasite infection when they are either resistant or susceptible, RNA samples from Schistosoma mansoni exposed juvenile BS-90 snails, maintained through several generations either at the permissive (32°C), or non-permissive temperature (25°C) were sequenced. Bioinformatic analyses of RNAseq datasets revealed a preponderance of stress related transcripts in parasite-exposed BS-90 snails maintained at the permissive temperature compared to similarly exposed reference stock maintained at room temperature. For example, at 2 hours post-exposure, a 77-fold induction of Hsp70 transcript was observed in susceptible BS-90 snails maintained at 32°C, corroborating earlier results that showed that this transcript was induced differentially between juvenile resistant and susceptible parasite-exposed snails. Differential expression of other stress genes, Hsp 90 (12-fold induction), Hsp 83 (40-fold induction) and Hsp 68 (5-fold induction) was also detected in these BS-90 snails responding to S. mansoni at the permissive temperature. These data, taken together with previous results provides further evidence for the role of stress in the snail-host and schistosome interaction.

Immunomodulatory proteins of Schistosoma haematobium

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Approximately 120 million individuals are infected with Schistosoma haematobium in sub-Saharan African alone. S. haematobium adult worms lay eggs throughout the urogenital tract that induce a pronounced inflammatory response. To date very little is known about how S. haematobium is able to produce such a robust immune response while evading immune clearance and immunity in many individuals. One plausible explanation lies in parasite secreted immunomodulatory proteins, however, due to the historical lack of a robust S. haematobium animal model, very little is known about the role of these proteins in urogenital pathology. We have recently cloned a homolog of the IL-4-inducing principle of S. mansoni eggs (IPSE), a protein originally identified in S. mansoni, the sister species of S. haematobium responsible for hepatic and enteric schistosomiasis. S. mansoni IPSE has been shown to bind IgE on the surface of basophils in an antigen independent manner, and drive basophil degranulation. To date it is not clear why it is advantageous for the parasite to secrete a protein capable of activating effector cells associated with anti-parasite responses. Intriguingly the S. haematobium IPSE homolog shares only 63% identity with its S. mansoni counterpart, suggesting that the protein may have evolved to suit each species’ infectious niche. Furthermore studies with recombinant S. haematobium IPSE and S. mansoni IPSE suggest that the immunoglobulin isotype binding profiles of IPSE differ across species. Despite IPSE’s sequence divergence and differences in immunoglobulin binding, several important protein features appear to be conserved across species, including a nuclear localization sequence. Using a novel model of S. haematobium egg-induced pathology we have shown that immunization with IPSE prior to S. haematobium egg injection alters urothelial inflammation. Together, these results suggest that S. haematobium IPSE also functions as an immunomodulatory protein, and plays an important and distinct role in regulating tissue pathology in urogenital schistosomiasis.

Gender dependence of p53-related abnormalities in mouse bladder urothelium due to Schistosoma haematobium egg-induced inflammation

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The bladder urothelium is dramatically altered during Schistosoma haematobium infection (urogenital schistosomiasis). These alterations include hyperplasia, ulceration, dysplasia, squamous metaplasia and frank carcinogenesis. Defining the pathways that drive these urothelial changes will contribute to a deeper understanding of how S. haematobium egg-induced expulsion, hematuria, and bladder cancer develop in humans. Defects in the function of the tumor suppressor gene p53 are evident in many cancers, including bladder cancer generally and schistosomal bladder cancer specifically. To identify any role p53 might play in urothelial alterations due to urogenital schistosomiasis, we employed transgenic mice with tamoxifen-inducible cre recombinase activity in cells expressing...
uroplakin-3a, a urothelial-specific gene (Upk3a-GCE mice). We confirmed specificity of cre expression in Upk3a-GCE mice by crossing them with TdTomato-floxed-EGFP reporter mice and administering tamoxifen to their progeny. As expected, these progeny switched from TdTomato to EGFP expression in their bladder urothelium. We then crossed Upk3a-GCE mice to p53-floxed mice. The resulting progeny (Upk3a-GCE+/wt;p53fl/fl) were given tamoxifen or vehicle control to render them urothelial p53-haploinsufficient or -intact, respectively. We then injected S. haematobium eggs or control vehicle into the bladder walls of these mice. Three months later, mice were sacrificed and their bladders subjected to histological analysis (H&E staining). Male p53-intact, egg-injected mice exhibited similar histological changes as their p53-haploinsufficient counterparts, including urothelial hyperplasia and ulceration. In contrast, female p53-intact, egg-injected mice featured no urothelial ulceration, whereas their p53-haploinsufficient counterparts often had significant ulceration. Additionally, some egg-injected p53-haploinsufficient females exhibited regions of squamous metaplasia. Thus, intact p53 activity seems to be required, in a gender-specific manner, for urothelial homeostasis during S. haematobium infection in this model. Ongoing work includes (1) examining histological changes in Upk3a-GCE+/wt;p53fl/fl mice beyond 3 months after egg-injection, and (2) measuring alterations in the cell cycle status of the urothelium as a consequence of schistosomiasis.

**CD4+ T-CELL COUNTS IN WOMEN WITH UROGENITAL SCHISTOSOMIASIS**

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Schistosoma haematobium causes urogenital schistosomiasis and has been shown to be associated with HIV in cross-sectional studies. Schistosomiasis infection has been hypothesized to affect the HIV viral load and HIV disease progression. Schistosomiasis treatment has been shown to increase CD4 counts in HIV negative individuals. Between May and October 2013, a CD4 count was done in 797 young women who were invited for a gynecological examination. One urine sample was collected from all, and microscopy for schistosome ova was done. HIV testing was done in 769 women, of which 123 were HIV positive (16.0%). The mean CD4 count was 864 x 10^6 cells / L. It was lower in the HIV negative group than in the HIV positive group (931 vs. 511 p < 0.001). Urinary schistosomiasis was not associated with a lower CD4 count (862 in urine negative vs. 871 in urine positive women, p=0.75). Likewise, there was no significant association between CD4 counts between women with moderate or severe degree genital schistosomiasis and women without genital lesions (846 vs. 829, respectively, p=0.70). Further there was no significant difference between schistosomiasis positive and negative women were found after stratifying for HIV (data not shown). This cross-sectional study did not show any significant difference in CD4 T-cell counts when comparing women with and without urogenital schistosomiasis.

**PATTERNS OF REACTIVITY TO SCHISTOSOMA MANSONI EGG GLYCAN ANTIGENS IN A POPULATION OF TREATMENT-NAÏVE KENYAN SCHOOL CHILDREN**

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Children in schistosomiasis-endemic areas develop partial resistance to infection as they age. This resistance is associated with immune responses, including IgG and IgG, to parasite antigens. Anti-glycan antibodies can kill parasite larvae in vitro and mediate resistance in some animal models of helminth infection, but their significance in human Schistosoma mansoni infection is still unclear. Plasma from S. mansoni-infected children demonstrate specific reactivity with several epitopes on schistosome glycan microarrays. To explore the relationship of such antibodies with naturally-acquired partial immunity, we measured IgG and IgM to mock- and periodate-treated S. mansoni soluble egg antigen (SEA), and two parasite cross-reactive glycoproteins, keyhole limpet hemocyanin (KLH) and horseradish peroxidase (HRP), in plasma from a population of treatment-naïve Kenyan school children. The ratio of antibody reactivity with periodate-resistant (primarily non-glycan) versus total epitopes in SEA increased, and antibodies to KLH and HRP glycans decreased slightly as children aged. These trends were especially pronounced throughout adolescence. The anti-glycan antibodies detected included a variety of IgG subtypes. Our results suggest that immune recognition of the glycan epitopes examined in this study are negatively associated with age, but some may warrant further investigation as diagnostics or indicators of the length of exposure to schistosomes. Future studies on anti-glycan antibodies to other epitopes or other isotypes/subtypes in naturally-acquired immunity, and on whether anti-glycan antibodies may be involved in resistance to reinfection after praziquantel treatment could be informative for vaccine development.

**CHILDREN WITH CEREBRAL MALARIA LACK SERORECOGNITION OF A DISTINCT PFEMP1 SUBSET**

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Plasmodium falciparum erythrocyte membrane protein-1 (PFEMP1) antigens play an important role in parasite sequestration and host immune system evasion. Acquired antimalarial immunity is at least partially due to antibodies directed against highly variable antigens like PFEMP1 that are present on the red blood cell surface. However, the PFEMP1 antigenic domains that drive this immune-mediated protection have not been identified. We have previously shown that infected erythrocytes from persons with cerebral malaria express a distinct “stealth” PFEMP1 group that do not bind the endothelial receptor CD36. We hypothesized that children with cerebral malaria lack serorecognition to a subset of PFEMP1s.

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that is subsequently recognized in convalescence. A protein microarray was printed with 171 fragments of PfEMP1s based on the 3D7 reference genome. For comparison, 268 diverse apical membrane 1 (AMA1) fragments, 20 merozoite surface protein 1 (MSP1) fragments, and 30 Rh5 fragments were also included on the array, based on sequences derived from field samples. Reactivity was measured in 195 serum samples from Malian children, including 43 cases of cerebral malaria and age-matched controls who were healthy or had uncomplicated malaria. Children with cerebral malaria had lower seroreactivity to stealth and non-stealth PfEMP1 antigen variants than both healthy controls and uncomplicated malaria controls. Seroreactivity to AMA1, Rh5, and MSP1 variants tested did not increase from acute cerebral malaria illness to convalescence, but seroreactivity to four stealth PfEMP1 fragments increased, suggesting that a lack of immunity to a subset of PfEMP1s may be associated with vulnerability to cerebral malaria.

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CYTOKINE RESPONSES TO THE VAR2CSA VACCINE CANDIDATE IN CORD BLOOD FROM BENINESE NEWBORNS

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The STOPPAM consortium conducted 2 longitudinal cohort studies in pregnant women in Benin and Tanzania in order to evaluate the immunopathological consequences of infections with Plasmodium falciparum during pregnancy for the newborns. In order to study the exposure of the foetal immune system to parasite-derived antigens in utero, we evaluated both, cytokine (IL10, IL12, IL13, IL17, IFN-γ, TNF-α) responses and T cell IFN-γ specific responses to the vaccine candidate antigen DBL5 domain of VAR2CSA as a function of placental infection with P. falciparum. In Come, southwestern Benin, we conducted a longitudinal prospective study of ~1000 pregnant women. Women at ≤24 weeks of pregnancy were enrolled and followed at each antenatal visit until delivery. For the immunological sub-study of the cord blood mononuclear cellular (CBMC) responses to VAR2CSA-DBL5 in vitro, a group of 200 pregnant women was selected on delivery at the basis of their history of infection with P. falciparum (uninfected during pregnancy/infected during pregnancy but uninfected at delivery/infected at delivery). Those harbouring P. falciparum infections at delivery were matched by gravidity and gestational age with mothers with no infection and those with no history of infection earlier in the pregnancy. The amounts of IL10, IL12, IL13, IL17, IFN-γ and TNF-α produced in response to mitogen (PHA) and to VAR2CSA-DBL5 were quantified in supernatants of stimulated CBMC. The ex vivo frequencies of IFN-γ secreting CD4 and CD8 T cells in response to PHA and VAR2CSA domains were also evaluated. At the time of writing, all data have been collected, cytokine concentrations have been evaluated and multivariate analyses are under way. Results will be discussed in the context of cytokine profiles that reflect the in utero acquisition of a specific cellular memory response to the vaccine candidate.
 IMMUNE CHARACTERIZATION OF PLASMODIUM FALCIPARUM PARASITES WITH A SHARED GENETIC SIGNATURE: VARIANT SURFACE ANTIGENS AND VAR REPERTOIRES

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As malaria transmission intensity has declined in some regions, Plasmodium falciparum parasite populations are displaying decreased clonal diversity resulting from the emergence of many parasites with identical genetic signatures. We have monitored genetically identical parasite clusters from 2006-2013 in Thiès, Senegal, and we have characterized the immune response against these parasites. We focus on one cluster of identical parasites that was present in 24% of clinical isolates in 2008 and declined to 3.4% of clinical isolates in 2009. We studied the susceptibility of 2 representative common genetic signature (CGS) parasites and 1 representative non-CGS parasite and measured the infected RBC IgG reactivity for 109 individual plasmas distributed between both years by variant surface antigen (VSA) flow cytometry. By VSA flow, the non-CGS parasites are similarly recognized by plasma IgG from 2008 and 2009, but reactivity is increased in 2009 compared to 2008 for the CGS parasites. We characterized the var genes expressed by CGS parasites by var Ups qRT-PCR and by sequencing using degenerate DBL1alpha domain primers. We observed that the CGS parasites expressed the same var Ups classes, and the same dominant var repertoire as identified by both DBL1alpha sequence analysis as well as RNAseq. Additionally, we used network analysis to compare the diversity of the var repertoires with that of globally diverse parasites. We generated a var sequence network that shows that the var repertoires of CGS-1 and CGS-2 overlap substantially, while the repertoire of the non-CGS parasite is unique at the level of globally diverse parasites. Taken together, our work indicates that these CGS parasites express similar var genes, more than would be expected by chance in the population, and there is year-to-year variation in immune recognition of these CGS parasites at the level of surface expression of VSAs. We are currently expanding these findings to other large clusters such as one that emerged in 2009 and persisted at a high frequency (26-30%) for multiple years before disappearing from the population in 2013.

SINGLE-CELL TRANSCRIPTIONAL ANALYSIS OF MALARIA-SPECIFIC CD4+ T LYMPHOCYTES FOLLOWING PFSZ VACCINATION AND PROTECTION IN HUMANS

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Intravenous immunization with highly purified, radiation-attenuated parasites (PFSZ Vaccine) is safe, immunogenic and confers high-level protection against controlled malaria infection in humans. Protection was associated with a dose-dependent increase in PFSZ-specific antibodies, CD4+ and CD8+ T cell responses. Heretofore, multi-parameter flow cytometry has been used to characterize the magnitude and quality of PFSZ-specific T cell responses following vaccination or infection. To substantially expand the analysis of such responses, we performed high-resolution, quantitative transcriptome analysis of PFSZ-specific CD4+ T cells. Accordingly, PFSZ-specific CD4+ T cells expressing the costimulatory marker CD154(CD40L) were sorted following in vitro activation with sporozoites. We first demonstrate that ~30 percent of PFSZ-specific CD154+CD4+ T cells do not produce IFN-γ, IL-2 or TNFα, the most common cytokines used to assess T cell responses. This finding highlights the increased sensitivity of the CD154 capture assay for broader assessment of antigen-specific responses. Furthermore, isolation of live malaria-specific CD4+ T cells permits downstream mRNA analysis using valved microfluidic chips from Fluidigm. Quantitative expression of ~100 genes can be rapidly analyzed from isolated samples of single antigen-specific T cells. Initial transcriptome analysis of protected subjects revealed that malaria-specific CD4+ T cells express a unique gene expression signature that is distinct from influenza-specific CD4+ T cells in the same individual. These data will serve as an internal control to compare virus- and parasite-specific responses. We are currently analyzing the gene signature of PFSZ-specific CD4+ T cells from vaccinated and protected subjects prior to challenge vs. nonvaccinated controls during infection. Overall, this analysis should advance our understanding of the heterogeneity of parasite-specific CD4+ T cell responses at the single-cell level and provide insights into how CD4+ T cells may influence protection against human malaria infection.

IMMUNOLOGICAL PROFILING AFTER SPOROZOITE IMMUNIZATION UNDER CHEMOPROPHYLAXIS IN THE CONTROLED HUMAN MALARIA INFECTION MODEL

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A unique tool to study malaria immunology and efficacy of immunisation strategies form Controlled Human Malaria Infections (CHMI) and has proved to be a reproducible, predictable and safe method of inducing Plasmodium falciparum (Pf) malaria. An efficient method for induction of complete protection in humans was achieved by exposing human subjects to Pf-infected mosquitoes while taking blood-stage suppressive chloroquine prophylaxis. When tested in clinical trials, this protocol induced > 95% clinically and parasitologically sterile protection against a standard challenge infection. Longlasting CPS-induced protection was primarily mediated by immunity to sporozoite and liver stages rather than to asexual blood-stages. This opens opportunities to explore mechanisms of protective immunity, allowing the search for immune correlates/ signatures of protection and clinical development of a whole sporozoite based vaccine. Humoral and cellular immune responses associated with protection to Plasmodium falciparum parasites will be presented.
GENE PROFILING IN NAÏVE AND SEMI-IMMUNE COLOMBIAN INDIVIDUALS SUBJECTED TO EXPERIMENTAL CHALLENGE WITH PLASMODIUM VIVAX SPOROZOITES

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In the context of a Plasmodium vivax malaria vaccine program, a total of 16 Colombian malaria naïve (n=7 from Cali) and semi-immune (n=9 from Buenaventura) volunteers were subjected to an experimental P vivax sporozoite infectious challenge using direct infected Anopheles mosquito bites (2-4) and followed up to determine the prepatent period, immune response and clinical outcome of the infection. Volunteers were closely monitored and treated as soon as malaria infection was detected by microscopy. The study offered a unique opportunity to assess the gene expression profile induced by P. vivax malaria infection in naïve and previously infected human volunteers. Blood samples were used for immunological analyses and RNA preserved in Tempus tubes was isolated for transcriptomic analyses. We used a Fluidigm nanofluidic qRTPCR array to profile the expression of 92 genes in the 16 individuals across 6 time-points following infection. The genes were chosen to represent 10 axes of variation that describe major components of transcriptional variation in peripheral blood. Strong covariance of transcript abundance was observed for 8 of these axes, 2 of which correspond to the first two overall principal components of variation. The results show that there is strong up-regulation of an interferon-response axis at the peak of parasitemia, but a down-regulation of the inflammatory response at the same time. Another set of transcripts was observed to be significantly up-regulated both in the naïve samples and at the peak of parasitemia, but across the entire experiment the difference between the naïve and pre-immune samples was minor relative to among individual variation. Nevertheless, these results strongly suggest that whole transcriptome profiling will uncover a set of genes that respond differently to infection as a function of the degree of prior exposure to malaria.

GAMETOCYTE CLEARANCE IN MELANESIAN CHILDREN TREATED FOR PLASMODIUM FALCIPARUM AND P. VIVAX MALARIA WITH ARTEMISININ COMBINATION THERAPY

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We present a detailed analysis of gametocyte clearance in Melanesian children infected with Plasmodium falciparum or P. vivax and treated with either Artemether-Lumefertrine (AL) or Artemisinin-Naphthoquine (AN). In addition, a detailed comparison of three methods, namely standard light microscopy (LM), magnetic fractionation (MF) and reverse transcriptase polymerase chain reaction (RTPCR) for detection of Plasmodium falciparum and Plasmodium vivax gametocytes is presented. Children (0.5-5 years) from the north coast of Papua New Guinea were randomly assigned to either AL or AN treatment upon presentation at the health centre with either P falciparum or P vivax malaria. LM was conducted by 2 trained microscopists with discordant reads judged by an expert microscopist. MF was conducted as previously described on the same day using 200 µL of blood. Samples for RTPCR were placed directly into RNA-later and stored at -80°C until analysis. MF and RTPCR were similarly sensitive and specific, and clearly superior to LM detection of gametocytes. P falciparum gametocyte clearance characteristics were found to be different between AL and AN, mostly due to a longer predicted gametocyte sequestration time in the AN group. However AN treatment provided longer protection from gametocytaemic relapse and/or reinfection. P. vivax gametocytes were found to be cleared very rapidly and along with the asexual blood stages upon treatment with AL or AN, highlighting the fundamental differences between the P. falciparum and the P. vivax parasite species. This study represents the first direct comparison of LM, MF and RTPCR on a large number of field isolates. It provides clear evidence that magnetic fractionation is superior to light microscopy and can be used to detect gametocytaemic patients under field conditions with similar sensitivity and specificity as RTPCR. Furthermore this study illustrates fundamental differences between ACT mediated clearing of P falciparum and P vivax gametocytes and describes differences in the effect of AL and AN on P falciparum gametocytes.

DEVELOPMENT AND EVALUATION OF A SIMPLIFIED MOLECULAR DIAGNOSTIC PLATFORM FOR MALARIA: THE DIRECT ON BLOOD PCR-NALFIA SYSTEM

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Molecular tools allow for specific and sensitive malaria diagnosis, but current formats, like PCR with gel-electrophoresis, are difficult to implement in resource poor settings. Therefore, a simple, fast, sensitive and specific molecular diagnostic platform, direct on blood (db)PCR combined with nucleic acid lateral flow immunosay (NALFIA) to detect amplified PCR products of Plasmodium, including species differentiation, and human GAPDH (internal amplification control) was developed. This platform does not require DNA extraction and circumvents complex read-out system. The platforms was evaluated under laboratory conditions, a multi country ring trial and in two malaria endemic countries (Burkina Faso and Thailand). Analytical sensitivity and specificity of the dbPCR-NALFIA in a single laboratory evaluation was >95% and the test was able to detect less than 1 parasite/µl blood. All four laboratories in the ring trial reported ease of use of the system and could successfully perform the protocol. Overall laboratory inter-variability was low and the agreement of reported results was high. Overall k-value was 0.89 (95% CI: 0.83 - 0.94; p<0.001). Overall test sensitivity and specificity was >95% with very small confidence intervals. Field evaluations by local staff without prior training in performing the dbPCR-NALFIA in malaria endemic countries, Thailand and Burkina Faso, were performed. In Burkina Faso (P falciparum environment) the relative sensitivity was 94.8% and relative specificity 82.4% compared to microscopy and 93.3% and 91.4% compared to RDT. In Thailand (P vivax environment) the relative sensitivity and relative specificity was 93.4% and 90.9 respectively compared to microscopy and 95.6% and 87.1% compared to RDT. These numbers are an underestimation of test performance as the results are not PCR corrected. The prototype dbPCR-NALFIA test will now be moved forward in diagnostic test development (supported by EU funding: www.diagmal.eu) to provide a molecular diagnostic test to detect malaria in for example near elimination settings. The final format will include a closed transfer unit to reduce possible workspace contamination with ampicilins. Funding: EU FP7 grant 601714: Translation of the direct-on-blood PCR-NALFIA system into an innovative near point-of-care diagnostic for malaria
1805

DEVELOPMENT OF A SINGLE NUCLEOTIDE POLYMORPHISM BASED BARCODE FOR THE IDENTIFICATION AND TRACKING OF PLASMODIUM VIVAX

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For global eradication of malaria to occur an enhanced understanding of the population structure of Plasmodium vivax is needed. An inability to maintain P. vivax continuously in culture has caused research on this parasite to lag behind that of the other Plasmodium species. Recent advances in molecular methods have enabled the P. vivax genome to be assayed directly from infected humans, providing the tools needed to develop genotyping methods. Single nucleotide polymorphism (SNP) genotyping provides a robust, inexpensive, field-deployable technology that allows malaria parasites to be tracked and identified by creating a unique genetic signature or barcode for each parasite. Using our experience in developing a SNP genotyping tool with quantitative PCR and High Resolution Melting (qPCR-HRM) for P. falciparum, we have developed a SNP barcode for P. vivax. The candidate SNPs were selected with a high minor allele frequency (MAF) from available P. vivax genome sequence data and were located at sites including intergenic, intragenic, or were 4-fold degenerate coding sites. Here, we report a pilot screen of a 95 SNPs by genotyping a set of 89 P. vivax containing clinical samples from geographically distinct parasite populations from the Americas (Brazil, French Guiana, Africa (Ethiopia) and Asia (Sri Lanka). Candidate SNPs were winnowed to a 41-SNP barcode based on robustness and reproducibility of the genotyping calls, and the ability to accurately detect polygenomic infections. The assays are robust with a detection range from 10 ng to 0.001 ng with an average assay efficiency of 90% among clinical samples tested. All 41 assays had an average minor allele frequency (MAF) > 0.1. Based on principle component analysis the clinical samples form distinct clusters that correspond to their geographic origin. Interesting, these analysis revealed a high level of polygenomic samples among all populations with Brazil (84%), Sri Lanka (95%), Ethiopia (78%), and French Guiana (62%).

1806

THE APICOPLAST OF PLASMODIUM FALCIPARUM PROVIDES A NOVEL TARGET FOR MOLECULAR DIAGNOSIS OF MALARIA USING POLYMERASE CHAIN REACTION AND LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAYS

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Despite a recent increase in procured rapid diagnostic tests (RDTs), and rate of diagnostic testing in the public sector of the African region, malaria still remains a global health burden with an estimated 627,000 deaths worldwide in 2012. WHO recommends all suspected malaria cases to be confirmed by microscopy or RDT prior to treatment however, millions of people with suspected malaria still do not receive diagnostic tests. As these methods do not rapidly and accurately detect sub-microscopic infections which can also contribute to transmission, high throughput molecular assays such as polymerase chain reaction (PCR) are used to detect asymptomatic and/or low-grade infections. Isothermal amplification methods were recently developed to address some of the major shortcomings of PCR. The most common target amplified in these molecular assays is the conserved small subunit ribosomal RNA 18S locus, which in the Plasmodium falciparum chromosomal genome exists in five to eight copies, depending on the strain. In this study, we report the development and optimization of the apicoplast of P. falciparum as a target for molecular diagnosis of malaria using a single step PCR (ssPCR), nested PCR (nPCR) and loop-mediated isothermal amplification (LAMP) assay. P. falciparum sequences from 15 Gambian isolates and 8 laboratory clones were aligned against the PlasmoDB reference sequence (ID: emb|K95275.2) and primers were designed from a highly conserved region of the consensus sequence, approx. 1.5kb segment of the gene coding for a ribosomal RNA protein (AP|0010|rRNA). The primers were validated in silico and mapped unto the consensus sequence with a web based tool. The assays were optimized for temperature and concentration of primers, deoxyribonucleotides (dNTPs) and magnesium chloride (MgCl2). 272 archived DNA samples from across West Africa and S.E Asia were analyzed against a reference PCR method targeting the 18SrRNA gene. Preliminary results show perfect agreement for ssPCR and nPCR compared with the reference PCR method, while Sensitivity of 100 % (95% CI: 94 % to 100 %), Specificity of 84 % (95% CI: 68 % to 94 %), Positive Predictive Value (PPV) of 91 % (95% CI: 81 % to 97 %), Negative Predictive Value (NPV) of 100 % (95% CI: 89 % to 100 %) and Kappa index of 0.86 (95% CI: 0.76 to 0.97) were obtained for LAMP. Based on the results, the apicoplast genome appears to be a suitable target for sensitive detection of Pfalciparum.

1807

GEOGRAPHICAL DISPERSION AND GENETIC CHARACTERIZATION OF PFHRP2 NEGATIVE PLASMODIUM FALCIPARUM PARASITES IN THE PERUVIAN AMAZON: IMPLICATIONS FOR RAPID DIAGNOSTIC TESTS (RDTs) BASED ON DETECTION OF HRP2

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There is prior evidence of Plasmodium falciparum parasites from clinical cases lacking pfhrp2 and pfhrp3 genes in the Peruvian Amazon Region. As various countries in South America move to introduce malaria rapid diagnostic tests (RDTs) as an alternative for diagnosis, the geographical distribution and genotypic characterization of parasites lacking pfhrp2 and pfhrp3 genes will have major implications for procurement choices for RDTs in the region. Ninety-three P. falciparum samples, collected in different communities from the Peruvian Amazon Region between 2009 and 2010, were used in this study. Genomic DNA was used to amplify 18s rRNA and pfmsp2 to confirm the diagnosis and DNA quality, respectively; pfhrp2, pfhrp3, and their flanking genes in order to assess the frequency of their deletions. Microsatellite analysis was performed using seven neutral microsatellites (MS) and seven novel MS loci flanking the pfhrp2 gene on chromosome 8 (-41kb, -10kb, -4kb, 1.4kb, 2.5kb, 5.2kb and 15kb). The data showed deletion of the pfhrp3 gene in 53.76% (50/93) and pfhrp2 gene deletion in 33.33% (31/93) of the samples. The proportion of the parasite populations that lacked these genes was quite variable from community to community. Among the flanking genes, PF3D7_0831900 (Mal7P1.230) showed the highest deletion frequency.
PLACENTAL MALARIA HISTOPATHOLOGY STUDIES
AN EXTERNAL QUALITY ASSURANCE PROTOCOL FOR PLACENTAL MALARIA HISTOPATHOLOGY STUDIES
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Placental histology can contribute to studies on the diagnosis, prevention and treatment of malaria in pregnancy (MIP). Infections prior to delivery can be identified by detecting hemozoin deposition, and parasitized erythrocytes can be seen in cases where peripheral blood is negative. However histology is prone to artifacts, and requires considerable training in slide reading. There is a general need for external quality control and quality assurance for MIP histopathology studies, where poor preparation can lead to the appearance of a hemozoin-like material which may be mistaken for evidence of prior malaria infection and debris can be mistaken for parasites, leading to false positive reads. In addition, very low level infections may be present in the placentas of semi-immune women, leading to false negatives if insufficient fields are examined or if the slide reader is inexperienced. We developed a protocol to formally provide confidential, blinded, retrospective review of slides with feedback in order to ensure high quality study data and to work towards building local expertise. A random subset of 10% of negative and 25% of positive slides are recommended for review by a single, blinded expert reader. Slides are formally scored on both the presence of parasites and hemozoin in fibrin to ensure high quality study data and to work towards building local expertise. Random subset of 10% of negative and 25% of positive slides are recommended for review by a single, blinded expert reader. Slides are formally scored on both the presence of parasites and hemozoin in fibrin to ensure high quality study data and to work towards building local expertise.

IMPACT OF MALARIA RAPID DIAGNOSTIC TESTS ON CARE OF FEBRILE PATIENTS: CROSS-PROJECT RESULTS FROM THE ACT CONSORTIUM
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The introduction of malaria rapid diagnostic tests (RDTs) is aimed to increase the proportion of febrile patients tested for malaria, but there are gaps in knowledge of what happens to patients who receive an RDT compared to those who do not. Multi-project data from the ACT Consortium provide an opportunity to examine the impact of RDTs on patient care outcomes under different contexts and settings. Twelve projects from seven countries contributed data, including projects in public health facilities, private retailers, and community health worker settings. Each compared different sets of diagnostic methods, resulting in 30 arms for analysis, which were grouped into four diagnostic categories: presumptive, microscopy, RDT, or enhanced RDT (in which RDTs were provided alongside supportive interventions). Patient care outcomes such as RDT use, antimarial prescription, antibiotic prescription, referrals, patient satisfaction, and consultation out-of-pocket costs were summarised for each arm and compared between categories. Preliminary results indicate a lower prescription of ACTs and a generally higher prescription of antibiotics where RDTs were used, compared to arms where only presumptive diagnosis was available. This difference was more marked in enhanced RDT arms. Referral to higher level care was also more frequent among RDT arms. However, the impact of RDTs was not always consistent and may depend on study setting and design, type of sector, characteristics of patients and providers, and measures of RDT implementation and support. Exploration of these contextual factors is underway, with complete results expected to be available in August 2014. While many studies have reported a positive impact of RDTs on patient outcomes, the effect is complex and likely to vary by setting and context. Further scrutiny is needed to better understand the impact of RDTs on patient care beyond their role as an important diagnostic tool.

SCHISTOSOME POPULATION GENOMICS USING SINGLE ARCHIVED MIRACIDA
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Population genomic analyses of schistosomes have not previously been possible due to the difficulty of sampling adult worms and the high repeat content and the large size (360-400Mb) of their genomes. We have developed a robust, inexpensive approach for capture and sequencing of the ~15Mb Schistosoma mansoni exome that can be used for single larval miracida. The approach uses whole genome amplification of miracida preserved on FTA cards, solution-based capture of exome sequences using 120bp RNA baits, and Illumina sequencing, and can be extensively multiplexed to reduce costs and simplify sequence library preparation. To demonstrate the utility of these methods we sequenced exomes from 45 single miracidia collected from a Brazilian location in three lanes of an Illumina HiSeq. We captured >99% of the exome sequences targeted.
obtained between 30-80x read depth per miracidia, and robustly called >70,000 SNPs. The method also efficiently captures exomes from the related parasite, S. rodhaini, providing outgroup sequences and opening up the possibility of detailed dissection of interspecific hybridization within schistosome populations. We are currently using these methods to characterize African S. mansoni populations from the SCAN collection at the British Natural History Museum. The exome data will be used to characterize SNP variation at candidate vaccine and drug resistance loci, to examine geographic differentiation in allele frequencies, and to identify genome regions under strong directional and balancing selection. We believe that this approach will have multiple uses for schistosome epidemiology, population biology and evolutionary genomics.

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POPULATION AND COMPARATIVE GENOMICS OF AFRICAN SCHISTOSOMA MANSONI

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Schistosomiasis is among the most important parasitic diseases, with over 200 million people infected and 300,000 deaths annually across Africa, Asia, South America and the Caribbean caused mainly by three closely related species. Around 90% of cases are in sub-Saharan Africa, where Schistosoma mansoni is one of the two most clinically important species, and the principal cause of intestinal schistosomiasis. A draft reference genome is available for S. mansoni and is being actively curated and improved, based on an isolate from Puerto Rico that has been maintained in research labs for many years. Here, we present genome sequence data and assemblies from seven adult male S. mansoni that were recently collected from the field with minimal lab passage, including six diverse African isolates - the first genomic data from the region of greatest public health interest. We confirm that the S. mansoni reference sequence is a suitable substrate for genomic analysis of African populations. We use this genomic diversity data to investigate signatures of natural selection on the S. mansoni genome, and apply two coalescent-based models to infer the population history of S. mansoni on two continents. Our results show that the New World strains have smaller past effective population sizes (Ne) than African strains, suggesting the possible occurrence of a past population bottleneck. We estimate the divergence time between the African and New World populations, finding support for the hypothesis that S. mansoni colonised the New World via the 16-19th century West African slave trade. In the light of this potential population bottleneck, we investigate systematic differences between South American populations and African populations in both genome structure (copy number variants) and single nucleotide polymorphisms (SNPs).

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USING MICROSATELLITE MARKERS TO DETERMINE SCHISTOSOMA MANSONI GENETIC DIVERSITY UNDER CONTRASTING CHEMOTHERAPY CONTROL STRATEGIES IN LAKE VICTORIA, TANZANIA

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National schistosomiasis control programs rely on mass drug administration using the drug praziquantel (PZQ). The widespread use of a single drug raises concerns of drug tolerance spreading in the parasite population. In the absence of specific markers for PZQ tolerance, neutral genetic markers such as microsatellite loci, and analysis of population genetics can be used to monitor changes in parasite populations and the effect of PZQ-reliant schistosomiasis control on the parasite's adaptation and evolution. In the Lake Victoria region of Tanzania (Mwanza), as part of a larger treatment study on intestinal schistosomiasis caused by Schistosoma mansoni, we are collecting parasite genetic material from school children from 16 villages with contrasting PZQ treatment pressures: annual Community-Wide Treatment (CWT-highest treatment pressure) and biennial School-Based Treatment (SBT-lowest treatment pressure). The infection prevalence at the start of the study was over 25%. In each village larval miracidia samples were collected prior to treatment from infected school children (n=30). For baseline (2012) 18,649 S. mansoni miracidia were collected from 263 children in 16 villages. In Year 2 (2013), 4,724 S. mansoni miracidia were collected from 95 children in the 8 annual CWT villages. Future follow-up collections are planned for May 2014 (16 villages). Samples were collected on to FTA cards and stored in SCAN (http://scan.myspecies.info/). Samples are being analysed using a new set of multiplex panels developed from 20 previously published microsatellites for S. mansoni. Comparison of genetic diversity indices such as number of alleles per locus, allelic richness, observed and expected heterozygosity will be made between baseline and post treatment samples and between annual CWT and biennial SBT control intervention strategies. Initial analysis of baseline-collected material shows little difference in the genetic diversity indices between the two different treatment arms. Year 2 miracidia samples are currently being analysed to determine if there has been a change in genetic diversity compared to baseline in the 8 CWT villages. This study utilizes field-collected material and microsatellite multiplex panels to determine the differential impact of annual CWT vs biennial SBT chemotherapy strategies on parasite clearance and population genetic outcomes.

1813

REPEATED TREATMENTS ARE REQUIRED TO AFFECT SCHISTOSOMA POPULATION STRUCTURE

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Repeated rounds of praziquantel treatment are able to reduce prevalence and morbidity of schistosomiasis, but parasite populations recover within a few years. To understand the dynamics of this recovery, two rural Brazilian communities were surveyed and treated in 3 different years: 2009, 2012 and 2013. On average, 96% of the residents participated in each year, and those who were positive on at least one of 3 stools were treated. Schistosoma mansoni eggs were collected from stool and genotyped using 11 microsatellite markers. Parasite differentiation was evaluated at the level of infrapopulations and component populations. Component populations were defined by host characteristics, village of residence or year of study. During this time, human population demographics changed little. New arrivals were 16% and 5% of the populations in 2012 and 2013, respectively. In both years ~12% moved elsewhere. After 2 rounds of treatment, prevalence decreased by 64% and intensity by 57%. Children 15-20 years old showed the greatest decline, while adults between 51-60 showed the least. Reinfection was 34% in 2012 and 18% in 2013, while incidence was 22% and 15%, respectively. The decreasing rates suggest that these treatments have an effect on transmission. Individual infrapopulations were moderately differentiated (D = 0.055-0.077) on reinfection, indicating the pre-treatment multilocus genotypes were not fully reacquired. Differentiation between the 2 villages decreased from 0.046 to 0.031, consistent with an increase in gene flow between them. Parasites from new immigrants were little differentiated from natives (D = 0.012). Between consecutive years, there was little differentiation (D = 0.008), but comparing 2009 to 2013, differentiation increased notably (D = 0.014). Population structure began to change only after 2 rounds of treatment when total parasite burden decreased by >10 fold. This seems
to be the tipping point for producing a genetic bottleneck or reducing effective population size. Intensive therapy is required to significantly impact the parasite's genetic potential.

### 1814

**EPIGENETIC CONTROL OF ENDOGENOUS AND EXOGENOUS (RETRO)TRANSPOSABLE ELEMENTS IN SCHISTOSOMA MANSONI**

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The epigenetic landscape includes modifications on the chromatin template that establish and propagate patterns of gene expression and silencing, not based on differences in the DNA sequence. Four major epigenetic phenomena are involved: 1) DNA methylation; 2) histone modifications; 3) nuclear gene repositioning; and 4) regulatory non-coding RNAs. Whereas early reports suggested absence of DNA methylation of the schistosome genome, it has now been shown that cytosine methylation regulates schistosome oviposition and embryo development. In addition, post-translational core histone modifications in epigenetic control of transcription of schistosome genes have been described, as have a set of non-coding RNAs -- integral components of the epigenetic machinery, including epi-miRNAs, i.e. miRNAs that regulate expression of enzymes involved in chromatin remodeling and DNA methylation. To evaluate the effect of the DNA methyltransferase inhibitor 5'-azaCytidine (5'-Azac) on the expression of both long terminal repeat (LTR) and non-LTR retrotransposons, schistosomules of Schistosoma mansoni were cultured in 100 µM and 500 µM of 5'-Azac. The parasites were harvested 2 or 7 days after treatment, RNA was isolated and the expression of the Bougainvillia (LTR-retrotransposon) and SR2 (non-LTR-retrotransposon) multi-copy endogenous mobile genetic elements of schistosomes analyzed by qRT-PCR. The expression level of these retrotransposons was upregulated 10 to 20 times in the presence of 5'-Azac. In addition, expression of reporter transgenes increased in schistosomules that had been transformed with virions of pseudotyped murine leukemia virus following culture in media supplemented with 5'-Azac. These findings demonstrated that endogenous mobile elements, which comprise ~45% of the genome of this schistosome are controlled by epigenetic marks. In addition, they indicated a central influence of methylation status on transgene activity, suggesting one avenue forward for enhancing transgenesis of this tropical neglected tropical disease pathogen.

### 1815

**SCHISTOSOMA MANSONI: ROLE OF BIOGENIC AMINES IN NEURONAL CONTROL OF MOTOR FUNCTION**

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Schistosoma mansoni is the main causative agent of schistosomiasis, a disease which infects over 200 million people worldwide. Treatment of the disease is primarily with praziquantel. With the lack of an available alternative, and the widespread use of the drug, there is a fear of the development of resistance. Biogenic amines (BAs) are the largest family of classical neurotransmitters in the schistosome nervous system. They are typically involved in motor control and are important to host infection and worm survival. The goal of this study is to determine the role of BA neurotransmitters in schistosomes, focusing on tyrosine derivatives, which include phenolamines and catecholamines. Using confocal immunolocalization, receptors responsive to the catecholamine, dopamine (DA), SmGPR3 and SmD2, were shown to localize to the main nerve cords of the central nervous system (CNS), and the peripheral nervous system (PNS), respectively. Both receptors localized to neurons innervating worm musculature, indicating a possible role in motility for the receptors. Octopamine (OA), a phenolamine, was also immunolabeled in the adult parasite, and showed widespread labeling in the main neurons of the worm CNS, the first indication that OA is present in schistosomes. In other studies we tested the role of several BAs on schistosomes motility. Treatment with both OA and DA caused marked changes in worm motility as compared to the control. Next, we performed RNAi targeting proteins predicted to be involved in DA and OA signaling in larvae and adult schistosomes, and effects of downregulation were assessed. Several of the RNAi-targeted animals showed strong changes in frequency of body movements and in worm morphology as compared to the control. Together these studies highlight the importance of tyrosine derived BAs in the control of motor activity in schistosomes.
works propose the use of paratransgenesis as a novel vector control tool, which exploits genetically engineered symbionts to deliver anti-parasite molecules to the vector. In order to identify potential determinants of mosquito biology as well as putative candidates for paratransgenesis, we have characterized the reproductive microbiome of two major African malaria vectors Anopheles gambiae and An. coluzzii from field population in Burkina Faso. Specifically, we performed high throughput sequencing of the bacterial 16S gene in the male and female reproductive tracts of mosquitoes. We identified two bacteria genera that are present in all the analyzed specimens, representing the core taxa of mosquito reproductive organs with possible symbiotic interactions with the vector. Nevertheless, although a general core microbiome was identified, we observed a general high diversity among different specimens that might indicate that the reproductive microbiome is highly dynamic and might be influenced by external factors. Indeed, we identified some taxa whose abundance was significantly associated with the environment where the mosquitoes lived. Finally, we identified intracellular bacteria that were previously believed not to colonize natural populations of Anopheles. Remarkably, these bacteria are capable of spreading into insect populations and negatively impact mosquito vectorial capacity by reducing their lifespan and boosting the immune response against parasites. To our knowledge this was the first identification of these intracellular bacteria in malaria mosquitoes, which opens new promising opportunities to exploit these organisms as vector control agents against malaria vectors. These results started to elucidate the composition of malaria mosquito reproductive tract microbiome offering novel opportunities to exploit symbiotic bacteria in the fight against malaria.

1818
PERIOSTIAL HEMOCYTE AGGREGATION IN ANOPELLES GAMBAE OCCURS FOLLOWING DIVERSE IMMUNE STIMULI AND IS ACCOMPANIED BY CHANGES IN MOSQUITO HEART PHYSIOLOGY
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Malaria parasites and other mosquito-borne pathogens must traverse the insect hemocoel prior to being transmitted. This process is affected by hemolymph circulation, as flow influences both pathogen movement and the movement of mosquito-produced immune factors. Using the African malaria mosquito, Anopheles gambiae, as our study system, we recently identified a novel immune tissue, called peristomal hemocytes, that exemplifies the co-adaptation of the insect immune and circulatory systems. Specifically, in response to infection, circulating hemocytes (immune cells) migrate to the valves of the mosquito heart, where they sequester and kill pathogens. We have previously reported the aggregation of hemocytes on the surface of the heart following Plasmodium and Escherichia coli infection, however, little is known about the breadth of this immune response or about how heart physiology changes following infection. In the present study we tested whether peristomal hemocyte aggregation occurs following diverse immune stimuli, whether this response is uniform across the length of the heart, and whether infection affects mosquito heart physiology. We found that peristomal hemocyte aggregation occurs following all types of infections tested, confirming the fundamental role of this immune response. Moreover, peristomial hemocyte aggregation is not uniform along the length of the heart, as larger hemocyte aggregates consistently form in abdominal segments 4, 5, and 6. Finally, peristomial hemocyte aggregation is accompanied by a decrease in heart contraction rates. In summary, these data further describe a recently discovered immune tissue in mosquitoes, and demonstrate how the immune and circulatory systems have co-adapted to fight infection.

1819
BREAKING THE LAW OF EFFECTIVE TEMPERATURE: ECOLOGICAL CONTEXT MATTERS FOR DEVELOPMENT RATE VARIATION MOSQUITO VECTORS
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The resurgence and spread of arboviruses in recent years underscores the continued need to understand the biology of its mosquito vectors including, Aedes aegypti and Culex pipiens complex. Despite advances in the development of vaccines, control of the mosquito vector populations remains the most effective control measure. In order to understand how the environment impacts disease transmission, a thorough understanding of the impact of environmental variables on mosquito biology is needed. Historically, the emphasis has been on temperature and the linear association with insect development rate. This association is widely observed across ecotones, and is often referred to as the law of effective temperature. We tested the hypothesis that the law of effective temperature is contingent on the ecological context of the larval environment in mosquito development. Through a combination of statistical modeling of published rearing experimental research and a larval rearing experiment under gradients of conditions in environmental chambers, we find that intraspecific density and dietary resources mediate the importance of temperature in explaining variation of mosquito development rate. Our results support the hypothesis of environmentally contingent impacts of temperature on mosquito development. These findings have broad implications for the modeling of mosquito population dynamics, climate change and vectorborne disease transmission, and our understanding of nature of mosquito life history evolution.

1820
PARASITE CO-INFECTION AND STRAIN DIFFERENCES AS DRIVERS OF PATHOGENIC VARIATION IN THE CHAGAS DISEASE PARASITE TRYPANOSOMA CRUZI WHEN INFECTING ITS INSECT VECTOR, RHODNIUS PROLIXUS
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Avoiding the over-exploitation of resources in a food patch while still obtaining all the resources needed to live and reproduce is a common challenge among all creatures. For many parasites that live inside another organism this is especially tricky because they do not have the option of moving to a new patch should they be too pathogenic and overexploit their current one. Classical theory suggests that the avoidance of host death should favor intermediate levels of parasite pathogenicity (i.e., negative effects) to the host, however that this is not the case: parasites actually exhibit a wide range of pathogenic effects on their hosts. We investigated parasite co-infection and strain differences as drivers of pathogenic variation in the Chagas disease parasite Trypanosoma cruzi and its sister species, Trypanosoma rangeli when infecting their insect vector, Rhodnius prolixus. Using insect survival, reproduction, and parasite load (qPCR amplification of parasite DNA extracted from each insect) as proxies for parasite pathogenicity, we found that T. cruzi-T. rangeli co-infection significantly reduces the survival of R. prolixus up to 30 days post-infection, but increases reproduction. We also found that T. cruzi pathogenicity in R. prolixus is highly variable, with R. prolixus death at 90 days ranged from 5-80% depending on T. cruzi strain, and at times was far more pathogenic than T. rangeli, a parasite believed to be highly pathogenic to triatomines. Furthermore, insects with higher parasite loads tended to have higher fecundity, presenting evidence for terminal investment in infected bugs.
Our results suggest that the pathogenic variation often found in T. cruzi infection of vertebrate hosts extends to its invertebrate hosts as well, with strain variability and co-infection with T. rangeli as two of the main drivers.

1821

THE EFFECTS OF FORCED-EGG RETENTION ON THE BLOOD-FEEDING BEHAVIOR AND REPRODUCTIVE POTENTIAL OF CULEX PIPIENS (DIPTERA: CULICIDAE)

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High rates of West Nile virus (WNV) transmission to humans are associated with exceptionally hot and dry summers. This is paradoxical since the eggs of Culex vectors of WNV depend on the persistence of containers with water, which decline during droughts. We examined the effects of forced-egg retention on the reproductive success of female Culex pipiens as well as behavioral responses, such as likelihood of secondary blood meals. As controls we examined the effects of female age and delayed mating. We found that early mating is essential to achieve reproductive success and, consistent with an “all-or-none” ovipositing strategy, Cx. pipiens females are able to retain considerable reproductive potential while searching for oviposition sites. Specifically, although forced-egg retention resulted in significant decreases in fitness, the decline was moderate for 5 weeks and most can be accounted for by increases in female age. Consequently, no females took blood more than once per gonotrophic cycle, which eliminates the possibility that heightened vectorial capacity due to multiple blood-feedings increases WNV transmission during periods of drought. Instead, our findings suggest that during droughts populations of Cx. pipiens have time to locate the remaining water holes, which are associated with human populations and WNV-competent bird species.

BEHAVIORAL CHANGE IN ANOPHELES FUNESTUS: AN OBSTACLE TO MALARIA ELIMINATION

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In the long road that leads towards malaria elimination, vector control was undoubtedly an essential component of success. Two major strategies have marked the vector control: the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). However, the effectiveness of these tools is being challenged by the emergence of insecticide resistance and behavioural resistance which thwarting the goal of decreasing malaria transmission. In this study, we focus on behavioural changes of malaria vectors that can hamper the efficacy of vector control interventions in Dielmo, a Senegalese rural village where a longitudinal study of malaria has been conducted. In this village, universal coverage with LLINs was done in July 2008, and in July 2011 all these LLINs were renewed. Adult mosquitoes were collected by human landing catches (HLC) from July 2011 to April 2013 and hourly from 19:00 and 07:00. Collecting mosquitoes was also done by pyrethrum spray catch (PSC) during this period. From January to April 2013, mosquito catches were continued until 11:00 and the entomological different parameters were investigated. This study shows that Anopheles funestus which have disappeared after first introduction of LLINs (July 2008) comes back in malaria transmission in Dielmo. An. funestus remains anthropophilic and endophilic but adopt a behavioural change in biting activity after introduction of LLINs. The human biting rate of mosquitoes collected from 07:00 to 11:00 was eight times higher than the one from 19:00 to 07:00. So the alarming phenomenon is the positive mosquitoes found in the day capture (mean CSP rate of 1.28%) while since distribution of LLINs in this village, no An. funestus has been found positive to CSP. These disturbing observations show the capacity of Anopheles to adapt and circumvent strategies aimed at reducing malaria transmission. In an arms race between malaria control programs and the vector populations, the behaviour change in Anopheles threatens to thwart the goal of decreasing malaria transmission.

1823

INFECTION OF LABORATORY-COLONIZED ANOPHELES DARLINGI MOSQUITOES BY PLASMODIUM VIVAX AND TEMPORAL CHANGE IN GENETIC VARIATION IN AN. DARLINGI

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Anopheles darlingi Root is the most important malaria vector in the Amazonia region of South America. However, continuous propagation of An. darlingi in the laboratory has been elusive, limiting entomological, genetic/genomic, and vector–pathogen interaction studies of this mosquito species. We report the establishment and maintenance of an An. darlingi colony (since July 2013) derived from wild-caught mosquitoes obtained in the northeastern Peruvian Amazon region of Iquitos in Loreto Department. We demonstrate that the numbers of eggs, larvae, pupae, and adults continue to rise at least to the F9 generation. In addition, comparison of feeding Plasmodium vivax by artificial membrane feeding of F4–F9 to F1 generation mosquitoes showed the comparable presence of oocysts and sporozoites, with numbers that corresponded to blood-stage asexual parasitism and gametocytemia, confirming P. vivax susceptibility in the colonized mosquitoes. Additionally, analyses of fourteen microsatellites markers were performed on a subsample of An. darlingi offspring to detect genetic variation and expected reduction in heterogeneity through generations. These results provide new avenues for research on An. darlingi biology and study of mosquito–Plasmodium interactions and malaria transmission in the Neotropics, including new genomic analysis and assessment of transmission biology of malaria parasites.

1824

RATIONAL DESIGN OF OXAMNIQUINE DERIVATIVES THAT KILL SCHISTOSOMES

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Schistosomiasis, a major cause of morbidity, infects >200 million people worldwide. Schistosomiasis control is based on a monotherapy consisting of repeated doses of praziquantel (PZQ). Drug resistance is a concern, especially as it is expected to increase in treatment coverage in sub-Saharan Africa (250 million doses per year for each of the next 5 years). New anti-schistosomal drugs are needed to reduce reliance on a single drug. A new drug could be used in combination with PZQ to minimize the probability of resistance arising to either drug. The goal of this research is to modify an existing anti-schistosomal drug oxamniquine (OXA) to make it more efficacious. We recently identified the gene encoding the Sm-sulfotransferase (SmSULT) responsible for drug activation and determined
the structure of the SmSULT•cofactor•OXA ternary complex at 1.75 Å resolution. These analyses provide detailed information of the mechanism of action of OXA against Sm, while structural analyses of drug–protein interactions direct redesign of OXA. We designed and synthesized 12 OXA derivatives based on four key design aspects: 1) the structural requirements of OXA and its derivatives based on available space in the substrate binding cavity in SmSULT and the key residue interactions from crystallographic studies, 2) the required ortho-electron withdrawing moiety necessary for the sulfonation process, 3) the design of analogs that fall within favorable “drug-like” physical chemical property ranges and 4) the development of efficient and convergent syntheses, allowing for the greatest amount of structural diversity and chemical space to establish structure–activity relationships (SAR). In vitro worm killing assays indicated that three of these analogs were as good as or better than OXA itself. These new compounds with antischistosomal activity have been soaked into the SmSULT•PAP crystals and their mode of binding elucidated. This information will be used to synthesize the next generation of OXA derivatives.

1825

ROLES OF ATP BINDING CASSETTE (ABC) MULTIDRUG TRANSPORTERS IN SCHISTOSOME PHYSIOLOGY, DRUG SUSCEPTIBILITY AND PARASITE-HOST INTERACTIONS

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Although its value in the treatment and control of schistosomiasis is well established, praziquantel (PZQ) has significant limitations. Most notably, it is largely ineffective against immature schistosomes. It is also essentially the only drug available for a disease afflicting hundreds of millions. New therapeutics or adjuncts to enhance PZQ activity and overcome possible drug resistance are urgently needed. We hypothesize that ATP binding cassette (ABC) multidrug transporters offer attractive candidate targets for new or repurposed drugs that either act as anthelmintics on their own, or that enhance parasite susceptibility to existing anthelmintics. ABC transporters such as P-glycoprotein (Pgp) mediate efflux of metabolic toxins, xenobiotics, and signaling molecules, and are associated with drug resistance in many organisms, including parasitic helminths. They exhibit broad substrate specificity and are inhibited by several drugs currently in clinical use. ABC transporters are also implicated in a variety of normal physiological activities such as excretion, maintenance of permeability barrier function, and modulation of immune responses. They transport many potent signaling molecules with high affinity, including several with immunomodulatory activity. Schistosomes exposed to PZQ increase expression of ABC transporters such as Pgp (SMDR2) and multidrug resistance associated protein (SmMRP1), and worms with reduced PZQ sensitivity show higher basal expression of these transporters. PZQ is also both an inhibitor and likely substrate of schistosome Pgp. Disruption of transporter expression (by RNAi) or function (by inhibition) enhances the activity of PZQ against adult parasites, and renders PZQ-refractory juvenile worms susceptible to the drug. Schistosome ABC transporters also appear to be important for normal schistosome egg production. We are currently exploiting molecular and pharmacological tools to understand the mechanism by which schistosome ABC transporters alter PZQ susceptibility and to assess the role of these transporters in the parasite’s modulation of host immune responses. These experiments could lend important insights into schistosome physiology and possibly provide targets for novel antischistosomal agents.

1826

SHORT-TERM ANTIBIOTIC TREATMENT INTERRUPTS THE EXCHANGE OF POLYMORPHIC VESICLES BETWEEN WOLBACHIA AND THEIR FILARIAL HOST

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Wolbachia endosymbionts are crucial for growth, reproduction, and survival of many medically important filarial parasites. Wolbachia have a tightly regulated lifecycle within filarial nematodes. While Wolbachia density is low in microfilariae and vector stage larvae, much higher numbers of the endobacteria are observed in developing larvae and young adult worms recovered from mammalian hosts. We have recently reported that Wolbachia release polymorphic outer membrane vesicles (OMV) that may be essential for their mutualistic relationship with filarial worms. OMV may transport bacterial products that are required for parasite growth and development. Tetracyclines (TET, antibiotics that inhibit bacterial protein synthesis) clear Wolbachia from filarial worms over a period of weeks. This treatment first sterilizes the parasites and eventually kills them. The present study was performed to elucidate the early effects of TET treatment on the morphology of Wolbachia and filarial worms. Gerbils with i.p. Brugia malayi infections were treated with TET on days 19 and 20 post-infection (i.p. injection, 5 mg/kg). Immature female worms recovered on day 21 were studied by transmission electron microscopy using high pressure freezing/freeze substitution fixation. OMV were largely absent near Wolbachia in TET-treated worms, while about half of the Wolbachia in untreated control worms were associated with OMV. Wolbachia in treated worms were often surrounded by membranes that appeared to come from the endoplasmic reticulum. Lateral chords in treated worms were heavily vacuolated with increased glycogen granules compared to untreated worms. Thus TET treatment appears to block OMV production by Wolbachia and promote encapsulation of the bacteria by internal host cell membranes.

1827

POPULATION GENOMICS OF Wuchereria bancrofti ELIMINATION FROM PAPUA NEW GUINEA

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Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, Brugia and Wuchereria, with W. bancrofti (Wb) responsible for ~90% of LF cases. Until recently, Wb has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Here, we report 13 new Wb genomes from the Dreikikir region of Papua New Guinea (PNG). We utilized multiple displacement amplification to amplify and sequence 13 juvenile stage (L3) Wb worms from four patient infections. We report the discovery of 60,000 novel single nucleotide polymorphism (SNPs) and 200 polymorphic microsatellite loci from the genomes. Within patient infections we find that genetic diversity is high, yet concentrated in specific regions of the genome, with large tracts of intervening homozygous sequence. We also identify candidate regions that harbor genes with extended haplotypes and shifted frequency spectrums, signals of either ongoing or recent positive selection. We discuss our results in the context of the recent mass drug administration, and identify SNP loci ideal for future monitoring of elimination success.

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**1828**

**NOVEL TEGUMENT EXPRESSED KUNITZ TYPE PROTEASE INHIBITOR FROM SCHISTOSOMA MANSONI**

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Schistosomiasis is one of the most prevalent and serious parasitic diseases of tropical and subtropical regions with 779 million people being at risk and 207 million infected. Kunitz type proteins belong to the I2 family of protease inhibitors and are involved in diverse biological processes in invertebrates. One secretory type sequence (SmKI) having similarity to Kunitz type protease inhibitors was identified from recent mining of the genome of *Schistosoma mansoni*. Recombinant SmKI was expressed in *E. coli*, purified and an antisera against SmKI was produced in mice and subsequent immunolocalization and western blotting carried out. Gene expression levels were determined within key lifecycle stages of *S. mansoni* using real-time PCR. Serine protease inhibitory assays were also used to determine the inhibitory effect of the rSmKI on trypsin, chymotrypsin, neutrophil elastase (NE), pancreatic elastase and Cathepsin G. Real time PCR indicated SmKI is highly expressed in adult worms which reside in the mesenteric venules of the definitive host. Immunolocalization showed the Kunitz protein is present in the tubercles of the male tegument and along the tegument of the female worm. Notably, western blots showed the level of SmKI was higher in the excretory secretory products of adult worm pairs than in soluble worm antigens. Further, SmKI inhibited trypsin, chymotrypsin and NE, with the highest inhibitory activity recorded against trypsin. Initial screens indicated that rSmKI interfere with both intrinsic and extrinsic blood coagulation pathways as well, indicating another important function. Thus, the SmKI protein may play an important role in schistosome survival in blood by inhibiting NE as well as playing a key role in evading host immune responses. As SmKI is secreted and exposed to the host immune system, we consider that SmKI may be a useful candidate as novel vaccine target to control schistosomiasis. Assays are underway to further understand the function of SmKI and vaccine/challenge experiments will be undertaken to evaluate its protective efficacy.

**1829**

**PLASMODIUM VIVAX LIVER STAGE DEVELOPMENT AND HYPNOZOITE FORMATION IN THE FRG HUHEP MOUSE MODEL**

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The ability of *Plasmodium vivax* to form dormant liver stages (hypnozoites) that can be activated weeks or months after initial infection to cause relapse of malaria is of crucial importance for the unprecedented epidemiological success of the parasite. Yet, little progress has been made to understand the unique biology of hypnozoite formation and activation. Due to the parasite’s strong preference for nonhuman primate and human tissue, the availability of models to study *P. vivax* liver stages is extremely limited. Here we report that the FRG KO mouse model transplanted with human primary hepatocytes (huHep) efficiently supports the development of *P. vivax* liver stages as well as the formation of hypnozoites for *P. vivax* isolates of *P. vivax*. Using a series of *P. vivax* specific polyclonal and monoclonal antibodies, we were able to evaluate the liver stage progression and maturation in the infected liver. The ability of the exo-erythrocytic merozoites to establish blood stage infection upon transfusion with human reticulocytes is being currently evaluated. Furthermore, *P. vivax* infections in the FRG huHep mice carried beyond the time of the liver stage maturation showed that persistence and activation of *P. vivax* hypnozoites can be further investigated in the model to determine the biological basis for liver stage dormancy. Successful evaluation of the antimalarial drugs with known activities on *P. vivax* liver stage infection (Primaquine and Atovaquone) confirmed that the FRG huHep/*P. vivax* infection model could be used as an efficient platform for testing new antimalarial drugs in vivo in quest to accelerate the development of interventions for the radical cure of *P. vivax* infection.

**1830**

**CHARACTERIZATION OF COENZYME A BIOSYNTHESIS PATHWAY REVEAL ESSENTIAL DISTINCTIVE FUNCTIONS DURING PLASMODIUM DEVELOPMENT IN BLOOD AND MOSQUITO**

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Coenzyme A (CoA) is an essential universal cofactor and carrier of acyl groups for all prokaryotic and eukaryotic cells. In nearly all non-photosynthetic cells, CoA biosynthesis depends on the uptake and phosphorylation of vitamin B5 (pantothenic acid or pantothenate). Earlier Studies showed the importance of pantothenate acquisition and phosphorylation for *Plasmodium* survival within erythrocytes. Recently, pantothenate plasma membrane transporter (PAT) was functionally characterized in *Plasmodium falciparum*. PAT was shown to be refractory to deletion and was localized to the parasite plasma membrane. However, very little is known about the in vivo cellular functions of CoA biosynthesis pathway in malaria parasite life cycle stages. We have targeted all enzymes of this pathway for deletion in the mouse malaria model *P. yoelii*. We show that first enzymes of this pathway are dispensable for asexual and sexual blood stage (BS) development but they are essential for mosquito stages development and sporozoite production. However, the last enzymes of this pathway are essential for both BS and mosquito stages development. These results indicate that the first substrates and intermediate products of this pathway can be supplemented by alternative novel pathways inside the blood but not inside the mosquito midgut. Collectively, our data show that CoA de novo biosynthesis is essential for both BS and mosquito stages. This is the first in vivo functional characterization of CoA biosynthesis pathway in a protozoan parasite.

**1831**

**A NOVEL RNA APTAMER SYSTEM FOR FUNCTIONAL GENETICS IN PLASMODIUM FALCIPARUM**

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Malaria is a parasitic disease that is widespread in tropical and subtropical regions, and a major cause of human morbidity and mortality. The most severe form of malaria is caused by the parasite, *Plasmodium falciparum*. A limited set of antimalarial drugs is used to treat the disease, but drug resistance is an increasing problem. Hence, identification of novel anti-malarial drugs is a high priority. While our understanding of *Plasmodium* biology has increased in the post-genomic era, tools for doing functional genetics remain quite limited. This impedes progress in identifying key parasite genes and processes that can be prioritized for drug development efforts. To address this need, our laboratory previously developed a novel small molecule-regulated protein-RNA interaction (TetR-aptamer system) that facilitates robust and inducible regulation of target gene translation in eukaryotic organisms including *Plasmodium*. Here, we present the application of protein engineering approaches to integrate our synthetic control system with native *Plasmodium* translational regulatory mechanisms. In so doing, we achieve substantially increased regulatory dynamic ranges (up to 200-fold) compared to a 5-10 fold range of the original system. With a view to identifying new potential drug targets, we are using this system to study several parasite genes. We envision that
this enhancement in regulatory dynamic range will facilitate functional interrogation of larger numbers of parasite genes with greater confidence that associated biological outcomes can be readily identified.

1832

EXPLORING COMPLEX MALARIA INFECTIONS WITH SINGLE GENOME SEQUENCING

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We have recently developed a single cell genomics platform capable of generating whole genome sequence from a single parasitized red blood cell. This has been extensively validated and can generate SNP calls genome-wide with high (>99%) accuracy, even in species such as Plasmodium vivax for which long term culture is not possible. This method provides a powerful approach for empirically determining the composition of multiple genotype parasite infections, and provides information that is not accessible using standard illumina sequencing of parasite infections. We have applied this approach to complex malaria infections by sequencing 17 single genomes from multiple-clone infections of Plasmodium falciparum (n=1) or P. vivax (n=2). After stringent quality control we scored an average of 62,720 P. vivax SNPs and 61,080 P. falciparum SNPs from each single cell sequence allowing us to map within host divergence between single parasite genomes at exceptional resolution. We use this data to highlight how single cell sequencing can be used to reconstruct genome-wide drug resistance haplotypes from individual infections. Such “phasing” data is expected to be of critical importance for determining the outcome of drug treatment, but cannot currently be determined from bulk sequencing of infections. Second, we have examined the size of blocks of haplotype sharing between genomes within infections and compared these with population data from single clone infections. We observed that parasite genotypes within infections tend to be closely related. Application of single cell genomics and can reveal patterns of relatedness at a fine scale, both within and between malaria infections.

1833

USING GENOMICS TO TRACK PROGRESS TOWARDS MALARIA ELIMINATION

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WHO recommendations for regions of low and moderate endemicity call for program re-orientation at milestones marked by changes in disease prevalence. However, genetic changes in parasite populations may occur before changes in prevalence are measurable. To identify these genomic changes, we examined Plasmodium falciparum samples collected from patients in Thies, Senegal from 2006 to 2013. We genotyped 24 independent SNPs from across the parasite genome - the molecular barcode - to separate annual collections into monogenomic (single parasite genome) and polygenomic (multiple parasite genome) infections. We also performed whole genome sequencing on 190 parasites from monogenomic infections. Following increased control efforts beginning in 2008, we observed large changes in population allele frequencies each season, suggesting enhanced random genetic drift expected from a reduced effective population size. We developed tools to visualize parasite inter-relatedness by molecular barcode and sequencing and identified increasing levels of identity by descent in both. SNP genotyping of monogenomic samples showed clusters with identical molecular barcodes, including several collections where 25-30% of samples shared the same barcode. Whole genome sequence analysis revealed that approximately half of the independent isolates shared between 10 and 88% of their genomes with other sequenced samples, including one obviously hybrid parasite. To our knowledge, this is the first observation of increasing identity by descent in an African population. We show evidence of significant parasite population changes undetectable by standard epidemiological methods. Early identification of population genomic changes associated with changes in transmission offers refined criteria for milestones tracking progress towards elimination. The decreasing costs of genomic analysis make this a feasible option for surveillance of malaria control efforts.

1834

DENGUE VIRUS NONSTRUCTURAL PROTEIN 1 CONtributes TO VASCULAR LEAK IN VITRO AND IN VIVO, WHICH CAN BE BLOCKED BY ANTI-NS1 ANTIBODY

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Dengue virus (DENV) is a mosquito-borne flavivirus consisting of 4 serotypes that causes ~100 million cases of dengue annually. DENV nonstructural protein 1 (NS1) is secreted by infected cells and is found by rNS1 could be inhibited by NS1-immune serum. We hypothesized that NS1 itself may have direct pathogenic effects. We found that fnhar mice inoculated intravenously with 10 mg/kg of DENV2 NS1 combined with a sublethal dose of DENV2 succumbed 3-4 days post-infection equivalently to mice receiving a lethal dose of DENV2. Mice inoculated with 10 mg/kg DENV2 NS1 alone exhibited morbidity but 100% survived, as did control mice receiving a sublethal dose of DENV2. We then tested the direct toxicity of NS1 on endothelial integrity in a trans-endothelial electrical resistance (TEER) in vitro assay. When 100% of mice vaccinated with DENV1 NS1 survived lethal DENV-2 challenge. Because NS1 vaccination blocked DENV pathogenesis, we hypothesized that NS1 itself may have direct pathogenic effects. We examined the protective efficacy of NS1 immunization against lethal DENV2 infection in a mouse model of vascular leak. Interferon αβ receptor-deficient C57BL/6 (fnhar) mice were injected intraperitoneally 3X over 6 weeks with 20 μg of DENV2 recombinant NS1 (fnrNS1) combined with different adjuvants, including alum, Sigma adjuvant system (SAS), CpG DNA, Addavax and/or monophosphoryl lipid A (MPLA). Two weeks after the third immunization, mice vaccinated with DENV2 rNS1 with either SAS + CpG or Addavax + MPLA were fully protected against lethal peripheral challenge with DENV2, whereas mice vaccinated with DENV2 rNS1 with alum or CpG DNA alone were not protected. In addition, heterologous cross-protection was observed, as 75% of mice vaccinated with DENV1 NS1 survived lethal DENV-2 challenge. Because NS1 vaccination blocked DENV pathogenesis, we hypothesized that NS1 itself may have direct pathogenic effects. We therefore vaccinated fnhar mice intravenously with 10 mg/kg of DENV2 NS1 combined with a sublethal dose of DENV2 succumbed 3-4 days post-infection equivalently to mice receiving a lethal dose of DENV2. Mice inoculated with 10 mg/kg DENV2 NS1 alone exhibited morbidity but 100% survived, as did control mice receiving a sublethal dose of DENV2. We then tested the direct toxicity of NS1 on endothelial integrity in a trans-endothelial electrical resistance (TEER) in vitro assay. When 100% of mice vaccinated with DENV1 NS1 survived lethal DENV-2 challenge. Because NS1 vaccination blocked DENV pathogenesis, we hypothesized that NS1 itself may have direct pathogenic effects. We examined the protective efficacy of NS1 immunization against lethal DENV2 infection in a mouse model of vascular leak. 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The role of protection and pathogenesis, and how NS1 contributes to dengue disease. Cytokines and other soluble mediators that correlate with severe disease. The interaction of the virus and host immune response, including cross-reactive structural alterations modify the normal architecture of the vessel wall, vascular homeostasis. In certain diseases, e.g., dengue, functional and the endothelial glycocalyx control the barrier function critical for the shock syndrome may be due to endothelial dysfunction. In the damage to the capillary endothelium seen in severe dengue suggests profound shock and potentially fatal complications is a critical determinant of severe disease. Dengue is the most prevalent arboviral disease in humans and a major public health problem worldwide. Systemic plasma leakage leading to hypovolaemic shock. However microvascular/endothelial dysfunction is a hallmark of severe disease. Increased vascular permeability, sometimes leading to hypovolaemic shock. However microvascular/endothelial dysfunction are difficult to assess clinically. A more refined analysis of well-characterized DENV-2 clinical isolates from the five DENV-2 genotypes demonstrated that all viruses infected IFN signaling in human cells, but sylvatic viruses displayed a superior ability to inhibit STAT1 phosphorylation. We analyzed inhibition of STAT1 phosphorylation by sylvatic strains in non-human primate cell lines and to our surprise there was no blockage. To determine if these observations were specific to sylvatic strains we performed our IFN inhibition assay with a prototypical DENV2 Asian strain and confirmed that inhibition of STAT1 phosphorylation by dengue viruses does not occur in non-human primate cell lines. However, dengue virus was capable of inhibiting IFN signaling in both human and Rhesus macaque primary dendritic cells. IFN-α production was detected in supernatants of dengue virus infected Rhesus macaque dendritic cells and contrast to published studies that have suggested that dengue virus can inhibit IFN-α production in human cells. The observed differences in inhibition of the IFN-α/β pathway in human and non-human primate cells may be cell type specific or could result from the transformation process. Nevertheless, these studies provide awareness of differences in the manipulation of the IFN system by dengue virus in human and non-human primate cells.

Dengue can present with a broad spectrum of clinical phenotypes. The hallmark of severe disease is increased vascular permeability, sometimes leading to hypovolaemic shock. However microvascular/endothelial dysfunction are difficult to assess clinically. A more refined analysis of well-characterized DENV-2 clinical isolates from the five DENV-2 genotypes demonstrated that all viruses infected IFN signaling in human cells, but sylvatic viruses displayed a superior ability to inhibit STAT1 phosphorylation. We analyzed inhibition of STAT1 phosphorylation by sylvatic strains in non-human primate cell lines and to our surprise there was no blockage. To determine if these observations were specific to sylvatic strains we performed our IFN inhibition assay with a prototypical DENV2 Asian strain and confirmed that inhibition of STAT1 phosphorylation by dengue viruses does not occur in non-human primate cell lines. However, dengue virus was capable of inhibiting IFN signaling in both human and Rhesus macaque primary dendritic cells. IFN-α production was detected in supernatants of dengue virus infected Rhesus macaque dendritic cells and contrast to published studies that have suggested that dengue virus can inhibit IFN-α production in human cells. The observed differences in inhibition of the IFN-α/β pathway in human and non-human primate cells may be cell type specific or could result from the transformation process. Nevertheless, these studies provide awareness of differences in the manipulation of the IFN system by dengue virus in human and non-human primate cells.
MONOCYTE RECRUITMENT TO THE DERMIS AND DIFFERENTIATION TO DENDRITIC CELLS INCREASES THE NUMBERS OF TARGETS FOR EARLY DENGUE VIRUS REPLICATION

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The four serotypes of dengue virus (DENV1-4) cause the most prevalent arthropod-borne viral disease in humans. Although Aedes mosquitoes transmit DENV via the skin and studies have identified Langerhans cells as targets of DENV replication in the epidermis, no information exists about the immune response and DENV infection in the dermis. DENV suppresses the interferon response, replicates, and causes disease in humans but not in wild-type mice. Here, C57BL/6 mice lacking the interferon-α/β receptor (Ifnar−/−) had normal frequencies of hematopoietic cells in the skin, were susceptible to intradermal DENV2 infection, and developed disease that displayed key features of severe dengue in humans. For the first time, we identified dermal dendritic cells (DCs), macrophages, and monocytes as targets for DENV replication in the dermis of Ifnar−/− mice. We made the following observations. (1) CD103+ DCs and macrophages were present in the steady-state dermis and were the first DENV-infected cells in the skin 12 hours post-inoculation (hpi); they then decreased in frequency over time and no longer contributed to DENV replication after 48 h. (2) Substantial numbers of CD11b+ Ly6C− DCs were present and were continuously DENV-infected between 12 and 72 hpi. (3) Ly6Chigh monocytes were actively recruited to the DENV-infected dermis as early as 12 hpi and, by 48 h, differentiated to Ly6C+ monocyte-derived DCs (moDCs). Ly6Chigh monocytes and Ly6C+ moDCs became DENV-infected 48–72 hpi and were then the major subsets for DENV replication in the skin. Finally, adoptive transfer of Ly6Chigh monocytes from Ifnar−/− and WT mice confirmed recruitment of circulating monocytes to the DENV-infected dermis, differentiation to Ly6C+ moDCs, and DENV infection of de novo recruited cells. Our study identifies dermal DCs and macrophages as the initial targets for DENV replication in the skin. Further, we establish a novel mechanism of how DENV exploits the immune response in the dermis by recruiting monocytes and moDCs, which then become the major targets for virus replication in the skin.

DENGUE VIRUS INFECTION INHIBITS THE CGAS/STING/IRF3 PATHWAY IN INFECTED HUMAN CELLS

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Dengue virus (DENV) has become the most relevant arthropod-borne virus that affects humans. To productively infect the host, DENV needs to inhibit the host innate immune system, particularly the type I interferon (IFN) system. Our group and others have demonstrated that DENV interferes with both, the production and signaling of type I IFN pathways through the expression of viral proteins that specifically target host proteins involved in these essential responses to pathogens. Our group showed that the DENV protease complex NS2B3 is able to interact and cleave the adaptor STING to inhibit the activation of IRF3 in infected cells. A recent report showed an anti flavivirus activity of the newly described pattern recognition receptor cGAS, which, after activation generate a second messenger (cGAMP) that in turn activates the adaptor STING and the subsequent induction of type I IFN. In order to investigate the role of cGAS during DENV infection we evaluated the ability of this protein to be activated and trigger type I IFN during DENV infection. We also investigated the role of the NS2B3 protease complex in the inhibition of type I IFN production induced by cGAS. We have found a novel mechanism of type I IFN inhibition by the DENV protease through the interference of the cGAS/STING/IRF3 pathway. Also, over expression of cGAS impaired DENV replication. Alternatively, silencing of cGAS in human dendritic cells (DCs) resulted in a higher accumulation of DENV RNA after infection. These results suggest an active role of cGAS as a sensor during DENV infection and confirm the role of DENV protease as a master regulator of the type I IFN response in DENV infected cells.

DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL FOR SECONDARY DENGUE VIRUS INFECTION USING MARMOSETS (CALLITHRIX JACCHUS): DETECTION OF VIRUS IMMUNE-COMPLEX USING Fcγ RECEPTOR EXPRESSING CELLS

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Infection with one dengue virus (DENV) serotype does not offer protection against secondary infection against a heterologous serotype. Antibodies to dengue virus (DENV) possess two competing activities: antibody-mediated virus neutralization that leads to protection and infection-enhancement that may cause severe complications. In this study, marmosets (Callithrix jacchus) were infected with DENV-2 and subsequently inoculated with DENV-1, DENV-2 or DENV-3 to evaluate the model utility as an infection model for DENV infection. Viremia levels were determined by RT-PCR, BHK and FcγR-expressing cell lines. Antibody response was determined by IgM and IgG ELISA, and neutralizing antibody titers were determined by using BHK cells and FcγR-expressing BHK cells. All marmosets consistently developed viremia after secondary heterologous infection and primary infection. Viremia was absent during secondary homologous challenge. As compared to primary infection, viremia during secondary heterologous challenge persisted longer. Higher levels of viremia were detected using FcγR-expressing cells as compared to FcγR-negative cells during secondary heterologous challenge in marmosets, suggesting presence of infectious virus-immune complex during secondary infection. However, levels of viremia were similar after primary challenge using FcγR-expressing cells and FcγR-negative cells. IgM and IgG antibody response in primary and secondary inoculation were consistent to those of human DENV infection. Marmosets also exhibited thrombocytopenia, leukopenia and increase in AST, ALT and LDH levels during DENV infection. The animal model also demonstrated enlarged liver and kidney during secondary DENV infection. Neutralizing antibodies were serotype cross-reactive in FcγR-negative cells but were specific to primary serotype in FcγR-expressing cells. During secondary infection, marmosets demonstrate viremia and antibody responses consistent with those of human DENV infection. Strong antibody responses induced after secondary heterologous infection possess high neutralizing antibody titers against all four DENV serotypes. The results suggest the potential of marmosets as a useful animal model for DENV infection.
MOSQUITO INFECTIVITY AND GAMETOCYTE CARRIAGE AMONG PATIENTS PRESENTING WITH UNCOMPlicated FALCIPARUM MALARIA IN NORTHERN CAMBODIA

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Halting the spread of malaria relies on identifying the transmission reservoir and providing effective transmission blocking therapy. Membrane feeding studies have shown that a substantial proportion of Plasmodium falciparum infected African children are capable of infecting mosquitoes in the absence of smear-detectable gametocytes. Less is known about transmission in Southeast Asia. We sought to determine the infectivity of adults with P. falciparum infection in northern Cambodia relative to gametocytemia. As part of a therapeutic efficacy study of dihydroartemisinin-piperaquine, half of patients with uncomplicated P. falciparum were randomized to receive a 45mg single dose primaquine on the 3rd day of dosing. Patient blood was membrane-fed to Anopheles dirus mosquitoes prior to treatment and on days 4, 7, and 14 following treatment. At Day 9 after membrane feeding, 50 mosquitoes were dissected for oocyst detection, while another 50 were saved at Days 9 and 16 for parasite detection by real-time PCR. Among 108 patients studied, 7 (6.5%) patients carried smear-detectable gametocytes at baseline (median 66 gametocytes/µL, range 5-728), and only 2 of 7 successfully infected mosquitoes. Both transmitting mosquitoes had high levels of gametocytemia (705 and 728 gametocytes/µL) resulting in high oocyst prevalence (26% and 70% of mosquitoes with average 2 and 56 oocysts/midge, respectively). Of the remaining patients without smear-detectable gametocytes, only 1 was infectious to mosquitoes, resulting in 8% oocyst prevalence with an average of 1 oocyst/midge. These results show a 30-fold greater transmission potential in patients with microscopic P. falciparum gametocytemia. PCR analysis for submicroscopic gametocytemia and oocyst positivity is currently in progress. However, our findings suggest that in an area with low P. falciparum endemicity where gametocyte carriage is relatively rare, only a small minority of patients with symptomatic malaria contribute to the bulk of human-to-mosquito transmission.

IVERMECTIN FOR MALARIA CONTROL: INSIGHTS FROM MODELLING

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Ivermectin (IVM), used alongside mass treatment strategies with an artesiminin combination therapy (ACT), has been suggested as a possible tool for reducing malaria transmission. Mosquitoes feeding on humans that have recently ingested ivermectin have a reduced lifespan, meaning they have a lower probability of completing sporogony and they complete fewer gonotrophic cycles. We use human pharmacokinetic data and mortality data for mosquitoes taking bloodmeals containing IVM to quantify the mosquitocidal effect of IVM. This is incorporated into a transmission model to estimate the impact of IVM in combination with mass treatment strategies with an ACT on transmission metrics. Adding IVM increases the reductions in parasite prevalence achieved and delays the re-emergence of parasites compared to mass treatment alone. This transmission effect is obtained through its effect on vector mortality. IVM effectiveness depends on coverage with the highest impact achieved if given to the whole population rather than only those with existing detectable parasites. Our results suggest that including IVM in a mass treatment strategy can reduce the time taken to interrupt transmission as well as help to achieve transmission interruption in transmission settings in which mass treatment strategies alone would be insufficient. We also investigate the optimal implementation of ivermectin administration in a range of intervention scenarios, for example whether it best used alongside dihydroartemisinin-piperaquine or artemether lumefantrine in a mass treatment intervention, whether there is any benefit of using primaquine alongside ivermectin, whether ivermectin could be beneficial if used as a stand-alone drug prior to the peak transmission season, and how the vector ecology and existing vector control interventions in a specific region impact the efficacy of ivermectin. Overall, we find that including IVM in mass treatment strategies could be a useful adjunct to reduce and interrupt malaria transmission.
MIND THE GAP: ASSOCIATION BETWEEN HOUSE STRUCTURE AND MALARIA IN UGANDAN CHILDREN

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Good house construction can lower malaria risk by reducing house entry by vectors. We assessed how house design may affect mosquito house entry and malaria risk in Uganda. 100 households were enrolled in each of three sub-counties: Walukuba, Jinja district; Kihhi, Kanungu district; and Nagongera, Tororo district. Light trap collections were made monthly in all homes. All children aged six months to ten years were followed prospectively to measure parasite prevalence routinely every three months and malaria incidence by passive case detection. Homes were classified as modern (cement, wood or metal walls; and tiled or metal roof; and closed eaves) or traditional (all other homes). We will present the association between house design and human biting rate, malaria infection and clinical malaria and discuss the potential of housing as an intervention against malaria, from low to very high transmission areas.

MAINTAINING UNIVERSAL COVERAGE OF LONG LASTING INSECTICIDAL NETS: IMPACT OF CONTINUOUS DISTRIBUTION ON HOUSEHOLD OWNERSHIP IN EASTERN REGION, GHANA

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Distribution of long lasting insecticidal nets (LLINs) is considered a key intervention for the prevention of malaria. Mass distribution is required to rapidly scale up LLIN coverage while continuous distribution systems are essential to sustain the results achieved. In the Eastern Region (ER), the National Malaria Control Programme and implementing partners supported mass LLIN distributions between December 2010 and April 2011. Continuous distribution (CD) activities were started in October 2012 and included antenatal care services, the expanded program on immunization and primary schools. The outcome was evaluated through cross sectional surveys, conducted at baseline in April 2012, 12-16 months after the campaign and at endline in December 2013, after one year of CD implementation. For each survey round, a representative sample of 900 households in ER was selected using a two-stage cluster sampling design. Household heads were interviewed using a structured questionnaire. Household ownership of at least one LLIN was 91.3% (95%CI 88.4 to 93.6) at baseline and fell to 88.4% (85.2 to 91.3) at endline 18 months later but would have been only 81.0% (76.3 to 84.9) without the LLIN from CD. Population access to an LLIN within the household decreased from 74.5% (71.1 to 77.6) at baseline to 66.5% (62.9 to 69.9) but would have been 57.4% (53.0 to 61.8) without the CD contribution. Households reached by any of the CD channels were primarily those who had not been reached by the campaign with any or sufficient ITN. In addition, the different CD channels largely complemented each other with little overlap in the first year. The continuous distribution of LLIN through primary schools and routine health services did not quite maintain the household coverage after one-year of implementation due to its late start almost two years after the campaign. Results show, however, that a CD approach is feasible.

INCREASE IN INTRA-HOUSEHOLD ACCESS TO AND USE OF INSECTICIDE TREATED NETS (ITNs) IN SENEGAL

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With the increase in funding to support malaria control, malaria endemic countries have increased the availability of insecticide treated nets (ITNs) to their populations. Senegal used primarily social marketing until 2008, when a mass distribution campaign targeting children under 5 was conducted, covering 5 of 11 regions. A nationwide campaign targeting children under 5 was conducted in 2009. A rolling universal coverage campaign started in 2010, in which every sleeping space was counted and households received sufficient ITNs to cover every sleeping space, accounting for existing nets in good condition. Between 2010 and early 2013, the universal coverage campaign was implemented in all regions of Senegal. Over this time, 690,000 ITNs were distributed in 2006; 1,065,141 in 2007; 1,586,522 in 2008; 2,532,018 in 2009; 1,258,663 in 2010; 2,465,770 in 2011; and 983,725 in 2012. We used nationally representative survey data to track the evolution of intra-household access to ITNs (a newly recommended indicator), calculated as twice the number of ITNs divided by the number of persons in the household (not to exceed 100%). Based on this indicator, only 11% and 19% of the population had access to an ITN in 2005 and 2006, respectively. In 2008, after the subnational distribution to children under 5 years, access was 36%. The post-campaign survey in 2009 indicated an increase in ITN access to 57%. Universal coverage was completed in four of 14 regions in 2010, resulting in access of 41% nationwide, and 70% in the campaign-covered regions. Access was 63% in 2012, with all but two regions covered. National-level household ownership of at least one ITN from these surveys was 20% (2005), 36% (2006), 60% (2008), 82% (2009), 63% (2010), and 72% (2012), while use by the general population was 6% (2005), 12% (2006), 23% (2008), 34% (2009), 29% (2010), and 41% (2012). Two-thirds of those with access to an ITN reported using it the previous night. Household ITN ownership is an inflated measure of ITN access. Intra-household access is a more appropriate indicator for assessing the gap between ownership and use. Access to nets closely reflects the number of nets distributed annually, and examination of access over time demonstrates the challenge of increasing and maintaining access to ITNs. Additional resources and robust routine distribution strategies are needed to maintain high access to ITNs during the interim periods between larger mass distribution campaigns.

BED NET DURABILITY ASSESSMENTS: EXPLORING A COMPOSITE MEASURE OF NET DAMAGE

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The durability of Long Lasting Insecticidal Nets (LLINs) in field conditions is less well understood making it challenging to determine overall net protectiveness. The 2011 World Health Organization Pesticide Evaluation Scheme (WHO/PEGS) guidelines provide a simple, standardized method using
a proportional hole index (PHI) for assessing net density. We evaluated the accuracy and utility of this measure using LLINs collected over three years in Nampula Province, Mozambique following a mass distribution campaign in 2008. For each LLIN the type of damage, diameter, and distance from the bottom of the net were recorded for every hole. Holes were classified into four size categories and a PHI was calculated based on the WHOPE's guidelines. The areas of WHOPE's defined hole size categories were compared to circular and elliptical areas based on actual diameters of each hole; and the PHI was compared to cumulative damaged surface area of the LLIN. The damaged area of small, medium, and large holes was overestimated and the area of extra-large holes was underestimated using the WHOPE's categories compared to actual measured areas (Wilcoxon signed rank test of differences p<0.0001 for all sizes). Approximating holes as circular overestimated hole surface area by roughly 1.5 to 2 times or more. For a range of hypothetical PHI thresholds associated with a “failed LLIN” found in current literature, roughly 75 to 80% of failed LLINs can be detected by only considering large and extra-large holes (which are easier to identify and count). Future research studies may refine the PHI to better approximate overall surface area interrupted. Furthermore, research is needed to identify appropriate PHI thresholds to deem a net no longer protective. Once a cutoff is selected, logistically simpler methods of determining the effective lifespan of LLINs can help guide replacement strategies for malaria control programs.

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COMMUNITY ACQUIRED BACTEREMIA AMONG CHILDREN IN AREAS OF LOW AND HIGH MALARIA TRANSMISSION IN RURAL WESTERN KENYA

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In many African settings, malaria continues to decline as a cause of febrile illness in children, highlighting the need for improved understanding of alternative causes of fever associated with high mortality. We evaluated the prevalence, etiologies, and correlates of bacteremia in outpatient children at two rural hospitals in Western Kenya. Children aged 6 months-15 years presenting consecutively with fever to Kisii hospital; an area of low malaria endemicity (entomological infection rates [EIR]<1.5) and Homa Bay hospital; a malaria endemic area (EIR≥300), were enrolled between 2012 and 2013. Detailed socio-demographic and clinical data were collected and all children tested for malaria using smear microscopy and Paracheck Pf® rapid diagnostic tests, HIV using antibody or PCR testing, and bacteremia using BACTEC™ 9250 blood culture system. Isolates were identified and tested for antibiotic resistance using MicroScan Walkaway40B. Correlates of bacteremia were evaluated using multivariate logistic regression. Overall, 1476 children were enrolled, 742 from Homa Bay and 732 from Kisii. Children from Homa Bay were younger (mean age±SD: 33.9±18.6 vs. 36.8±24.6 months) and more likely to be malaria-infected (49.2% vs. 8.6%), HIV-infected (4.2% vs. 1.2%) or HIV-exposed (19.3% vs. 3.4%) and more severely ill based on presence of ≥1 IMCI danger signs (51.5% vs. 16.9%). Only 48 children (3.3%) had bacteremia (3.1% in Kisii and 3.4% in Homa Bay). Salmonella spp. (19 NTS and 19 Typhi) were the predominant cause of bacteremia, accounting for 79.2% (38/48) of all isolates, and the distribution of pathogens did not differ between sites. Bacteremia was associated with HIV infection (aOR=4.5; 95% CI: 1.1-19.3) and lower education of caregiver (aOR=2.6; 95% CI: 1.2-5.7); and inversely associated with malaria infection (aOR=0.4; 95% CI: 0.1-0.9). Bacteremia appears to be a relatively uncommon cause of fever in outpatient children in Western Kenya. Given the infrequent availability of blood culture, targeted testing of high-risk children, including those with HIV, may be a useful strategy to reduce mortality among febrile children.

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SALMONELLA TYPHI-SPECIFIC EFFECTOR/MEMORY CD8+ T CELL RESPONSES ELICITED IN A WILD-TYPE S. TYPHI CONTROLLED HUMAN INFECTION MODEL

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Salmonella enterica serovar Typhi (S. Typhi) is a human restricted pathogen which causes significant morbidity and mortality, particularly in developing countries. A better understanding of the immune responses which result in protection from S. Typhi infection is imperative for the development of improved attenuated vaccines. Current knowledge is limited due to the lack of appropriate clinical and preclinical models. Recently, a controlled human infection model was re-established in which volunteers received 104 colony forming units of wild-type S. Typhi (Quailes strain) orally. Twelve volunteers were evaluated for their cell-mediated immune (CMI) responses. ex vivo PBMC isolated before and up to 1 year after challenge were exposed to 3 S. Typhi-infected targets, i.e., autologous lymphoblastoid cell-lines (B-LCL), autologous blasts and HLA-E restricted A2B-LCL cells. CMI responses were evaluated using 14-color multiparametric flow cytometry to detect simultaneously 5 intracellular cytokines/chemokines (i.e., IL-17A, IL-2, IFN-γ, TNF-α and MIP-1b) and a marker of degranulation (CD107a). Pre-challenge CD107a expression and cytokine production by S. Typhi-specific CD8+ T effector memory (TEM) following exposure to S. Typhi-infected targets were higher in most volunteers diagnosed with typhoid (TD) compared to those who were not. Direct correlations were observed between the levels of responses before challenge and time to disease onset for CD107a, IFN-γ and MIP-1b following stimulation with S. Typhi-infected targets. After challenge, decreases in immune responses were observed prior to the time of disease onset, followed by a sharp increase in most TD volunteers. Multifunctional cells (i.e., concomitantly producing 3-5 cytokines/chemokine and/or expressing CD107a) were dominant at all time-points. These data suggest that S. Typhi-specific responses prior to challenge, as well as the magnitude, kinetics and quality (“multifunctionality”) of these responses might play a critical role in the development of typhoid fever.
S. Typhi-specific T<sub>lym</sub> were determined. We identified significantly higher gut homing (integrin-α4β7 expressing) S. Typhi-specific T<sub>lym</sub> prior to challenge in volunteers diagnosed with typhoid (TD) than in those who were not (No TD). At early time-points following challenge, circulating integrin-α4β7 expressing S. Typhi-specific T<sub>lym</sub> decreased in TD volunteers, indicating likely homing and a resulting accumulation in the gut. Additionally, S. Typhi-specific T<sub>lym</sub> from TD volunteers demonstrated up-regulation of activation molecules following challenge, including expression of Human Leukocyte Antigen (HLA)-DR and Lymphocyte function-associated antigen (LFA)-1/CD11a as early as 1–4 days post-challenge compared to No TD volunteers. Furthermore, significantly higher expression of the chemokine receptor CXCR3, a molecule associated with homing to sites of active inflammation, was observed on the surface of S. Typhi-specific T<sub>lym</sub> in TD volunteers 1–4 days post-challenge compared to No TD volunteers. Taken together these results suggest that activation of T<sub>lym</sub> that home to the site of S. Typhi infection may play a role in disease pathogenesis, possibly through suppression of S. Typhi-specific effector T cell responses.

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CHARACTERIZATION OF CANDIDATUS BARTONELLA ANCASHI: A NEW AGENT ASSOCIATED WITH CARRION’S DISEASE

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“The genus” Bartonella consists of Gram negative, facultative intracellular, vector-borne bacteria, which infect a wide range of mammalian hosts. B. bacilliformis, B. henselae, and B. quintana have long been recognized as pathogens of human importance, while other species, such as B. claridgeaeae and B. rochalimae, are newly recognized human pathogens. During a 2003 clinical treatment trial a novel Bartonella species, Candidatus B. ancashi, was uncovered. This treatment trial was conducted, in Ancash, Peru (where B. bacilliformis is endemic), to test the efficacy of azithromycin as a treatment for B. bacilliformis. During this trial, two patients were found to be infected with a Bartonella species disparate from other Bartonella species based on gH2 sequence typing. Subsequently, this new agent, Candidatus B. ancashi, was more completely characterized by 1) observations of in vitro microscopic, phenotypic, colonial morphology, and growth characteristics, 2) multilocus sequence typing (MLST), multispacer typing (MST), and whole genome analyses, and by 3) the development of species-specific qPCR assays to identify Candidatus B. ancashi’s presence in possible vectors (Lutzoma spp). Gram-staining and transmission electron microscopy showed the isolates to be small, Gram-negative bacilli with variable expression of unipolar flagella. Biochemical testing provided a single phenotype for all the isolates, which is consistent with other Bartonella spp. Fully genome sequencing and subsequent genome analyses confirmed these isolates to be genetically identical to one another, yet distinct from other Bartonella species. Genome analyses revealed B. bacilliformis to be the closest relative to Candidatus B. ancashi, although unlike B. bacilliformis, the genome of Candidatus B. ancashi encodes virulence determinates not seen in B. bacilliformis. Surprisingly, whole genome mapping showed major gene rearrangements between the isolates. Additional genome analyses uncovered a possible link between the rearrangements and flagella expression. Based on the results from these studies, we believe Candidatus B. ancashi is a novel pathogen of human importance.

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INVESTIGATION OF GENOTYPE VARIATIONS IN ORIENTIA TSUTSGAMUSHI OBTAINED FROM PATIENTS WITH MILD SEVERE SCRUB TYPHUS

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Orientia tsutsugamushi is a Gram negative, obligate intracellular bacterium that is transmitted by the bite of infected chiggers (Leptotrombidium sp) and is the causative agent of scrub typhus. Scrub typhus presentation varies from a mild illness to severe disease, including pneumonitis, meningitis, encephalitis, disseminated intravascular coagulation, and in some cases death. Additionally, Orientia tsutsugamushi is found in Southeast Asia, the southwestern Pacific Islands, Korea, and parts of Russia, China, Japan, Australia, New Zealand, Pakistan, India, and Afghanistan, where over 1 billion persons are at risk for infection and approximately one million are infected annually. While the genus Orientia contains only two known species Orientia tsutsugamushi and Orientia chuto, the former is extremely genetically diverse, with >100 genotypes currently recognized. A study is currently underway to examine the relationship between disease severity and genotype. 322 clinical isolates were collected from 6,740 adult patients who presented with suspected scrub typhus in Vientiane (n=4875), Luang Namtha (n=1335), and Salavan (n=530) provinces of the Lao People’s Democratic Republic (Lao PDR) from 2004 until 2012. The patients (n=322) were divided by disease severity, with 69 patients exhibiting severe disease and 253 patients exhibiting moderate disease. Severe disease included patients with reduced consciousness (Glasgow Coma Score < 15), shock (systolic pressure < 80 mmHg), jaundice (clinical observations), meningitis/encephalitis (clinical observations), and/or difficulty breathing (respiration rate > 30 breaths/minute). While moderate disease included patients with malaise, fever, headache, and/or rash, who were sick enough to seek medical attention. Additionally, 30 clinical isolates from various locations, within the endemic area for Orientia tsutsugamushi, will be used to improve O. tsutsugamushi genotyping methods. Single Nucleotide Polymorphism (SNPs) analyses will be employed to look for differences between the isolates from various locations in the endemic region and between the isolates that cause severe disease and those isolates that cause a moderate illness in Lao PDR. Through this study, we hope to, identify predictors for severe disease as well as create a more accurate evolutionary phylogeny for Orientia tsutsugamushi.

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SPEEDIER LEPTOSPIRA DIAGNOSIS USING HEMOCULTURE FLUIDS

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Leptospirosis is a common bacterial zoonosis worldwide, with infections occurring after exposure to contaminated water. Despite this global problem, diagnosis is difficult with culture results taking up to three months and Microscopic Agglutination Test (MAT) serology being retrospective by nature. Molecular assays are ~55% sensitive and 90% specific to detect infection on admission blood samples, with low bacterial density complicating detection. Leptospirosis was shown to survive and multiply in blood culture media and we hypothesised that extracting DNA from incubated hemoculture fluid (HCF) from blood
culture bottles, followed by quantitative real-time PCR (qPCR) could improve the sensitivity and speed of leptospira diagnosis. We assessed this retrospectively, using pre-incubated HCF of leptospira positive (n=109) and negative (n=63) (as determined by culture, PCR directly on clinical samples and MAT on convalescent serum) febrile patients in Vientiane, Laos PDR. After optimization, receiver-operator-characteristics analysis was employed to identify the most suitable qPCR-threshold and corresponding diagnostic values. The final method showed promising sensitivities of 82% (95%CI: 71-90), 66% (95%CI: 55-76) and 59% (95%CI: 49-68) compared to culture, culture+PCR or culture+PCR+MAT, as the respective reference standards. The specificities were >95%, for all three comparisons. This approach may enable the diagnosis of leptospirosis infection without the submission of additional samples and the incubation step may further increase the sensitivity without compromising the specificity. The optimized protocol and its usefulness in a routine laboratory setting will be further evaluated prospectively during May-October 2014 and these data will be presented.

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INNOVATING DIAGNOSIS OF BACTERIAL BLOODSTREAM INFECTIONS IN MALARIA-ENDEMIC SETTINGS: FROM DISEASE METABOLOMICS TO RAPID DIAGNOSTIC TESTS

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The increasing use of malaria rapid diagnostic tests has revealed that febrile illnesses are often caused by other pathogens than Plasmodium. Amongst them, bacterial bloodstream infections (bBSI) are increasingly recognized as an important cause associated with a high mortality, especially in the African setting. Currently, diagnosis of bBSI is clinical as microbiological culture testing is usually not available and if available, takes 2 to 3 days for a result. Missed diagnosis can result in preventable deaths, while overdiagnosis results in inappropriate or unnecessary use of antibiotics. There is an urgent need to develop rapid diagnostic tests targeting bBSI. We hypothesize that the pathophysiological processes triggered by bBSI induce characteristic changes in the > 4000 different blood metabolites. Our objective is to harness these characteristic metabolite features for bBSI diagnosis. We are conducting a first metabolomics study to examine whether blood plasma contains metabolites that could be useful to diagnose bBSI in a malaria-endemic setting. We quantified 1600 polar and lipid metabolites in plasma from 83 children with severe febrile illness admitted to a rural district hospital in Burkina Faso using liquid-chromatography mass-spectrometry. The patients included (i) 12 bBSI cases confirmed by blood culture, (ii) 34 severe malaria cases with a positive thick blood film and (iii) 37 cases with negative blood culture and negative blood film. A distinct metabolite profile was identified in children with culture-confirmed bBSI compared to children with severe malaria. A first diagnostic model including 10 polar metabolites has a sensitivity of 80% (95% CI: 44.4-96.9%) and specificity of 76.5% (95% CI: 62.5-87.2%) to identify culture-confirmed bBSI. Mining of the lipid data is ongoing to fine-tune this diagnostic model for differential diagnosis of bBSI and severe malaria. We will present the predicted diagnosis of the 83 patients by the final metabolite diagnostic model(s), and compare to the results obtained with blood culture, PCR-based SepsitTest™, malaria thick blood smear and HRP2 rapid diagnostic test. This study demonstrates the potential of plasma metabolites to identify causality in children with severe febrile illness. We will discuss the translation to metabolite-based rapid diagnostic tests and their potential impact on clinical management of severe febrile illness in malaria-endemic settings.
OVERCOMING THE CHALLENGES OF CLINICAL DATA MANAGEMENT IN LOW AND MIDDLE-INCOME COUNTRIES: A CONTEXT-ADAPTED DATA MANAGEMENT PLAN AND LIFECYCLE

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More and more non-commercial clinical research is carried out on a collaborative basis in resource-limited contexts to address priority health problems in low and middle income countries (LMICs). Despite the existence of FDA- and ICH-GCP guidelines for clinical research, there is a need for a practical approach in all processes including clinical Data Management (DM) to facilitate the collection and analysis of high-quality data, despite the contextual and financial constraints. Since 2010, the members of the Association for Data Management In the Tropics (ADMIT https://admit.tghn.org) share knowledge and tackle common issues experienced like standardization, Electronic Data Capture and Data sharing. One of the first initiatives for ADMIT was to tackle the need for uniformity in terms of context-adapted standard operating procedures (SOPs). We defined the essential DM processes within a research project. The members of the network were assigned authorship to prepare two SOPs which were presented and peer reviewed during a workshop within the wider group. A harmonization process was undertaken to ensure uniform structure, terminology and the level of detail across the suite of SOPs. During the harmonization, the alignment of the individual processes inspired the creation of an overall lifecycle for DM. As a result, a usable Data Management Plan (DMP) is now available incorporating the suite of these SOPs. (In the oral presentation.) We will present the characteristics of this DMP and describe how it may help with collaborations on non-commercial research projects where DM processes are spread across different places. We recommend the DMP to be used to ensure a uniform approach to DM, strengthening partnerships and knowledge exchange. We hope that this may be a starting point for standardization in DM in LMICs and possibly to formulate practical recommendations for regulatory and GCP guidelines. The next challenge is to look at the definition of the roles and responsibilities needed to resource these DM activities and the development of training packages.

LACTIC ACIDOSIS AND RESPIRATORY DISTRESS ARE FREQUENT IN CEREBRAL MALARIA AND SEVERE MALARIAL ANEMIA, BUT PREDICT MORTALITY ONLY IN CEREBRAL MALARIA

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We investigated the pathogenesis of lactic acidosis (LA) and deep breathing (DB) and their associated mortality in 249 children with cerebral malaria (CM) and 216 children with severe malarial anemia (SMA) in Kampala, Uganda. Platelet count and hemoglobin, lactate and histidine rich protein-2 (HRP-2) levels at admission were assessed. Children with a hemoglobin of <5 g/dL were transfused (all SMA, 59 CM). LA was more frequent in children with SMA (43.1%) than CM (32.1%, P=0.02), and DB was similar in the two groups (CM, 8.8%, SMA, 7.4%, P=0.6), but mortality was higher in children with CM (12.5%) than SMA (0.5%, P<0.001). In children with CM, mortality was increased in children with LA (odds ratio (OR), 2.2, 95% confidence interval (CI), 1.0, 4.7, P=0.04) or DB (OR 5.0, 95% CI, 1.9, 13, P=0.001), but in children with SMA neither LA nor DB was associated with mortality. Children with CM had higher HRP-2 levels and lower platelet counts than children with SMA, while children with SMA had lower hemoglobin levels than children with CM (all P<0.001). In children with CM, both LA and DB were associated with decreased platelet counts and increased HRP-2 levels (all P<0.01), while in children with SMA, LA and DB were not associated with platelet counts, and only LA was associated with increased HRP2 levels (P=0.02). Conversely, hemoglobin levels were inversely associated with lactate levels in children with SMA (P<0.001) but not CM. In children with CM and DB, each natural log increase in HRP-2 levels was associated with an 8.6 fold increased risk of mortality (95% CI, 1.1, 67.0, P=0.04). DB and LA in CM are associated with parasite sequestration with platelet adhesion, while in SMA they are associated with low hemoglobin. These differences may in part explain the high mortality associated with DB and LA in CM, and the lack of DB- or LA-associated mortality in children with SMA who receive a blood transfusion.
ANEMIA AND TRANSFUSION REQUIREMENTS AMONG CHILDREN WITH SEVERE MALARIA TREATED WITH ARTESUNATE AT A RESOURCE-POOR HOSPITAL IN UGANDA

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Treatment of non-immune travelers with malaria using parenteral artesunate is associated with late-onset hemolysis. Most cases of severe malaria occur in children in sub-Saharan Africa, where the hematologic effects of artesunate have not been well documented. We report a prospective case series of 92 children with severe malaria, all treated with parenteral artesunate, managed at a resource-poor hospital in Africa, with detailed longitudinal data on hemoglobin (Hb) levels. The median (range) age was 2 (1-8) years and 43 (47%) were female. Fifteen patients had tea-coloured urine at admission and 14 were visibly jaundiced. The median (IQR) admission Hb level was 69 (56-80) g/L and 17 patients (19%) had severe anemia (Hb<50 g/L). During hospitalization, 69 patients (76%) received one or more transfusions of packed red blood cells or whole blood, for a total of 114 transfusions. The median (IQR) total volume of blood administered was 10.4 (5.6-20) mL/kg. Patients with jaundice at presentation received significantly larger volume (p=0.014) and volume (p=0.043) of transfusions. Fatal outcome in 8 patients was associated with severe anemia in 6/8 cases. Follow-up Hb measurement was performed on 35 patients (38%) at day 14 after initial hospital admission; the remaining patients had no clinical evidence of anemia (no pallor, tachycardia, hyperdynamic circulation, parental report of lethargy, or easy fatigability) at the follow-up visit. The convalescent Hb was median (range) 90 (60-138) g/L, which was significantly higher than the paired admission levels (median increase +28 g/L, p<0.001). The day 14 Hb level was higher than any level measured during hospitalization in 22 (63%) patients, but decreased or remained the same in 13 (33%). Among children with a decrease in Hb level by day 14, the magnitude of the Hb change ranged from -8 to -66 g/L, but none reached the threshold for severe anemia (lowest day 14 Hb was 60 g/L). None required transfusion after hospital discharge. In this representative cohort of young children with severe malaria in a hyper-endemic setting treated with artesunate, anemia was common at admission, required one or more transfusions in a majority of patients, and on average was improving by day 14. However, a substantial proportion of children had persistent or worsening anemia at follow-up. Further study is needed to determine whether this effect is attributable to artesunate.
SEVERE MALARIA INFECTIONS IMPAIR GERMINAL CENTRE REACTIONS AND INHIBIT EFFICIENT ANTIBODY RESPONSES TO INFECTION

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Naturally acquired immunity to malaria develops only after many years of repeated exposure to Plasmodium parasites. Protective immunity predominantly targets blood-stage parasites and requires antibody responses. Despite the key role that antibodies play in protection against malaria, the cellular processes leading to the slow acquisition of immunity remain unknown. Children in high transmission settings that experience frequent malaria clinical episodes are characterized by a delayed development of parasite-specific memory B cells, suggesting that the inflammatory factors contributing to disease hinder these responses. To address that hypothesis we used a severe malaria infection model to investigate the development of germinal centres (GC), memory B cells and plasma cells. C57BU6 mice were infected with P. berghei ANKA, followed by treatment with anti-malarial drugs or immunized with equivalent antigenic loads of irradiated parasites. Reduced numbers of GC B cells and T follicular helper cells (Tfh) were found in mice experiencing an active infection compared to immunized control animals. Despite normal IL-21 secretion, Tfh cells from infected mice displayed an unusual phenotype characterized by low surface expression of PD-1 and CXCR5, required for their successful localization in GCs. Consistently, confocal microscopy experiments revealed that clinical malaria inhibits the establishment of GC reactions in the spleen. The frequency of memory B cells and relative antibody affinity of long-lived plasma cells emerging from GCs was also examined. Unlike immunization with irradiated parasites, active infections appeared to compromise these processes. Pro-inflammatory cytokines involved in the induction of severe malaria episodes were found be partly responsible for the inhibition of B cell responses. Thus these data indicate that clinical malaria negatively impact the development of long-term humoral immunity by disrupting critical early stages in the development of B cell responses.

LOSS AND DYSFUNCTION OF Vα2+ Γδ T CELLS IS ASSOCIATED WITH CLINICAL TOLERANCE TO MALARIA

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The Vα2+ subset of γδ T cells possess intrinsic reactivity to malaria antigens, but their role in acquired immunity to malaria is unclear. To evaluate γδ T cell responses in children living in a highly malaria-endemic area, peripheral blood mononuclear cells (PBMCs) were obtained from 78 HIV-uninfected 4-year old children enrolled in a longitudinal cohort study in Tororo, Uganda. The incidence of symptomatic malaria in this cohort was 5.4 episodes ppy (IQR 3.2-7.0) and peaked at 25 months of age with a subsequent gradual decline in malaria episodes and a corresponding increase in asymptomatic parasitemia. PBMCs were stimulated with Plasmodium falciparum-infected red blood cells (iRBC) and assessed by multiparameter flow cytometry and gene expression microarray. We noted a striking inverse association between frequencies of Vα2+ cells and the prior cumulative incidence of malaria (Rho=0.39, P=0.003). Repeated episodes of malaria were also associated with decreased cytokine production (Rho=-0.41, P=0.002) and decreased proliferation (Rho=-0.58, p=0.009) of Vα2+ cells in response to malaria antigen stimulation, suggesting that children who have survived repeated clinical malaria episodes exhibit dysfunction as well as loss of Vα2+ cells. Whole transcriptome analysis of sorted, unstimulated V2+ cells revealed increased expression of immunoregulatory genes in children with heavy prior malaria, including genes encoding Tim-3, BAFF, and CD57, suggesting that repeated infection may lead to the upregulation of immunoregulatory pathways that dampen the innate V2 inflammatory response. Finally, loss and dysfunction of pro-inflammatory Vα2+ γδ T cells was associated with a reduced likelihood of symptoms upon subsequent P falciparum infection. Together, these results suggest that repeated malaria infection during childhood results in progressive loss and dysfunction of Vα2+ γδ T cells that may facilitate immunological tolerance of the parasite.
LONGITUDINAL ANTIBODY RESPONSES TO ANTIGENS ON THE SURFACE OF PLASMODIUM FALCIPARUM GAMETOCYTE-INFECTED ERYTHROCYTES IN GHANAIAN SCHOOL CHILDREN

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Little is known about the immune responses directed at circulating Plasmodium falciparum gametocytes in humans, knowledge of which would be useful in the development of intervention strategies to reduce and block malaria transmission. Consequently, antibody responses to surface antigens of P. falciparum gametocyte-infected RBCs (GSA) were determined in plasma samples from malaria asymptomatic Ghanaian school children between the ages of 5-17 years. These children were screened for malaria parasites and treated with dihydroartemisinin piperaquine and followed up weekly for one month. Gametocytes were produced from a laboratory adapted parasite line, 3D7 and a recent patient isolate from Kenya (HL1204). From a cohort of 113 children, 56% of the children exhibited marked antibody responses to GSA (immune response above the median within the cohort per sampling time) that recognized GSA on a proportion of mature gametocyte-infected RBCs of 3D7 by flow cytometry. These responsive individuals were identified by measuring both the proportion of mature gametocytes recognised by antibodies and the intensity of the antibody binding to GSA. Longitudinal data provided an additional 10% developing GSA responses during the 1 month follow-up. Children with GSA antibodies present at enrolment, were less likely to develop new gametocytaemia at subsequent visits (odds ratio = 0.29, 95% CI 0.06 - 1.05; P = 0.034). 3D7a is a laboratory adapted parasite line so a selection of positive plasma samples was tested against mature gametocyte preparations from HL1204 and strong plasma antibody binding was again shown. No binding to the surface of RBCs infected with immature gametocytes of HL1204 was detected. In conclusion, a proportion of malaria infected asymptomatic children harbour plasma antibodies which strongly recognized antigens on the surface of mature gametocyte-infected RBCs. Strong plasma antibody responses were associated with the control of gametocytaemia in vivo. Ghanaian GSA responses recognized antigens on both 3D7 and a Kenyan parasite line, suggesting that conserved antigenic determinants are present on the surface of gametocyte-infected erythrocytes.

CHARACTERIZATION OF MALARIA PARASITE LINES (PLASMODIUM FALCIPARUM) SELECTED BY LONG-TERM CULTURE IN THE PRESENCE OF INHIBITORY ANTIBODIES TO APICAL MEMBRANE ANTIGEN-1 (AMA1)

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Vaccination has been the most effective medical intervention (after sanitation, hygiene, and nutrition) for preventing and eliminating infectious diseases. The development of resistance to anti-malarial drugs by the parasite has spurred the search for effective vaccines. It remains unclear, however, if the antigenically highly polymorphic malaria parasite will also evolve resistance to vaccines. Antigenic escape, for example, was observed for Combination B (MSM and CFM) and CFM.1 (AMA1) malaria vaccines, and the parasite could also shift to alternative invasion pathways that circumvent the requirement of the vaccine antigen. Thus, it is important to study the effects of long term persistence of inhibitory levels of antibodies induced by a Plasmodium falciparum blood stage vaccine - using in vitro and if possible in vivo animal models of malaria. We have recently shown that AMA1 strain-specific antigenic escape could be overcome by inducing broadly inhibitory antibodies using a Quad-allelic formulation of AMA1 (QuadVax, or QV: 3D7+ FVO+HB3+W2mef allelic forms) which elicited high levels of invasion inhibitory antibodies in rabbits against not only all four vaccine strains but also against 22 antigenically diverse non-vaccine strains (Dutta et al. 2013, PLOS Pathogens). We now used anti-QV antibodies to exert immune pressure on two parasite strains (3D7 and W2mef) in long-term cultures. Parasites were maintained in culture for six months in the presence of ~50% inhibitory concentration of anti-QV rabbit serum while the control parasites were maintained in parallel in the absence of antibodies. During the cultures, parasite lines were frozen at various time-points and at the end of the 6 months of culture the parasites were cloned by limiting dilution. Selection pressure was finally removed and anti-AMA1 selected clones were compared to the parental or control selected parasites. Comparative data will be presented regarding (a) growth and invasion rates in the presence or absence of anti-AMA1 antibodies, (b) parasite DNA sequences, (c) invasion into enzyme treated red cells, and (d) quantity and location of AMA1 and its proteolytic processed products.

A SYSTEMATIC CHARACTERIZATION OF MALARIA-ASSOCIATED ATYPICAL MEMORY B CELLS

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Antibodies (Ab) play a critical role in malaria immunity, but Ab-mediated protection is only acquired after years of repeated infections, leaving children in endemic areas vulnerable to repeated bouts of febrile malaria. Many Plasmodium falciparum antigens are diverse and clonally variant, contributing to the inefficient acquisition of protective Abs. However, mounting evidence suggests that Plasmodium-induced dysregulation of B cell function may also play a role. Several studies have shown that malaria exposure is associated with an expansion of atypical memory B cells (MBCs) which are distinguished from classical MBCs by the expression of inhibitory receptors. A similar subset of B cells has been described in individuals infected with HIV and HCV, yet the origin and function of this B cell subset remains unclear. We performed a comprehensive investigation of atypical B cells collected from individuals exposed to intense malaria in Mali. Sorted naïve B cells (CD19+ CD21+ CD27-), classical MBCs (CD19+ CD21- CD27+), and atypical MBCs (CD19+ CD21+ CD27+) were subjected to genome wide expression profiling, VDJ sequence analysis (Ab heavy and light chain gene usage and somatic hypermutation rate), KREC analysis (replicative history), as well as proliferative and cytokine production analysis following in vitro stimulation. We found that classical and atypical MBCs have distinct expression profiles, but are similar in heavy and light chain variable gene usage as well as replicative history. Atypical MBCs have, however, lower levels of somatic hypermutation in heavy and light chain sequences, indicating less antigen-dependent selection compared to classical MBCs. We further show how these B cell subsets differ in proliferative and cytokine production capacity, and how the expression of inhibitory receptors on atypical MBCs impairs their proliferation. This thorough characterization of B cell subsets in malaria-exposed individuals has generated new hypotheses on how chronic Plasmodium exposure leads to B cell dysregulation and the inefficient acquisition of protective antibodies.
ENHANCED MULTIFUNCTIONAL CD4+ T CELL MEMORY RESPONSES TO MALARIA ANTIGENS IN MALIAN CHILDREN CO-INFECTED WITH SCHISTOSOMA HAEMATOBIUM

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Durable immunologic memory to malaria is limited in young children, where repetitive and ongoing exposure to malaria is required to achieve and maintain immunity. We have previously demonstrated that schistosomiasis-positive (SP) Malian children, aged 4-8 years, are protected from malaria compared to matched schistosomiasis-negative (SN) children. The effect of concomitant S. haematobium upon acquisition of memory to malaria antigens is unknown. We examined antigen-specific T cell frequencies in 48 Malian children aged 4-14 to malaria blood-stage antigens, Apical Membrane Antigen 1 (AMA1) and Merozoite Surface Protein 1 (MSP1) and to schistosoma antigens, Soluble Worm Antigenic Preparation (SWAP) and Schistosoma Egg Antigen (SEA) during a malaria episode and at convalescence 6 months later. CD4+ T cell memory cytokine (IFN-γ, TNFα, IL2 and/or IL17A) responses specific to schistosoma antigens was measured in 18/23 SP children at one or both time points, compared to 4/23 SN children (p < 0.0001). At the time of malaria infection, CD4+ T cells from 12/24 SN children and 15/23 SP children (p=0.29) stimulated with malaria antigens demonstrated significantly increased levels of cytokine production. In contrast, 7/23 SN children and 16/23 SP children (p=0.009) had responses in paired convalescent samples. 46.2% of cytokine-secreting CD4+ T cells expressed a single cytokine after stimulation with malaria antigens during the malaria episode. This fell to 40.9% at follow-up with a compensatory rise of multifunctional cytokine secretion over time (double+; 30.7 to 32.5%, triple+; 20.6 to 23.1%, and quadruple+; 2.4 to 3.8%) consistent with memory maturation. The majority (53.2%-59.5%) of cytokine responses were observed in CD45RA- and quadruple+: 2.4 to 3.8%) consistent with memory maturation. The effect of concomitant S. haematobium upon acquisition of memory to malaria antigens is unknown. We examined antigen-specific T cell frequencies in 48 Malian children aged 4-14 to malaria blood-stage antigens, Apical Membrane Antigen 1 (AMA1) and Merozoite Surface Protein 1 (MSP1) and to schistosoma antigens, Soluble Worm Antigenic Preparation (SWAP) and Schistosoma Egg Antigen (SEA) during a malaria episode and at convalescence 6 months later. CD4+ T cell memory cytokine (IFN-γ, TNFα, IL2 and/or IL17A) responses specific to schistosoma antigens was measured in 18/23 SP children at one or both time points, compared to 4/23 SN children (p < 0.0001). At the time of malaria infection, CD4+ T cells from 12/24 SN children and 15/23 SP children (p=0.29) stimulated with malaria antigens demonstrated significantly increased levels of cytokine production. In contrast, 7/23 SN children and 16/23 SP children (p=0.009) had responses in paired convalescent samples. 46.2% of cytokine-secreting CD4+ T cells expressed a single cytokine after stimulation with malaria antigens during the malaria episode. This fell to 40.9% at follow-up with a compensatory rise of multifunctional cytokine secretion over time (double+; 30.7 to 32.5%, triple+; 20.6 to 23.1%, and quadruple+; 2.4 to 3.8%) consistent with memory maturation. The majority (53.2%-59.5%) of cytokine responses were observed in CD45RA- and quadruple+: 2.4 to 3.8%) consistent with memory maturation.

REVISITING THE BURDEN OF TYPHOID FEVER IN LOW AND MIDDLE-INCOME COUNTRIES TO INFORM POLICY DECISIONS

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Typhoid fever still causes significant burden in low and middle income countries where access to safe water and sanitation is compromised. There have been several efforts to quantify the global burden of typhoid fever, but the estimates have not considered the heterogeneity in risk levels within countries. Since the World Health Organization has recommended risk-based use of vaccines against typhoid, we attempted to revisit the burden on typhoid fever in low and middle income countries after adjusting to the risk levels at population. The typhoid disease burden was estimated based on community representative typhoid incidence studies applied to 2010 population after correcting for the operational issues related surveillance, limitations of diagnostic tests and risk difference due to exposure to unimproved water. Incidence estimates, correction factors and mortality estimates were derived from systematic literature review. Scenario analyses for risk factors, blood culture sensitivity and case fatality rates were conducted accounting for the uncertainty in these estimates and compared to previous disease burden estimates. Findings: The risk-adjusted estimate of typhoid fever in low and middle income countries was 11.9 million cases (CI: 9.9 - 14.7 million) and 129,000 deaths (CI: 75,000 - 208,000). In comparison, without the risk- adjustment, the burden estimate would be 20.6 million cases (CI: 17.5 - 24.2 million) with 223,000 deaths (CI: 131,000 - 344,000). Scenario analyses indicated that the risk-factor adjustment and updated diagnostic test correction factor derived from systematic literature review were the drivers of difference between current estimate and past estimates. Interpretation: The risk-factor adjusted typhoid fever burden estimate is inherently more conservative than previous estimates that did not account for study site selection bias or fractions of the populations residing in urban slums or rural areas lacking access to improved water supplies. However, by distinguishing and discriminating the risk differences, it allows better estimation of the population level impact and evaluation of cost effectiveness of risk-based vaccination strategies recommended by World Health Organization.

CAUSES OF MORTALITY IN WOMEN OF REPRODUCTIVE AGE LIVING IN AN URBAN SLUM (KIBERA) NAIROBI

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Women of reproductive age (15-49 years) are confronted by dual burden of health concerns related to maternal conditions: infectious diseases and emerging and emerging diseases associated with non-communicable diseases. More than 30% of all deaths in resource-limited settings occur in these women, as compared to 15% in resource-rich settings. There is a paucity of mortality data and causes of death among females of reproductive age in low income countries. We present findings from verbal autopsies among women of reproductive age living in Kibera, an urban slum in Nairobi, Kenya. Verbal autopsies were conducted among women of reproductive age who were participants in a population-based surveillance system and who died between January 2009 and December 2013. Details regarding the death were obtained from close relatives and cause was assigned using the InterVA-4verbal autopsy model (version 4.02). We identified 157 deaths with an overall mortality rate of 5.4 per 1000 person-years of observation. The median age at the time of death was 31.5 years with the highest (40%) proportion of deaths occurring among women 30-39 years of age. Causes of death were identified in 51% of the individuals. Maternal deaths as defined by WHO were less frequent compared to non-maternal deaths (7% vs. 93%, respectively; χ²=80.0, p<0.001) in this population. Among the non-maternal deaths, 62% were due to infectious diseases, with HIV/AIDS associated illness being the leading cause (43%). Non-communicable diseases were associated with 38% of non-maternal deaths, of which cancers and cardiovascular disease were common (43% and 32%, respectively). Communicable diseases were found to be a major cause of death among women of reproductive age; however, non-communicable diseases are increasing in frequency among this population. These findings highlight the need to address and reduce the risk of deaths resulting from both communicable and non-communicable diseases, along with efforts to reduce maternal deaths among women of reproductive age.

DISABILITY- AND QUALITY-ADJUSTED LIFE YEARS: MEASURING HEALTH OR--?

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Disability-adjusted life years (DALYs) and quality-adjusted life years (QALYs) have risen to prominence over the past years and are frequently...
used as denominators in cost-effectiveness analyses. They have become a powerful “currency” in health economics, health policy and public health decision making. By combining life years lost due to premature mortality with disability- or quality-adjusted life years reflecting morbidity and attributing these summary measures to specific health conditions and interventions, DALYs and QALYs aim at quantifying health losses and health gains respectively. An explicit assumption is that DALYs and QALYs allow for comparison of different causes of health losses and health gains and that they are therefore suitable to guide global national and local decision making on where to invest scarce resources. However, based on a literature review, we argue that the DALYs and QALYs lack a clear definition of the concepts “health”, “disability” and “health-related quality of life” and therefore also of their disability- and quality-adjustments for individuals’ life years spent in less than perfect health. Mainly based on the highly topical International Classification of Functioning, Disability and Health of the World Health Organization, we developed a conceptual framework to delineate what the DALYs and QALYs do, and do not measure. Important similarities and differences between the two measures are revealed. Critical questions about the conceptualization of DALYs and QALYs are discussed and we conclude that if these questions are not addressed there is a continued risk of inefficient decision making and ill-informed advocacy.

**VALIDATION OF CAUSES-OF-DEATH USING VERBAL AUTOPSY DATA COLLECTED FROM NAVRONGO HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN GHANA:**

**2007-2011**

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Determination of cause-specific mortality rate in developing countries is a major difficulty due to poor vital registration systems, deaths occurring outside health facilities, and deaths not being medically certified. Verbal autopsy conducted on these deaths have proven to be one of the reliable methods of compiling cause of death data. We sought to validate the causes of death assigned by physician coders and provide a cause of death structure to improve estimates on cause specific mortality in Ghana. Longitudinal VA data from the Navrongo Health and Demographic Surveillance System (NHDDS) from 2007-2011 was used. Physicians were retrained on death certification and coding using ICD 10 codes and Sample Vital Registration with Verbal Autopsy (SAVVY) methods. VA forms were recorded using the SAVVY methods. In all, 7086 VA forms were retrieved and recoded using SAVVY methods. Males constituted 56% of total deaths and 60% of deaths occurred outside health facilities. The main causes of neonatal deaths were neonatal sepsis (31.5%), birth asphyxia (18.1%) and low birth weight with prematurity (15.4%). Malaria (37.0%), diarrhoea (13.1%) and acute respiratory infection (11.6%) were the leading causes of death among children aged 1-11 months. The main causes of death for 1-4 year olds were malaria (52.2%), diarrhoeal diseases (9.0%), and unspecified infectious diseases (4.7%). Among children 5-15 years, the main causes of death were malaria (22.5%), accidental drowning, submersion and falls (14.6%) and meningitis (7.7%). For those above 15 years, unspecified non-communicable diseases (14.7%), malaria (6.9%) and cerebro-vascular diseases (6.5%) were the main causes of death in the districts. Variability between two coders using SAVVY method was fair (49.7%; P<0.001) with a higher value for neonatal deaths compared to adults. Despite the limitations of VA data the method provides an understanding of the cause of death structure at the population level in developing countries comparable with global estimates that is not possible with existing sources of data.

**ENHANCING PUBLIC HEALTH RESPONSE TO CLIMATE-SENSITIVE INFECTIOUS DISEASE OUTBREAKS IN FLOOD-PRONE AREAS OF BANGLADESH: ARE PRIMARY HEALTHCARE FACILITIES READY TO RESPOND?**

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Global climate change is increasing Bangladesh’s vulnerability to natural disasters including floods and raising the potential for infectious diseases including floods and raising the potential for infectious
outbreaks. Preparing healthcare for outbreak management is complex and requires comprehensive understanding of the systems’ capacities and challenges. We evaluated preparedness of government primary care facilities in flood-prone sub-districts to respond to outbreaks. We asked 69 primary care managers to complete a self-administered survey to assess 7 core capacities critical for outbreak response. We determined percentages of facilities reporting each capacity; assessed managers’ perceived preparedness level; and conducted 2 focus group discussions (FGDs) with 24 managers to explore contextual factors driving preparedness. Qualitative data were analyzed using thematic content analysis. All 69 facilities lacked preparedness plans, emergency stockpiles, funds and authority to buy drugs or supplies locally; all lacked dedicated evaluation, isolation and vehicles for infectious patients; 58% lacked sufficient clinicians and 20% lacked adequate field staff. Rapid response teams and digital communication with referral centers existed in all, limited infection control practice in 75%, and diagnostic laboratory in 80% facilities. Unlike lower stockpiling, infrastructure and human resource capacities, higher surveillance, communication, infection control and laboratory capacities were observed. Sixty-three (91%) managers felt their facilities had limited preparedness. There was moderate (71%; Kappa=0.3; P=0.001) correlation between measured and perceived preparedness. FGDs identified insufficient training of providers, low motivation and rapid turnover of clinical staff, and lack of epidemiologists at facilities as impediments to human resource capacity. Using a mixed methods approach, this assessment identified deficiencies in all capacities, indicated major gaps in stockpiles, infrastructure and human resource as priority areas for investing constrained resources and offered baseline data for monitoring progress in preparedness. This may be a feasible methodology to evaluate health systems in low-income countries. Development of a contingency plan that secures emergency financing and improves surge capacity by establishing a system to engage existing field workers in outbreaks can sustainably enhance primary care preparedness.

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A SYSTEMATIC REVIEW ON THE EFFECTIVENESS OF Strategies TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES: PRELIMINARY RESULTS ON HEALTH OUTCOMES

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Health workers (HWs) play essential roles in delivering health care. In low- and middle-income countries (LMICs), however, HW performance is often inadequate. To characterize the effectiveness of strategies to improve HW performance in LMICs, we conducted a systematic review of 15 electronic databases, 30 document inventories of international organizations, and bibliographies of 510 articles. We included studies meeting accepted criteria for methodological adequacy (e.g., trials with comparison groups) of any strategy on any health topic in any language, published or not. After screening, data from relevant reports were double-abstracted and entered into a database. This analysis focuses on studies that measured health outcomes (morbidity and mortality rates). Effect sizes were calculated as percent change over time in the intervention group minus percent change over time among controls. We screened >105,000 citations, 829 reports met inclusion criteria, and 60 studies measured health outcomes (28 on morbidity only; 24 on mortality only; and 8 with both). Many strategies have been tested, usually with multiple intervention components. The median effect size (MES) across all studies was an improvement of 9 percentage-points (%-points) (interquartile range [IQR]: 0, 39). Among 45 studies focused on facility-based HWs, the strategy with the greatest health impact was HW training + group problem solving (MES = 49 %-points, IQR: 24, 77). Often used strategies, such as HW training and supervision, alone or in combination, had lower effect sizes (typically ranging from no effect to 16 %-points). Among 15 studies focused only on community HWs, the strategy with the greatest health impact was consumer supports (e.g., patient education) + HW training + providing drugs or equipment (MES = 55 %-points, IQR: 15, 62). Contextual and methodological heterogeneity made comparisons difficult. Results from this review, which will be finalized by the end of 2014, should inform decision-making on how best to improve HW performance and health outcomes in LMICs.

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PREVALENCE OF TRACHOMA IN BRAZILIAN SCHOOLCHILDREN

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The aim of the study was to estimate the prevalence and describe the distribution of trachoma among schoolchildren in Brazil. We conducted a cross-sectional study, using cluster sampling of the schoolchildren population, living in Brazilian municipalities with Human Development Index lower than the national mean. This prevalence survey was conducted by the Brazilian Ministry of Health, in the period 2002-2008. 3,144 schools, with 176,224 schoolchildren, from 1st to 4th grades, located in 1,491 municipalities, were selected. The selected schoolchildren underwent an external ocular examination, with a magnifying glass (2.5X), to detect clinical signs of trachoma according to the WHO grading criteria. The prevalence of trachoma, by state and national level, and their respective 95% confidence intervals were estimated. Chi-square and chi-square for trends tests were used to compare categorical variables. 8,526 cases of trachoma were detected, resulting in a prevalence of 5.00% (95%CI 5.05; 4.95). Most cases were mild (TP prevalence = 4.92%). Prevalence of intense inflammatory trachoma (TI) and trachomatous scarring (TS) was low: 0.03% and 0.05% respectively. There was no significant difference between the sexes. The prevalence of trachoma was 10.8% among children under 5 years of age, decreasing as age increased (chi square for trend p < 0.00001). There was a significant difference in prevalence between urban and rural areas, 4.3% versus 6.2% respectively (p < 0.001). Cases were detected in 1,189 municipalities (80% of the municipalities in the sample), in all 27 states of the country. In 37% of the selected municipalities, the prevalence was higher than 5%. The study has shown that trachoma is still endemic in a large proportion of the poorer Brazilian municipalities, contradicting the belief that the disease had been controlled in the country. The survey provided a baseline for evaluating planned interventions aimed at achieving the goal of certification of elimination of trachoma as a cause of blindness in Brazil by 2020.

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BACTERIAL LOAD AND PATHOGEN DIVERSITY IN OCULAR INFECTION WITH CHLAMYDIA TRACHOMATIS IN A TRACHOMA-HYPERENDEMIC ISLAND SETTING

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Trachoma is caused by ocular infection with Chlamydia trachomatis (Ct). Acute conjunctival infection may recur and progress to a chronic inflammatory process causing conjunctival scarring and blindness. The
ASSESSING THE BURDEN OF PEDIATRIC ACUTE RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE — AMERICAN SAMOA, 2011-2012

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In August 2013, LBJ Tropical Medical Center and the American Samoa (AS) health department notified CDC of a perceived high burden of pediatric acute rheumatic fever (ARF) and rheumatic heart disease (RHD). ARF is an immunologically mediated sequelae of inadequately treated group A Streptococcus pharyngitis and, potentially, pyoderma. Recurrent or severe ARF can cause permanent cardiac damage and RHD. Long-term prophylactic penicillin injections post-ARF diagnosis can prevent RHD. We aimed to describe pediatric ARF and RHD and prophylaxis in AS and the pyoderma-ARF association. We used ICD-9 codes and hospital prophylaxis registries from AS’s only medical system to identify all patients aged ≥18 years with a physician-recorded ARF or RHD diagnosis during 2011-2012. We recorded penicillin compliance and pre-ARF pharyngitis and pyoderma diagnoses (≥6 weeks preceding) for cases. Two age- and sex-matched control subjects per case-patient were selected from non-ARF/RHD patients examined during 2011-2012. We calculated ARF 2011-2012 incidence and RHD prevalence by using 2010 U.S. Census data. We used univariate statistical tests and conditional logistic regression for case-control comparisons. During 2013, RHD prevalence was 3.2 cases/1,000 children. ARF incidence was 1.1 (2011) and 1.5 (2012) cases/1,000. Of 65 children diagnosed with ARF during 2011-2012, a total of 32 (49%) subsequently received RHD diagnoses. Median ARF diagnosis age was 11 (range: 2-18) years. Pharyngitis history was more common among case-patients (18%) than control subjects (0%; P <0.01), but preceding pyoderma was not. Post-ARF penicillin prophylaxis compliance (65%) was suboptimal. RHD causes considerable childhood morbidity in AS. Although the pyoderma-ARF association remains unclear, attempts to curb AS’s RHD burden should address improved pharyngitis diagnosis and treatment and increased ARF prophylaxis compliance.

ACCURACY OF THE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS (IMCI) ALGORITHM IN IDENTIFYING CULTURE-CONFIRMED DIARRHEAL PATHOGENS REQUIRING ANTIBIOTIC THERAPY

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Diarrhea is the second leading cause of death in children under 5, with most deaths occurring in settings where microbiology facilities are unavailable or cost prohibitive. The World Health Organization (WHO) developed the Integrated Management of Childhood Illness (IMCI) guidelines to manage sick children based on clinical signs and history. For children with diarrhea, the guidelines recommend empiric antibiotics for children with suspected shigellosis (presence or history of bloody stool) or suspected cholera (age ≥2 years, severe dehydration, and living in a cholera endemic area). We assessed the diagnostic performance of the IMCI guidelines for diarrhea management as compared to stool bacterial culture. Children aged 6 months to 5 years presenting to two Western Kenya District hospitals between December 2011 and September 2013 with acute diarrhea were enrolled. Stool samples were tested using standard methods for bacterial culture. Multiplex PCR was used to further classify diarrheagenic Escherichia coli. Among 973 enrolled children,
EVALUATION OF INTEGRATED MANAGEMENT OF ADOLESCENT AND ADULT ILLNESS DISTRICT CLINICIAN MANUAL EMPIRIC ANTIMICROBIAL THERAPY RECOMMENDATIONS FOR SEVERE INFECTIONS IN NORTHERN TANZANIA

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We assessed the effectiveness of Integrated Management of Adolescent and Adult Illness District Clinician Manual (IMAi) empiric antimicrobial therapy recommendations for septic shock, severe respiratory distress without shock, and severe pneumonia in hospital settings in northern Tanzania. IMAi recommended empiric therapies were retrospectively evaluated against laboratory-confirmed etiology of illness data for participants in a febrile illness cohort study who met IMAI criteria for the three clinical syndromes. Therapies evaluated included IMAI emergency antibacterials (ceftriaxone or ampicillin plus gentamicin) for septic shock and severe respiratory distress without shock, and ceftriaxone plus a macrolide for severe pneumonia. Among 423 participants hospitalized with febrile illness, 171 cases met IMAI criteria for the three syndromes. 25 septic shock, 37 severe respiratory distress without shock, and 109 severe pneumonia. Forty-four (10%) of 423 participants died in-hospital. Ceftriaxone was the single-most effective agent in all three syndromes, being effective for 12 (48%) septic shock, 5 (14%) severe respiratory distress without shock, and 18 (17%) severe pneumonia illnesses. For each syndrome 17-27% of participants had an etiologic diagnosis non-responsive to ceftriaxone, but responsive to other available antimicrobial regimens, namely amphotericin for cryptococcosis and histoplasmosis; anti-tuberculosis therapy for bacteremic disseminated tuberculosis; or tetracycline therapy for rickettsioses and Q fever. IMAI recommendations for empiric ceftriaxone to treat septic shock, severe respiratory distress without shock, and severe pneumonia are warranted. Etiologies not explicitly addressed in IMAI guidance for these syndromes, such as cryptococcosis, histoplasmosis and tetracycline-responsive bacterial infections, were common. Prospective assessments of IMAI are needed to confirm these results and improve syndromic management algorithms.

FLY ME TO THE PLUME: VIDEO-TRACKING ANALYSIS OF ANOPHELES GAMBIÆ FLIGHT BEHAVIOR AT HUMAN-BAITED BEDNETS

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Understanding how mosquitoes interact with insecticide-treated bednets (LLINs) is fundamental to advancing the design and performance of LLINs, and to ensuring they continue to be effective and sustainable tools for malaria prevention. We have developed an innovative video system that enables nocturnal flight activity of multiple mosquitoes at a human occupied bednet to be captured at high resolution, and individual mosquito flight paths and details of movements to be tracked and analysed over periods of 60 minutes or more. Following initial laboratory studies, the system has been deployed in an experimental hut at a field site in Tanzania, where we are investigating behaviour of local Anopheles sp. populations entering the hut in response to human baits in untreated and insecticide-treated bednets. Analysing flight tracks, we have classified mosquito activity into four broad types, termed ‘swooping’, ‘visiting’, ‘bouncing’ and ‘resting’. Mosquitoes flew more slowly and flight paths were more tortuous when nets were baited. Responding to human bait, most activity was spent in flight. The majority of contacts made with the net surface were very brief (duration less than 4 seconds) and activity occurred primarily on the top surface of the net over the sleeper's torso, with less activity seen at the supine human's feet. This finding is consistent with previous studies suggesting that hostseeking mosquitoes orient towards a ‘plume’ of host attractants, funnelled upwards by the ‘chimney’ effect of the bednet walls. We compared activity on untreated nets with Permanet 2.0 (deltamethrin-treated LLINs) to examine how treatment

IMPACT OF FUTURE CLIMATIC CONDITIONS ON VIRAL, BACTERIAL AND PROTOZOAN ENTERIC PATHOGENS

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Understanding the seasonality of infectious diseases is important, in order to prepare for case loads, plan vaccination campaigns, and to anticipate impacts of climate change. Diarrheal diseases are often cited as one of the major health impacts of climate change, but there much uncertainty remains in the estimates associated with the relationship between climatic drivers and diarrheal disease. One source of this uncertainty is the variety of pathogens associated with infectious diarrhea, each of which has different life cycle characteristics such as survival outside of the host. Therefore it is important to understand how the seasonality of different etiological agents of diarrheal disease vary. In this systematic review and meta-analysis, we examined the impact of climatic variability on three representative diarrheal disease pathogens of different taxa: pathogenic E. coli, norovirus, and cryptosporidium. Incidence data for each pathogen were taken directly from tables or extracted from graphs in the published papers. Year-specific monthly temperature and precipitation from each location at the time of disease data collection were assembled from publicly available datasets. We examined the relationship between climatic variables and incidence of each pathogen for each location using generalized log-linear Poisson regression models, and we also pooled all datasets for each pathogen to calculate an overall association between monthly cases and mean monthly temperature, using a generalized estimating equation. We then used the model results to examine what proportion of the total of cases attributable to these three pathogens would be attributable to any one of the pathogens given increases in temperature of 1-4°C, in increments of one degree. We found that a one-unit increase in temperature was associated with increases in incidence of pathogenic E. coli (IRR = 1.08, 95% CI = 1.04-1.11) and Cryptosporidium (IRR = 1.03, 95% CI = 1.03-1.04) and decreases in Norovirus (IRR = 0.92, 95% CI: 0.90-0.94). These results highlight the importance examining taxa-specific climate-disease relationships for enteric diseases. As temperatures increase under future warming scenarios, bacterial and protozoan pathogens are expected to represent an increasingly large fraction of the burden of diarrheal disease. This has important implications for development of control strategies.

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influenced mosquito behaviour. Results investigating flight patterns and visiting patterns at the net surfaces, changes in activity patterns over time and LLIN repellency will be presented and implications for current and future LLIN-based approaches will be considered.

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**SWARMING BEHAVIOR OF ANOPELLES GAMBIAE MALES INCREASES FEMALE INSEMINATION RATE IN CAGED POPULATIONS: AN OPPORTUNITY TO STUDY MOSQUITO MATING SYSTEMS**

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Long-term control of Anopheles gambiae, the major malaria vector in Africa, is difficult to achieve and maintain with traditional control methods. Genetically Modified (GM) A. gambiae strains that bear sterility genes are potentially powerful new tools to control the disease. Natural male mating behavior is a key component in GM mosquito control strategies in order to spread transgenes into the wild type population. Mating behavior involves the ability of males to form swarms, which is crucial to inseminate females in the wild. In spite of its biological importance and relevance for vector control, swarming is a poorly understood process because it is hard to study in the wild and difficult to stimulate under laboratory conditions. Here we describe features that promote male A. gambiae G3 strain swarming in large cages. In 15.6 m3 cages, a dark foreground and contrasting illuminated background with a contrasting mark on the ground stimulated swarm formation during artificial twilight. G3 males have not lost their capability to swarm although this strain has been colonized since 1975. We asked whether swarming behavior would affect mating performance of wild-type (WT) G3 and I-Ppol transgenic A. gambiae sexually sterile males competing for G3 females. We performed competitive matings and recorded female insemination rate and proportion of matings by WT and GM males. The presence of swarming stimuli was associated with an increase in mating frequency from 77.4 to 97.4 %. There was no change in competitiveness by transgenic males as a function of swarming stimuli. The increase of mating frequency in the presence of swarming stimuli highlights the importance of swarming in A. gambiae mating behavior. Reproducing A. gambiae swarms in controlled conditions provides the possibility to dissect the mating behavior of this species and explain the mechanisms controlling it, which is innovative in mosquito research. We will discuss the results and the possible applications of our findings to investigation of A. gambiae biology and to support vector control strategies.

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**ANTIMALARIAL AND ANTI-DENGUE PROPERTIES OF A NATURAL CHROMOBACTERIUM MOSQUITO MIDGUT COMMENSAL**

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Plasmodium and dengue virus, the causative agents of the two most devastating vector-borne diseases, malaria and dengue, are transmitted by the Anopheles gambiae and Aedes aegypti mosquito vectors, respectively. We have identified a novel Chromobacterium species in adult A. aegypti collected in Panama, Csp_P, that can effectively colonize the midgut of A. gambiae and A. aegypti mosquitoes when introduced through an artificial nectar meal. We have shown that this isolate exerts entomopathogenic activity against both of the mosquito species along with in vivo and in vitro anti-Plasmodium and anti-dengue activities. Interestingly, the well-characterized Chromobacterium violaceum does not exert such effect. Upon bioassay-guided fractionation of supernatants of a Csp_P culture, we were able to map the anti-parasitic and antiviral properties to a fraction significantly enriched in a previously characterized cyclic dehydropeptide lactone. This bacterial secondary metabolite was previously pursued as an antifungal, being part of a complex of closely related molecules. We have produced n-butanol-based extracts of Csp_P cultures that retain in vitro activity against blood-stage Plasmodium and dengue virus, as well as against the yeast Saccharomyces cerevisiae. We are currently pursuing mass spectrometry analysis to characterize the compounds behind the antipathogenic activity of our extracts, along with efforts to identify the gene cluster responsible for production of such compounds by means of both comparative genomics and a transposon-mediated random mutagenesis screening. To our knowledge, this is the first identified bacterium that exerts broad spectrum entomopathogenic and antipathogenic activities, thereby rendering it an interesting candidate for the development of novel vector-borne disease control strategies.

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**THE EFFICACY OF LONG-LASTING NETS WITH DECLINING PHYSICAL INTEGRITY MAY BE COMPROMISED IN AREAS WITH HIGH LEVELS OF PYRETHROID RESISTANCE**

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Long-lasting insecticide-treated mosquito nets (LLINs) are a primary malaria prevention tool in sub-Saharan Africa but emergence of insecticide resistance threatens their effectiveness. Cross-sectional surveys of LLINs were conducted in houses of seven and four villages in Gem and Bungoma...
Indoor use of attractive toxic sugar bait (ATSB) for control of mosquitoes and for resistance management

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Attractive toxic sugar bait (ATSB), a mixture on insecticide and sugar solution, sprayed onto vegetation has been successful in controlling Anopheles mosquitoes outdoors. Indoor application of ATSB has yet to be explored. This study determined whether ATSB stations positioned inside the home would kill host-seeking mosquitoes and constitute a new approach to control of malaria. Classes of insecticides new to malaria vector control were mixed with sugar solution and tested as toxic baits against Anopheles in feeding bioassay tests. The most promising ATSB candidates were then trialed in experimental huts in Tanzania against free flying, host seeking mosquitoes. The ATSB stations were hung from ceilings of huts next to untreated mosquito nets occupied by human volunteers. In feeding bioassays, chlorfenapyr (a pyrrole), boric acid and tolfenpyrad (a mitochondrial electron transport inhibitor), mixed in a guava juice-based bait, each killed more than 90% of pyrethroid-susceptible An. gambiae s.s. and pyrethroid-resistant An. arabiensis at less than 1% w/v. In the experimental hut trial, the mortality rates of the three ATSB treatments were comparable to long lasting insecticidal nets (LLINs) tested against the same species in the same area. Indoor ATSB constitute a novel application method for insecticide classes that act as stomach poisons and have not been exploited for mosquito control hitherto. Combined with LLIN, indoor use of ATSB has the potential to serve as a strategy for managing insecticide resistance.

Factors mediating mating success in male Anopheles gambiae mosquitoes

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Human malaria, a major public health burden in tropical and subtropical countries, is transmitted exclusively by female Anopheles mosquitoes. Malaria control strategies aimed at inducing sexual sterility in natural vector populations are an attractive alternative due to increasing levels of insecticide resistance. However, the development of these strategies is hampered by a profound lack of knowledge regarding the most basic elements of Anopheles mating ecology. Females mate only once, and the suite of mating induced physiological and behavioral changes are precocious in large part upon the transfer of a mating plug containing steroid hormones (SH). Here we report mechanisms of pre and pericopulatory sexual selection in An. gambiae mosquitoes and a role for SH as a possible selective mechanism. High-speed video analysis of mosquito mating swarms revealed definitive evidence of female choice, as females employ specific rejection and acceptance behaviors. Furthermore, video analysis revealed behavioral mechanisms of male competition. We show that successfully mating males are not only larger, but through an ELISA assay we demonstrate that they have significantly higher SH titers in their reproductive accessory glands relative to their unsuccessful counterparts. The mechanisms behind female discrimination is currently being investigated. Additionally, females mated to males with reduced SH levels have lower fecundity and fertility compared to females mated with controls. Given that previous work has demonstrated the importance of male SH in female reproductive phenotypes, fitness in both sexes of An. gambiae appears at least partially SH dependent. This work provides critical insights into the mating ecology of a major disease vector and implicates SH as a key factor determining fitness across sequential episodes of sexual selection. Moreover, these results extend our understanding of swarming and monogamous insect mating systems.
proteins from Wolbachia strain wOo (33% and 27% of their theoretical proteomes, respectively). Of the filarial proteins, 1,038 (22%) were common to all stages, whereas microfilariae exhibited the greatest number of stage-specific proteins (~920), despite direct harvesting of this material from adult female uteri. Proteins identified by gelLC-MS alone accounted for <20% of the total for any single stage, but showed enrichment for membrane transporters, polyubiquitin and respiratory chain components. Preliminary analyses suggested that the relative abundance of galectins, calpionins, myosins and antioxidant proteins varied between lifecycle stages. In nodule fluid, >2,000 proteins were identified (77% bovine, 23% filarial, 0.1% bacterial), with strong representation of bovine antimicrobial proteins and filarial transthyretin-like proteins. These data provide a rich resource for comparative analyses of filarial protein expression throughout the lifecycle, as well as supporting research efforts directed at the development of a filarial vaccine, new drugs and diagnostic biomarkers.

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TOWARDS IDENTIFICATION AND VALIDATION OF BIOMARKERS FOR THE QUANTIFICATION OF LOA LOA MICROFILARIAE (MF) USING PROTEOMIC ANALYSES OF BODY FLUIDS FROM MICROFILAREMIC LOA-INFECTED INDIVIDUALS

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Among parasitic helmiths, Loa loa (L) presents a challenge for the mass drug administration programs in areas co-endemic for Wuchereria bancrofti and Onchocerca volvulus because of the severe adverse events (SAE’s) in cases of very high Li microfilaremia. To identify microfilarial-derived Li-specific biomarker(s) that could provide the basis for a microfilarial quantitative immunoassay, we characterized the excretory/secretory (E/S) proteome of Li mf as well as the Li-specific proteins found in urine and plasma of Li-infected and -uninfected individuals using LC-MS/MS. From 20 x 10^6 mf purified from the blood of Li-infected patients and cultured in vitro, 1,273 proteins (representing 8.2% of the Li putative proteome) were identified. To further identify if any of these ES proteins were present in body fluids, proteomic analyses of urine and plasma of Li-infected individuals (depleted of the top 12 to 20 human abundant proteins in plasma) resulted in the identification of 18 (from urine) and 29 (from plasma) Li proteins found only in Li-infected individuals that were identified by having at least 2 unique peptides. 4/18 antigens found in urine and 13/20 found in plasma have been selected for biomarker validation based on limited homology to other filarial species and, specific reactivity to polyclonal antibody raised to Li mf ES. In addition, 9 of these tested to date were found to be immunogenic in humans (based on antigen-specific IgG4 reactivity by serum from mf+ Li-infected plasma (n=30) and not by those from uninfected plasma (n=20)). Development and testing of rapid antigen capture immunoassays are underway to provide an alternative to more standard methods of mf quantification.

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VACCINATION WITH BRUGIA MALAYI-103 AND BRUGIA MALAYI-RAL-2 CONFER SIGNIFICANT PROTECTION AGAINST SUBCUTANEOUS CHALLENGE OF B. MALAYI INFECTIVE LARVAE IN MONGOLIAN GERBILS

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Two Brugia malayi proteins, Bm-103 and Bm-RAL-2, are orthologous proteins of confirmed vaccine candidates of Onchocerca volvulus. The Ov-103 was identified first as a microfilariae surface associated protein, but later was found to be also expressed in the cuticle, hypodermis and multivesicular bodies of infective stage larvae of both filarial parasites. Ov-RAL-2 and Bm-RAL-2 are immunodominant proteins expressed in the hypodermis of all stages. Bm-103 was cloned and expressed in Pichia pastoris and Bm-RAL-2 was cloned and expressed in Escherichia coli. These recombinant proteins were tested for their efficacy as a vaccine in the B. malayi - Mongolian gerbil animal model of lymphatic filariasis. Vaccination was via 3 intraperitoneal injections separated by 2 week intervals. Animals were challenged subcutaneously with 100 infective larvae third stage larvae (L3) and worm recovery was performed 42 or 90 days post infection. Vaccination with Bm-103 administered with alum showed 40% worm reduction in comparison to alum controls. Vaccination with Bm-RAL-2 showed 43% worm reduction in comparison to controls. A fusion protein of Bm-103 and Bm-RAL-2 was created, cloned and expressed in E. coli. Vaccination of gerbils with the Bm-103-Bm-RAL-2 fusion protein induced a 51% worm reduction in comparison to controls. Vaccination of gerbils with the two antigens, Bm-103 and Bm-RAL-2, each injected separately resulted in worm reduction of 69%. The development of embryograms to study the fecundity of female worms harvested from control and vaccinated gerbils are currently underway and will bring insights on impact of vaccination on fertility of female worms. In all vaccination experiments, a strong antigen-specific IgG response was detected by ELISA to the recombinant proteins. Moreover, in vitro killing assays using peritoneal exudates cells (PEC) in the presence of gerbil anti-serum against Bm-103 and Bm-RAL-2 showed active killing of L3 larvae in comparison to L3 larvae cultured with appropriate controls sera suggesting that an antibody dependent cell mediated cytotoxicity (ADCC) maybe a potential mechanism of protection. The results suggested that further experiments using these proteins alone or in combination are warranted.

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A COMPARATIVE STUDY OF POST-DIETHYLCARBAMAZINE TREATMENT REACTIONS IN ONCHOCERCIASIS AND LOIASIS

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Severe post-treatment reactions occur following diethylcarbamazine (DEC) treatment of filarial infections, including onchocerciasis and loiasis. Although release of the intracellular bacteria, Wolkbachia, has been implicated in the pathogenesis of these reactions, Loa loa infection to those in patients infected with Onchocerca volvulus. The study included three groups: Group I [LOA-NIH], subjects with MF+ loiasis treated with DEC (8-10 mg/kg/day for 21 days) at NIH; Group II [LOA-CAM], subjects with MF+ loiasis treated with DEC...
(8 mg/kg in a single dose) in Cameroon; Group III [ONCHO], subjects with MF+ onchocerciasis treated with DEC (200 mg/day for 7 days) in Ghana. Complete blood counts and previously collected serum were available at 0h, 4h, 8h, 1-7d and 14d post-initiation of treatment for LOA-CAM and ONCHO and at variable time points for LOA-NIH. The early pattern of eosinophilia post-DEC (a decrease from baseline during the first 24 hours followed by a significant increase over the next 3-5 days) was similar in all 3 groups and the rise was preceded by a transient increase in serum IL-5 levels. In contrast, the % baseline ANC increased significantly post-DEC only in the ONCHO group (P<0.05 at days 1,2 and 3 compared to LOA-CAM and LOA-NIH. Serum IL-10 levels increased transiently in all 3 groups, reaching peak values at 1-2 days post-DEC. Although serum TNF-alpha, levels increased at 1-2 days post-DEC in all subjects in the ONCHO group, there was no consistent pattern in the subjects with loiasis. To conclude, parasite antigen release and the resultant Th2-driven eosinophilia may be a major driver of post-DEC reactions in both onchocerciasis and loiasis. The increased TNF-alpha and neutrophilia seen post-DEC in onchocerciasis is likely due to the concomitant release of Wolbachia during microfilarial killing.

ENDOTHELIAL CELLS RELEASE SOLUBLE FACTORS THAT PROLONG THE SURVIVAL OF FILARIAL WORMS IN VITRO
Holly Evans, Edward Mitre
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A major barrier for current mass drug administration (MDA) efforts to control lymphatic filariasis is the inability of current medications to kill adult worms when given as a short course. The development of novel drugs is complicated by the inability to maintain worms for long periods of time in vitro, making effective screening of new drugs difficult. In an attempt to improve in vitro culture methodology for filarial worms, we have conducted a series of experiments using microvascular (MF) obtained from gerbils infected with Litomosoides sigmodontis, a filarial parasite of rodents. While the culture of L. sigmodontis MF in Dulbecco's Modified Eagle Medium supplemented with 10% FBS results in an average survival of only 7 days, co-culturing MF with a mouse endothelial cell line (EOMA) expanded survival to 40 days. Not all cell lines have this property, as MF co-cultured with a rat basophilic cell line (RBL-2H3) survived for only 5 days. Culturing EOMA cells in transwell plates extended MF survival to the same degree as direct co-culture, suggesting that the factors microvascular require are soluble in nature. Heat inactivation of EOMA conditioned media at 56°C reduced MF survival by approximately 50%. However, heat inactivation at 100°C reduced survival to 3 days, signifying that MF require both heat labile and heat stable factors. EOMA cells require FBS to produce these factors, as conditioned media collected from EOMA cells grown in the absence of FBS fail to prolong survival. Importantly, these findings also pertain to adult worms. Both rodent L. sigmodontis and human Brugia malayi adult worms also show significantly extended survival when cultured in EOMA conditioned media. We are poised to begin biochemical and comparative analyses to elucidate the chemical nature of these essential factors. Identification of such factors will advance our ability to cultivate filarial pathogens in vitro and may provide insights for the development of new anti-filarial compounds.
associated (3525 transcripts), whereas only 65 transcripts show L3-biased expression. Pairwise comparison of the L3 and L4 stages reveals 3157 significantly differentially expressed genes (1170 L3 upregulated, 1987 L4 upregulated) and provides important information regarding transcriptional changes required for this transition. As anticipated, significantly fewer reads mapped to wDí genes than to Di genes in each life cycle stage. Interestingly, synthesis of the critical metabolite, heme, by wDí appears to be synchronized with the production of heme-binding proteins in Di in a stage-specific manner. Comparative analysis to human filarial nematodes provides further information on the evolutionary biology of these parasites, while also highlighting opportunities for further drug targeting initiatives. A better understanding of how these genomes function in concert with one another is required for unraveling the complex relationship of the nematode with its endosymbiont, Wolbachia, as well as with its canine and mosquito hosts.

EXPERIMENTAL PLASMODIUM FALCIPARUM GENETIC CROSSES IN HUMAN LIVER-CHIMERIC MICE

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Forward genetic studies using experimental genetic crosses are an incredibly powerful tool to pinpoint the genetic basis of phenotypic variation and are typically more powerful than population analysis. For Plasmodium falciparum however, only three experimental crosses have been carried out due to the hurdles associated with the process – for ethical reasons humans can not be used and until recently, only splenectomized chimpanzees allowed for the transition from the sporozoite stage to the asexual blood stage of the life cycle necessary for progeny amplification and analysis. Nevertheless, the recombinant progeny from the three crosses led to the discovery of genetic determinants for drug resistance, erythrocyte invasion and blood stage parasite growth. The recent decision by the NIH to cease chimpanzee biomedical research suggests further forward genetic studies are impossible. However, we have shown that a mouse harboring human hepatocytes (the FRG KO huHep mouse) infused with human erythrocytes can support P. falciparum sporozoite infection, the completion of liver stage development and the transition to asexual blood stage replication. This thus suggests that P. falciparum experimental crosses can be achieved in the FRG KO huHep mouse and here we show here that this is indeed possible and thus this mouse model can replace the previously essential chimpanzee. To achieve our goal, we generated P. falciparum gametocytes from the NF54 chloroquine sensitive and GB4 chloroquine resistant parasite lines and used these in mixed feeds to mosquitoes to generate recombinant sporozoite progeny – produced after zygote formation and sexual recombination. Sporozoites were injected into FRG KO huHep mice harboring human erythrocytes and after the liver stage-to-blood stage transition, blood stage parasites were maintained in vitro. Parasite cloning and downstream microsatellite analysis revealed the presence of unique recombinant progeny. Furthermore, drug selection demonstrated the creation of recombinant progeny with unique drug resistance patterns not shared by the parental populations. Thus we provide evidence of successful experimental genetic crosses. This methodology should allow for P. falciparum “systems genetics” – the study of complex genetic traits in which genomic data and clinical phenotypes are obtained using global “omic” technologies.

MOLECULAR BASIS FOR SIALIC-ACID DEPENDENT RECEPTOR RECOGNITION BY THE PLASMODIUM FALCIPARUM INVASION PROTEIN ERYTHROCYTE-BINDING ANTIGEN-140 (EBA-140/BAEBL)

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Erythrocyte-binding antigen 140 (PfEBA-140/BAEBL) is a Plasmodium falciparum erythrocyte invasion ligand that engages Glycophorin C (GPC) on host erythrocytes during malaria infection. PfEBA-140 is a member of the erythrocyte-binding ligand (EBL) family, which contains the four sialic acid dependent invasion proteins utilized by P. falciparum. Each of these ligands recognizes a different erythrocyte receptor despite being composed of a highly conserved domain architecture. To elucidate the foundations of receptor specificity within the EBL family and define the structural basis of GPC engagement, we determined two crystal structures of the PfEBA-140 minimal binding domain unbound and in complex with a glycan containing the essential sugar component of GPC that is recognized during erythrocyte engagement. The two domains comprising the minimal binding region contain unique structural elements that are likely determinants of receptor specificity. Two glycan binding pockets were observed, one per domain, and the bound sialic acid was modeled into each site. Erythrocyte binding experiments elucidated important glycan contact residues and identified distinct functional roles for the individual sugar binding sites. Our studies provide a structural framework for GPC recognition, form a foundation for future studies of the interaction between PfEBA-140 and erythrocytes, and offer insight into deficient receptor binding and putative receptor switching described for polymorphisms in PfEBA-140. Preventing erythrocyte engagement is an excellent opportunity to inhibit merozoite invasion. Our results will thus aid in the design of rational therapeutics and vaccines that target erythrocyte invasion ligands.

EVOLUTION BEFORE OUR EYES: GENOME MUTATION IN PLASMODIUM FALCIPARUM


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Genetic mutation is a central process in evolution, and is involved in the emergence of drug resistance and the generation of antigen sequence diversity in the malaria parasite Plasmodium falciparum. We studied P. falciparum mutation by regularly sub-cloning parasites cultured in vitro to isolate single infected red blood cells, so that mutations arising in asexually dividing cells could be detected using whole genome sequencing. We produced the most comprehensive description of P. falciparum mutation to date, studying four lab strains, artesiminin resistant Cambodian field isolates and parasites with experimentally induced mutations in DNA repair genes. In total, we analysed >300 genomes from parasites cultured for a total of >1,000 days, capturing hundreds of mutations. We found that: (1) Point mutations are distributed throughout the genome and occur at a similar rate between strains regardless of the drug sensitivity status of the line, contrary to previous studies suggesting that drug resistant parasites are hypermutable; (2) There is a strong mutation bias with G/C to A/T transition mutations over-represented. We estimate this would equilibrate at a similar AT ratio to that observed in the P. falciparum genome (~80%); (3) InDels occur predominantly in AT rich low-complexity regions at a higher rate than point mutations, likely due to DNA polymerase slippage events; (4) Structural variation is focused in and around var genes, which encode highly polymorphic PfEMP1 surface-expressed antigens, and this mitotic recombination generates sequence diversity by producing mosaic var genes. With 10^10 parasites in a single infected individual, our data indicate that every nucleotide in the P. falciparum genome will undergo point mutations and millions of new mosaic var genes will be produced
every 48-hour life cycle. In summary, we have produced a comprehensive catalogue of P. falciparum mitotic genome mutation at all scales from point mutations to interchromosomal translocations, adding considerably to our understanding of parasite genomics and evolution.

1900
IDENTIFYING NOVEL TRAFFICKING COMPONENTS OF THE PLASMODIUM FALCIPARUM VIRULENCE FACTOR PFEMP1 THROUGH QTL ANALYSIS
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Plasmodium falciparum erythrocyte membrane protein 1 (PFEMP1) is the main parasite virulence factor due to its central role in mediating the cytoadherence of infected red blood cells (iRBCs) to the host microvascular endothelium. Directly targeting PFEMP1 as a therapeutic strategy is greatly limited due to the protein’s hypervariable nature, which gives rise to approximately 60 different variants. However, interfering with the trafficking of PFEMP1 to the iRBC surface is an attractive approach, as hypervariability becomes inconsequential and previous studies have demonstrated that reduced surface PFEMP1 levels significantly weaken cytoadherence, likely lessening the severity of malaria symptoms and permitting parasite clearance by the spleen. Interestingly, the in vitro culture-adapted parasite line 3D7 is inherently defective in exporting PFEMP1 to the iRBC surface. Presuming that PFEMP1 export from the parasite to the iRBC surface is controlled at the genetic level, we hypothesized that 3D7 harbors one or more genetic determinants of impaired PFEMP1 trafficking. To test this possibility, we examined the surface PFEMP1 levels of 17 progeny clones from the genetic cross between 3D7 and the ‘trafficking-competent’ parasite line HB3. This was assessed using Western blotting and a two-color, triple-layer flow cytometry assay with plasma from malaria-immune Malian adults. Normalized to HB3, we found that 3D7 displays 75% less PFEMP1 on the iRBC surface, with progeny phenotypes ranging from 37% more to 88% less PFEMP1. QTL analysis using 3,597 genome-wide SNP markers identified a significant locus with a LOD score of 4.82 on chromosome 12 that explains approximately 50% of the phenotypic variance. This locus contains a single gene, Pf3D7_1245600, encoding a putative kinesin. The role of this gene in the trafficking of PFEMP1 is being confirmed in allele-exchange experiments, where the defect is rescued in 3D7 and introduced in HB3. The results of this study may strengthen our understanding of malaria pathogenesis and provide new targets for much needed therapeutics.

1901
PROTEOMIC COMPOSITION AND SURFACE ACCESSIBLE TARGETS OF PLASMODIUM SPOROZOITES
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The transmission of salivary gland sporozoites from an Anopheline mosquito initiates the malarial infection of a new mammalian host, which has prompted the development of various therapeutic interventions targeted against sporozoites. While several sporozoite proteins have been historically well studied, systematic proteomic analyses have been hampered by the presence of overwhelming amounts of proteins, nucleic acids, and lipids derived from the dissected mosquito vector. We have overcome this obstacle by developing streamlined purification methods that result in fully infectious sporozoites with very low levels of vector, bacterial, and fungal contamination. These approaches have enabled the most comprehensive total proteomics of two human-infective malaria species (Plasmodium falciparum and P. vivax), as well as the model rodent-infective species P. yoelii. Moreover, these purified sporozoites are also sufficiently devoid of soluble mosquito material to permit the assessment of the surface-accessible proteome of the salivary gland sporozoite. This was done using an amine-reactive crosslinker bearing a cleavable biotin group, which enables high affinity purification and yet leaves a covalent modification of accessible lysines for high confidence identification. We have developed an extensive and stringent washing strategy to minimize the binding of non-specific proteins, which has yielded a greatly expanded surface-accessible proteome above and beyond our previously published list. Several of these candidates have been confirmed with transgenic parasites and the generation of specific antisera. Finally, we have treated purified sporozoites with molecular mimics of mammalian body conditions to observe any differences in protein accessibility or secretion onto the parasite surface in response to these stimuli. Taken together, these characterizations provide a sizeable list of surface accessible proteins that may be valuable new targets for antibody-based interventions.

1902
AGE SPECIFIC INCIDENCE RATES OF MALARIA SUGGEST DIFFERENT RATES OF NATURALLY ACQUIRED IMMUNITY TO MALARIA ACROSS HUMAN HOST GENOTYPES
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In endemic areas, the incidence of clinical malaria declines with age, reflecting the role of naturally acquired immunity (NAI) in modulating malaria. Asenso is a malaria endemic area in western Kenya. We investigated whether NAI develops at different rates among children carrying a range of malaria-associated genes by comparing age-specific malaria incidence in children, stratified by genotype. We recruited a birth cohort in March 2012-December 2013 (n=700) from a population under passive surveillance for clinical malaria since August 2006. Additionally, we recruited birth cohort member siblings <12 years of age into a sibling cohort (n=780) for a combined cohort of 1480 children. Clinical malaria was defined as fever with a malaria-positive blood film in the absence of bacterial co-infections causing fever. Participants were typed for polymorphisms in 40 malaria-associated genes. Age-specific malaria incidence was significantly lower for sickle cell trait (AS) compared to sickle normal (AA) individuals (P<0.001), marginally significant for homozygous (-/-/α) compared to normal (αααα) alpha thalassemia individuals (P=0.04) and not significant for αααααα compared to heterozygous (-/-/αα) individuals. Incidence rates peaked earlier in AA (4-5 years, 0.77 episodes per child per year) compared to AS children (9-10 years, 0.8episodes per child per year). For alpha thalassemia, incidence rates peaked earlier in -/-/αα (2-3 years, 0.73 episodes per child year), compared to -/-/αµαµα individuals (6 years, 1 episode per child per year) and αααααααα (4-5 years, 0.79 episodes per child year). Analyses for the 40 malaria susceptibility genes are ongoing. Preliminary results show shifts in the peaks of age-specific incidence rates by genotype. These findings suggest different rates of NAI among children of different genotypes. Results may be useful in understanding genotype-specific effects of interventions such as malaria vaccines that can modulate NAI.
QUANTIFYING THE INDEPENDENT EFFECTS OF AGE AND EXPOSURE ON TWO COMPONENTS OF MALARIA IMMUNITY: RESTRICTION AND TOLERANCE

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While epidemiologic data consistently suggest that clinical immunity to Plasmodium falciparum develops over time in exposed children, lack of accurate measures of exposure and protection have limited our capacity to understand the development of immunity to infection and disease. The objective of this study was to define the acquisition of immunity against P. falciparum malaria among children living in endemic areas of varying malaria transmission. In particular, we were interested in measuring the development of clinical tolerance (lack of symptomatic disease given parasitemia, evaluated here by parasite density at which subjects developed objective fever) and parasite restriction (ability to kill or otherwise control the growth of parasites, evaluated here by parasite density). We used data from representative cohort studies being conducted in 100 households from each of three sub-counties in Uganda: Walakuba (aEIR= 3.3), Kibeho (aEIR=31.5) and Nagongera (aEIR=315). The study comprises continuous passive surveillance, active surveillance every 3 months, and monthly mosquito collections in all households. Thus, the dataset used for this analysis included data on over 3400 episodes of clinical malaria and 1400 episodes of asymptomatic parasitemia occurring in 739 children aged 6 months to 11 years of age over two years of follow up. Results from generalized additive models, allowing for flexible interactions between variables of interest, are consistent with strong independent effects of both age and exposure on the development of both tolerance and restriction. Tolerance develops gradually beginning early in life and is not strongly modified by variable exposure. In contrast, restriction starts to develop later in life (4-6 years of age) and depends strongly on cumulative exposure. Further analyses will explore the role of recent and persistent exposure on both of these components of immunity. These findings provide unprecedented insight about the roles of age and exposure on the development of immunity to malaria.

SEVERE MALARIAL THROMBOCYTOPENIA: A RISK FACTOR FOR MORTALITY

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The significance of thrombocytopenia to the morbidity and mortality of malaria is poorly defined. We compared the platelet profiles of patients with and without malaria in southern Papua, Indonesia. Between April 2004 and December 2012 data were available on patient demographics, malaria diagnosis, haematological investigations and clinical outcome in a referral hospital. Of 922,120 patient episodes a total of 215,479 (23.4%) had clinical malaria. Patients with Plasmodium falciparum mono-infection had the lowest platelet counts with an adjusted odds ratio (AOR) for severe thrombocytopenia (platelet count <50,000 µl-1), compared to those without malaria, of 6.03 [95% Confidence Interval (CI) 5.77-6.30]. The corresponding risks were 5.4 [95% CI 5.02-5.80] for mixed infections, 3.73 [95% CI 3.51-3.97] for P. vivax and 2.16 [95% CI 1.78-2.63] for P. malariae; p<0.001. In total 1.3% (2,701/215,479) of patients died. Compared to patients with neither severe anaemia nor severe thrombocytopenia, those with severe anaemia alone had an AOR for death of 5.21 [95%CI 4.53-5.98], those with severe thrombocytopenia alone had an AOR of 4.65 [95%CI 4.10-5.28] and those with both risk factors an AOR of 16.44 [95%CI 13.70-19.74]; p<0.001. In conclusion, severe thrombocytopenia is associated with malarial related mortality. Prospective studies are warranted to define its utility in defining the clinical management of patients with malaria.
Seasonal Malaria Chemoprevention (SMC) is recommended for malaria control in children under 5yrs of age where transmission is highly seasonal. In some areas the disease burden may justify extending the age range for SMC. We investigated the effectiveness of SMC in children under 10 years of age in Senegal. SMC with sulfadoxine-pyrimethamine plus amodiaquine was introduced in three districts in a step-wedge design. Fifty-four health posts were randomized to implement SMC starting in 2008, 2009, or 2010, or to remain without the intervention. A surveillance system was established to record all deaths and all malaria cases diagnosed at health facilities, and a pharmacovigilance system was put in place to detect adverse drug reactions. A poisson regression model was used to estimate the effectiveness of SMC in reducing malaria incidence in treated children, with a random effect to account for variation in incidence between health posts. To determine whether SMC was able to reduce malaria transmission, incidence of malaria in age groups too old to receive SMC, was compared between health posts in which SMC was delivered to children, and health posts without SMC, using random-effects poisson regression. SMC was administered to about 14,000 children under 5yrs in 2008, 90,000 children under 10yrs in 2009, and to 155,000 children under 10 yrs in 2010. No serious adverse events attributed to SMC were detected despite a high level of surveillance. Where SMC was delivered, the number of malaria cases in children under 10 years was reduced by 69% (95%CI 65%, 72%). Malaria incidence in older age groups was reduced in areas where SMC was delivered to children, by 29% (21%, 35%). In conclusion, in some regions of the Sahel and sub Sahel, the age distribution of malaria may justify extending the age range for SMC. Including older children in SMC programmes is safe and effective, and may contribute to reducing transmission.

**1906**

**EXTENDING THE AGE RANGE FOR SEASONAL MALARIA CHEMOPREVENTION (SMC): EFFECTIVENESS OF SMC IN CHILDREN UNDER 10 YEARS OF AGE DELIVERED THROUGH THE DISTRICT HEALTH SERVICE IN SENEGAL**

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Seasonal Malaria Chemoprevention (SMC) is recommended for malaria control in children under 5yrs of age where transmission is highly seasonal. In some areas the disease burden may justify extending the age range for SMC. We investigated the effectiveness of SMC in children under 10 years of age in Senegal. SMC with sulfadoxine-pyrimethamine plus amodiaquine was introduced in three districts in a step-wedge design. Fifty-four health posts were randomized to implement SMC starting in 2008, 2009, or 2010, or to remain without the intervention. A surveillance system was established to record all deaths and all malaria cases diagnosed at health facilities, and a pharmacovigilance system was put in place to detect adverse drug reactions. A poisson regression model was used to estimate the effectiveness of SMC in reducing malaria incidence in treated children, with a random effect to account for variation in incidence between health posts. To determine whether SMC was able to reduce malaria transmission, incidence of malaria in age groups too old to receive SMC, was compared between health posts in which SMC was delivered to children, and health posts without SMC, using random-effects poisson regression. SMC was administered to about 14,000 children under 5yrs in 2008, 90,000 children under 10yrs in 2009, and to 155,000 children under 10 yrs in 2010. No serious adverse events attributed to SMC were detected despite a high level of surveillance. Where SMC was delivered, the number of malaria cases in children under 10 years was reduced by 69% (95%CI 65%, 72%). Malaria incidence in older age groups was reduced in areas where SMC was delivered to children, by 29% (21%, 35%). In conclusion, in some regions of the Sahel and sub Sahel, the age distribution of malaria may justify extending the age range for SMC. Including older children in SMC programmes is safe and effective, and may contribute to reducing transmission.

**1907**

**COMPARISON OF SEASONAL MALARIA CHEMOPREVENTION COVERAGE IN NORTHERN NIGERIA VIA DOOR-TO-DOOR, HEALTH FACILITY AND RETAIL SECTOR DELIVERY**

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In 2012, WHO recommended seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine and amodiaquine (SP-AQ) to prevent malaria in children under five in the Sahel sub-region. As the National Malaria Elimination program in Nigeria plans to apply the recommendation to protect 6 million children in its 9 northern states, identifying the most effective ways to deliver the drug is critical. From August-November 2013, SP-AQ was delivered monthly to under-fives in 3 local government areas of Kano State using 3 delivery mechanisms: door-to-door using community drug distributors, at public health facilities (fixed points), and for a low cost in the private sector. A household survey was conducted in December 2013, one month after the final round of SMC distribution, to assess the coverage achieved through each distribution method. Data were collected on demographics, malaria knowledge, treatment practices, and SMC awareness. We estimated the partial SMC coverage (proportion of under-fives receiving at least one of the four monthly doses) and full coverage (proportion of under fives receiving all doses), and identified factors associated with coverage using multivariable logistic regression models. 176,281 doses of SP-AQ were distributed over four months. The survey collected data from 5,291 children and 3,206 caregivers in 3,079 households. Adjusted partial coverage was significantly higher via door-to-door distribution (86.5%) than via health facility (46.7%) or private sector (27.9%). Full coverage was also highest in the door-to-door delivery arm (56.3%) compared to health facility (19.4%) and private sector (12.2%). Children aged 1-4 years old were significantly more likely than those <1 year old to receive SMC (p<0.001), and child use of an insecticide-treated bed net was significantly associated with partial coverage (OR=1.4). Door-to-door delivery achieved the highest coverage although a substantial population did not receive SMC. The findings are informing plans for 2014 SMC scale-up across Kano State including community mobilization and sensitization strategies and other fixed-point distribution opportunities.

**1908**

**THE EFFECTIVENESS OF INSECTICIDE TREATED BEDNETS IN HAITI: RESULTS FROM A CASE-CONTROL STUDY**

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Insecticide-treated bednets (ITNs) are a cornerstone of malaria prevention, but little evidence exists on their effectiveness in Haiti where the primary vector, Anopheles albimanus, has been documented as exophilic and with variable biting times. We conducted a case-control study to assess ITN effectiveness in Haiti, following a national ITN distribution campaign in 2012. Patients presenting to outpatient departments were systematically screened for fever or history of fever. Eligible patients were administered a brief questionnaire and blood was collected for a malaria rapid diagnostic test (RDT) and dried blood spots. From September 2012-February 2014, 9,318 patients, including 379 (4.1%) RDT-positive patients, were enrolled across 17 health facilities in five departments in Haiti. Retrospectively matching up to four RDT-negative controls per RDT-positive case by age group, sex, location of residence, and enrollment period yielded 365 cases and controls. We found no difference in the proportion of cases and 1,204 RDT-negative controls. Slightly more than half (57.1%) of patients reported owning any bednet, with no difference among matched cases and controls. We found no difference in the proportion of cases and controls who reported using any bednet (34.5% vs. 32.9%, p=0.39) or a campaign ITN (21.9% vs. 19.5%, p=0.30) the previous night, or always using a campaign ITN in the two weeks before their illness (18.4% vs. 18.5%, p=0.84). In a multivariable conditional logistic regression model, consistent use of a campaign ITN was not related to RDT positivity. The only variable related to RDT positivity in the model included body temperature (Odds Ratio = 1.40, 95% Confidence Interval: 1.25, 1.57 per one-degree Celsius increase). Additional entomologic investigation found that all Anopheles mosquitoes tested from the study areas were susceptible to permethrin, the insecticide used on campaign ITNs. Our results based on RDT status do not provide evidence to support ITNs as an effective malaria prevention strategy in Haiti. Additional results using a PCR-based case definition will be presented.
**Aedes aegypti Female Mosquitoes with Alanine Aminotransferase Deficiency Face a Stressful Metabolic Challenge During Blood Digestion**

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We have recently evaluated the exposure of *A. aegypti* females to L-cycloserine (LCS), a well-known inhibitor of alanine aminotransferase (ALAT) in animals. Our results indicated that 10 mM LCS interferes with *Aedes aegypti* blood metabolism causing motor impairment and a 35% of mortality with an acute effect during the first 6 hours after treatment. Interestingly, only 11% of the total mortality was observed between 24 and 72 hours after feeding. In order to follow up this finding, the expression pattern of two genes encoding ALAT (1 and 2) was first analyzed in sucrose- and blood-fed *A. aegypti* tissues by qRT-PCR. ALAT1 and ALAT2 transcript levels exhibited a distinct expression pattern in mosquito tissues dissected during a gonotrophic cycle. Next, RNAi-mediated gene silencing was used to knock down endogenous levels of each transcript in mosquito tissues. Injection of female mosquitoes with either dsRNA-ALAT1 or dsRNA-ALAT2 or both (dsRNA-ALAT1/2) significantly decreased the expression of ALAT1 or ALAT2 or ALAT1/2 in fat body (FB) and Malphigian tubules (MT) at 24 hours after blood feeding, when compared to dsRNA-firefly luciferase-injected control. As expected, the expression of ALAT1 was not modified in tissues from dsRNA-ALAT2-injected females and vice versa. Western blot analysis demonstrated that the protein levels of ALAT were also significantly reduced in tissues of dsRNA-ALAT-injected females when compared to control mosquitoes. Moreover, the knockdown of *A. aegypti* ALAT1 or ALAT2 or ALAT1/2 caused unexpected phenotypes such as a delay in blood digestion, a massive accumulation of uric acid in the midgut posterior region, and a significant decrease in nitrogen waste excretion during the first 48 hours after blood feeding. Concomitant with these results, the expression of genes encoding both the ammonia transporter and xanthine dehydrogenase were significantly increased in FB and MT of dsRNA-ALAT-injected females. These findings highlight the efficient and complex mechanisms that blood-fed mosquitoes use to avoid ammonia and free radical toxicity.

**Anopheles gambiae Small-RNA Pathways Immunity to Different Pathogens**

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Many mosquito species are vectors of human pathogens, such as viruses responsible for Dengue or Chikungunya, or malaria parasites. Mosquitoes from the *Aedes* genus mainly transmit viruses, whereas the *Anopheles* genus is exclusively responsible for transmission of human malaria. The basis for these pathogen-vector specificities is currently unknown. Mosquito small RNA pathways have many functions such as regulation of genes in the development or immunity. Previous studies in *An. gambiae* have shown that (i) regulation of anti-Plasmodium immunity by microRNA was essential for correct mosquito protection, and that (ii) the siRNA pathway is essential for the control of arboviral infection after intrathoracic inoculation. Using next generation sequencing and functional genomics to examine *Anopheles gambiae* mosquitoes infected with O’Nyong Nyong virus and *Plasmodium* parasites, we were able to (i) discover new *Anopheles* microRNAs and characterize specific microRNAs that are regulated upon infection; (ii) show that the siRNA pathway does not contribute to antiviral defense during early arboviral infection in the midgut while it is protective at later stages in the systemic compartment; (iii) and implicate the siRNA pathway in evasion of immunity by malaria parasites in the midgut. These results expand our understanding of the small RNA immunity machinery in an important African vector.

**Structural Divergence of Heterochromatin Between Incipient Species of Anopheles Gambiae**

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The major African malaria vector *Anopheles gambiae* is known to be undergoing incipient speciation into two molecular forms – M and S – recently named *An. coluzzi* and *An. gambiae*, respectively. Heterochromatin plays a vital role in several important biological functions, and is known to be associated with longevity and individual fitness of organisms as well as with postreproductive isolation between species. The genome of *Anopheles gambiae* was first sequenced in 2002 and has been updated since but still contains important information gaps with regards to the repetitive DNA content in heterochromatin. In order to achieve a better understanding of differentiation within *An. gambiae*, it is essential to develop a physical map containing information about the repetitive DNA and determine the differences in heterochromatin between the incipient species. Using multiple strains of M and S forms, we mapped repetitive DNA sequences including satellite DNA and ribosomal DNA (rDNA) with respect to bands of pericentric heterochromatin of mitotic chromosomes. Satellite DNA probe Ag53A hybridized to the pericentric heterochromatin/rDNA locus junction in both forms. However, unexpectedly, satellite DNA AgY53B hybridized at the base of proximal band in the M form but at the tip of the band in the S form, indicating a possible shift or inversion in the satellite DNA position during divergence of these forms. Satellite DNA AgY477 depicted a similar pattern, hybridizing to different positions between the forms. Idiograms based on above information were prepared for the M and S forms as well as other members of the *An. gambiae* complex, serving as a tool in better understanding of evolution of the repeat rich regions in the *An. gambiae* genome. Our results revealed that the rapid evolution of heterochromatin is not restricted to species with the postreproductive isolation. The structural reorganization of satellite DNA observed between the M and S forms suggests a possible role of heterochromatin in initial diversification of malarial vectors.

**X-Chromosome Localized Recombination Hotspots Undermine Existing Molecular Diagnosis of Anopheles gambiae and An. coluzzi Under High Hybridization**

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*Anopheles gambiae* and *An. coluzzi* are defined based on SNPs in the multi-copy IGS chromosome-X linked rDNA region. In most of their range of sympatry, where they rarely hybridize, multiple areas of the genome are differentiated, with highest divergence found toward centromeres. However, genomic divergence varies markedly with levels of introgression.

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In a putative secondary contact region in Guinea Bissau, where hybrid females have been observed at frequencies >20%, differentiation is largely limited to a region proximal to the X-centromere. This region may play a critical role in the speciation process, but the evolutionary forces acting upon it are poorly understood. The aim of our work was to evaluate interspecific genomic differentiation and frequency of recombination along the X-centromere in the Guinean hybridization region, focusing the analysis on X-chromosome hemizygous males to permit unambiguous haplotype analysis. We genotyped 263 males for the IGS diagnostic locus and for two additional markers about 1 Mb from it: i) the insertion of a SINE retrotransposable element specific for An. coluzzii, which is widely used as a species diagnostic; and ii) a 57 bp-insertion in intron 4 of cytochrome CYP4G16 (CYP) gene specific for An. gambiae. Moreover, using Illumina and Sequenom genotyping we characterised almost 800 SNPs (34 of which are species-specific and located in the X centromeric region) in 59 males. We observed: i) lack of inter-specific differentiation in the overall genome, with the exception of chromosome-X centromere; ii) intra-individual mixed IGS-arrays in 12% of the whole male sample, suggesting the occurrence of introgression events; iii) unexpected recombination among IGS, SINE and CYP in 24% of the males, and between SINE and CYP in 13% of them despite the close proximity of these 2 loci (7 Kb). Moreover, results from SNP-genotyping showed: i) some, although low, levels of recombination in the X-centromere; ii) introgression in the IGS-region in the absence of recombination in the X-centromere; iii) a hot-spot of recombination nearby the SINE-insertion. The results highlight (1) the likely importance of reduced recombination in maintaining integrity of the X-genomic island of divergence with high gene flow, consistent with genetic-hitchhiking-based speciation models and (2) the poor reliability of existing diagnostics (IGS, SINE) for the two species in the secondary contact region.

1913

AN INTEGRATED CHROMOSOME, GENETIC LINKAGE AND GENOME MAP FOR THE SOUTHERN HOUSE MOSQUITO CULEX quinquefasciatus

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Culex quinquefasciatus, a southern house mosquito, is a member of geographically widespread mosquito complex species with high variation in behavioral patterns and ability to transmit diseases, including lymphatic filariasis and West Nile fever. Only 10.4 % of the 579 Mb genome is currently assigned to the chromosomes based on genetic linkage mapping. Although cytogenetic maps for the polytene chromosomes for this mosquito were developed, their utilization for the genome mapping remains difficult because of the low number of high quality spreads in chromosome preparations. We constructed idiograms for mitotic chromosomes of Cx. quinquefasciatus based on their banding patterns at early metaphase. These idiograms represent the first cytogenetic map developed for mitotic chromosomes of Cx. quinquefasciatus. Genetic contigs associated with 14 major genetic markers, 18S rDNA and 10 largest contigs were anchored to the exact positions on Cx. quinquefasciatus chromosomes using fluorescent in situ hybridization. The order of genetic markers was consistent with the previously developed genetic linkage map. Some new insights were provided into chromosome evolution in mosquitoes. For example, FISH result of 18S rDNA suggests an inverted position of the ribosomal locus in chromosome 1 of Cx. quinquefasciatus compared with Ae. aegypti. This locus was mapped close to the centromere above the heterochromatin band in Cx. quinquefasciatus but in the middle of the 1q arm below the heterochromatin band in Ae. aegypti. Our study in progress linked chromosome and genetic linkage maps with 4.8% of the Cx. quinquefasciatus genome.

1914

MICROSATELITE AND DNA SEQUENCE POPULATION GENETICS EVALUATION OF THE SOUTHWEST PACIFIC MALARIA VECTOR ANOPELES KOLIENSI (OWEN)

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The southwest Pacific malaria vector Anopheles koliensis is one of 13 cryptic members of the Anopheles punctulatus group that can only be identified by molecular tools. Its distribution in Papua New Guinea (PNG) has only recently been described and it is found predominantly throughout inland lowlands and river valley flood plains below 300m and is common throughout the continual wet Sepik, Ramu, and Markham River valleys on the north side of PNG’s central ranges as well as throughout the northern and southern lowland region of PNG’s Papuan Peninsula. An. koliensis utilizes both natural larval habitat (ground pools and swamps), as well as human modified habitat (vehicle wheel tracks and drains). In this study, we drill into the population genetic of An. koliensis in PNG - a region of incredible biogeography - to detail the spatial and genetic connectivity of this malaria vector species. We evaluate nuclear and mitochondrial DNA sequence as well as develop and analyse 12 microsatellites. We find a species with overt genetic and geographic population structure that can be explained, in most cases, by natural barriers. We do not find evidence to support the existence of intraspecific rDNA genotypes previously described but we do find An. koliensis to be a single species with a long history in New Guinea.

1915

CHARACTERIZATION OF MALE REPRODUCTIVE FACTORS ESSENTIAL FOR MATING SUCCESS IN THE LIFE CYCLE OF ANOPHELES GAMBIAE MOSQUITOES

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Mating is a vulnerable step in the lifecycle of Anopheles gambiae mosquitoes, as females of this species mate only once. Seminal fluids produced in the male accessory glands (MAGs) and transferred during mating are likely to induce permanent refractoriness to further insemination in females. Moreover, these factors are likely to play a key role in other post-mating processes, including egg laying and fertility. However, the identity of these male molecular triggers is largely unknown. As changes in levels of individual semen components after mating may indicate factors crucial for mating success, we performed a time course transcriptional analysis of MAG genes at 3 time points (3h, 12h, 24h) after mating, representing the period between 2 mating events in the field. Mated tissues were compared to those from virgin males in 4 replicates using whole-genome microarrays. Surprisingly, a total of 4,319 genes were differentially expressed after mating (p<0.05 FDR). Gene enrichment analysis revealed a number of functional groups significantly enriched in the dataset. During early time points after mating, genes associated with RNA transcription, translation and post-translational modifications were enriched, suggesting an induction of pathways essential for the replenishment of MAG content. At the latest time point enrichment was observed in genes involved in protein export, indicative of males preparing for the next mating event. Interestingly, genes involved in hormone biosynthesis were also highly enriched, supporting previous findings that male hormones may play a critical role in An. gambiae reproduction. We then investigated the function of male hormones that are replenished after mating. Tampering with the synthesis of these hormones in males...
had dramatic effects on the reproductive physiology of mated females, which showed strongly reduced fecundity and fertility. These results reveal previously unknown pathways that are key to mosquito reproductive physiology, and offer novel targets for vector control efforts aimed at reducing mosquito reproductive success.

**1916**

**AN INPUT ON PREVENTIVE STRATEGIES FROM THE FIELD: IRON LEVELS AND INTERMITTENT PREVENTIVE TREATMENT (IPTP) CALENDAR ARE ASSOCIATED WITH PLASMODIUM FALCIPARUM PARASITEMIA DURING THE FIRST YEAR OF LIFE**

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Malaria is the disease with the highest infant mortality and morbidity worldwide. In 2012 the WHO reported over 207 million cases and more than 627,000 deaths. In Benin malaria is the leading cause of mortality (23%) among children under 5 years. There are significant differences in P. falciparum parasitemia among children during the first year of life. We aim at describing the factors contributing to malaria episodes and to high parasitemia during the first year of life in Benin. Therefore we have investigated the association of maternal exposure to the vector in utero (determined by pregnancy associated malaria (PAM) and the intermittent preventive treatment (IPTp)) to infant parasitemia analyzing as well nutritional, environmental and socio-economic risk factors. 1000 pregnant women and 400 of their children were followed during pregnancy and the first year of life in Allada (Benin), between 2010 and 2012. At inclusion socio-demographic status and gyneco-obstetric history were investigated. Extensive medical and biological exams were realized with both doses of IPTp and at delivery for the mothers and at 6, 9, and 12 months for the infants. Further exams were realized at each emergency consultation. All patients were treated in case of disease. Random coefficient models assessed the relationship between the different parasitemia measures in infants and other variables. A novel approach consisting in pathway analysis was used to analyze the evolution pattern of parasitemia. Maternal age at both IPTp doses, infant weight, mother parasitemia at delivery, number of emergency consultations and total body iron were correlated with infant parasitemia. Placental malaria was not correlated with infant parasitemia when adjusting for mother parasitemia at delivery. We find for the first time that IPTp has not only an effect on LBW but also on infant parasitemia. Therefore IPTp calendar should cover extensively the pregnancy and protect both the mother and the infant. Total body iron is also correlated with infant parasitemia. WHO recommends supplements with iron and folic acid when the prevalence of anaemia exceeds 40%. However the Pemba study and a Cochrane review conclude to an increased risk for malaria among supplemented children in the context of limited malaria coverage. Our results confirm the association between iron and malaria and plead for protective measures in the context of iron supplementation.

**1917**

**MALARIA-TRANSMISSION INTENSITY AND THE PROTECTIVE EFFECT OF INTERMITTENT PREVENTIVE TREATMENT (IPTP): POLICY IMPLICATIONS FOR THE ANTENATAL CARE OF PREGNANT WOMEN IN SUB-SAHARAN AFRICA**

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The World Health Organization (WHO) recommends the provision of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) to pregnant women resident in areas of moderate (stable) or high malarial transmission to prevent low birthweight (LBW), neonatal deaths and maternal anaemia. The incidence of malaria below which IPTp-SP no longer provides a cost-benefit is unknown, but it is important to estimate as countries make progress towards malaria elimination. We conducted a review of IPTp-SP studies and matched the protective efficacy of IPTp-SP against LBW to a proxy measure of malarial incidence in women of the same studies, the prevalence of malaria in children. The latter was calculated in two ways. We first extracted prevalence estimates in children from the Malaria Atlas Project (MAP) database and, for the second measure, we selected and then pooled the point prevalence data that underlay the MAP estimates using random-effects models. We then applied meta-regression models of the protective efficacy of IPTp-SP against LBW to the estimates of malarial prevalence in children calculated in both ways, and stratified results by gravity. Among multigravidae, the protective effect of IPTp-SP against LBW was no longer significant in areas where the malarial prevalence in children was < to 9% when we applied MAP estimates, and < to 8% using our pooled estimates. The latter analysis showed a significant linear trend (P=0.043). Malarial transmission intensity could not explain variations in the efficacy of IPTp-SP among paucigravidae. IPTp-SP no longer protects against the incidence of LBW among multigravidae in geographical areas of 20 sub-Saharan countries where the parasite prevalence among children is < 8%. In contrast, our analysis among paucigravidae suggests that two or more doses of SP is protective against LBW in transmission settings that are below the current recommendation set by the WHO.

**1918**

**MISSED OPPORTUNITIES FOR DELIVERING PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY DURING ANTENATAL CARE AND COMPARISON WITH DELIVERY OF NEONATAL TETANUS PREVENTION: AN ANALYSIS OF HOUSEHOLD SURVEYS IN SUB-SAHARAN AFRICA**

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Coverage of intermittent preventive treatment for malaria during pregnancy (IPTp), a potentially lifesaving intervention, remains low in Africa, despite high antenatal care attendance among pregnant women and recommendations for IPTp to be given at each antenatal care visit after the first trimester. To highlight areas of potential improvement, we assessed trends in IPTp coverage over time, and identified the missed opportunities for delivery of IPTp at antenatal clinics. Data from 66 national household surveys conducted in 31 sub-Saharan African countries between 2000 and 2012, with relevant questions on antenatal care (ANC) were used to determine the coverage of ANC visits, IPTp and tetanus toxoid (TT). Missed opportunities for IPTp were calculated by comparing the number of IPTp doses received to the number of ANC visits during which IPTp could be given. To account for visits occurring in the first trimester when IPTp is not given, one visit was subtracted from the total number of visits for women who reported having their first visit in the first trimester. The median proportion of pregnant women receiving at least 2 doses of IPTP was 1.0% (IQR 0-10.3%) during 2000-2007 and 27.2% (IQR 13.9-42.1%) during 2008-2012. Missed opportunities for IPTP delivery occurred in a median 99.2% (IQR 90.2-100%) of ANC visits from 2000-2007 and 76.8% (IQR 65.6-92.9%) from 2008-2012. The median proportion of primigravida women receiving at least 2 doses of TT is much higher: 50.2% (IQR 34.4-64.0%) during 2000-2007, and 59.2% (IQR 48.6-64.9%) during 2008-2012. With the exception of two countries, the proportion of primigravida women receiving at least 2 doses of IPTp is lower than the proportion receiving at least 2 of TT: the median absolute difference is 41.7% from 2000-2007 (IQR 29.2-54.1%) and 36.7% (IQR 23.7-40.6%) from 2008-2012. Although IPTp coverage has increased slightly over time, levels remain disappointingly low, and missed opportunities for IPTp occur at the majority of ANC visits. Although...
both are delivered through the ANC, delivery of IPTp occurs much less frequently than delivery of TT, suggesting that barriers to IPTp delivery could be overcome. Further work is required to determine the specific factors that are driving the surprising discrepancies between IPTp and TT coverage, with an eye toward improving IPTp coverage through potential linkage with the TT administration infrastructure.

1919

PHARMACOKINETICS OF ARTEMETHER-LUMEFANTRINE IN PREGNANT AND NON-PREGNANT WOMEN WITH UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN WESTERN KENYA

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Pregnancy increases the vulnerability to malaria infection in all women living in areas of malaria risk. The World Health Organization recommends the use of artesiminin-based combination therapies (ACTs) for treatment of acute uncomplicated falciparum malaria in the second and third trimesters of pregnancy. The pharmacokinetic properties of antimalarial drugs are often affected by pregnancy, resulting in lower drug concentrations and a consequently higher risk of treatment failure. While artemether-lumefantrine is used in Kenya and most Eastern Africa countries as first line treatment for malaria in pregnancy in the second and third trimesters, its pharmacokinetics in pregnant African women with malaria is not well characterized. This study evaluated the population pharmacokinetics of artemether, dihydroartemisinin, lumefantrine and desbutyl-lumefantrine in 45 pregnant and 25 non-pregnant women with uncomplicated malaria in Western Kenya. All patients were treated with the standard fixed dose artemether-lumefantrine 20/120mg tablets over 3 days. Frequent venous blood sampling was obtained over the treatment period for pharmacokinetic evaluation. Estimates for pharmacokinetic and variability parameters will be obtained through nonlinear mixed effects modeling. Simultaneous modeling of parent drug and metabolite will be used for both artemether and lumefantrine. Absorption and clearance of artemether-lumefantrine in pregnant compared with non-pregnant African women with uncomplicated malaria and the implications of findings will be presented.

1920

A STUDY OF THE PHARMACOKINETICS OF PRIMAQUINE IN LACTATING WOMEN AND BREASTFED INFANTS FOR THE RADICAL TREATMENT OF UNCOMPPLICATED MATERNAL PLASMODIUM VIVAX

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Women of reproductive age in malarial areas of the world suffer due to lack of evidence on the safety of effective antimalarials in pregnancy and lactation. Plasmodium vivax recurrences are more common during pregnancy but the only widely available medication for radical treatment of P vivax, primaquine, is contraindicated in pregnancy. The postpartum period presents a key opportunity for radical treatment of P vivax, but there are no studies quantifying primaquine excretion in breast milk and the dose that breastfed infants would be exposed to is unknown. We are conducting the first-ever study of the pharmacokinetics of primaquine lactating women and their breastfed infants during a 14-day radical treatment of P vivax. Twenty-four healthy lactating women at risk for recurrent malaria (i.e. with a history of P vivax) and their infants (at least 28 days old) are being recruited for detailed pharmacokinetic study. Prior to enrolment, G6PD deficiency is excluded by rapid qualitative fluorescent spot test and G6PD genotype from PCR spot. Anemic patients are treated and enrolment is delayed until normal HCT is established. Hemoglobin typing is analyzed and fetal hemoglobin in infant blood is quantified. Primaquine is administered to eligible mothers at a dose of 0.5 mg/kg/day and is directly observed. Primaquine and carboxyprimaquine levels are measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) on venous and capillary plasma, urine, saliva and breast milk samples from the mothers, as well as capillary plasma samples from the infants. There have been no drug-related adverse events to infants, though several women have experienced mild to moderate methemoglobinemia (not requiring treatment). Preliminary data shows low but measurable levels of primaquine in both breast milk and infant plasma. The final results of this study could have profound impacts on malaria control and women’s health in the tropics.

1921

SAFETY OF ARTEMETHER-LUMEFANTRINE EXPOSURE IN EARLY PREGNANCY: AN OBSERVATIONAL COHORT

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There is limited data available regarding safety profile of artesminsins in early pregnancy. They are therefore not recommended by WHO as a first line treatment for malaria in first trimester due to associated embryo-foetal toxicity in animal studies. The aim of the study was to assess birth outcome among pregnant women inadvertently exposed to AL during first trimester in comparison to those of women exposed to other antimalarial drugs or no drug at all during the same period of pregnancy. Pregnant women with gestational age < 20 weeks were recruited from Reproductive and Child Health (RCH) clinic or from monthly house visits (demography surveillance), and followed prospectively until delivery. A structured questionnaire was used to interview participants. 2167 pregnant women were recruited and 1783 (82.3%) completed the study until delivery. 319 (17.9%) used antimalarials in first trimester, of whom 172 (53.9%) used artemether-lumefantrine (AL), 78 (24.4%) quinine, 66 (20.7%) sulfadoxine-pyrimethamine (SP) and 11 (3.4%) amodiaquine. Quinine exposure in first trimester was associated with an increased risk of miscarriage/stillbirth (OR 2.5; 1.3 - 5.1) and premature birth (OR 2.6; 1.3 - 5.3) as opposed to AL with (OR 1.4; 0.8 - 2.5) for miscarriage/stillbirth and (OR 0.9; 0.5 - 1.8) for preterm birth. Congenital anomalies were identified in 4 exposed groups namely AL only (1/164 [0.6%]), quinine only (1/70 [1.4%]), SP (2/66 [3.0%]), and non-antimalarial exposed group (19/1464 [1.3%]). Exposure to AL in first trimester was more common than to any other antimalarial drugs. Quinine exposure was associated with adverse pregnancy outcome, which was not the case following other antimalarial intake. Since AL and quinine were used according to their availability rather than to disease severity, it is likely that the effect observed was related to the drug, and not to the disease itself. Detailed information on developmental milestone up to 12 months is ongoing to rule out any adverse effect on infancy as a result of AL exposure in first trimester. Even with this caveat, a change of policy from quinine to AL for the treatment of uncomplicated malaria during the whole pregnancy period could be already envisaged.
SAFETY AND EFFICACY OF FOUR ARTEMISININ-BASED COMBINATION TREATMENTS IN AFRICAN PREGNANT WOMEN WITH MALARIA

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Pregnant women are at increased risk of Plasmodium falciparum malaria which is associated with increased maternal, foetal and neonatal morbidity and mortality. Thus, malaria-infected pregnant women need prompt and effective treatment. Artemisinin-based combination treatments (ACT) are recommended for pregnant women in their second and third trimesters of pregnancy though information on their safety and efficacy in African pregnant women is limited. A Phase 3, non-inferiority, multicentre, randomized, open-label clinical trial compared the efficacy and safety of four ACTs, namely amodiaquine-artesunate, dihydroartemisinin-piperaquine, artemether-lumefantrine, and mefloquine-artesunate, in women with malaria and in the second or third trimester of pregnancy. A total of 3,423 pregnant women were recruited in Burkina Faso, Ghana, Malawi and Zambia. After being treated with one of the 4 ACTs at day 0, 1 and 2, women were reviewed at days 3, 7, 14, 21, 28, 35, 42, 49, 56 and 63, and whenever they were sick. There were 3 early treatment failures, 2 in Malawi and 1 in Zambia. Eight hundred twenty five women (24.1%) had a recurrent infection during the follow up, 81 (2.4%) of them identified as recrudescences after genotyping. No major safety problems were observed during the follow up. This is the largest trial on ACT use during pregnancy ever done in sub-Saharan Africa. Its preliminary results are reassuring.

EPIDEMIOLOGICAL AND MOLECULAR FEATURES OF DENGUE, ZIKA AND CHIKUNGUNYA CONCURRENT OUTBREAKS IN THE PACIFIC, 2014

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During more than a century dengue has been the only mosquito-borne virus considered as major public health concern for Pacific nations. However, during the past 5 years the epidemiology of arboviruses in the Pacific region has shown terrific changes. The situation gradually switched from the predominant circulation of a single dengue virus (DENV) to active transmission of multiple DENV serotypes and genotypes as observed in French Polynesia from 2013 and in New Caledonia and Fiji since the beginning of 2014. In the mean time, Chikungunya virus (CHIKV) appeared for the first time in New Caledonia with autochthonous cases sporadically reported from 2011 up to 2013, and large outbreaks occurring in Papua New Guinea in 2012, Yap Island in 2013 and in Tonga in 2014. Another unexpected event was the emergence of Zika virus (ZIKV) in French Polynesia at the end of 2013. ZIKV caused in French Polynesia the largest outbreak ever documented, and in a context of active circulation of DENV serotypes 1 and 3. At the beginning of 2014, ZIKV outbreaks also emerged in New Caledonia and Cook Islands. As of April 2014, outbreaks of “dengue-like illnesses” were under investigation in several other Pacific islands suggesting that the situation was evolving from bad to worse. We will describe here the early laboratory investigations that contributed to the identification of the aetiological agents of the outbreaks that recently occurred in the Pacific, notably based on the use of filter paper-spotted serum and saliva collected on cotton swab as a source of viral RNA. Based on phylogenetic data we will discuss how these viruses were introduced from continental regions into the Pacific and how they spread from one Pacific island country to another. We will also discuss the particular features of these outbreaks, notably in the occurrence of unusual clinical manifestations, like observed in French Polynesia during the ZIKV outbreak.
1925

CLINICAL STUDIES OF DNA VACCINES FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME

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Hemorrhagic fever with renal syndrome (HFRS) is endemic in Asia, Europe and Scandinavia, and is caused by infection with the hantaviruses Hantaan (HTNV), Seoul (SEOV), Puumala (PUUV), or Dobrava (DOBV) viruses. We developed candidate DNA vaccines for HTNV expressing Gn and Gc genes of HTNV or PUUV and evaluated them in an open-label, single-center Phase 1 study. Three groups of nine subjects each were vaccinated on days 0, 28, and 56 with the DNA vaccines for HTNV, PUUV, or mixture of both vaccines using the Ichor Medical Systems TriGridTM Intramuscular Delivery System (TDS-IM). All vaccinations consisted of a total dose of 2.0 mg DNA in an injected volume of 1 mL saline. For the combined vaccine, the mixture contained equal amounts (1.0 mg) of each DNA vaccine. There were no study-related serious adverse events (SAEs). Neutralizing antibody responses were detected in 5/9 and 7/9 of individuals who completed all three vaccinations with the HTNV or PUUV DNA vaccines, respectively. In the combined vaccine group, 7/9 of the volunteers receiving all three vaccinations developed neutralizing antibodies to PUUV. The three strongest responders to the PUUV vaccine also had strong neutralizing antibody responses to HTNV. These results demonstrate that the HTNV and PUUV DNA vaccines delivered by TDS-IM separately or as a mixture are safe. In addition, both vaccines were immunogenic, although when mixed together, more subjects responded to the PUUV than to the HTNV DNA vaccine, suggesting immunological interference. Consequently, we have developed an optimized HTNV DNA vaccine that shows no interference in hamsters when mixed with the PUUV vaccine. A Phase 2a clinical study will be initiated in 2014 to assess dose and schedule with the combined, optimized HTNV and PUUV DNA vaccines. An additional Phase 1 study is being planned to compare intradermal and intramuscular delivery of the mixed DNA vaccines.

1926

AN INEXPENSIVE SYSTEM FOR PRODUCING STRUCTURALLY STABLE REPLICATIVE RNA VIRUS-BASED NANOPARTICLE VACCINES

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Tropical infectious disease vaccine development requires particular attention to cost-efficiency and minimized storage conditions to be optimally useful in third world countries. In this study, the use of a plant-produced, trans-encapsidated non-pathogenic RNA virus as a vaccine vector addresses these issues. Plant-based manufacturing allows large scale production with greatly reduced expense. Transencapsidation with Tobacco mosaic virus (TMV) coat protein provides superior chemical and environmental protection to any RNA sequence containing a TMV encapsidation site. A transencapsidated RNA virus that expresses vaccine antigens in human cells would be highly attractive for vaccine use due to its ability to induce innate immune activation pathways to aid immunogenicity of its antigen payload. To avoid the potential safety concerns using a human virus, we have used the insect RNA virus, Flock House virus (FHV), which replicates in both human and plant cells but is not pathogenic to either. The RNA2 of the bipartite FHV genome codes for FHV coat protein and is not necessary for replication. We created an FHV RNA1/eGFP vector and noted strong expression of eGFP in inoculated Nicotiana benthamiana plants. This was followed by inserting the TMV encapsidation site into the FHV RNA1 and inoculating plants in combination with a high expression vector derived from Fovtial mosaic virus to express TMV coat protein. The production of in planta nanoparticles was verified by transmission electron microscopy and these yielded an immune response in vaccinated mice that was superior to that of in vitro assembled nanoparticles. In this study, we validated the use of in planta encapsidated RNAs as an immune activator in the absence of adjuvants. We can now use this system to create sturdy and inexpensive vaccines for tropical infectious diseases.

1927

SEROPREVALENCE OF NGARI AND BUNYAMWERA VIRUSES IN SELECT PARTS OF RIFT VALLEY AND NORTHERN KENYA

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Ngari and Bunyamwera viruses are among the few mosquito-borne human pathogens in the Orthobunyavirus genus, Family Bunyaviridae, associated with febrile illness. Ngari virus has been associated with hemorrhagic fever during Rift Valley fever outbreaks in Africa. Ngari virus is a reassortant virus composed of the S and L segments from Bunyamwera and the M segment from Bafatia virus. While isolations of both viruses have been made from mosquito and tick vectors in the transmission foci in Kenya, no human serosurveys have been conducted. We report findings from a retrospective serosurvey of febrile ill patients attending three health facilities located in Sanguai, Kotile (in Garissa) and Naivasha in Kenya. Bunyamwera and Ngari virus specific antibodies were detected by plaque reduction neutralization tests in 84 (24.3%) of 345 persons tested; Prevalence rates were 11.9% for Bunyamwera virus and 15.9% for Ngari virus. Multivariable analysis revealed age and location as risk factors for Bunyamwera and Ngari virus infections. Patients presenting with febrile illness in identified endemic regions should be vigorously investigated to determine the public health impact of these infections especially during seasons of high mosquito abundance.

1928

ITAYA VIRUS: A NOVEL ORTHOBUNYAVIRUS ASSOCIATED WITH HUMAN FEBRILE ILLNESS IN PERU

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The Orthobunyavirus genus in the family Bunyaviridae comprises more than 170 viruses, at least 30 of them associated with human disease. Caraparu virus, a member of the group C (Orthobunyavirus genus), was first isolated from a sentinel monkey in Brazil and subsequently isolated from febrile patients in Bolivia, Brazil, Peru and Trinidad. Febrile surveillance studies conducted by the U.S. Naval Medical Research Center Unit No. 6 identified Caraparu virus (and other group C viruses) as an important cause of febrile illness in the Amazon region of Peru. We conducted genetic analyses of previously uncharacterized bunyavirus strains isolated from febrile patients in Peru, and identified a novel reassortant virus containing the S and L segment of Caraparu virus and the M segment of an unidentified Group C virus. Neutralization test using mouse antisera prepared against the prototype Caraparu strain BeAn 3994 and the novel reassortant virus showed that there was more than a 4-fold difference in titer between these viruses, indicating that the new reassortant was serologically distinct from the prototype Caraparu strain. Serological analyses also confirmed that the novel reassortant was antigenically
distinct from Peruvian Caraparu strains. This new reassortant virus, which we named Itaya virus, was first isolated during 1999 from a 25-year-old male febrile patient in Iquitos, Peru, and subsequently isolated during 2006 from a 59-year-old male febrile patient in Yurimaguas, another city within the Amazon region of Peru. Geographical distance between these two cases indicates that Itaya virus may be widely distributed within the Peruvian Amazon. The recognition of a new Orthobunyavirus human pathogen in the Amazon region of Peru reinforces the need to continue and expand viral disease surveillance activities in tropical regions of South America.

1929

MAPPIng THE ZOOntotic NICHe OF EBOLA VIRUS DISEASE IN AFRICA

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Ebola virus disease (EVD) is a complex zoonosis that is highly virulent in humans. The largest recorded outbreak of EVD is ongoing in West Africa, outside of its previously reported and predicted niche. We assembled location data on all recorded zoonotic transmission to humans and Ebola virus infection in bats and primates (1976-2014). Using species distribution models these occurrence data were paired with environmental covariates to predict a zoonotic transmission niche covering 22 countries across Central and West Africa. Vegetation, elevation, temperature, evapotranspiration and suspected reservoir bat distributions define this relationship. At-risk areas are inhabited by 22 million people, however the rarity of human outbreaks emphasises the very low probability of transmission to humans. Increasing population sizes and international connectivity by air since the first detection of EVD in 1976 suggest that the dynamics of human-to-human secondary transmission in contemporary outbreaks will be very different to those of the past.

1930

USE OF A NOVEL CHAGAS URINE NANOPARTICLE TEST (CHUNAP) FOR DIAGNOSIS OF CONGENITAL CHAGAS DISEASE

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Detection of congenital Trypanosoma cruzi transmission is considered one of the pillars of control programs of Chagas disease, because 25% of new infections occur by this route with an estimated of 15,000 infected infants per year in Latin America. Current programs to detect congenital Chagas disease in Latin America utilize microscopy early in life and serology after 6 months. These programs suffer from low sensitivity by microscopy and high loss to follow-up later in infancy. We developed a Chagas urine nanoparticle test (Chunap) to concentrate, preserve and detect T. cruzi antigens in urine for early, non-invasive diagnosis of congenital Chagas disease. This is a proof-of-concept study to provide an initial indication that Chunap allows for the early diagnosis of congenital Chagas disease. Poly-N-isopropylacrylamide nano-particles functionalized with trypsin blue were synthesized by precipitation polymerization and characterized with photon correlation spectroscopy. We evaluated the ability of the nanoparticles to capture, concentrate and preserve T. cruzi antigens. Urine samples from congenitally infected and uninfected infants were then concentrated using these nanoparticles. The antigens were eluted and detected by Western Blot using a monoclonal antibody against T. cruzi lipophosphoglycan. The nanoparticles concentrated T. cruzi antigens by 100 fold (western blot detection limit decreased from 50 ng/ml to 0.5 ng/ml). The sensitivity of Chunap in a single specimen at one month of age was 91.3% (21/23, 95% CI: 71.92%-98.68%), comparable to PCR in two specimens at 0 and 1 month (91.3%) and significantly higher than microscopy in two specimens (34.8%, 95% CI: 16.42%-57.26%). Chunap specificity was 96.5% (71/74 endemic, 12/12 non-endemic specimens). Particle-sequestered T. cruzi antigens were protected from trypsin digestion. Chunap has the potential to be developed into a simple and sensitive test for the early diagnosis of congenital Chagas disease.

1931

A THERAPEUTIC NANOPARTICLE VACCINE AGAINST TRYPANOSOMA CRUZI IN A MOUSE MODEL OF CHAGAS DISEASE

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Chagas disease is a neglected tropical disease of great importance in the Americas, with 7-8 million people infected. The causative agent is Trypanosoma cruzi, and results in an acute febrile illness that progresses to chronic chagasic cardiomyopathy in 30% of patients. In endemic areas, Chagas disease is the leading cause of cardiovascular death between ages 30-50. Current pharmaceutical treatments are plagued by significant side effects, poor efficacy, and are contraindicated in pregnancy. There is an urgent need for new treatment modalities. A therapeutic vaccine for Chagas disease has potential advantages that include cost savings, reduced adverse effects, and the potential to be used as a replacement for current therapies or when paired with chemotherapy. Prior work in mice has identified an efficacious T. cruzi antigen (Tc24). To elicit a protective cell-mediated immune response to the Tc24 protein, we have utilized a nanoparticle delivery system in conjunction with CpG motif-containing oligodeoxynucleotides (ODN) as an immunomodulatory adjuvant. When tested in a BALB/c mouse model, a dose response study demonstrated a positive relationship between dose of vaccine and Tc24-specific IFN-γ response. Our nanoparticle vaccine, comprised of Tc24 and CpG ODN encapsulated in poly(lactic-co-glycolic acid) (PLGA) nanoparticles, produced the most robust Tc24-specific CD8+ T cell immune response. When tested for therapeutic efficacy in T. cruzi infected BALB/c mice, improved survival was seen in the vaccine group compared to the control groups. Additionally, there was a significant reduction in the number of parasites in the cardiac tissue of the vaccine group compared to the PBS sham vaccine group, indicating protection from parasite-driven cardiac damage. The mice that survived to the end of the study had almost undetectable numbers of parasites in the cardiac tissue. These data demonstrate the immunogenicity and efficacy of a Tc24/CpG ODN nanoparticle vaccine and are convincing evidence for a potential new therapeutic vaccine against Chagas disease.
1932

ASSEMBLING NEW CHEMICAL BOXES AS AN OPEN SOURCE OF STARTING POINTS FOR DRUG DISCOVERY AGAINST KINETOPLASTID PARASITES CAUSING NEGLECTED TROPICAL DISEASES

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Neglected Tropical Diseases (NTDs) are a group of infectious diseases categorized by the particular neglect they have suffered in terms of investment in control measures, when compared with malaria and tuberculosis, “the big two” within the diseases. The NTDs encompass a broad range of viral, bacterial, and parasitic infections. Kinetoplastids are a group of flagellated protozoa that include the species *Leishmania* and *Trypanosoma*, some of which are human pathogens with devastating health and economic effect. The most common human diseases caused by kinetoplastids are included within the list of 17 core NTDs declared by WHO. They are human African trypanosomiasis, caused by subspecies of *T. brucei*; Chagas disease, caused by the infection with *T. cruzi*; and various *T. cruzi*-caused diseases (Visceral Leishmaniasis, Chagas disease and human African onchocerciasis). The collaboration, with support from the Wellcome Trust and the WHO, has made significant progress, in building new methods and infrastructure to carry out drug discovery for these parasites. Our advances has resulted in the development of 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.

1934

CLINICAL EVALUATION OF CL DETECT™ RAPID TEST FOR CUTANEOUS LEISHMANNIASIS: PERFORMANCE CHARACTERISTICS WHEN COMPARED TO SMEAR MICROSCOPY AT MULTIPLE TEST SITES

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This study focused on establishing the performance characteristics of the CL Detect™ rapid immunoassay when compared to smear microscopy. The assay is intended for the diagnosis of Cutaneous Leishmaniasis in far-forward, rugged environments. The test is based on the detection of the thiol specific antioxidant protein (TSA, Peroxidoxin) present in amastigotes and promastigotes of *L. major* and other Leishmania spp. It uses a capture polyclonal antibody to TSA in combination with a gold conjugated monoclonal antibody directed to *L. major* amastigotes but reactive with TSA. Testing was performed on samples from skin lesions collected with a dental broach device. In each case dipstick reactivity was compared to the lesion parasite load determined by microscopy and quantified using the WHO scale. A total of 168 patients ranging in age from 18-79 years who were suspected CL lesions were enrolled with written informed consent at 2 sites endemic for *L. major* infections in central Tunisia (Sidi Bouzid, Gafsa). 149 were positive by CL Detect™ and microscopy while 16 were negative by both tests and 3 were positive by dipstick but not microscopy. Of these three, 1 was positive by culture. Of the 16 negatives by dipstick and microscopy 2 were positive by culture. In the Icahn School of Medicine, Mount Sinai specificity study, 150 samples were tested by CL Detect™ and microscopy. These included patients ranging in age from 18-92 years with other skin lesions and non-CL infections. In the specificity study 144 of 150 were true negatives for parasites by both CL Detect™ and microscopy for a specificity of 96.0%. Six samples negative by microscopy were low positive by rapid test but negative by microscopy. Cross reactivity studies with other bacteria, parasites, viruses and fungi confirmed specificity for Leishmania spp. Interference, stability and reproducibility studies indicate that CL Detect™ is a robust assay. The pairing of this test with a safe and easy to use drug treatment has the potential to greatly enhance the management of CL patients in far-forward rugged environments.
**1935**

**ACCESS TO DIAGNOSIS AND TREATMENT FOR CHAGAS DISEASE IN THE UNITED STATES: A HEALTH SYSTEMS PERSPECTIVE**

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Chagas disease, caused by infection with *Trypanosoma cruzi*, is a vector-borne disease with an estimated 300,000 cases in the United States (US). Screening of blood donors for infection with *T. cruzi* was started in 2007 in the US. Currently, benznidazole and nifurtimox are available to treat this infection through direct request from the US Centers for Diseases Control and Prevention (CDC) under compassionate use protocols. This study analyzed epidemiological trends in diagnosis and treatment for Chagas disease in the US and assessed national and state barriers to access. Data on the distribution of cases of Chagas disease identified in blood donors and drug releases were obtained from the AABB and CDC, respectively. Semi-structured in-depth interviews were conducted with 30 key informants at the national level and in 6 high-burden states (CA, FL, VA, NY, MA, and TX) where treatments were provided. Interview responses were analyzed according to the health system’s dimensions of regulation, financing, payment, organization, and persuasion. Data indicate that 1,908 cases were identified in the blood donation system from 2007-2013 and that CDC provided 422 courses of benznidazole or nifurtimox during this period. Interview data revealed that local ad-hoc procedures were used by individual physicians with an interest in the disease to increase access to medicines for Chagas disease, especially through cross-financing of patient care activities using grants and donations. The primary barriers to access at the national level include limited diagnostic and institutionalized referral and care processes (Organization), lack of financing for patient care activities in most states (Financing), and limited awareness and training among physicians and patients (Persuasion). This study demonstrates that access to treatment for Chagas disease in the US is limited. The lack of licensing for the two medications used in treatment was only one of several barriers to access, highlighting the need for a health systems perspective when scaling up access to these essential medicines.

**1936**

**IMMUNOGENICITY OF TRYPANOSOMA CRUZI VACCINE CANDIDATE ANTIGENS TSA-1 AND TC24 IN MEXICAN CHAGASIC PATIENTS**

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Chagas disease affects 8–10 million persons worldwide, and at least 1–2 million in Mexico. Current drugs have a limited efficacy and the development of a preventive and/or therapeutic vaccine is an important goal. Based on previous work in mouse and dog models, trypomastigote surface antigen-1 (TSA-1) and 24-kDa-trypomastigote secretion/excretion antigen (Tc24) are good candidates for a vaccine against *Trypanosoma cruzi*. We evaluated here the recall immune response against these vaccine antigens in Chagas disease patients, as a first step to assess their immunogenicity in humans. We used peripheral blood mononuclear cells (PBMC) from chagasic patients (*n*=8) and healthy controls (*n*=8) that were stimulated *in vitro* with TSA-1 and Tc24 recombinant antigens. After 120 hours of stimulation, we evaluated cell proliferation, identified CD4+ and CD8+ memory cell subpopulations, and IFN-gamma and IL-10-producing cells by flow cytometry. We observed a specific proliferative response to TSA-1 and to a lesser extent to Tc24, with a central memory T cell phenotype and antigen-specific INF-gamma and IL-10 production in several Chagas disease patients. Additional patients and controls will be enrolled until September 2014. These preliminary results suggest that the selected antigens are immunogenic in humans and may thus be good candidates for further development of a Chagas disease vaccine.

**1937**

**EARLY IL-10 PRODUCTION BY CD4+ T CELLS IN THE SKIN IS FUNCTIONALLY SUPPRESSIVE**

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Communities in endemic areas affected by Schistosomiasis, a parasitic disease caused by *Schistosoma mansoni*, are repeatedly infected when they come into contact with contaminated water. *S. mansoni* cercariae penetrate the skin and elicit a strong inflammatory response, which after repeated exposure becomes both exacerbated and skewed towards type 2 immunity, with high levels of IL-10, IL-4, eosinophilia and alternative activation of macrophages. In compass with this phenomenon at the site of infection, local skin draining draining lymph node (sdlN) cells progressively lose their ability to respond to *S. mansoni* cercarial antigens, becoming unable to proliferate or produce cytokines. Our evidence shows that the effect multiple infections have on sdlN responsiveness is mediated by IL-10, which is found at significantly higher levels in the skin after repeated exposure to the parasite. sdlN cells from IL-10 deficient mice retained their ability to respond to schistosomula antigens, while the immune response in the skin was significantly more pro-inflammatory. After the initial exposure to the parasite, CD4+ T cells and F4/80+MHC-IIhigh monocytes produced most of the IL-10 in the skin. Strikingly, CD4+ T cells in the skin made IL-10 as early as day 1 after the initial exposure. This initial response was directed against commensal antigens that would penetrate the skin during cercariae invasion. However, by day 4 after the first exposure, non-regulatory CD4+ T cells respond to *S. mansoni* antigens, expanding considerably after multiple infections and accounting for most of the detected IL-10. Furthermore, IL-10 producing CD4+ T cells from the skin have the ability to inhibit the proliferation of sdlN CD4+ cells. In summary, CD4+ T cells in the skin produce IL-10 and prevent cells in the lymph nodes from responding to repeated infections with the parasite, whilst they contribute to a type 2 immune response environment in the skin. Skin commensals are partly responsible for this type of response, as they penetrate the skin when *S. mansoni* cercariae invade the tissue.

**1938**

**A CENTRAL ROLE FOR TYPE I IFN IN THE INDUCTION OF TH2 RESPONSES BY DENDRITIC CELLS**

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Although dendritic cells (DCs) are critical for induction of Th2 immunity against helminths or allergens, relatively little is known about how they become activated and function in response to Th2-polarizing antigens. We have discovered a previously unrecognized role for Type I IFN (IFN-I) in the optimal activation and function of DCs following exposure to strongly Th2-polarizing antigens from the parasitic helminth *Schistosoma mansoni*. To date, IFN-I has primarily been associated with anti-viral immunity, and its role in Th2 settings is currently unclear. DCs lacking the IFN-I receptor displayed a dramatically impaired ability to induce Th2 cytokines in vivo, but unimpaired ability to support Th2 polarization in vitro. Further, Th2-polarizing DCs depended on IFN-I signaling for efficient migration to the draining LN. We are now investigating whether IFN-I is also required for effective localization within the draining LN and interaction with LN-
resident T cells. Together, our data suggest a key role for IFN-I to enable Th2 induction by DCs against helminths in vivo. Future work will address the wider role of IFN-I in Th2 inflammation, including during helminth infection, and the activation of allergic responses in the airways.

1939

VSG-SEQ: A QUANTITATIVE METHOD FOR TRACKING THE IN VIVO DYNAMICS OF ANTIGENIC VARIATION IN TRYPANOSOMA BRUCEI

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Using a repertoire of over 2000 different variant surface glycoprotein (VSG) genes within its genome, Trypanosoma brucei, the causative agent of African sleeping sickness, changes its dense VSG surface coat to avoid detection by the immune system of its mammalian host. The dynamics of antigenic variation in T. brucei during an infection, however, are poorly understood. How many variants appear over the course of an infection? Is there a pattern to VSG expression over time? Although some of these questions have been broached using Sanger sequencing of VSG cDNA, technical limitations have prevented a high-resolution, quantitative study of VSG expression during T. brucei infection. Here we present VSG-seq, the first method for quantitatively examining the diversity of expressed VSGs in a population of trypanosomes, isolated either from culture or from blood. This next-generation sequencing approach requires very little material and is quite sensitive, detecting VSGs expressed on less than 0.1% of a population of trypanosomes. Using samples isolated from mouse infections, expressed VSG sequences can be assembled accurately de novo, demonstrating that this approach can be used for the high-resolution study of VSG expression in any strain of T. brucei, whether in the lab or in the field. We have used VSG-seq to study the kinetics of VSG populations throughout T. brucei infections. These studies reveal more complex switching dynamics than previously expected and hint at the possibility of new mechanisms for increasing antigenic diversity in vivo.

1940

BLOODSTREAM FORM TRYPANOSOMA BRUCEI MEMBRANE NANOTUBES AND EXTRACELLULAR VESICLES MEDIATE INTERCELLULAR INTERACTIONS AND HOST ANEMIA

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Trypanosoma brucei cycles between an insect vector and a mammalian host, causing human African sleeping sickness and Nagana in cattle. We identified extracellular vesicles (EVs) from wild type bloodstream form (BF) cells by electron microscopy. Purified vesicles have been shown to propagate a distinctive morphological phenotype when added to wild type cells. Fluorescently labeled EVs bind to the trypanosome flagellar pocket and are subsequently endocytosed. We have developed an RNAi cell line that can be induced to produce an excess of EVs. When used in transwell separation experiments, these cells showed EVs mediate phenotype transfer. Proteomic analysis of EVs from both wild type and RNAi cells revealed ~50 shared proteins. In addition, we observe that purified EVs are capable of membrane fusion and transferring variant surface glycoprotein to human red blood cells. This fusion and protein transfer alters the physical properties of the red blood cell membrane, potentially leading to anemia seen during infection. Imaging of T. brucei cells reveal the formation of long membrane nanotubes at the posterior end of the cells that are able to bind other trypanosomes. These membrane nanotubes originate from budding of the flagellar membrane and form a helical wrapping structure that resembles “beads on a string.” These “beads” closely resemble the structure of cell-associated EVs. In addition, live cell imaging suggests that these nanotubes can disassociate into what appear to be free vesicles. We hypothesize that membrane nanotubes are the structures with which trypanosomes produce EVs. This demonstrates that T. brucei is capable of cellular communication and may have significant impact to understanding infection, immune evasion, and differentiation of this parasite.

1941

MONOCYTE-DERIVED ALTERNATIVELY ACTIVATED MACROPHAGES RETAIN PLASTICITY AFTER ACTIVATION AND DIFFERENTIATION

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Alternatively activated macrophages (AAM) induced by type 2 responses promote clearance of helminth parasites, wound healing, and tissue remodeling. AAM can accumulate through recruitment and differentiation of monocytes, or through proliferation of tissue resident macrophages. We have recently shown that AAM derived from monocytes (monoAAM) are phenotypically and functionally distinct from AAM that arise from tissue macrophages (tissAAM). monoAAM are F4/80low and express the mitochondrial protein Ucp1, but low levels of MR1 and PDL2, while tissAAM are F4/80high and expressed high levels of MR1 and PDL2. Here, we find that monoAAM recruited to sites of inflammation remain plastic after activation and differentiation. In short-term transfer experiments, monoAAM donor cells transferred into naive recipients retain expression of MR1, but lose expression of PDL2. In long-term transfer experiments, monoAAM can convert to F4/80high macrophages with a phenotype similar to tissAAM. Hence, monoAAM remain plastic after activation, which is dependent on Stat6 signaling in donor AAM, as well as accessory cells in the recipient mice. We have also found that AAM in the hepatic granulomas of mice infected with the parasitic helminth Schistosoma mansoni accumulate primarily through the recruitment of inflammatory monocytes. Future experiments will determine if these monoAAM will further adopt the phenotype of tissAAM in the livers of infected mice after long-term residence in the granulomas.

1942

METABOLIC REGULATION OF TYPE 2 IMMUNITY CONTROLS TISSUE REPAIR

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Host metabolism is profoundly affected by gastrointestinal (GI) nematodes. Many GI nematodes feed upon the tissues and blood of their hosts, resulting in anemia, malnutrition, and generalized immunosuppression. It is debatable whether these features of worm infection are due to parasite and/or host-derived factors. In this study, we investigated whether adenosine monophosphate kinase (AMPK) controlled the outcome of GI nematode infection through controlling the inflammatory response. AMPK is a heterotrimeric enzyme complex of αβγ subunits that restores cellular energy through oxidative phosphorylation. Given that AMPK activity is regulated by phosphorylation of the catalytic α subunit, we generated CD11cα-xAMPKα1flox/flox (DC-AMPK-/-) mice to study the importance of AMPK in alveolar macrophage and dendritic cell function. DC-AMPK-/- mice infected with the hookworm Nippostrongylus brasiliensis (N.b.) generated abnormal Type-2 immune responses and failed to regenerate areas of hookworm-damaged tissue 9 days post-primary infection. In comparison to littermate controls, DC-AMPK-/- mice were unable to generate intestinal goblet cell metaplasia and failed to expel adult worms from the intestine. Moreover, N.b.-induced lung injury was more severe in DC-AMPK-/- mice and the restoration of pulmonary function was significantly delayed compared to controls. Dysregulated responses generated in DC-AMPK-/- mice were associated with increased Type-1 responses (IL-12, IFN-γ), greater numbers of T,17 cells, and defects in the generation of alternatively activated macrophages. Taken together, our data are consistent with an
THE TOXOPLASMA DENSE GRANULE PROTEINS GRA17 AND GRA23 MEDIATE THE MOVEMENT OF SMALL MOLECULES BETWEEN THE HOST AND THE PARASITOPHOROUS VACUOLE


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**Toxoapexon gondii** is a widespread protozoan pathogen in the phylum Apicomplexa that resides intracellularly within a parasitophorous vacuole (PV) that is selectively permeable to small molecules through an unknown mechanism. We have identified GRA17 as a novel Toxoapexon-secreted protein, which localizes to the parasitophorous vacuole membrane (PVM) and is conserved across PV-residing apicomplexans. GRA17 mediates the passive transport of small molecules across the PV. The PVs of GRA17-deficient parasites have aberrant morphology, reduced permeability to small molecules, and structural instability. GRA17-deficient parasites proliferate slowly and are avirulent in mice. GRA17 functions synergistically with a related protein, GRA23. Exogenous expression of GRA17 or GRA23 alters the membrane conductance properties of Xenopus oocytes in a manner consistent with a large non-selective pore. GRA17 and GRA23 provide the first molecular basis to explain the PVM permeability to small molecules.

QUANTIFYING LABILE HEME IN LIVE MALARIA PARASITES USING NOVEL FRET BIOSENSORS

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The heme cofactor is centrally important in malaria parasite physiology. On one hand, it is a byproduct generated in large quantities during parasite-mediated hemoglobin degradation during the blood stage of malaria infection. Parasites must detoxify excess heme to limit oxidative damage, and do so by polymerizing it into non-reactive hemozoin. Several successful antimalarial drugs such as the aminoquinolines inhibit this process, presumably leading to the toxic accumulation of labile heme, although this has not been directly demonstrated or quantified. On the other hand, the parasite genome encodes a complete heme biosynthetic pathway that is essential for other stages of parasite development. This suggests heme is a necessary cofactor that is toxic in excess. Despite the central role of heme to parasite metabolism and its link to antimalarial drug potency, little is known about heme dynamics in normal parasite physiology or how these dynamics change under stresses imposed by heme-interacting drugs. To address these questions, we have developed and characterized a family of novel, genetically-encoded FRET biosensors for quantifying labile heme in live parasites. In vitro spectroscopic characterization of the purified protein sensors demonstrates their ability to reversibly bind heme, and to exhibit significant heme-dependent changes in FRET.

Our studies with blood-stage parasite lines expressing these biosensors indicate that micromolar concentrations of labile heme are maintained in the parasite cytosol throughout development. Furthermore, exposure to chloroquine, but not pyrimethamine, leads to accumulation of cytosolic labile heme, thus directly linking heme dysregulation to the in situ effects of aminoquinolines. We believe these studies will advance our understanding of how heme perturbation is linked to antimalarial drug potency, and help to mechanistically inform future drug development efforts.

CONDITIONAL EXPRESSION OF PFRIPR CONFIRMS ITS ESSENTIALITY FOR PLASMODIUM FALCIPARUM MEROZOITE INVASION INTO HUMAN ERYTHROCYTES

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Plasmodium falciparum merozoites actively invade human erythrocytes during blood stage malaria, the stage that causes clinical symptoms. Invasion of erythrocytes is a complex process that involves a cascade of protein-protein interactions between merozoite ligands and erythrocyte receptors. To date, many merozoite ligands have been described as essential based on the inability to knockout the proteins in vitro. One of these is P. falciparum Rh5 interacting protein (PFRipr). Here we present evidence for the efficient knockdown of PFRipr using a dimerizable Cre recombinase (DiCre) system. PFRipr is an essential invasion protein that forms a complex with P. falciparum reticulocyte binding-like homologues 5 (PFR5s). Previous studies have shown that the PFRipr/PFR5 complex plays a critical role in merozoite attachment and invasion as anti-PFRipr antibodies block merozoite invasion. We generated parasites expressing DiCre, which is activated by the addition of rapamycin leading to the deletion of PFRipr. Knockdown of gene and protein levels up to 90% within one cycle of the blood stage (about 48 hours) was achieved. This led to a growth reduction in the following cycles, which relates to a reduction in invasion efficiency as determined by flow cytometry, live-imaging and superresolution microscopy. In summary, conditional regulation of PFRipr confirms the essential role of this protein and further elucidates its functions.
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