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, axenial 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=0.0014 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.

1499

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 (MMMPs) 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, MMMPs 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 MMMPs 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 MMMPs change frequently there is a need for sustained public health efforts to reach this population, especially within an elimination context.

1500

IMPLEMENTING ENHANCED HIGH-RESOLUTION SURVEILLANCE USING SPATIAL DECISION SUPPORT SYSTEMS TO GUIDE TARGETED RAPID RESPONSE IN MULTI-DRUG RESISTANT AREAS OF VIETNAM

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Emerging artemisinin resistant malaria in the Greater Mekong Subregion (GMS) has important implications for public health. A project was established to research, develop and implement enhanced surveillance and targeted appropriate intervention measures to stop the spread of multi-drug resistant malaria through elimination of the disease in the region. The aims of this project are to pilot a spatial decision support system (SDSS) approach to conduct high-resolution surveillance to guide swift and targeted responses. Pilot sites were established in selected communes in Vietnam with associated customised SDSS developed. Publically available topographic geographic information system data were uploaded into the SDSS to provide baseline information. Household and forest transmission location data were located and enumerated through field-based geographical reconnaissance using handheld computers. Passively detected malaria cases were geo-referenced to the suspected transmission location sites upon diagnosis. Using case location data in the SDSS, active transmission foci were automatically classified and response areas of interest (AOI) generated. Supporting data (including population, location and number of sleeping locations within the AOI) were automatically produced in the SDSS and sent to village health workers and district level units to mobilize appropriate responses. Complete pilot data for presentation are expected in September 2014. This new approach utilizing novel geo-spatial tools to support targeted, appropriate and aggressive response measures to support malaria elimination in areas of global significance will be presented.

1501

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-patent 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-patent 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 parasitemia 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 harvest season in late 2013. The study assessed access to and use 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. The main barrier for not using a LLIN was that it was considered too stiff (29%). A fifth of respondents also said they were allergic to the insecticide. Half of the farm workers said they would be willing to pay a small amount for their own net, suggesting an opportunity for subsidized vouchers for LLINs.

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 regimens, 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.
MAPPING GLOBAL MALARIA CONNECTIVITY FOR STRATEGIC ELIMINATION PLANNING

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Calls for the eradication of malaria require the development of global and regional strategies based on a strong and consistent evidence base. Evidence from the previous global malaria eradication program and more recent transborder control campaigns have shown the importance of accounting for human movement in introducing infections to areas targeted for elimination. Here, (micro)census, survey and cellphone-based human movement data and models were analysed with network analysis tools to map globally the connectivity of both countries and subnational administrative units through population movements. 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 here of both communities of subnational regions and countries linked by high levels of population exchange, and ‘natural’ migration boundaries that display reduced movement of people and infections between regions has practical utility. These inform the design of malaria elimination strategies by identifying regions afforded protection from re-colonisation by natural ‘firebreak’ regions of reduced connectivity. For more isolated areas, a regionally-focussed control or elimination program is likely to stand a better chance of success than those receiving high levels of isolated areas, a regionally-focussed control or elimination program is likely to stand a better chance of success than those receiving high levels of visitors and migrants from high transmission regions. Moreover, we demonstrate how the mobility and malaria connectivity framework 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 GAMETOCYTOCIDAL ACTIVITY AGAINST P. FALCIPARUM IN SUB-SAHARAN AFRICA

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Primaquine is currently available as an antimalarial drug for the treatment of gametocytes. It is the only drug that can prevent the re-emergence of malaria in patients infected with P. vivax and P. ovale. The drug is currently used to prevent relapse of P. vivax parasites in patients who have received a standard treatment of artesunate and amodiaquine. However, primaquine is associated with adverse effects, including hemolytic anemia in G6PD deficient individuals. This study aims to determine the lowest efficacious dose of PQ without inducing hemolytic anemia in G6PD deficient individuals.

Primaquine was studied in a Phase 2a dose escalation study of safety and efficacy of low single-dose primaquine in non deficient G6PD male in Ouelesseboougou Mali. The study was conducted in a group of 50 participants aged 5 to 50 years with Plasmodium falciparum and P. vivax infections. Participants were randomly allocated to one of the following treatment groups: placebo, 0.5 mg/kg, 0.125 mg/kg, and 0.0625 mg/kg. The study was conducted from April to June 2011.

The results showed that the 0.5 mg/kg dose group had the highest efficacy, with a 100% reduction in infectivity on day 2 and 100% reduction in day 7. In the control group, compared to day 0 there was no reduction in infectivity on day 2 or day 7, 22.8% (95% CI 100%, 100%) and 40.0% (95% CI 86%, 100%), respectively. In the 0.125 mg/kg dose group, compared to day 0, there was 86.3% (95% CI 39.4%, 100%) reduction on day 2 and 100% reduction in day 7. In the 0.5 mg/kg dose group, compared to day 0 there was a 100% reduction in day 2 and 90.1% (95% CI 64.7%, 100%) reduction on day 7. In summary, our preliminary results indicate a higher reduction in infectivity in the 0.5 mg dose group (100% reduction at day 2) without safety concerns.

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 hyperendemic 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 Pf500/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 there was 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
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. Scenario 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 a 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. Furthermore, FRESAPP allows users to plot the trends in malaria burden using a variety of interactive displays (i.e., bubble, bar, and line charts). FRESAPP allows users to include and adjust for various covariates of interest (i.e., incident rate, population size, P. falciparum proportion, time, etc.) using different mathematical transformations. Users can contrast trends against epidemiological thresholds for each reporting level, which are automatically updated with each week of data entry. Furthermore, FRESAPP users are able to estimate costs of implementing interventions by using a cost-effectiveness algorithm that is adjustable by population size, distance, and coverage rates. In order to allow data managers to update the system without altering their current reporting protocol, FRESAPP was developed using a combination of four free tools: Motion Chart, Google Earth, Google maps, and R software. Given its open source free format, FRESAPP may contribute to enhancing the local readiness and response capacity at each of the levels of the Health System hierarchy in most malaria endemic countries. FRESAPP may also potentially be used for other reportable diseases, therefore facilitating improved public health decision-making.

A FREE SURVEILLANCE APP FOR PLANNING MALARIA ELIMINATION INTERVENTIONS AND OUTBREAK RESPONSES AT THE COMMUNITY LEVEL IN MALARIA ENDEMIC COUNTRIES

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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” (FRESAPP), 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 making tools 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, FRESAPP allows users to plot the trends in malaria burden using a variety of interactive displays (i.e., bubble, bar, and line charts). FRESAPP allows users to include and adjust for various covariates of interest (i.e., incident rate, population size, P. falciparum proportion, time, etc.) using different mathematical transformations. Users can contrast trends against epidemiological thresholds for each reporting level, which are automatically updated with each week of data entry. Furthermore, FRESAPP users are able to estimate costs of implementing interventions by using a cost-effectiveness algorithm that is adjustable by population size, distance, and coverage rates. In order to allow data managers to update the system without altering their current reporting protocol, FRESAPP was developed using a combination of four free tools: Motion Chart, Google Earth, Google maps, and R software. Given its open source free format, FRESAPP may contribute to enhancing the local readiness and response capacity at each of the levels of the Health System hierarchy in most malaria endemic countries. FRESAPP may also potentially be used for other reportable diseases, therefore facilitating improved public health decision-making.

EVALUATING REACTIVE CASE DETECTION ACTIVITIES IN RANONG PROVINCE, THAILAND

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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 Thailand 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 (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

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A vaccine for geographically focused malaria elimination campaigns must provide sustained sterile protection against infection. The Sanaria® PFSPZ Vaccine was developed to address this need. It is composed of aseptic, purified, cryopreserved, viable 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 INFECTIOUS DISEASES IN RURAL CLINIC FOR REFUGEES AND DISPLACED POPULATION ON RWANDA-DR CONGO BORDER: ANALYSIS OF 10,051 PATIENTS

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Migrants and internally displaced in sub-Saharan Africa are subject of increasing threat of infectious diseases (IDs) due to contaminated water supplies (cholera, typhoid fever), food (salmonellosis, shigellosis), malnutrition (tuberculosis-TB, HIV) and absence of housing (pneumonia, upper respiratory tract infections - RTI). Cross sectional study in area close to DRC border (Sud-Kivu) and Rwanda (Bisesero) in two clinics serving for 50 000 population (of them 25 000 internally displaced and refugees in UNHCR camps) was performed to asses occurrence of major ID in 2013. Bigugu clinic is located in altitude of 2350 m and Bisesero United Nations High Commissioner for Refugees (UNHCR) camp is in 1150 m above sea level. Of 10 051 patients, only 31 (0,3%) had malaria, and 26 of them (0,26%) had true highland malaria (without down country travelling history), confirmed both macroscopically and with rapid diagnostic test (RDT). Commonest IDs were upper RTI representing (72-89%) of all visits, followed by diarrheal and gastro-enteric diseases (13-19%). Also, 26-77% of all children were infected by geohelminths. Only one case of neoninfestation was recorded. Urinary tract infections and sexually transmitted diseases were rare as well (1-4%). Among 10 051 outpatient visits in two rural clinics, serving for UNHCR registered refugees from DRC in Rwanda and internally displaced population near Sud-Kivu Province. Malaria was extremely rare due to high altitude, and diarrheal and gastroenteric infections were relatively rare, too. Of all ID, upper RTI were the commonest, while neuroinfections (such as bacterial or viral meningitis and sleeping sickness) were only exceptional. Very high proportion of RTI was associated with malnutrition and very low socio-economic status in areas of high altitudes above sea level with low temperature.
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). Monthly incidences 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.a.s. (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 altitude (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. Franciscus 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 moresuccessfully 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 experimented significant changes when parasite population before treatment was compared with parasite population in the second year follow up (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.
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SEASONALITY IN MALARIA TRANSMISSION - IMPLICATIONS FOR CASE-MANAGEMENT WITH LONG-ACTING ARTEMISININ COMBINATION THERAPIES

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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 two 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.

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DETECTING FOCI OF MALARIA TRANSMISSION: IMPLICATIONS OF SAMPLE SIZE AND CHOICE OF MALARIA METRIC

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Identifying malaria foci in endemic settings can be challenging. Many pragmatic decisions must be made, including the sample size to employ and which malaria metric to use. However, it is not known how these decisions affect the ability to detect foci of infection, particularly the boundaries of foci identified. If control or elimination programs are targeted to foci of infection that do not accurately reflect the true nature of transmission in the community, ensuing interventions may incorrectly be perceived as being ineffective. To determine the impact of sample size and choice of malaria metric, data for 17,500 individuals residing in 3,200 compounds, approximately one third of the population, collected during a cross-sectional survey in the western Kenyan highlands were used to identify foci of transmission in the community. All structures in the area were digitized to provide a total census of the area and several structures can comprise a compound. Model-based geostatistical methods were used to analyze the spatial variation of parasite prevalence, as determined by polymerase chain reaction (PCR), a measure for current infection, and by seropositivity, a measure of malaria exposure. Informative thresholds of risk were defined in order to identify foci in the spatial distribution of the two outcomes. The impact of the sample size on both the accuracy of prevalence estimates and the ability of the model to identify foci was assessed through a simulation study. Preliminary findings suggest that foci defined by the two outcome measures were measures were only moderately correlated (r=0.43) with only 36% of structures identified by both outcome measures. Among 14 discrete foci identified as having increased risk by one or both outcomes 6 clusters were identified by both metrics, although only 3 had good overlap. Five clusters (592 structures) were identified based on PCR but missed using seroprevalence, and 3 clusters (1214 structures) were missed using PCR but identified using seroprevalence. In terms of sample size, initial findings indicate that halving the sample size would have a minimal impact on model efficiency for generating the predicted surface for both outcome measures.

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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 detected 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<0.001). 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<0.001 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. While

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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. 21% (102/491) were terminated after confirmed malaria infection by clinical laboratory test (study end point), and one participant died from a non-malarial infection. The malaria attack rate was found to be as low as 18% to above 24%. Plasmodium falciparum was the predominant species detected. This study revealed one of very few non-immune populations with sufficient malaria exposure to conduct malaria prevention studies of a reasonable size, made possible by the heterogenous disease distribution in the country. TPDF recruits from non-endemic areas may be an ideal non-immune population for future malaria prophylaxis, vaccine and treatment trials.

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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 confirmed 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 5-9, 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 in 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.

1527

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, Kolle, Pongonon and Sotuba). Thick smears were made and read onsite by experienced microscopists. Genomic DNA was extracted using Qiagen 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.

1528

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 based 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 3,282 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.

**1530**

**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.

**1531**

**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 were 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 parasites 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 90% 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.

**1532**

**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
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 parasitolological 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.
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 MIS 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 decreased 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 medians=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 fever 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 distributed in 17 pilot medium endemicity districts. Diagnosis and treatment patient-level data are collected at monthly intervals in all study facilities. Multivariate logistic regressions will be performed to assess the impact of clinical mentorship on confirmatory diagnosis and on adherence to test results adjusted for covariates such as age, health facility level 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.

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COLLABORATIVE DATA MANAGEMENT FRAMEWORK FOR BORDER MALARIA RESEARCH IN SOUTHEAST ASIA ICEMR CENTER

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Document and data archiving for the research activities are the tedious but important tasks through the whole scientific research project. It is a challenge for the scientist to maintain a solid, flawless and well organized data structure and data entry procedures especially for the project included international collaboration within multi-cultures and languages. Southeast Asia ICEMR center is focused on bolder malaria transmission between several Southeast Asia countries. We collaborated with several Universities, government agencies, local hospital and clinics, and field research labs in the remote border area of China, Myanmar, and Thailand. How to standardize the field survey and data entry procedures and semi-real-time to share research results is the goal for data manager and coordinators to conquer. Therefore, we design multi-language survey forms and data entry system as the tools to provide consistent interface and user experience for all research staffs. In order to expedite the data sharing and secured data services, we utilized several open source applications and cloud computing services to sustain our database system. To minimize the catastrophe of computer hardware/software collapse or network traffic congestion, the failover and load balancing features were setup in main server and several mirror servers and offsite remote backup scheme were implemented. To maintain the maximum data quality and structure, the standard operating procedures (SOPs) for field survey, data entry, data QA/QC were developed and documented. Currently the database system is online since July 2012.

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MALARIA PREVALENCE AND SPOROZOITIC INDEX IN SUBURBAN AREA IN KINSHASA BEFORE ITN MASS DISTRIBUTION, DR CONGO

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Malaria is a parasitic disease due to Plasmodium transmitted by a female mosquito of Anopheles genus. It is a public health problem which causes a high mortality in less 5 years children. Estimated deaths by malaria word report are around 60000. This study interest is to update data on malaria prevalence in Kinshasa suburb area, in order to provide data for the stratification and control of the disease. The objectives of the study were to assess malaria prevalence into sub urban Kinshasa area population, to determine the most concerned population group, to identify plasmodial species and to determine sporozoite index. An analytical cross-sectional survey was conducted in the village of LUZIZILA to 329 people whose age ranged between 6 months and 76 years for the period from August 10 to 25, 2013 Blood sample for a thick, a thin smear was conducted to determine the prevalence of malaria and determining plasmodial species. The sporozoite index was determined by ELISA Results The overall prevalence of positive thick smears was 53.2%. In the age group under 5 years, prevalence was higher with 68.8%. Between 6 and 15 years it was 55% beyond 15 years the prevalence stood at 37.3%. P. falciparum was found in 97.7% of cases in thin smear slides The sporozoite index was 11%. In conclusion, the prevalence found place Luzizila in an hyperendemic area. After the distribution we can expect a reduction of these indices
MALARIA INFECTION IS ASSOCIATED WITH PREGNANCY LOSS IN OUELESSEBOUGOU, MALI

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In malaria endemic areas, pregnant women are more susceptible to malaria infection compared to their non-pregnant counterparts. While the relationships between pregnancy malaria (PM) and outcomes such as severe maternal anemia and low birth weight are well established, there have been limited studies on the relationship between PM and pregnancy loss particularly in areas of high malaria transmission. We evaluated the relationship of fetal loss to malaria infection among pregnant women in Ouelessebougou Mali from November 2010 to January 2014. Pregnant women were enrolled during the antenatal consultation visits and 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 represents 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 Plasmodium, 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 red blood cells between 2 and 6º 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 Plasmodium 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 Plasmodium. ELISA detected 53.6% (40.7 - 65.9) of samples reagent for Plasmodium, with Reactivity Index ≥1.0. SD Bioline detected antibodies against Plasmodium 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 NORTHWESTERN 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 Obrego and 71 from Quebecotillo. 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 microbiopst as quality control. The DNA extraction from the filter paper was done by the Chelex-100 method and the PCR was basied 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 Obrego 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 presences 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 Chessor 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 and vector control.

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NONINVASIVE SURVEILLANCE OF ZOONOTIC MALARIA
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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.

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A COMPARATIVE CASE CONTROL STUDY OF THE DETERMINANTS OF CLINICAL MALARIA IN THE GAMBIA
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The massive deployment of life saving malaria interventions has not only resulted in a decline in disease burden but a change in the risk of infection and disease. The study reassesses the importance of known risk factors and reviews socio-demographic determinants of malaria risk in the population. We conducted a case-control study involving 150 children aged 6 months to 12 years with slide-confirmed malaria recruited from the outpatient clinics of three health facilities (cases) in the Greater Banjul area, The Gambia. One hundred and fifty controls were matched on age, residence, and were negative for malaria. We collected information on the use of long lasting insecticidal nets, occupation of parents, housing structure, knowledge of malaria and socio-demographic factors. The mean age of study participants was 6.8 (SD 3.3) years with 147 (49%) being males. Significant determinants of malaria risk were parent’s occupation: mother as trader (OR 0.18, 95% CI 0.04 - 0.73, p = 0.017), father as trader (OR 0.02, 95% CI 0.002 - 0.193, p = 0.001), civil servants (OR 0.04, 95% CI 0.008 - 0.257, p = 0.001) or handymen (OR 0.03, 95% CI 0.005 - 0.182, p < 0.001). Children sleeping in rooms with windowpanes had a 76% reduction in their odds of malaria (OR 0.24, 95%CI 0.07 - 0.82, p = 0.022. Household socio-economic status plays an important role in management of illnesses. The ability of mothers to engage in an occupation increases household resources to access healthcare and on time. The balance between the type of mother’s occupation and her time available to supervise the child is an interesting emerging issue that needs further investigation.

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ANTIBODIES TO PLASMODIUM VIVAX MSP1-19 RECOMBINANT ANTIGEN IN BLOOD DONORS FROM BRAZILIAN LOW ENDEMIC AREAS
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In non-endemic and low endemic areas, transfusion-transmitted malaria (TTM) is a rarely reported event. However, four TTM were detected in São Paulo State, in Southeastern Brazil, including one death. Infected donors were identified as asymptomatic carriers with displacements to the Atlantic forest biome in São Paulo State. Due to the immune status of these donors, the parasite densities are low and undetectable in the thick blood smear or rapid diagnostic tests requiring the use of other methods for detection in blood banks outside the endemic areas. In this study, since Plasmodium vivax is the most prevalent species in Brazil, we assessed the prevalence of anti- P. vivax MSP1-19 IgG antibodies among blood donors from Southeastern Brazil. Initially, for validation, ELISA-PvMSP1-19 was assayed with 197 sera from patients with positive thick-blood smear for P. vivax yielding 96.95% sensitivity. A specificity of 100.0% was achieved in serum specimens from 101 normal individuals and 98.21% in 168 serum specimens from other diseases patients. After validation, 1,974 blood bank serum samples were tested: 1,309 from São Paulo and 665 from Rio de Janeiro. These samples were collected after the donors had been screened by clinical parameters, provided they were considered fit to donate and had signed the informed consent form. Regarding samples from São Paulo, 1.15% (N=15) positivity was achieved. In Rio de Janeiro samples, the positivity was 1.65% (N=11). The reactivity index (RI) of the positive samples ranged from 8.98 to 1.16 (São Paulo) and 13.03 to 1.08 (Rio de Janeiro). The detection of specific antibodies is not necessarily a marker of parasitemia or disease, but the detection of anti- P. vivax IgG antibodies in blood bank donors in non-endemic areas constitutes an alert that impel us to review the adopted criteria for screening of the donors aiming to reduce the risk of TTM in these areas without losing donations.

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HOUSEHOLD-LEVEL SOCIAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH BED NET OWNERSHIP AND DIFFERING MALARIA PREVALENCE IN SOUTHERN MALAWI
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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 status, house location, building materials, and nearby environmental characteristics were collected by cross-sectional survey from 398 households in two rural Traditional Authorities (TAs) of southern Malawi, Sitola and Nsamala, which were in the catchment area of Machinga District Hospital (MDH). The proportion of households in Sitola reporting bed net ownership was significantly lower (OR 0.59, 95% CI 0.39-0.89), and the prevalence of malaria among under-5s significantly higher (OR 4.20, 95% CI 2.74-6.46) than in Nsamala, this despite higher bed net use among owners in Sitola (OR 2.56, 95% CI 1.00-6.57). Households in Sitola were also much more likely to be located within 50 m of active agriculture (OR 9.60, 95% CI 4.93-18.68), of brick-making sites (OR 4.59, 95% CI 2.34-9.01), and of water sources (OR 11.87, 95% CI 3.49-40.40). Households in the two TAs did not differ with respect to housing materials,
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 1.42-7.65). 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 sporozoite 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 global 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±194 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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DETERMINANTS OF RISK OF MALARIA PARASITEMIA IN BUNKPURUGU-YUNYOO DISTRICT, NORTHERN GHANA, INCORPORATING REMOTE SENSING AND SURVEY DATA

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Ghana’s malaria control strategy prioritizes the northern savannah regions due to persistent hyperendemicity. In Bunkpurugu-Yunyoo district, previously reported anemia and parasitemia surveys were conducted serially in 3 rainy seasons (RS) and 3 dry seasons (DS) in 2010-13, covering 11,945 children under five from 179 communities. In spite of high coverage for insecticide-treated bed nets (>75% use in each RS) and indoor residual spraying (IRS) with pyrethroid pesticides in years 2 and 3 (>98% households sprayed), investigators found unexpectedly high and geographically heterogeneous malaria prevalence. To better define and explain this heterogeneity, this study enhanced the survey dataset with remotely sensed data, then analyzed by ecologic zones, delineated as urban (Zone 1, n=1131); rocky uplands (Zone 2, n=5234, >750 ft altitude); transition (Zone 3, n=3456), and riverine plains (Zone 4, n=2124, <550 ft). The RS odds ratios for microscopic malaria parasitemia in children living in Zones 2, 3, and 4, as compared with Zone 1, were respectively 3.9 (95% CI: 2.8-5.4), 7.6 (95% CI:5.7-10.3), and 11.1 (95% CI: 8.0-15.6; all p values here and in the following <0.0001). Zone 4 parasitemia prevalence across the 3 years was 65.6-72.1% in the RS and 39.8-54.9% in DS. Among 17 variables with statistically significant odds ratios (OR) for malaria risk, 12 exhibited a zonal gradient favoring reduced risk in Zone 1 vs. Zone 4, with Zone 2 intermediate. In the RS these included lower wealth quintile (OR=3.6; 95% CI:2.7-4.7), caregiver’s lack of education (OR = 2.7; CI: 2.1-3.3), ethnicity (OR = 3.9; CI: 3.2-4.8), lack of health insurance coverage (OR 3.0; CI=2.4-3.6), higher vegetation index (OR=1.6; CI: 1.1-2.3), lower human influence index (OR 5.2; CI:3.8-7.1); and >3 km distance to nearest health facility (OR=2.4; CI:1.9-3.1), among others. DS findings were similar. No consistent zonal gradient was found for the malaria control measures (ITN use, ACT use, IRS). Findings suggest that, in spite of high coverage with ITNs and pyrethroid-based IRS, high malaria prevalence in northern Ghana may be found in locations where reduced socioeconomic status and isolation coincide with low-lying terrain. Such areas may require additional and/or modified methods for vector and parasite control.
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-polyorphic markers for differentiation of gametocytes. We have evaluated capillary electrophoresis-based differentiation of 6 length-polyorphic 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 pfg377 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 emphasises 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 gametocyto genesis of a specific parasite clone over the duration of its infection.

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 caseloads. 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.

THE ROLE OF THE INHIBITORY FC RECEPTOR, FCIIb IN HOST IMMUNE RESPONSE AND SUSCEPTIBILITY TO MALARIA IN PAPUA NEW GUINEA

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Binding of human Fragment crystallizable gamma receptor 2b (FcγRIIb) on B cells to antigen (Ag)-containing immune complexes (ICs) mediates a critical inhibition of host immune responses. Homozygosity for a FcγRIIb missense mutation (p.Ile232Thr) that reduces inhibition is associated with protection against severe malaria in Kenyan children. Protection against severe malaria in p.232Thr/Thr (TT) Kenyan children may result from a more robust humoral immune response and/or enhanced phagocytosis of 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%). 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 higher repertoire of 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.
UNREVEALING 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 contemporaneous 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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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. In this context, parasite antigen-specific cellular responses, in particular to the vaccine candidate antigen VAR2CSA, have received little attention. Here we evaluated both, cytokine (IL10, IL13, IL17, IFN-γ, TNF-α) responses and the T cells IFN-γ-specific responses to the DBL5 domain of VAR2CSA. 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. Peripheral blood mononuclear cellular (PBMC) responses to VAR2CSA-DBL5 in vitro were determined in subgroups of 150 women at inclusion and 100 at delivery. In each subgroup those harbouring P. falciparum infections were matched by gravidity and gestational age with mothers with no infection at inclusion and those with no history of infection earlier in the pregnancy. The amounts of IL10, IL13, IL17, IFN-γ and TNF-α produced in response to mitogen (PHA) and to VAR2CSA-DBL5 were quantified in supernatants of stimulated PBMC. The ex vivo frequencies of IFN-γ secreting CD4 and CD8 T cells in response to PHA and VAR2CSA domains were evaluated in the same maternal PBMC groups. 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 firstly in the context of cytokine profiles that reflect the acquisition of a specific cellular memory response to the vaccine candidate according to gravidity or to previous P. falciparum infection. Secondly, we will discuss cytokine profiles as potential markers of protection in the context of infection, anaemia and birth weight.
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 less severe clinical disease with repeated parasite exposures and these data may inform vaccine strategies.

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.

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 time 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 Models--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.

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 (Pf) sporozoites (SPZ) has been shown to induce sterilizing protection against malaria infection in naive 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-blind randomized phase 1b trial of the Sanaria®
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 $2.7 \times 10^5$ PfSPZ or normal saline placebo. In addition twelve volunteers received two vaccinations to ascertain safety of the vaccine, and none 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.

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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 exo 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 against clinical malaria is also associated with lower serological reactivity as response. Thus the association between previous exposure and 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.

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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 (Vk2.10, Vk247 and Pvi-vax-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 (Fishier 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.

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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 liver stages 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 parasitides 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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**TRANSCRIPTOMЕ 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 immunosorbent 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 Pl. 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 reactogenicity 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. yoelli 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 immunity engenders protective cross-stage immunity against a direct lethal blood stage infection. 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 immunity engenders protective cross-stage immunity against a direct lethal blood stage infection.

INTERLEUKIN 8 AND TRANSFORMING GROWTH FACTOR-BETA (TGF-β) 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 (TGF-β) plays an important role in malaria pathogenesis and outcome. TGFβ, 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 TGF-β in 136 individuals with malaria and correlate the production of these cytokines with the severity of the disease. IL-8 and TGF-β 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±755.1 pg/ml) in malaria infected individuals and its value was significant in parasitemia levels (43200.0, p<0.05). In contrast TGF-β levels were found to be lower in malaria patients (23,672±30,703.8 pg/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 TGF-β levels and packed cell volume was negatively correlated (r = -0.27). These findings suggest that fine mechanisms regulate the interaction between TGF-β 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 P. falciparum infection; however, at the individual level a minority of children exhibited accelerated IgG decay rates following 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 PLASMODIUM FALCIPARUM-INDUCED IMMUNE RESPONSES

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Plasmodium falciparum and Schistosoma haematobium are often overlap in tropical and subtropical countries and impose tremendous disease burdens. Evidence suggests that S. haematobium modifies the risk of febrile malaria; however, the nature of this putative immunomodulation remains unclear. To investigate this question we analyzed 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 S. haematobium-infected and uninfected individuals, all of whom were negative for P. falciparum by PCR. PBMCs were analyzed before and after in vitro stimulation with lysate of 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, 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-γ producing CD4+ T cells in S. haematobium-infected individuals. These preliminary data suggest that S. haematobium modulates the innate and adaptive immune response to P. falciparum infection. In an ongoing longitudinal study we are studying the impact of concurrent or recent S. haematobium infection on the host immune response to subsequent P. falciparum infection.

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AVIDITY OF NATURALLY ACQUIRED ANTI-PLASMODIUM VIVAX MSP1-19 ANTIBODIES IN INDIVIDUALS PRESENTING DIFFERENT CLINICAL EXPRESSIONS OF MALARIA

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Plasmodium vivax is the most prevalent species in Brazil accounting for around 85% of clinical cases. 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 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 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 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.IIL2RgcKO background that develop a functional human immune system and respond to vaccination. Using human B cells from humanized HLA mice immunized with irradiation-attenuated Plasmodium falciparum sporozoites we have generated a panel of hmAbs against P. falciparum CSP. Herein we present data on anti-CSP hmAb immunochacterization and in vitro and in vivo anti-parasitic activity.

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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

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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 UNCOMPROMICED MALARIA AT PRIVATE CLINICS IN MAKitDYE DIVISION, KAMPALA DISTRICT
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Over 60% of the population in developing countries seeks 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 settings 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 Inteventes. A total of 180 exit interviews were conducted with careakers 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 ARTEMESININ COMBINATION THERAPIES
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Artemesinin 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 endline. 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 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 (mm) in Nigeria and 28.3mm in Kenya, but only 2.1mm 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 $110.2mm in Nigeria, $39.9mm in Kenya and $4.9mm in Madagascar. Annual economic costs for 2011 were $57.2mm in Nigeria, $17.7mm in Kenya and $2.2mm 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 antimalarials 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 stocking 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.

1584
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 analysed 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 NEOtRALIZING MONOCONAL 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 PdBPII 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 PdBPII 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 PdBPII for a subunit vaccine designed to protect against blood-stage *Plasmodium vivax* malaria.

EVALUATION OF FUNCTIONAL IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING ANTIGEN PFS25 PRODUCED IN *E. COLI* ADJUVANTED IN VARIOUS NANO PARTICLES AND ADJUVANTS

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Pfs25, expressed on the surface of gametes, zygotes and ookinetes, is an established target antigen for malaria transmission blocking vaccines. Previously, we have reported that codon harmonized recombinant Pfs25 (CH-rPfs25) produced in *E. coli* elicited highly potent transmission blocking antibodies using Montanide ISA51, Alum and CFA as adjuvants. In the current study, we have undertaken extensive evaluation of various nanoparticles/adjuvants via different routes of immunization to identify safer and effective adjuvants. Mice were immunized with CH-rPfs25 via IM route (10 µg, three doses at 4 week intervals). Mice immunized with CH rPfs25 in Alum via IP and IM routes induced comparable antibody titers (640,000). Since the protein was equally immunogenic by IP and IM routes, other adjuvant formulations were tested by IM route only. CH-rPfs25 was adsorbed to nano-emulsions (4% & 8%) and PLGA particles (2 different amounts). CH-rPfs25 formulated in 4% NE gave highest antibody response (ELISA titer 1,280,000) as compared to 320,000 in 8% NE. Antibody titers with PLGA (10 and 20 mg PLGA) were only 160,000. We also evaluated NE formulations combined with M PLA and chitosan, and both demonstrated 640,000 antibody titers. Functional activity of antibodies was evaluated by standard membrane feeding assay using purified IgG (50-400 µg/ml) from immunized animals. 100 % transmission blocking activity (no oocysts detected) was observed at 400 µg/ml of IgG from Alum group (both routes IP and IM), NE (4%), and NE-MPLA. Purified IgG from various adjuvant groups at lower doses (100 µg/ml) still exhibited >90% transmission blocking activity, while 52-81% blocking was seen at 50 µg/ml. Our results suggest that CH-rPfs25 is strongly immunogenic by different routes and as formulations with Alum and NE. We are continuing these studies to develop effective vaccine formulations for further evaluation and investigations into immune correlates of relative immunogenicity of CH-rPfs25 in various adjuvants.

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 (p = 0.014). R21 was also assessed in combination with the Cha663 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.

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**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.

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**EFFICACY, SAFETY AND IMMUNOGENICITY OF HETEROLOGOUS PRIME-BOOST IMMUNIZATION WITH THE CANDIDATE MALARIA VACCINES CHAD63 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 IIIb 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.

1591

**ANTIBODIES AGAINST A PLASMODIUM FALCIARUM ROPHTRY NECK PROTEIN PFRON12 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* (PFRON12) was recently reported as a protein specifically
exhibited in schizont and localized to the rhoptry neck of merozoite by
immunoelectron microscopy (IEM). The characteristics of the PRION2
suggest that it is a potential blood-stage vaccine candidate, and here we
assessed its potential in this regard. We expressed a recombinant PRION2
protein by the wheat germ cell-free system to obtain anti-PRION2
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-PRION2 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 PRION2 forms a disulfide-bond-mediated
homodimer. Immunofluorescence assay and IEM revealed that PRION2
localized to the rhoptry neck of merozoite in schizonts and to the surface
of free merozoites. The biological activity of anti-PRION2 antibody
was tested by an in vitro growth inhibition assay, and the antibody
significantly inhibits the merozoite invasion of erythrocytes. Since anti-
PRION2 antibody inhibited the merozoite invasion in vitro, we decided
to investigate whether PRION2 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 PRION2,
indicating PRION2 is immunogenic in humans. Our findings suggest
that PRION2 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 (PFsp) 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
were randomized to receive 5 doses of 2.7x105 PFSPZ or normal saline
placebo. At this time, 207 DVIs with PFSPZ Vaccine or placebo have been
administered. The time from vaccine request by the clinical team to thaw
and formulation in a syringe to completion of injection was on average 6
minutes and injections on average took < 10 sec. Of the 207 vaccinations,
only one has required a second injection attempt. This was for inability to
locate a vein for the 1st dose in the pilot group. Vaccinations have been
well tolerated with no local reactogenicity. Seven episodes of solicited
systemic reactogenicity, all grade 1, have been reported. Complete data
on 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.

1593
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. Primaqune (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
does 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 (PFsp) 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 malaria ΔslarpΔb9 SP2s 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 slarpΔb9 GAP was suitable for producing aseptic, purified, cryopreserved PfSPZ. The parasites demonstrated all growth characteristics necessary for cGMP production. The Pf slarpΔb9 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 PfΔp52Δp36 SPZ as positive controls; the wild type Pf, PfΔp52Δp36, and Pf slarpΔb9 SPZ produced 0.17+1.0, 1.5-1.25 and 0, 6-day liver stage schizonts respectively. Pf slarpΔb9 SPZ were potent and viable. We will next use this genetically attenuated double-mutant parasite (Pf slarpΔb9) to manufacture, characterize and release a corresponding PfSPZ Vaccine, PfSPZ (ΔslarpΔb9) 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.

1595

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-Traversal 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 (PCelTOS) 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 PCelTOS 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 PCelTOS. To this end, various protein fragments were generated in E. coli or as synthesized peptides, and their reactivity with sera from PCelTOS/GLA-SE immune mice, rats, non-human primates and human subjects was determined. We are currently implementing in vitro 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.

1596

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 Plasmodium 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 PacBio 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 DBlpam4 domain, and a second primer set targets a 5 kb region spanning the DBLpam3 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 stimulation, 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/Alhydreg® 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 HIV/1/2 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 (> 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.
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 is, suggesting that additional suitable challenge strains can be easily identified.

1601

**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, kuffer 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 OUTBRED MICE FROM PLASMODIUM YOELII CHALLENGE FOLLOWING RECOMBINANT DNA-PRIME/AD5-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 1:7812) while those to Falstatin were more uniformly high (range 1:34576 to 1:1443217). High frequencies of CD8 T cells producing IFN-g 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-g. Interestingly, the frequencies of Falstatin-specific CD8 T cells producing IFN-g 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 component contribution to protection and its duration, as well as to identify an optimal platform eliciting high level durable T cell immunity.

D/HUAD5-PFCSLAM, A PLASMODIUM FALCI PARUM 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-vectorized vaccine platform (Ad) prime/boost vaccine expressing PFCSP and PfAMA1 antigens (PfCA) is well tolerated, immunogenic, and efficacious in a Phase 1 clinical study (27%, 4/15 volunteers steriley 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. PFCSLAM is a cocktail of PFCSP, PfSSSP2/TRAP, PLSA1, PfAMA1, and PfMSP1 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 PICA DNA/Ad vaccine is protective in humans. Here, BALB/c mice were immunized i.m. with either the individual components, the 5 antigen PC/SLAM mixture, or a 4-antigen PC/SLAM mixture, administered as a single Ad dose (1x10^6 pu) on study day (SD) 28, 2 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 ELISIA. These data support that the addition of antigens to the PICA 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.
results suggest that a priori knowledge of a functional epitope map could inform the selection of malaria subunit vaccine antigens that would generate protective antibody populations.

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A FULL-LENGTH PLASMODIUM FALCIPARUM RECOMBINANT CIRCUMSPOROZOITE PROTEIN EXPRESSED BY PSEUDOMONAS FLUORESCENS PLATFORM AS A MALARIA VACCINE CANDIDATE

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The circumsporozoite protein (CSP) of Plasmodium falciparum is a major surface protein, which forms a dense coat on the sporozoite’s surface. Preclinical research on CSP and clinical evaluation of a CSP fragment-based RTS,S/AS01 vaccine have demonstrated a modest degree of protection against P. falciparum, mediated in part by humoral immunity and in part by cell-mediated immunity. Given the partial protective efficacy of the RTS,S/AS01 vaccine in a recent Phase 3 trial, further improvement of CSP-based vaccines is crucial. Here we describe the preclinical evaluation of a full-length, recombinant CSP (rCSP)-based vaccine candidate against P. falciparum malaria suitable for current Good Manufacturing Practice (cGMP) production. Utilizing a novel high-throughput Pseudomonas fluorescens expression platform, we demonstrated greater efficacy of full-length rCSP as compared to N-terminally truncated versions, rapidly down-selected a promising lead vaccine candidate, and developed a high-yield purification process to express immunologically active, intact antigen for clinical trial material production. The rCSP, when formulated with various adjuvants, induced antigen-specific antibody responses as measured by ELISA and immunofluorescence assay (IFA), as well as CD4+ T-cell responses as determined by ELISPot. The adjuvanted rCSP vaccine conferred protection in mice when challenged with transgenic P. berghei sporozoites containing the P. falciparum repeat region of CSP. Furthermore, heterologous prime/boost regimens with adjuvanted rCSP and an adenovirus type 35-vectoried CSP (Ad35CS) showed modest improvements in eliciting CSP-specific T-cell responses and anti-malarial protection, depending on the order of vaccine delivery. Collectively, these data support the importance of a further clinical development of adjuvanted rCSP, either as a stand-alone product or as one of the components in a heterologous prime/boost strategy, ultimately acting as an effective vaccine candidate for the mitigation of P. falciparum-induced malaria.

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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 Circumsorozoite Protein (CSP) can protect against infection by Plasmodium sporozoites. Due to its repetitive nature and tandem disposition, the epitopes at CSP’s 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 (SD5) 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 SD5 MAb epitope specificity and show that it can bind both live and air-dried fixed sporozoites. Most importantly, we demonstrate that SD5 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 CSP’s 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.

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SAFETY AND PROTECTIVE EFFICACY OF INTRAVENOUS IMMUNIZATION WITH CRYOPRESERVED PLASMODIUM FALCIPARUM SPOROZOITES UNDER CHEMOPROPHYLAXIS - TUECHMI-002

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Exposure to 12-15 Plasmodium falciparum (Pf)-infected mosquitoes, administered three times at monthly intervals under continuous chemoprophylaxis with chloroquine is highly efficacious in preventing asexual blood stage infection following subsequent controlled human malaria infection (CHMI) in healthy, adult, malaria-naive individuals. To translate and expand this immunization strategy, mosquito bites need to be replaced by a pharmaceutical product that can be easily administered and exactly dosed. In addition, the chemoprophylactic regimen needs to be adjusted to the specific requirements of this approach. Our previous study (TUECHMI-001) showed that one mosquito bite corresponds to ~620 intravenously (IV) injected, cryopreserved Pf sporozoites (PISPZ) produced by Sanaria Inc. Here, we report first results on the safety, tolerability, immunogenicity and preliminary protective efficacy of escalating doses of Sanaria’s PISPZ (PISPZ Challenge) under chemoprophylaxis (PISPZ-CVac approach) with chloroquine. During immunization, PISPZ were injected by direct venous inoculation (DVI) three times at 4-week intervals. Volunteers receive staggered doses of 3,200 (Group A), 12,800 (Group B) or 51,200 (Group C) PISPZ per injection, corresponding to approximately 5, 20 or 80 mosquito bites. In every group, 9 volunteers received PISPZ and 5 placebo, while all received 10 mg/kg chloroquine 2 days before the first injection, followed by 5 mg/kg every week for a total of 10 doses. To assess efficacy of the immunization regimen, CHMI will be done 8 weeks after completion of chemoprophylaxis by DVI of 3,200 PISPZ Challenge. Subsequently, a PISPZ-CVac dose that shows at least 75% efficacy, good safety and tolerability will be tested using an experimental ultra-short chemoprophylaxis with azithromycin and chloroquine. Here, 2 g extended-release azithromycin will be given on the day of each PISPZ injection followed by 10 mg/kg chloroquine 5 days later. Complete results for the first two dose groups will be presented together with safety, tolerability and preliminary immunogenicity data of the highest dose (51,200 PISPZ).
LONGITUDINAL ANALYSIS OF HUMORAL AND CELLULAR IMMUNITY FOLLOWING VACCINATION WITH AN ATTENUATED PLASMODIUM FALCIPARUM SPOROZOITE 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-γ producing CD4 and CD8+ T cell responses. Here, we substantially expanded this analysis and performed a longitudinal assessment of antibody and cellular responses through 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, viable PfSPZ manufactured 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 1.35x10⁵ 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 2.7x10⁵ 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 4.5x10⁴ 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 aiming to halt 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 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.

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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.

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ANALYSIS OF DETERMINANTS OF INSECTICIDE TREATED NETS (ITNs) USE AMONG CHILDREN UNDER FIVE YEARS USING LOGISTIC REGRESSION

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Malaria causes between two 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 DHS 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.

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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 than in 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 method 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, urban and rural, 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.
INCREDING ROLE OF ANOPHELES FUNESTUS AND AN. ARABIENSIS IN MALARIA TRANSMISSION IN THE KILOMERO 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 EUSA respectively. Anopheles funestus susceptibility to insecticides was assessed using WHO guidelines. A total of 100,810 Anopheles funestus mosquitoes were assessed for species identity and sporozoite infection.

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 membrane feeding assay. A recombinant form of HSP70z (named HSP70z) was produced in Pichia pastoris in SMFA and iTB assay will be presented.

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 of personnel time and resources, require a mosquito colony and are not easily adaptable to high through-put. Thus, it is difficult, time consuming and expensive to assess large numbers of candidate anti-sera in SMFAs. Sanaria's technology platform generates live, aseptic, purified, cryopreserved P. falciparum sporozoites (PSPZ) that can be administered as a highly protective malaria vaccine. PSPZ are produced using Anopheles stephensi mosquitoes as bioreactors. Sanaria, in its quest for developing PSPZ-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 oocnetes 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 Pfs48/45 in SMFA and ITB assay will be presented.

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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.
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.

**1626**

**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/popups of mainly asymptomatic carriers. Targeting malaria transmission in low transmission settings requires a better understanding of this heterogeneity.
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.

1630

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 (ITWL) 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
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.

**1632**

**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 scale-up of interventions 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.

**1633**

**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. The fight against this disease should be of interest to all 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 of 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 tiny 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 family. This could be, an innovative approach to malaria control.

**1634**

**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 hanging 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 screened installed (used); the proportion of pregnant women who slept under LLINs; the proportion of children who slept under LLINs; 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 communities 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 (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 LLNs 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 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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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 vectorvivorship 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 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 mosquitoicidal 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
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 nuleotide 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.

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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 (aarr) was the most frequently detected (86.5%), followed by aggregative adherence regulator (aggR, 53.6%) and EAEC HIA homologue (eiiA, 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 (aarr(-) and aggR(+)) in a murine model and their impact on the host on diarrhea and growth impairment.

1640

SENDING PEACEKEEPING TROOPS INTO AN ONGOING CHOLERA OUTBREAK - THE CHILEAN EXPERIENCE DURING MINUSTAH (MISSION DES NATIONS UNIES POUR LA STABILISATION EN HAÏTI)

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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 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 returnees to non-industrialized countries such as Chile.

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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.

THE SPATIOTEMPORAL DYNAMICS AND PHYLOGENETICS OF \( \textit{Salmonella Paratyphi} \) A IN KATHMANDU, NEPAL

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Enteric fever is a life threatening systemic disease caused by the bacteria \( \textit{Salmonella} \) Typhi and \( \textit{Salmonella} \) 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.

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 \( \textit{Escherichia coli} \) was detected in 94 (70%) cases, \( \textit{Shigella} \) spp in 31 (23%), \( \text{Campylobacter} \) spp in 5 (4%), and \( \textit{Salmonella} \) 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.

SALMONELLA ENTERITIDIS SEROVAR ENTERITIDIS OUTBREAK AT A LODGE IN MOKOPANE, LIMPOPO PROVINCE, JANUARY 2014

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\( \textit{Salmonella enterica} \) 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 pathogen 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 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 mean 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 14 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-alpha, interleukin-6 (IL-6), and IL-1beta, and fecal alpha-1-antitrypsin (AAT), a marker of intestinal inflammation. The mean plasma osteocalcin at seven months was 41.2µg/L in boys and 36.4µg/L in girls, by 15 months this had fallen to 34.1 µg/L and 25.2 µg/L, respectively. The mean DPD/Cre 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 (WLAZ) at 6 months 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/Cre 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...
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.

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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 via 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 immunofluorescent 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 versus 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.

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INCORPORATING 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 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-poly saccharide 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.

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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 interventions to reduce cholera transmission in this region.

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BIOMARKERS OF ENVIRONMENTAL ENTEROPATHY FOR POSITIVELY ASSOCIATED WITH TOXIN-SPECIFIC B AND T CELL RESPONSES TO AN ORAL CHOLERA VACCINE IN BANGLADESHI 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 an 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 (AAT), a marker of intestinal absorption, as well as plasma 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
(LPS)-specific antibody responses by ELISA, toxin-specific memory T cell responses by flow cytometry following whole blood culture, and T cell culture cytokines by Luminex array. Using a multiple linear regression model, we assessed each vaccine-associated immune response outcome as separate dependent variables, and used log-transformed EE marker measurements, along with gender, blood group, and age, as independent variables. We found stool MPO to be a positive predictor of antibody responses to CT, plasma iFABP a positive predictor of gut-homing memory T cell responses, and stool AAT a positive predictor of interferon-gamma responses. No marker predicted antibody responses to LPS. Variance inflation factor for all independent variables were < 1.6, suggesting no multi-collinearity. In summary, we demonstrate that biomarkers of environmental enteropathy are positive predictors of toxin-specific immune responses to an OCV.

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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 3623(17.57%) in 2011. The prevalence reduced from 4.3x10^3 /10,000 population in 2008 to 1.2x10^4/10,000 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 24hours 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 Cochran 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
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.

CONTRIBUTION OF THE COMMUNITY HEALTH VOLUNTEERS

green 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), papGII and papGIII (pilus associated with pyelonephritis GI and GII), hlyA (haemolysin), af (afimbrial adhesin), sfa (5 fimbrae), iron (iron), iuc (aerobactin), cfa, and cfb (cytotoxic necrotizing factor), astA (enteroaggregative toxin), sap (shigellosis 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, sfa, iuc, cfa, cfb, 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 cfa; 21.6% (n=13) carried pap; 20% (n=12) carried papGIII; 18.3% (n=11) carried sfa; 16.6% (n=10) carried iron; 13.3% (n=8) carried iuc; 6.6% (n=4) carried cnf1; 10% (n=6) carried...
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 (SCC mec) 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 Cefotaxim 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 SCC mec type IV, the genotype most commonly associated with community-acquired MRSA (CA-MRSA). Five isolates were observed to be SCC mec 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 SCC mec IV, including two which preceded (12.0%), ten were found to be SCC mec IV, including two which preceded (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.

THE ROLE OF YERSINIA PESTIS PRESENBSILIZATION 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.

EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING ENTEROBACTERIACEAE ISOLATED FROM HEALTH CARE WORKERS’ CELL PHONES IN FIVE PERUVIAN INTENSIVE CARE UNITS: ANTIBIOTIC RESISTANCE PATTERN 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. Suspicous ESBL-producing bacteria in the microbiogram were confirmed by the phenotypic CLSI ESBL confirmatory test. We then performed PCR for detection of b1aTEM, b1aSHV 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.
CHARACTERIZATION OF ACINETOBACTER 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 carbapenams 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 relatioship 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 extremely 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 cloned 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 ensure 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 sepsimemia. 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 630-931 of LipB (rLigB-Rep). The over-expressed rLigB-Rep, which contains a six-histidine tag at the N-terminus, was primarily found 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 was used as a suitable genetic marker for rapid detection of Leptospira in the environmental monitoring program of public health importance.
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.

### 1665

**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 Cuilapa 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. Cases were higher in females (68.4%) than males (31.6%). Blood cultures positive for *Leptospira* were identified in 3.9% of cultures (n=57), and were found more commonly in females (68.4%) than males (31.6%). Blood cultures positive for *Leptospira* occurred most frequently in February followed by July. The following seven antibiotics were tested on isolates: cefepime, cefotaxime, ceftriaxone, gentamicin, nalidixic acid, ciprofloxacin and amikacin. Antibiotic resistance variations were measured monthly and appeared to have a seasonal dependence. In January Enterobacter isolates were strongly resistant to cefepime, in February to cefotaxime, in March to ceftriaxone, cephalexin and gentamycin, and in April to cefotaxime and nalidixic acid. In July isolates showed no resistance to amikacin and low resistance to ciprofloxacin, while in August and September strong resistance to cephalaxin. Identification of factors which lead to an apparent seasonal variation in antibiotic resistance patterns among Enterobacter will require further study that includes careful evaluation of demographic and therapeutic histories of patients from whom Enterobacter strains are isolated, speciation of these isolates and collection of larger numbers of isolates over longer periods of time in order to control for sampling variability.

### 1666

**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 2003) 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.

### 1667

**ANTIBIOTIC RESISTANCE PATTERNS OF ENTEROBACTER SPECIES ISOLATED FROM CHILDREN WITH CYSTITIS IN IRAQ**

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Awareness of antibiotic sensitivity patterns among gram negative bacterial pathogens is clinically important not only to empirically guide therapy but also to monitor the emergence of new drug resistant strains. We report here a survey of antibiotic resistance in Enterobacter species cultured from children with cystitis in Iraq. We collected 1,474 urine cultures from children aged 1-7 years treated at Al-Hakeem Hospital in Najaf governorate, Iraq between January and September of 2010. Enterobacter species were identified in 3.9% of cultures (n=57), and were found more commonly in females (68.4%) than males (31.6%). Cultures positive for *Enterobacter* occurred most frequently in February followed by July. The following seven antibiotics were tested on isolates: cephalexin, cefotaxime, ceftriaxone, gentamicin, nalidixic acid, ciprofloxacin and amikacin. Antibiotic resistance variations were measured monthly and appeared to have a seasonal dependence. In January *Enterobacter* isolates were strongly resistant to cefalexin, in February to cefotaxime, in March to ceftriaxone, cephalexin and gentamycin, and in April to cefotaxime and nalidixic acid. In July isolates showed no resistance to amikacin and low resistance to ciprofloxacin, while in August and September strong resistance to cephalexin. Identification of factors which lead to an apparent seasonal variations in antibiotic resistance patterns among *Enterobacter* will require further study that includes careful evaluation of demographic and therapeutic histories of patients from whom *Enterobacter* strains are isolated, speciation of these isolates and collection of larger numbers of isolates over longer periods of time in order to control for sampling variability.

### 1668

**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.

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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 mononeuropathies with wrist or foot drops, as well as mononeuritis multiplex, were among the common misdiagnoses for patients with leprous neuritis. 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, even for 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 leprous neuritis misdiagnosed as mononeuritis multiplex from sarcoidosis, a second of polyarthritus from leprosy mimicking rheumatoid arthritis, and lastly, a case of borderline tuberculosis 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 morbidities.

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TWO FATAL CASES OF MELIOIDOSIS ON THE THAI-MYANMAR BORDER

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Melioidosis, 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-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. The discussion of cases in as-yet-unrecognized foci of disease is of great public health importance and may help to better elucidate environmental and host determinants of infection. Our study highlights the need to both increase clinical awareness of melioidosis on the Thai-Myanmar border, and to better assess the true burden of disease in the region through improved case detection and rigorous B. pseudomallei prevalence studies.

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 paracoccidioidiomycosis. 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 RTPCR or anti-DENV IgM antibody detected by ELISA) dengue patients
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 previous civilian studies: African-born VFRs in the military sought pre-travel health care more often than their U.S.-born counterparts. Access to care and positive beliefs about the benefits of travel medicine services contribute to this finding. These findings have implications for the role of 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 ELISA-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 ± 11.92nM, 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 ± 36.97nM for Chloroquine, Mefloquine, Quinine, Amodiaquine and Piperazine 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 average age 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

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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 during 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 3,33 (IC 95%: 1,21; 9,17), altered state of consciousness 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 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, atorvastatin 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 atorvastin is a safe and well-tolerated drug that has modest anti-inflammatory effect in patients with moderate to severe active RA.

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**GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND DENGUE IN SINGAPOREAN MALES: A CASE-CONTROL STUDY**

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Clinical presentations of dengue infection range from asymptomatic, non-severe to severe disease. We aim to test the hypothesis that patients with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency may present with severe disease and hemolysis. We analyzed a cohort of adult dengue patients treated at Tan Tock Seng Hospital, Singapore from January 2005 to December 2008. Dengue infection was confirmed by positive polymerase chain reaction or dengue serology with World Health Organization (WHO) probable dengue definition. Singaporean males with documented G6PD status were defined as cases. For each case, three controls were selected by matching citizenship and year of infection. Hemolysis was defined as low hemoglobin concurrent with low serum haptoglobin, or high reticulocyte or lactate dehydrogenase or bilirubin. Dengue hemorrhagic fever and severe dengue were classified according to WHO 1997 and 2009 dengue guidelines. Compared with cases (n=30), controls (n=120) were significantly younger (median 26 vs. 35 years, p<0.05). During their clinical course, cases had significantly higher rates of jaundice (10% vs. 1%, P<0.05), serum bilirubin (median 27 vs. 10 mmol/L, p<0.001), aspartate transaminase (median 148 vs. 91 U/L, p<0.05), and lower hematocrit (45% vs. 46%, p<0.001), haemoglobin level (13 vs. 14 mg/dL, p<0.001). There was no difference in rates of dengue hemorrhagic fever (23% vs. 22%, p>0.05). However, cases had higher tendency to develop severe dengue and hemolysis than controls [(23% vs. 12%) and (14.29% vs. 2.77%) respectively] although the difference was not significant (p>0.05). The two groups had similar rates of blood and platelet transfusions, intravenous fluid and length of hospitalization (p>0.05). The observed differences should be prospectively validated in larger cohorts and in different populations.

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A RANDOMIZED, DOUBLE BLIND, CLINICAL TRIAL OF TWO DOSE REGIMENS OF VINS POLYVALENT ANTIVENOM FOR THE TREATMENT OF SNAKEBITE WITH NEUROTOXIC ENVENOMATION IN NEPAL

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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 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 been bitten by cobras (Naja spp) and 22 by kraits (Bungarus spp.). Those bitten by kraits experienced more primary outcomes (68.2% vs 27.1%, p=0.004), and recovered less often (40.9% vs 96.5%, p<0.001) or more slowly (mean time 18 hours vs 5 hours, p<0.001) than did patients bitten by cobras. These findings suggest that there is no difference in efficacy and safety between low and high initial dose of antivenom for neurotoxic snakebite, and that envenomation due to krait bites is less responsive to antivenom than that following cobra bites. 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 Diphtheria-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 LEPROSUM: 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 leprosum (ENL) reactions, or Type 2 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 splenic 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 recurrences. 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.

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
after analysis by ELISA and Western blot. Two main antigens of 24 and 35 kDa were found in sera from 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 against 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 against 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.

MAPPING THE POTENTIAL RISK OF MYCETOMA IN SUDAN USING MAXIMUM ENTROPY ECOLOGICAL NICHE 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 of mycetoma in environmental dimensions, and the results revealed that mycetoma in ecologically diverse landscapes under wide ranges of environmental conditions. We tested niche similarity across environments that are potentially suitable for disease emergence, raise the concerns for mycetoma-acacia association, and provide steps towards a robust, predictive risk map for the disease.

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 test 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.

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.

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-Nasriyah Endocrine Centre in Thi-Qar province, Iraq. The diabetic patients 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-Wafaa 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-Abbasseya 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 FILARIALISIS (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 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, washing 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 for validating new monitoring tools that may be developed to support 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 microfilariae 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 microfilaremia 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 PCR-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 hypendemic 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 microfilaria 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 46% (74/164). Thus, skin snip microscopy does not appear to be sufficiently sensitive to assess transmission in areas with low microfilariaemia 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 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 ≥15 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 ≥15 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.

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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 astmh.org
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 Kolondièba, one of the highly LF endemic district of Sikasso, Mali. To assess transmission interruption after 6 annual MDA in the district of Kolondièba 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. Microfilaraemia was significantly more frequent in the 15 years and above in Boundioba (1.95%, 4/205) as compared to Bougoula (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 MICROFILARIASIS 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 female adult males with occupations associated with rural areas, whereas those infested with the atypical parasite were rarely female 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.

1700

INTEGRATED FILARIAL MICRO-MAPPING TO DETERMINE IMPLEMENTATION STRATEGIES IN LOA LOA CO-ENDEMIC AREAS: THE ANGOLAN EXPERIENCE

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The co-distribution of L. loa (loiasis or tropical eye worm) is a significant impediment to the lymphatic filariasis (LF) and onchocerciasis elimination programmes in Angola, due to the potential risk of severe adverse events (SAEs) associated with the drug ivermectin when given to individuals with high L. loa microfilarial loads in the blood. This has significant implications for mass drug administration campaigns and alternative strategies may be required in selected areas. One of the highest L. loa risk areas is Dande Municipality, Bengo Province in Northern Angola, an area which historically has reported cases of LF and onchocerciasis. To better determine the safest treatment strategy in this area, this study conducted an integrated filarial micro-mapping survey to understand the geographical overlap of the three diseases. GIS-Remote Rapid Eye satellite data was also employed to provide the foundation for empirical information on vector and parasite populations. In total 23 villages, distanced approximately 10-15km apart, across peri-urban and rural areas were surveyed during January-February 2014. In each village, up to 100 individuals were assessed using the rapid assessment procedure for loiasis (RAPLOA) and rapid epidemiological mapping of onchocerciasis (REMO), and two questions on LF morbidity (presence of lymphedema, hydrocele). The study found low levels of endemicity of all three diseases (<20%), with different overlapping distributions, with most villages reporting at least one filariasis case. To confirm the hypo-endemic levels of LF and onchocerciasis, a further seroprevalence survey using rapid diagnostic tests in the same villages is planned for June 2014. This will provide additional micro-epidemiological information to help determine if the recommended alternative strategy of albendazole twice yearly and long-lasting/insecticide treated bednets (LLINs/ITNs) should be used for LF elimination, and if an alternative to ivermectin for hypoendemic onchocerciasis elimination, such as the drug doxycycline or vector control, needs to be considered.

1701

SERO-PREVALENCE AND RISK FACTOR SURVEY FOR LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA

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Papua New Guinea (PNG) has an estimated population of 7 million inhabitants; of which 4 million are predicted to be at risk of lymphatic filariasis (LF). LF is a debilitating disease caused by lymphatic-dwelling nematodes Wuchereria bancrofti, which is transmitted by Anopheles mosquitoes in PNG, similar to malaria. Some national and published data exists, however, the geographical distribution, burden of disease and associated risk factors are currently not well defined. This study investigated the prevalence and potential risk factors of LF in one endemic province of PNG through the administration of a prevalence survey and household questionnaire related to the LF transmission and the national LF programme. In April 2013, four villages in a southern remote area of Madang Province were selected. Approximately 100 individuals in each village were interviewed and examined for LF infection, using ICT rapid diagnostic kits, and evidence of clinical disease. This study found that 32 individuals out of 389 surveyed (8.2%) were LF antigen positive, and 3 individuals had lymphoedema (elephantiasis) of the leg (0.8%). All of those
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 finish 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.

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 microfilaricidal (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.

**1702**

**METHODS FOR ASSESSING LYMPHATIC FILARIASIS TRANSMISSION IN LOW ENDEMIC AREAS OF BANGLADESH: ONE STEP CLOSER TO THE ELIMINATION GOAL**

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Bengal 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.

**1703**

**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.

**1704**

**SIMULTANEOUS DETECTION OF ONCHOCERCA VOLVULUS AND O. OCHENGI IN INFECTED SIMULIUM FLIES USING A NEW MULTIPLEX REAL-TIME PCR**

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Onchocerca volvulus parasitic worms infect ~37 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 Onchocercidae genus with specific TaqMan probes to differentiate O. volvulus and O. ochengi from other Onchocercidae. A blinded study with 217 flies from O. volvulus and 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.

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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 coendemicity, 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 georeferenced 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 / high 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.

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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.

**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 basal, eosinophilic, 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.

**DIFFERENCES IN OV-16 ELISA IMMUNE RESPONSES AMONG CHIMPANZEEES 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 microfiladermia 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 microfiladermia. 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.

**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 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 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 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 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 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.
among controls with lymphatic filariasis (LF). The Ov16 cutoff values for IgG or IgG4 were 379 and 32 fluorescent units (MFI), with specificities 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.

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EVALUATION OF HLA IMMUNOINFORMATICS FOR THE IDENTIFICATION OF BRUGIA MALAYI PUTATIVE T CELL EPITOPE CONSERVED WITH Wuchereria bancrofti and LOA LOA

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The availability of filarial genome sequences and improved immunoinformatics tools promise 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 conservation with W. bancrofti and/or Loa loa. Proof-of-principle assays for all 20 putative epitopes were performed using rHLA binding assays tested with 4 out of the 8 HLA alleles predicted by the software: DRB1*0101, DRB1*0401, DRB1*1101, and DRB1*1501. Results from these competitive binding assays demonstrated allele-specific binding biases. The 6 putative sequences sharing conservation with W. bancrofti and/or L. loa were tested on PBMCs from patients living in LF endemic areas that had been exposed to W. bancrofti. Upon peptide stimulation, subset CD4+ and CD8+ T cell populations from patients infected with W. bancrofti were selected for determination of cytokine-specific responses by ELISPOT and flow cytometry. Results demonstrated the predicted peptides derived from the B. malayi secretome were capable of inducing T cell responses, which differed dependent on infection and disease status. These results suggested that the cross-conserved peptides were capable of binding to HLA from patients exposed to W. bancrofti.
EFFECT OF A SINGLE DOSE OF 8 MG MOXIDECTIN OR 150 µG/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 µg/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 ≥ 1 type of intestinal helminth: *Ascaris lumbricoides* 44 (3%), *Trichuris trichuria* 22 (1.5%), hookworm 783 (53.4%), *Schistosoma mansoni* 233 (15.9%), *Strongyloides* 4 (0.3%). 835 were co-infected with ≥ 2 types of intestinal helminths. Participants 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=34, EPG=386, 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.
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, which were not significantly different between them. The mean weight z-score for the study participants was -0.02 (±0.97), 0.65 (±1.19) and -0.39 (±0.89) for negative and positive cases respectively, which were significantly better between the two groups (P=0.02). The mean height z-score overall was -0.01 (±0.0), -0.01 (±0.95) and -0.12 (±0.07) for negative and positive cases respectively, which were not significantly between the two groups (P=0.087). 74.2% of the study participants experienced food insecurity and so were 72.4% and 74.6% of the negatives and positives cases respectively. Above-average dietary diversity was observed in 46.5% of the study participants, and was 48.8% and 37.9% 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 Sh and Sj than for Sm; 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 Sh-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 83.3%, 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 rice differed in micronutrient compositions and production method. 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 rice differed in micronutrient compositions and production method. 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 rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method. Children (6-15 y) received 1 type of rice, unfortified rice (placebo), or no rice differed in micronutrient compositions and production method.
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 *a. 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 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 insight 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.

1726 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.

1727 HELMINTH INFECTIONS DURING PREGNANCY MAY DECREASE NUTRITIONAL FITNESS OF THE OFFSPRING

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Helminth infections represent a significant disease burden in endemic regions of the world, and polyparasitism may have a bigger impact on overall health than any individual infection. We have previously shown that Schistosoma japonicum results in a profound pro-inflammatory response at the maternal-fetal interface during pregnancy and decreased invasion characteristics of placental trophoblast cells in vitro. Another critical function of the placenta is to regulate nutrient exchange between mother and fetus. Herein, we have shown that treatment in vitro of primary trophoblasts with schistosome soluble egg antigens (SEA), resulted in a significant drop in gene expression of specific amino acid transporters. These include the sodium-coupled neutral amino acid transporter 1 (SNAT1; 80% reduction) and large neutral amino acid transporter (LAT1; 70% reduction). To investigate the metabolic impact of helminth infections during pregnancy, we utilized samples from a cohort of pregnant women from Leyte, the Philippines. Most subjects had polyparasitic infections, including schistosomiasis and geohelminth infections, with prevalence rates of 70%, 79%, and 40% for Ascaris lumbricoides, Trichuris trichiura, and hookworm, respectively. Given the relatively low intensity of schistosome infection and the high prevalence rates of geohelminths, we assessed the relationship between the number of helminth infections and metabolic parameters in utero. After controlling for SES and gestational age, leptin levels were found to be lower in the cord blood of infants born to mothers with one or more helminth infections. In addition, cord blood leptin levels were positively associated with birth weight (107g heavier on average in those infants in the highest tertile of leptin levels), and increased leptin levels were associated with a reduced risk of fetal growth restriction. These data suggest that helminth infections can impact the transport of nutrients across the maternal-fetal interface, providing a possible link between fetal metabolic hormones and growth in utero.

1728 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 helminth 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.
RANDOMIZED CONTROLLED TRIAL OF TWO IVERMECTIN REGIMENS FOR STRONGYLOIDES STERCORALIS: EARLY FINDINGS

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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 setting 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 0.55/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. Over 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 Tucumán, 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 classical, immunological and parasitological aspects between patients with atypical cutaneous leishmaniasis (ACL) and classical cutaneous leishmaniasis (CL) of a region with high endemicity 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 INFγ (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.

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CHAGAS DISEASE IN MEXICO: SURVEILLANCE AND PERCEPTIONS OF BURDEN

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Chagas disease, a parasitic disease caused by Trypanosoma cruzi, disproportionately affects the poor throughout Latin America. We describe the spatial and temporal distribution of officially reported Chagas disease incidence and mortality in Mexico. The greatest burden appears to occur in southern states. Incidence rates and deaths were highest in adults (25-44 years and ≥45 years, respectively). We show increasing temporal trends for incidence (AR(2) p=0.002, 95% CI: 0.040-0.061) and mortality (MA(1) p < 0.0001, 95% CI: 0.012-0.021). While these results provide insight to the changing burden of Chagas in Mexico, under-reporting likely compromises our capacity to understand the epidemiology of this disease. The reported 500 new cases and 20 deaths in 2010 are in stark contrast to estimates of 69,000 new cases and 25,000 deaths per year from seroprevalence studies. As changes in Chagas surveillance improve our understanding of the full burden of this disease, it is likely that the reported and estimated
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 exact tests. 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 Artiodactyla 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 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 raccoon, 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 (14/116, 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.
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MAPPING THE PREVALENCE AND CASE DETECTION RATES OF GAMBIANE 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 statistical 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 ANTIBODYs 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-k39 (k39 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-k39 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 k39 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-k39 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.

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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 occurrence 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 conditions.
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.

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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 CD14+ 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.

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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.

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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 (p=0.0087) and Th1-associated chemokine receptors CXCR3 (p<0.0001) and CCR5 (p<0.001). There was no significant difference in Foxp3 or the Th2-associated chemokine receptor CCR4 in purified CD4 T cells from uninfected and infected animals. These data suggest both 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/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 mechanism(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 FGFR, IGFR 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-gamma 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 parasitism 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. Metalloprooneinase-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. Our study contributes to the understanding of immunopathology in CL and reveal 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...
ligand. In this study, we evaluated the immunogenicity and protection of pVAX-NH36 for the prevention and therapy of *L. mexicana* infection in dogs. We then established a model of infection in Beagle dogs. Then, Beagles received 3 doses of 250 microg of pVAX-NH36 as prophylaxis or therapy, while dogs from control group received saline solution. *L. mexicana* promastigotes were used for infection via intradermal. Immune response was evaluated measuring antibodies, IFNγ and IL-10 production, and DTH. Ulcer diameters and parasite burden were evaluated to assess protection. Canines receiving pVAX-NH36 showed higher IgG levels against NH36 in comparison with the control group. High IFNγ 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*.

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**LEISHMANIA SPECIFIC CD4 T CELLS RELEASE IFNγ 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, IFNγ, 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 (WBA) 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.

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**ISOLATION AND INITIAL CHARACTERIZATION OF TRYPANOSOMA CRUZI ISOLATES FROM A POPULATION OF CYNOLOGY 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.

From a locale. Similar to endemic human infection, infection in these macaques is thought to have been acquired early in life (age range at infection 1-15 years) and to persist chronically with an average infection time of 6.4 years. The *T. cruzi* isolates were obtained from hemocultures of 43 out of 64 (67%) macaques. An additional 8 hemocultures were positive for *T. cruzi* DNA by PCR, but failed to yield a culturable line, resulting in an overall hemoculture detection rate of 80%. This figure is comparable to the frequency of detection of infection by serial PCR (up to 3 samples) of whole blood (81%). Furthermore, parasite isolates were obtained by hemoculture from 8 of the 12 PCR-negative animals. The combination of whole blood PCR and hemoculture +/- PCR confirmed active infection in 94% of the seropositive animals. Genotyping of hemoculture-isolated *T. cruzi* revealed the presence of two lineages: Tcl and Tclv, which are the most common lineages identified by previous studies in infections originating in North America. To date, all tested hemoculture-isolated *T. cruzi* could be converted to metacyclic trypomastigotes and were orally infective in C57Bl/6 and IFN-gamma knockout mice with the Tclv lineage isolates exhibiting less virulence in both mouse models. All isolates appeared to establish chronic infection in mice and to induce immune responses consistent with those observed in infections by long-maintained laboratory-adapted strains of *T. cruzi*. Future studies will assess the susceptibility of these fresh isolates to clearance by treatment with benznidazole.
ENHANCEMENT OF MURINE VISERAL LEISHMANIASIS 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 naïve mice. There was little notable difference in the cytokine profiles between L. major exposed and naïve 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 L. major-infected matched control 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.

HUMAN SEROPREVALENCE OF LEPTOSPIROSIS AND RICKETTSIOSIS IN FOUR REGIONS OF PERU

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Leptospira and Rickettsia 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 Rickettsia (SFGR) and the typhus fever group Rickettsia (TFRG). 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 TFRG (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 TFRG (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.

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.
Leptospira spp. infection was also reported in a wide range of animal hosts. 11 out of the 26 (44.8%) countries reported the highest number sylvatic rabies cases, mainly transmitted by bats. Vaccination of humans and livestock is an effective prevention method, but vaccination is not usually undertaken until a human exposure is identified. The ecological and topographic factors underlying the distribution of rabies vectors are not well understood. Specifically for sylvatic rabies, there is little data on the factors influencing the 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 geographic 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 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.
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 involving use of 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, fluorquinolones 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 environs. 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 lamblia, 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 prevalence 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.
we have learned to date about hygiene conditions, infrastructure, and stalls selling bush meat and/or poultry. This presentation will describe what we have carried out monthly one-week full-day observations of vendor market catchment areas (as determined by exit interviews) to learn where interviews, consumer exit interviews and then a household survey in the well as eight markets in Kinshasa, DRC. We conducted key informant studying eight urban markets in Brazzaville and two in Dolisie, ROC, as transmission of zoonotic infections via exposure to live animals, animal where humans - consumers, vendors, managers - are at increased risk of wellbeing, presented by bacterial infections such as Brucellosis. In addition that herders are unaware of threats, both to health and economic 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 where humans - consumers, vendors, managers - are at increased risk of transmission of zoonotic infections via exposure to live animals, animal products, and poor biosecurity practices. USAID's PREVENT Project is studying eight urban markets in Brazzaville and two in Dolisie, ROC, as well as eight markets in Kinshasa, DRC. We conducted 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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Fewers 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), participants (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

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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, dyspnoea, fever (≥38.3°C) or hypothermia (<36.0°C), 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 received 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. amnon furnace, Chlamydophila pneumoniae, C. psittaci, Legionella pneumophila and L. longbeachea. 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 was positive for S. pneumoniae in 80/125 cases (64%), M. pneumoniae in 17/125 (13.6%), C. psittaci in 10/125 (8%), M. amnon furnace in 5/125 (4%), C. pneumoniae and L. pneumophila in 1/125 (0.8%) each and there were no cases of L. longbeachea. Clinical findings and outcomes will also be explored.

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PROFILE OF MYCOBACTERIUM TUBERCULOSIS DRUG RESISTANCE IN A TROPICAL REGION OF PERU 2007-2013
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Tuberculosis in Peru is a public health problem. In terms of South America is the second country in prevalence after Bolivia, and in America is the third after Haiti and Bolivia. In 2013, WHO reports that in Peru Multidrug-resistance Tuberculosis (MDR - TB) exceeds the reported cases that year by Colombia, Ecuador, Argentina, Chile and the United States throughout its territory, and ranks first in report for MDR - TB and Extreme drug resistance tuberculosis (XDR - TB). In Peru the regions with high prevalence of TB are Lima, Callao, Madre de Dios, but the profile of Mycobacterium tuberculosis drug resistance is not known in the region of Madre de Dios. Determine the profile of Mycobacterium tuberculosis drug resistance in patients admitted to retreat in Madre de Dios, Peru, 2007-2013 Observational, cross sectional study. Logbook and monitoring of TB patients in retreatment Regional Health Direction - Madre de Dios of 2007-2013, included 111 patients diagnosed with tuberculosis smear (+), they were tested for sensitivity at the National Institute of Health (NIH). Frequencies established variables, measures of central tendency and dispersion in qualitative variables were used. 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 isoniadiz resistance was 19.20 % (n = 92), rifampicin 79.09 % (n = 87), Streptomycin 18.18 % (n = 20) Ethambutol 11.81 % (n = 13) and pyracetamidine 7.27 % (n = 8). The initial cultures were basciloscopias 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 isoniadiz is the drug resistance most often generated in monoresistencia 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 5-17 years (55.1%) and ≥25 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<0.05) 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<0.05) with rainfall in any locations. Among the meteorological factors, SH had the highest contribution (2-15%) to the model in all locations except 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 was positively correlated with SH (R=0.2-0.6, p<0.05), 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 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.

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.
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 specimens were then tested with RT-PCR for enteroviruses and rhinoviruses (EV/RV) and enterovirus EV71. Among 586 ILI-patients (median age 5 year, 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 Coxackie virus 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 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 MEGA5.1 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.
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 1,364 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 type 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 worm burden, fecundity, and egg passage in the feces than previously appreciated. Regardless, 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, Tucumán) 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 electro-soaking (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 and short interfering RNAs. While both 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.

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 encoding leucine aminopeptidase (LAP) as the target, we refined delivery conditions, identifying electro-soaking (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 and short interfering RNAs. While both 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.
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 8kDa 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 AP5. 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 FASCIOILIASIS 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= 7.9-33.7) on day 4, possibly related to the expelling of the adult worms through the biliary tract. In the tested group, 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.
FIELD EVALUATION OF MINI-FLOTAC®, SPONTANEOUS SEDIMENTATION TECHNIQUE IN TUBE OF TELLO, RAPID SEDIMENTATION TECHNIQUE BY LUMBERRAS AND KATZ-KATZ 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 techniques. 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. SAS was achieved, and 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 butschlii (15.0%), Iodamoeba bantiana, Entamoeba histolytica/dispar (12.0%), Giardia lambia (11.4%), Fasciola hepatica (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 multiple techniques is an appropriate approach for highly endemic areas where intestinal multiparasitism is common. SSTT is highly sensitive for enteric protozoa, including when compared to standardized techniques such as the mini-FLOTAC®. Mini-FLOTAC® holds promise for the diagnosis of H. nana and F. hepatica in endemic areas. Notably, F. hepatica infection continues to be highly prevalent among children in Azángaro, thus prompt and realistic field interventions are needed.

GRANULOMATOUS INFLAMMATION OF THE BLADDER COMPROMISES THE OVERLYING UROTHELIAL BARRIER

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Bladder granulomas can form as a result of retained suture material from surgery, BCG treatment, urinary tuberculosis, and urogenital schistosomiasis. We previously demonstrated in a mouse model of urogenital schistosomiasis that multiple urotheletic barrier function genes (i.e., uropakins) were downregulated on a whole bladder level after bladder wall injection with Schistosoma haematobium eggs. Given that egg-injected bladders exhibit hyperplasia of the urothelium overlying resulting egg granulomata, we hypothesized that this hyperplastic response was a response to egg inflammation-induced, regional compromise of the molecular urotheletic barrier. Anesthetized mice underwent laparatomies and bladder exposure. Mice then underwent either bladder wall injection with S. haematobium eggs or a vehicle control. Five days later, mice were sacrificed and their bladders harvested and fixed. Frozen sections of each bladder were stained with Cresyl violet. Laser microdissection was used to harvest RNA from three regions of each bladder: 1) the “proximal” urothelium (urothelium overlying the egg granuloma site); 2) the “distal” urothelium (urothelium from the opposite side of the bladder relative to the granuloma site); and 3) granuloma tissue (subepithelial). Equal areas were harvested for each of the three tissue sites (478,000-1,000,000 µm2/site). RNA was isolated, reverse transcribed to cDNA, pre-amplified using the NuGen PicoS1 WTA System, and then subjected to qPCR for uropakin and housekeeping genes. Control vehicle-injected bladders exhibited subepithelial edema, normal urothelium, and some inflammation. Egg-injected bladders, in contradistinction, featured markedly thickened, hyperplastic urothelium overlying areas of significant egg-associated inflammation. The proximal urothelium in egg-injected bladders featured lower expression levels of uropakins relative to the distal urothelium. Bladder granulomas, such as those induced by urogenital schistosomiasis, may locally suppress the overlying urotheletic barrier. This suppression may be a mechanism by which chronic bladder inflammation results in urotheletic-related bladder dysfunction. Future work will further define these mechanisms and their physiologic significance.

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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 roles 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 S. 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 Sm38 were chosen for experimental validation. RNAi and pharmacological inhibition were used to elucidate the functional role of HMEs and MAPK signaling pathway proteins in S. mansoni. Mice were injected with schistosomula subsequent to RNAi and the development of adult worms observed. The data demonstrate that SmHDAC8 and SmJNK contributes to the parasite transformation and survival, whereas HDAC8, PRMT3, KDM1/KDM2, SmERK, and Sm38 seems to be involved in egg production as infected mice had significantly lower egg burdens and female worms presented underdeveloped ovaries. Additionally, SmJNK and Sm38 dsRNA treated worms exhibited tegumental damage. We also observed that Sm38 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.

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REPRODUCTIVE DEVELOPMENT

COMPROMISES THE OVERLYING UROTHELIAL BARRIER

GRANULOMATOUS INFLAMMATION OF THE BLADDER

ABSTRACT

Scabies is a chronic parasitic disease caused by Sarcoptes scabiei, a small mite that burrows into the skin and causes intense pruritus. The mites leave eggs in the intercellular spaces of the stratum corneum, which may lead to the formation of follicular papules, nodules, and scabrous plaques. These papules can become infected with secondary bacterial pathogens, leading to the formation of skin abscesses and furuncles. Scabies is characterized by intense pruritus, which can interfere with sleep and daily activities. The disease is treatable with topical ivermectin or benzyl alcohol, but relapses are common. In severe cases, scabies can lead to systemic complications such as pneumonia, sepsis, and disseminated intravascular coagulation. The disease is more common in developing countries and is associated with poverty and malnutrition. The diagnosis of scabies is based on clinical features and is confirmed by the detection of Scabies infestation.
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PROTEOMIC ANALYSIS OF BIOMPHALARIA GLABRATA HEMOCYTES DURING ENCAPSULATION OF SCHISTOSOMA MANSONI SPOROCYSTS IN VITRO

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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-ESI-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 non-Schistosoma sequences. Significandy more of Hsp70 peptides in 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 samples 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.

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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.

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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.

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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-floxed; p53flox/flox) 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-floxed; p53flox/flox 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 schistosomes ova was done. HIV testing was done in 769 women, of which 123 were HIV positive (16.0%). The mean CD4 count was 864 ± 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-NAIVE 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-naive Kenyan school children. The ratio of antibody reactivity with periodate-resistant (primarily non-glycan) versus total epitope 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 of 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
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 cell (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.

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RNA-SEQ ANALYSIS OF WHOLE BLOOD FROM MALARIA-SUSCEPTIBLE AND IMMUNE CHILDREN REVEALS AN EARLY PRO-INFLAMMATORY RESPONSE TO PLASMODIUM FALCIPARUM INFECTION THAT CORRELATES WITH CONTROL OF PARASITE GROWTH: A PROSPECTIVE STUDY IN MALI

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Non-sterile, antibody-mediated immunity that reliably protects from febrile malaria is acquired gradually through repeated Plasmodium falciparum infections; however, the nature of cellular immune responses at the onset of clinically apparent versus clinically silent blood-stage infections in children is unclear. In a prospective study in Mali, we collected whole blood RNA, PBMCs and plasma from healthy, uninfected children aged 6-11 years (n=79) before the 6-month malaria season and from the same children during their first P. falciparum infection of the ensuing season—detected retrospectively through bi-weekly active surveillance by PCR. We used RNA-seq to compare whole-blood transcriptomes of children whose clinically silent infections never progressed to fever (immune, n=21), children whose infections progressed to fever within 2-14 days (late fever, n=32) and children who were febrile at the time of infection (early fever, n=26). We found that baseline transcription profiles before the malaria season distinguished children whose future P. falciparum infections either progressed to fever or not, including upregulation of B-cell-receptor signaling pathways in immune children. Transcription profiles induced by the first detected P. falciparum infection of the season revealed upregulation of pro-inflammatory genes in immune versus late fever children, despite both groups having similar levels of parasitemia and the clinical absence of fever initially. In addition, this early upregulation of pro-inflammatory genes was associated with slower subsequent parasite growth rates in vivo. In ongoing work, we are testing hypotheses generated by this study at the protein level and in functional assays, using contemporaneous PBMCs and plasma samples from the same children. Molecular and cellular signatures that correlate with protection from malaria are yielding novel insights into the mechanisms underlying naturally acquired immunity to malaria. The resulting datasets may inform the development of interventions that prevent or mitigate malaria disease.
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 Thies, 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 variant 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 TRANSCRIPTIOINAL ANALYSIS OF MALARIA-SPECIFIC CD4+ T LYMPHOCYTES FOLLOWING PFSPZ VACCINATION AND PROTECTION IN HUMANS

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Intravenous immunization with highly purified, radiation-attenuated parasites (PFSPZ Vaccine) is safe, immunogenic and confers high-level protection against controlled malaria infection in humans. Protection was associated with a dose-dependent increase in PFSPZ-specific antibodies, CD4+ and CD8+ T cell responses. Heretofore, multi-parameter flow cytometry has been used to characterize the magnitude and quality of PFSPZ-specific T cell responses following vaccination or infection. To substantially expand the analysis of such responses, we performed high-resolution, quantitative transcriptome analysis of PFSPZ-specific CD4+ T cells. Accordingly, PFSPZ-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 PFSPZ-specific CD154+CD4+ T cells do not produce IFN-γ, IL-2 or TNFα, the most common cytokines used to assay 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 isolated from 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 PFSPZ-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 SPOROZITE 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 qRT-PCR 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 ARTEMESININ 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-Lumefantrine (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 immunosassay (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 circumstances complex read-out system. The protocols 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 amplicons. Funding: EU FP7 grant 601714: Translation of the direct-on-blood PCR-NALFIA system into an innovative near point-of-care diagnostic for malaria
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 polygenic samples among all populations with Brazil (84%), Sri Lanka (95%), Ethiopia (78%), and French Guiana (62%).

THE APOCLOPLAST 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 PlasmodDB 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 (AP001010: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 18S rRNA 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.

GEOPOLITICAL DISPERSION AND GENETIC CHARACTERIZATION OF PFMHRP2 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, -6kb, 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.
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 in addition to general quality. Following unblinding, discrepant slides are re-examined to determine source of discrepancy, and consensus opinion with the submitter is attempted by using high quality photomicrographs. All slides are returned to the submitter so that they can be used for further education. Error rates less than 20% are considered standard for passing a laboratory proficiency test, and further rounds of QC can be performed as needed. For histology studies of malaria in pregnancy, discordance over 10% should trigger consideration of additional training and targeted re-review of study slides. In pilot studies with experienced readers, there was concordance in 85/94 (90.5%) of submitted cases; discrepancies primarily included false positives for hemozoin due to artifact, and false negatives due to low levels of hemozoin. We anticipate that a standardized histopathology QC/QA protocol will be of value to pregnancy malaria research community, and have potential to strengthen local histopathology expertise.
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.

1811

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).

1812

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 SCHISTOSOME 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′-azacitidine (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 Boudicca (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.

**1816**

MICRORNAS IN THE EXCYSTATION OF FASCIOLA HEPATICA METACERCAE

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Host invasion by the trematode parasite Fascula hepatica is initiated by the activation of the metacercariae usually in the host stomach. The released juvenile forms actively transverse the gut wall towards the abdominal cavity and follow their journey to the biliary ducts of the liver. The activation is a rapid switch finely tuned by signals from the environment, that can be easily reproduced _in vitro_. miRNAs have emerged as relevant modulators of gene expression at the post-transcriptional level (either by translation blocking or mRNA degradation), playing essential roles in development. We aimed to study the miRNA expressed in the liver fluke metacercarial activation, and for that purpose we purified and sequenced the small RNA populations expressed at this developmental transition. After filtering the reads with homology to mRNA, repetitive sequences and other non coding RNAs, we ended up with several thousand reads that were compared to miRBase, Rfam and all the mRNA previously identified in other flatworms. Within the known miRNAs found, those common to all metazoans and protostomes were the most abundant. Some miRNA so far only detected in other flatworms were also found, highlighting the existence of flatworm specific miRNA families. Furthermore within sequences with no homology, novel F. hepatica-specific miRNAs were predicted. We observed subtle differences between dormant and activated metacercariae and further differences to newly excysted juveniles. While sequence conservation in mature miRNA is high across the metazoan tree, we observed that in general flatworm miRNA are more divergent than in other lineages, with strict conservation restricted to seed region. Whether this variability leads to changes in the regulated target genes associated to the parasitic way of life deserves further investigation.

**1817**

IDENTIFICATION OF SYMBIONTS IN THE REPRODUCTIVE TRACT MICROBIOME OF NATURAL ANOPHELES GAMBIAE POPULATIONS AND IMPLICATIONS FOR NOVEL VECTOR CONTROL METHODS

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Identification of symbionts in the reproductive tract microbiome of natural Anopheles gambiae populations and implications for novel vector control methods Recent findings show that mosquito-microbiota interactions are crucial determinants of mosquito fitness and vectorial capacity opening novel and promising areas of research in the utilization of symbiotic bacteria for the control of vector-borne diseases. Furthermore, recent
works propose the use of paratransgenesis as a novel vector control tool, which exploits genetically engineered symbionts to deliver anti-parasite molecules in 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 analysed 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 Anophelines. 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 ANOPHELES GAMBIAE 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 peristomial 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 sequestrate 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 peristomial 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 peristomial 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 ecototherms, 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.

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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 *Cx. pipiens* vectors 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 *Cx. 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 vascular 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.

### 1822

**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 parasitemia 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 anthelmints or 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 antischistosomals.

### 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 filarimal 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 Dreiikir 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.
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 vertebrates. 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 antiserum 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 rSmKI 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 Thai 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 transfection 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 any 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 individual parasitized red blood cells. This has been extensively validated and can generate SNP calls genome-wide with high (>99%) accuracy, even in species such as P. 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 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 98% 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 at high levels in patient serum during acute infection. We examined the protective efficacy of NS1 immunization against lethal DENV2 infection in a mouse model of vascular leak. Interferon α/β receptor-deficient C57BL/6 (Ifnar–/–) mice were injected intraperitoneally 3X over 6 weeks with 20 µg of DENV2 recombinant NS1 (NS1) 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 nNS1 with either SAS + CpG or Addavax + MPLA were fully protected against lethal peripheral challenge with DENV2, whereas mice vaccinated with DENV2 NS1 at 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 vaccine blocked DENV pathogenesis, we hypothesized that NS1 itself may have direct pathogenic effects. We found that Ifnar–/– 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 nNS1 was added to cultured human pulmonary microvascular endothelial cells (HPMEC) in a transwell system, the relative TEER value decreased compared to untreated or OVA-treated HPMEC cells. We next investigated if the in vivo lethality and in vitro disruption of endothelial integrity caused by nNS1 could be inhibited by NS1-immune serum. Ifnar–/– mice passively administered anti-NS1 serum after receiving sublethal DENV2 + NS1 protein were completely protected against death while those receiving control serum were not. In the HPMEC assay, the disruptive effects of nNS1 on TEER were prevented by NS1-immune serum but not serum from OVA-immunized or control mice. Thus, DENV NS1 appears to directly contribute to increased vascular permeability, which can be blocked by anti-NS1 antibody.

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DIFFERENCES IN TYPE I INTERFERON SIGNALING ANTAGONISM BY DENGUE VIRUSES IN HUMAN AND NON-HUMAN PRIMATE CELL LINES

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The type I interferon (IFN-α/β) response has been shown to be one of the most regulated systems during dengue virus infections. Moreover, attenuation of IFN-stimulated genes has been associated with severe disease. In this study we wanted to address conflicting reports of inhibition of IFN signaling by dengue viruses and if there were variations in viruses that differed in their pathogenicity. Using a method that combines flow cytometry and a four-parameter logistic regression model we compared the relative inhibition of IFN-α/β signaling between viruses. Our results showed that all dengue virus serotypes were capable of inhibiting IFN signaling in human cells. A more refined analysis of well-characterized DENV-2 clinical isolates from the five DENV-2 genotypes demonstrated that all viruses inhibited 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 VIRUS NON-STRUCTURAL PROTEIN-1 (NS1) INCREASES HUMAN PULMONARY ENDOThelial CELL PERMEABILITY IN VITRO

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Dengue is the most prevalent arboviral disease in humans and a major public health problem worldwide. Systemic plasma leakage leading to profound shock and potentially fatal complications is a critical determinant of dengue severity. Increased vascular permeability without morphological damage to the capillary endothelium seen in severe dengue suggests the shock syndrome may be due to endothelial dysfunction. In the endothelium, dynamic structures including intercellular junctional proteins and the endothelial glyocalyx control the barrier function critical for vascular homeostasis. In certain diseases, e.g., dengue, functional and structural alterations modify the normal architecture of the vessel wall, increasing plasma extravasation. Dengue pathogenesis involves a complex interaction of the virus and host immune response, including cross-reactive antibodies and T cells, complement activation, and elevated levels of cytokines and other soluble mediators that correlate with severe disease. However, the mechanism of vascular dysfunction in dengue disease is still unclear. Secreted and cell-surface-associated dengue virus nonstructural protein 1 (NS1) and anti-NS1 antibodies are implicated in contradictory roles of protection and pathogenesis, and how NS1 contributes to dengue pathogenesis remains uncertain. Here we evaluated the role of soluble NS1 (sNS1) in inducing endothelial dysfunction. Cultures of human pulmonary microvascular endothelial cells grown on a transwell permeable membrane system as a model of barrier function in vitro were exposed to sNS1 (0.2-20 µg/mL), and endothelial permeability was examined by continuously measuring the trans-endothelial electrical resistance (TEER). sNS1 induced a significant dose-dependent increase in endothelial permeability starting 2 hours post-treatment (hpt), at 5 and 20 µg/mL (20 and 50% decrease in TEER, respectively). This effect persisted for more than 24 h as compared to the TEER baseline values exhibited by untreated controls and treatment with unrelated protein (20 µg/mL OVA). Lower concentrations (0.2 and 1 µg/mL) showed less dramatic but still significant decreases in TEER that returned to baseline 6-12 hpt. Confocal microscopy revealed concomitant alterations in intercellular junctional proteins. Our findings suggest a new mechanism of sNS1 directly triggering endothelial vascular dysfunction that occurs in severe dengue disease.

MICROVASCULAR AND ENDOTHELIAL FUNCTION IN PREDICTING CLINICAL OUTCOME OF DENGUE

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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 prospective observational study recruiting a) patients presenting to the OPD with fever for <72 hours and a clinical diagnosis of possible dengue, and b) patients hospitalized with warning signs or established severe disease, is ongoing in two hospitals in Hanoi and Ho Chi Minh City, Vietnam. Clinical, laboratory, and haemodynamic assessments are performed daily for a maximum of 6 days, and again at follow-up 2 weeks later. Microvascular imaging using Sidestream Darkfield Imaging (SDF) and endothelial function testing using peripheral artery tonometry (EndoPAT) are performed at enrollment, defervescence/hospital discharge and follow-up. To date, 167 patients have been recruited, 92 in the outpatient arm and 75 in the inpatient arm. The median age is 27 years (range 5-65 years) and 47% are male. In the outpatient arm 29/67 (43%) of the confirmed dengue cases developed warning signs and 3/67 (4%) developed shock, while 25/92 (27%) were diagnosed as having other febrile illnesses (OFI). At enrolment, the reactive hyperaemic index (RHI), a marker of endothelial function, was lowest in the patients who went on to develop severe dengue (median [range] 1.54 [1.36-1.96]) followed by those who developed warning signs (1.78 [1.17-3.5]) and then uncomplicated dengue (2.18 [1.16-2.29]). In the OFI category the RHI was 1.63 [1.22-3.38]. Results for the inpatient arm showed a similar trend with the lowest RHI seen in severe dengue patients. The SDF images are being analysed; initial results show microvascular flow is impaired in early dengue with a lower proportion of perfused vessels, mean flow index and vessel density compared with follow-up. These preliminary results suggest microvascular and endothelial dysfunction are associated with dengue disease severity, and can be detected prior to severe clinical manifestations. These techniques may prove useful as outcome predictors and/or to monitor endothelium-directed therapies.
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) Ly6C+ 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). Ly6C+ 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 Ly6C+ monocytes from Ifnar-/- 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 FcR 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, leucopenia 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 transmitters 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/midgut, 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/midgut. 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 artemisinin 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.

THE EFFECT OF ARTEMISININ-COMBINATION THERAPY TREATMENT OPTIONS ON PLASMODIUM FALCIPARUM GAMETOCYTE CARRIAGE: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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The production of gametocytes during malaria is determined by a variety of parasite, human and environmental factors. ACTs rapidly clear the asexual parasite biomass in infected individuals, with potent gametocyticidal activity against early sexual stages of the parasites; they play a critical role in reducing the transmission of malaria and decreasing the spread of drug resistant parasites. We conducted a large pooled analysis of clinical data to examine the differential effect of ACTs on the transmission potential of Plasmodium falciparum. A systematic search of the literature was conducted to identify all studies published between 1960 and March 2014, in which patients were enrolled and treated with antimalarials and where gametocyte data were recorded. Individual patients data from over 100 studies (n>40,000 patients) were collated, curated and included in analysis. Data from 21 African and 6 Asian countries was analysed for gametocyte carriage following treatment with artemether-lumefantrine, amodiaquine-artesunate, dihydroartemisinin-piperaquine, and mefloquine-artesunate. An apriori data analysis plan was developed to identify factors associated with gametocyte prevalence and density prior to treatment and following treatment with an ACT. Criteria for the quality of gametocyte assessments have been ascribed to the various studies. In conclusion, the effects of asexual parasite density, age, transmission intensity and haemoglobin concentration on enrolment gametocyte prevalence and density will be presented. The differential effect of ACTs on post-treatment gametocyte carriage, density and carriage time will be examined in relation to ACT regimen, parasite clearance time, transmission intensity and human host factors. The results of this important study and their relevance for malaria elimination will be highlighted.
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; Khihi, 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 (LLIN) 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 endpoint 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 endpoint 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; 2,456,770 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.

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 of great importance for malaria control programs. Although LLIN bioefficacy has been investigated, the physical integrity of the net fabric is less well understood making it challenging to determine overall net protectiveness. The 2011 World Health Organization Pesticide Evaluation Scheme (WHOPES) guidelines provide a simple, standardized method using
a proportional hole index (PHI) for assessing net damage. 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 WHOES guidelines. The areas of WHOES 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 underestimated using the WHOES 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 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 Walkaway40®. 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 typhimurium) 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.

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 B lymphoblastoid cell-lines (B-LCL), autologous blasts and HLA-E restricted AEH B-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, INF-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, INF-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.

ACTIVATION OF SALMONELLA TYPHI-SPECIFIC REGULATORY T CELLS IS ASSOCIATED WITH TYPHOID DISEASE IN A WILD-TYPE S. TYPHI CONTROLLED HUMAN INFECTION MODEL
Monica A. McArthur, Stephanie Fresnay, Thomas C. Darton, Claire Jones, Claire S. Waddington, Cristoph J. Blohmke, Gordon Dougan, Brian Angus, Myron M. Levine, Andrew J. Pollard, Marcelo B. Sztein
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Salmonella Typhi (S. Typhi), the causative agent of typhoid fever, causes significant morbidity and mortality throughout the world. Currently available vaccines are only moderately immunogenic. To develop improved vaccines, identification of immunological responses associated with protection or disease is necessary. This has been hindered, in part, by the lack of an animal model that faithfully recapitulates human disease. Recently, a controlled human infection model with wild-type S. Typhi has made these critical studies possible. Peripheral blood mononuclear cells were obtained from volunteers (n=10) prior to and at multiple time-points after challenge with 108 colony forming units of wild-type S. Typhi (Quailes strain). Regulatory T cell (Treg) responses were measured by flow cytometry and activation status and homing potential of Treg cells were measured by flow cytometry and activation status and homing potential of Treg cells.
S. Typhi-specific T<sub>reg</sub> were determined. We identified significantly higher gut homing (integrin-a4b7 expressing) S. Typhi-specific T<sub>reg</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-a4b7 expressing S. Typhi-specific T<sub>reg</sub> decreased in TD volunteers, indicating likely homing and a resulting accumulation in the gut. Additionally, S. Typhi-specific T<sub>reg</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>reg</sub> in TD volunteers 1-4 days post-challenge compared to No TD volunteers. Taken together these results suggest that activation of T<sub>reg</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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**1851**

**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. claridgeiae 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 gltA 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 (Lutzomia 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 determinants 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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**1852**

**INVESTIGATION OF GENOTYPE VARIATIONS IN ORIENTIA TSUTSUGAMUSHI OBTAINED FROM PATIENTS WITH MODERATE AND 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 spp) 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, O. 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 O. tsutsugamushi and O. 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 O. 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 O. tsutsugamushi.

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**1853**

**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 leptospirosis diagnosis. We assessed this retrospectively, using pre-incubated HCF of leptospirosis 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, Lao 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% Cl: 71-90), 66% (95% Cl: 55-76) and 59% (95% Cl: 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.

1854

INNOVATING DIAGNOSIS OF BACTERIAL BLOODSTREAM INFECTIONS IN MALARIA-ENDEMIC SETTING: 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 > 4,000 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 SepsisTestTM, 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. We 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.

WHAT IS AN ASYMPTOMATIC CARRIER IN AN ENDEMIC AREA?

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Nowadays there is a greater consensus that is crucial to identify asymptomatic carriers in order to eliminate/eradicat malaria. The methods of evaluating clinical malaria in endemic areas, which include both passive (PCD) and active case detection (ACD), are not very effective in detecting asymptomatic infections, limiting treating asymptomatic carriers. Aggressive infection detection (AID) is other strategy where malaria parasites are searched in people of endemic areas, regardless of the presence of clinical symptoms, using PCR and thick smear (TS). However, applied on a large scale AID would be expensive and impractical. In this perspective, we try to understand who is an asymptomatic carrier in an endemic area through an open population cohort study, conducted in the Peruvian Amazon, and involved 2000 people from 8 communities around
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 administrated was 10.4 (5.6-20) ml/kg. Patients with jaundice at presentation received significantly larger number (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 convalsenial 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.
SM group receiving WHO guideline resuscitation (15mls/kg over 1 hour, repeated twice if indicated) mortality was 73% (8/11 patients). Following a protocol amendment to slower rehydration (10mls/kg over 1 hour up to a maximum of 50mls/kg) 3/8 died (38%) compared to a mortality of 2/10 in controls. Echocardiographic and haemodynamic data pre-bolus showed marked evidence of underfilling. Boluses lead to early rapid shock reversal, in those treated with slower rehydration shock reversal was more protracted. In all study participants we found no evidence of that mortality was due to fluid overload. Fluid boluses administered to children with SM (per WHO guideline)-resulted in early shock reversal, but this was not associated a survival benefit. Slow rehydration strategy in cases (SM) and control patients appeared to be well-tolerated. Further research is required to optimize fluid management and other supportive strategies to inform future guidelines.

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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 this 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 were also reduced. 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.

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ACUTE MALARIA INDUCES CTLA4+PD1+ EFFECITOR T CELLS WITH REGULATORY PHENOTYPE

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Acute Plasmodium falciparum (Pf) malaria induces proinflammatory T cell responses which have been shown to confer protection against malaria but also contribute to the development of severe disease. A tight regulation of T effector (T eff) responses is therefore crucial to protect the host. An important mechanism to fine-tune T cell responses in the periphery is the induction of co-inhibitory receptors such as CTLA4 and PD1 and their ligands but their role in the immune response in Pf malaria remains poorly understood. To test the hypothesis that co-inhibitory receptors modulate the T cell response in acute malaria, blood samples were obtained from patients with acute uncomplicated Pf malaria treated in Hamburg, Germany as well as from healthy volunteers. Flow cytometric analysis showed high expression of CTLA4 and PD1 on CD4+ T cells of malaria patients and the ligands for PD1, PD-L1 and PD-L2, were upregulated on monocytes, B cells and T cells. We then stimulated PBMCs with Pf-infected red blood cells (iRBCs) to detect antigen-specific cytokine production and proliferation. The majority of antigen-specific T cell were CTLA4+PD1+. IFNg was the most frequently detected cytokine and >50% of IFNg+ CTLA4+PD1+ T cells simultaneously produced IL10. In some donors T cell proliferation was inhibited by PD1 and blockade of PD1-ligation enhanced antigen-specific proliferation. We further isolated CTLA4+PD1+CD4+ T cells based on surface expression of PD1 and CTLA4 and investigated their inhibitory function in in-vitro proliferation assays stimulated with aCD3/28 or iRBCs. CTLA4+PD1+CD4+ T cells suppressed aCD3/28-induced as well as plasmodial-antigen-specific T-cell proliferation in a cell-extrinsic manner. In summary, acute Pf infection leads to induction of malaria-specific CTLA4+PD1+ T cells which coproduce IFNg and IL10 while inhibiting CD4+ T cell proliferation in a cell extrinsic manner. Induction of T eff+ cells with regulatory function might be an important mechanism to control T cell responses and prevent severe inflammation in acute malaria and potentially other acute infections.

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LOSS AND DYSFUNCTION OF VΔ2+ γΔ T CELLS IS ASSOCIATED WITH CLINICAL TOLERANCE TO MALARIA

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The VΔ2 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) or controls and assessed by multiparameter flow cytometry and gene expression microarray. We noted a striking inverse association between frequencies of VΔ2+ cells and the prior cumulative incidence of malaria (Rho=-0.30, 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<0.001) of VΔ2+ cells in response to malaria antigen stimulation, suggesting that children who have survived repeated clinical malaria episodes exhibit dysfunction as well as loss of VΔ2+ cells. Whole transcriptome analysis of sorted, unstimulated VΔ2 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 VΔ2+ 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 VΔ2+ γΔ T cells that may facilitate immunological tolerance of the parasite.

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LONGITUDINAL ANTI BODY RESPONSES TO ANTIGENS ON THE SURFACE OF Plasmodium falciparum GAMETO CYTE-INFECTED ERYTHROCYTES IN GHANAIAN SCHOOL CHILDREN

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Little is known about the immune responses directed at circulating Plasmodium falciparum gametocyte-infected erythrocytes 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 dihydro-artemisinin piperarquine 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 gametocytamia 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 gametocyteemia 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.

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.

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 (MSPF2) and FMP2.1 (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 inhibition of anti-malarial activity 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. This study informs an important decision point for future development of an AMA1 vaccine as well as malaria blood stage vaccine development in general.
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-CD62L- effector memory T cells with little variation depending upon the time point or the study cohort. We conclude that detectable CD4+ T cell memory response can be measured against both malaria and schistosoma antigens and that the presence of S. haematobium may be associated with enhanced functional T memory cell induction to malaria antigens.

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-4 verbal 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. 26%, 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 28% 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 death resulting from both communicable and non-communicable diseases, along with efforts to reduce maternal deaths among women of reproductive age.

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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 - 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 - 208,000).
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.

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VALIDATION 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 AT THE POPULATION LEVEL IN DEVELOPING COUNTRIES COMPARABLE WITH GLOBAL ESTIMATES.

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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 (NHDSS) 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 recorded 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%), diarrhea (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%), diarrheal 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, submersions 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.

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FALSIFIED MEDICINES IN AFRICA AND PUBLIC HEALTH - ‘NO ACTION-TALK ONLY’

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Poor quality medicines are neglected impediments to improving global public health. In June 2012 suspected falsified medicines labelled as the antimalarial ‘artemether-lumefantrine’ bearing the Affordable Medicines Facility-malaria (AMFm) logo and others labelled as the antihelminthic ‘mebendazole’ were seized in Luanda, Angola. The tablets were analysed by an array of analytical platforms including high performance liquid chromatography, ambient ionization mass spectrometry, Raman spectroscopy, X-ray powder diffraction analysis, nuclear magnetic resonance spectroscopy, isotopic-ratio mass spectrometry, botanical assays and packaging analysis, using the portable counterfeit detection device CD-3. No artemether or lumefantrine or other active pharmaceutical ingredients were detected in the ‘artemether-lumefantrine’ tablets. Brushtite and three different yellow dyes and few pollen grains were found. No mebendazole was detected in the ‘mebendazole’ formulation, but calcite and levamisole (270mg/tablet) were present. Both ‘products’ showed marked differences in packaging characteristics from genuine products. The discovery of falsified artemether-lumefantrine, labelled as an AMFm product and without any detectable antimalarial, is of considerable concern for malaria control. Presence of levamisole in falsified ‘mebendazole’ is also of great concern as it has been banned for human use. This seizure illustrates many of the current problems regarding poor reporting and transparency and inaction. Enhanced collaboration between African MRAs/police and the authorities in China to stop criminal transcontinental trade in falsified essential medicines is urgently needed. Delays in reporting and action must be reduced by mandatory notification systems and independent public health risk assessments. Despite multiple reports, public health research has failed to stimulate actions required to improve the quality of global drug supply.

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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.
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 diagnostic tools 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 (TF 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
reasons why active and scarring trachoma are so prevalent on the Bigaros Archipelago of Guinea Bissau are unclear. We collected 1507 population-based conjunctival swabs with corresponding detailed clinical phenotype. We used droplet digital PCR assay to detect and quantify Ct DNA on swabs. Associations between Ct load and clinical phenotype were examined using regression models. We used agent-based modeling to investigate the role of Ct load in trachoma transmission. Whole genome sequence analysis was used to identify variants in putative virulence-associated genes/loci. The geometric mean of estimated Ct load in clinically normal conjunctivae was 294 copies/swab (95% CI: 165-524). In clinically active trachoma it was 8562 copies/swab (95% CI: 5412-13546). In active trachoma Ct load increases with disease severity (for both follicular and inflammatory scores). The highest Ct loads were associated with the most severe clinical disease and the strongest associations were with increasing inflammatory grade (at maximal inflammatory score (P3) OR 30.9, 95% CI 9.39-101.5, p<0.0001). Genotypic differences in virulence-associated genes within this population of ocular Ct are suggested. The association between load and disease severity may be related to Ct strain diversity, where multiple strains are co-circulating. We used a novel mathematical modeling strategy to investigate the role of Ct load in trachoma transmission. This is the first application of these approaches in understanding the pathogenesis and transmission of Ct infection, which are fundamental to successful trachoma elimination and surveillance strategies.

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 sequel 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 (≥6weeks 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/100,000 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.

REPRODUCTIVE TRACT INFECTIONS AMONG PRIMARY SCHOOLGIRLS IN RURAL WESTERN KENYA

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Reproductive tract infections (RTIs) among adolescent girls remain a great public health concern in developing countries. However, the burden of RTIs in this key population is insufficiently understood. This study presents preliminary analysis of RTIs’ symptoms reporting and laboratory detection rates among girls aged 14-16 (median 14) years, in 30 primary schools, enrolled in a feasibility study on acceptance, use and safety of menstrual products in western Kenya. Vaginal self-swabbing samples were prospectively collected during symptom guided RTI testing (SGT) (March-October 2013) and cross-sectional end-of-study screening (EOSS) (November 2013). Samples were analyzed for Bacterial vaginosis (BV), Chlamydia trachomatis (CT), Neisseria gonorrhoea (NG), Trichomonas vaginalis (TV) and Candidiasis. Infected girls were referred for treatment. Data were analyzed using SPSS v21.0. Overall, 532 girls (SGT: 17, 3.2%; EOSS: 453, 85.2%; and overlap in both: 62, 11.7%) were included. Of a total 79 girls in SGT group, BV 13 (16.5%), Candidiasis 11 (13.9%), TV 5 (6.3%) and CT 2 (2.5%) were test confirmed. None tested positive for NG. BV was the most common in EOSS, 94 (13.8%), followed by Candidiasis 44 (8.5%), TV 13 (2.5%) and NG 3 (0.6%). Of 62 girls in both STG and EOSS, RTI detection rates varied (SGT-EOSS) for BV (19.4%-14.5%), Candidiasis (14.5%-12.9%), TV (6.5%-0%), CT remained constant. While only 82 (15.9%) girls reported symptoms for RTI at EOSS, laboratory testing showed 146 (28.3%) had at least one RTI. In conclusion, high detection rate of RTIs was observed among the rural adolescent schoolgirls. Symptom-based diagnosis of RTIs poorly predicted RTI in this population. These findings offer important insights for treatment and prevention of RTIs among schoolgirls.

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,
median age was 17 months (interquartile range 10-34), 16.5% were stunted, and 4.4% were HIV-infected. The most predominating bacterial isolate was EAEC (14.1%), followed by Campylobacter (6.6%), EPEC (6.2%), Shigella (4.6%), and ETEC (4.4%). IMCI correctly classified 3 of 45 lab-confirmed Shigella cases (sensitivity 6.7%), 2 cases of Shigella flexneri and 1 S. dysenteriae. Among 928 children without shigellosis, IMCI correctly classified 871 (specificity 93.9%). Of the 57 children incorrectly diagnosed with Shigella by IMCI, 73.7% had no other bacterial pathogen identified. Cholera was not detected although 11 (1.1%) children were classified as having suspected cholera based on IMCI criteria (specificity 98.9%); of these 11, 36.4% had no isolated bacteria. The IMCI guidelines appear reasonably specific but not sensitive in identifying children requiring antibiotic therapy. IMCI guidelines should be adapted to enhance sensitivity and to account for additional enteric pathogens associated with increased morbidity and mortality.

**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 GAMBAE FLY 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 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 directed 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
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-Pool 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.

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), boron acid and tolfenpyrad (a mitochondrial electron transport inhibitor), mixed in a guava juice-based bait, each killed more than 90% of pyrethroid-resistant 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 predicated in large part upon the transfer of a mating plug containing steroid hormones (SH). Here we report mechanisms of pre and peri-copulatory 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 gel-C-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, calponins, 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 transhyretin-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.

1891

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 helminths, Loa loa (Ll) 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 Ll microfilaraemia. To identify microfilarial-derived Ll-specific biomarker(s) that could provide the basis for a microfilarial quantitative immunoassay, we characterized the excretory/secretory (E/S) proteome of Ll mf as well as the Ll-specific proteins found in urine and plasma of Ll-infected and -uninfected individuals using LC-MS/MS. From 20 x 10^6 mf purified from the blood of Ll-infected patients and cultured in vitro, 1273 proteins (representing 8.2% of the Ll 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 Ll-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) Ll proteins found only in Ll-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 Ll 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+ Ll-infected plasma (n=30) and not by those from uninfected plasma (n=20)). Development and testing of rapid antigen capture immunooassays are underway to provide an alternative to more standard methods of mf quantification.

1892

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, hypoderms and multivesicular bodies of infective stage larvae of both filarial parasites. Ov-RAL-2 and Bm-RAL-2 are immunodominant proteins expressed in the hypoderms 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 embroygrams 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 germil 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.

1893

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, Wolbachia, has been implicated in the pathogenesis of these reactions, L. loa infection to those in patients infected with Onchocerca volvulus. The study included three groups: Group I (LOA-NIH), subjects with microfilaricmic (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 Wobachia during microfilarial killing.

1994

ENDOTHELIAL CELLS RELEASE SOLUBLE FACTORS THAT PROLONG THE SURVIVAL OF FILARIAL WORMS IN VITRO

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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 microfiliariae (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 over 7 days, co-culture 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 microfilariae 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.

1989

THE IMPACT OF MATERNAL HELMINTH INFECTIONS ON TH2 RESPONSES AND ATOPIC SENSITIZATION OF INDONESIAN YOUNG CHILDREN

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Immune responses to helminth infection and allergy are both characterized with TH2 responses. Manifestation of allergies in children of low-to-middle income countries were much lower compared to those of affluent countries. We aimed to investigate the impact of maternal filariasis infections and other factors on child’s development of type 2 responses and atopic sensitization at 4 years of age in an area endemic for filaria and soil-transmitted helminths. Data were collected from pregnant mothers on helminth infections, total IgE and Ascaris-specific IgE, education and socioeconomic status (SES). Total IgE and IL-5 in response to mitogen, and helminth antigens were measured in children at 2, 5, 12, 24 and 48 months of age. Ascaris and allergen-specific IgE and skin prick testing (SPT) were determined at 4 years of age. Strong TH2 responses were seen at 5 months of age and increased with time. Child’s helminth-antigen specific TH2 responses increased significantly with age and were associated with maternal filarial infection, while the increasing of child’s general TH2 responses with age were more associated with higher maternal total and Ascaris-specific IgE, as well as with low maternal education or SES. Child’s Ascaris-specific IgE were both associated with child’s general and helminth-specific TH2 responses. At 4 years of age when allergen reactivity was assessed by SPT, the high general TH2 responses did not translate into higher SPT. The risk factor for SPT reactivity was low maternal education which decreased the risk of SPT positivity to allergens (adjusted OR, 0.32; 95% CI, 0.12 - 0.87) independently of maternal filarial infection which tended to reduce the child’s risk for being SPT positive (adjusted OR, 0.35; 95% CI, 0.07 - 1.70). In conclusion, young children living in areas endemic for helminths developed a strong TH2 responses which was influenced by maternal or child’s exposure to helminth infections, but did not translate to a higher SPT reactivity to allergens. This result might explain why the prevalence of allergies in low-to-middle income countries were much lower compared to the more affluent countries.

1986

TRANSCRIPTIONAL PROFILE OF THE DIROFILARIA IMMITIS LIFE CYCLE

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Dirofilaria immitis (Di), or canine heartworm, is a filarial nematode evolutionarily related to those responsible for human parasitic diseases. The D. immitis genome, along with the genome of its obligate endosymbiont, Wobachia (wDi), was recently completed and published. We initiated a series of transcriptional profiling experiments to better understand the temporal transcriptional activity of Di and wDi throughout the nematode life cycle. Over 215 million single-end 50 bp reads were generated from total RNA from five Di life cycle stages. Based on hierarchical clustering of expression data, nearly 60% of all Di genes display stage-specific transcriptional patterns. Pairwise comparison of adult male (AM) and adult female (AF) samples reveals that over 9,000 genes display sex-biased transcriptional patterns. The L3 to L4 transition, which occurs upon entering the mammalian host, is critical to the Di life cycle and a potential point of intervention. Among all five life cycle stages examined, a significant portion of Di genes are L4-
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 wDi genes than to Di genes in each life cycle stage. Interestingly, synthesis of the critical metabolite, heme, by wDi 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.

1897

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 cannot be used and until recently, only spleenectomized chimpanzees allowed for the transition from the sporozoite stage to the axenial blood stage of the life cycle necessary for preynegy 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 hu-Hep mouse) infused with human erythrocytes can support P. falciparum sporozoite infection, the completion of liver stage development and the transition to axenial blood stage replication. This thus suggests that P. falciparum experimental crosses can be achieved in the FRG KO hu-Hep 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 hu-Hep 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.
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.

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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. Presumably 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 4.963 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 allelic-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 *Anopheles* 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. Asombo 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/α) compared to normal (αα/αα) alpha thalassemia individuals (P=0.04) and not significant for αα/αα compared to heterozygous-(α/-α) individuals. Incidence rates peaked earlier in AA (4-5 years, 0.77 episodes per child per year) compared to AS children (9-10 years, 0.8 episodes per child per year). For alpha thalassemia, incidence rates peaked earlier in α/αα (2-3 years, 0.73 episodes per child per year), compared to α/-α αα individuals (2 years, 1 episode per child per year) and α/αα individuals (4-5 years, 0.79 episodes per child per 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 ($\text{aEIR}=3.3$), Kibihi ($\text{aEIR}=31.5$) and Nagongera ($\text{aEIR}=315$). 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%) were associated with a platelet measurement, of whom 66,421 (30.8%) had clinical malaria. Patients with Plasmodium falciparum monoinfection 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 anemia nor severe thrombocytopenia, those with severe anemia 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 malaria related mortality. Prospective studies are warranted to define its utility in defining the clinical management of patients with malaria.
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 facilities 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.

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 1-4 year olds 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.

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 exophagic 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 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.
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ANOPHELES GAMBIAE SMALL-RNA PATHWAYS IMMUNITY DURING BLOOD DIGESTION

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 females 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.

Aedes aegypti female mosquitoes with alanine aminotransferase deficiency face a stressful metabolic challenge during blood digestion

Aedes aegypti females face stressful metabolic demands during blood digestion. The primary food consumed by these mosquitoes includes blood from vertebrates, which is rich in proteins. The ingestion of blood induces a metabolic crisis in mosquitoes, which is characterized by a rapid mobilization of amino acids and other nutrients for the synthesis of essential compounds. This process is highly energy-consuming and requires the upregulation of metabolic pathways to efficiently utilize the provided amino acids.

To cope with this challenge, mosquitoes have evolved mechanisms to modulate their metabolism. One such mechanism involves the inhibition of the alanine aminotransferase (ALAT) enzyme, which is responsible for the conversion of alanine to pyruvate. By knocking down ALAT activity, the mosquitoes can reduce the rate of alanine breakdown, thus conserving this amino acid for the synthesis of amino acids required for the synthesis of essential molecules.

毛巾.jpg
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 QUINQUEFACSIATUS

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Culex quinquefasciatus, a southern house mosquito, is a member of a 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 ideograms for mitotic chromosomes of Cx. quinquefasciatus based on their banding patterns at early metaphase. These ideograms 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 locus 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**

MICROSATELLITE AND DNA SEQUENCE POPULATION GENETICS EVALUATION OF THE SOUTHWEST PACIFIC MALARIA VECTOR ANOPHELES KOLIENSI (OWEN)

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The southwest Pacific malaria vector Anopheles koliensis is a sibling species of An. p. punctulatus that can co-exist with An. gambiae in PNG and Australia. The species is present in many parts of the world including PNG, Vanuatu, New Caledonia, and Australia. It is a common mosquito in urban areas and is closely related to An. gambiae in terms of morphology. The species is susceptible to malaria and dengue. It is a vector of Plasmodium falciparum and Plasmodium vivax. The species is also a vector of filariasis. It is a vector of dengue and chikungunya.

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.

**1915**

CHARACTERIZATION OF MALE REPRODUCTIVE FACTORS ESSENTIAL FOR MATING SUCCESS IN THE LIFE CYCLE OF ANOPELE 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 anemia. 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 underly 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 gravidity. Among multigravidae, the protective effect of IPTp-SP against LBW was no longer significant in areas where the malarial prevalence in children was < 9% when we applied MAP estimates, and < 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 multigravidae. 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 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 0.1-10.3%) 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 primigravid 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 primigravid 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 artemisinin-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 UNCOMPLICATED 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 artemisinins 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-piperazine, arteether-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.

1924

POTENT ANTI-MERS COV (MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS) FULLY HUMAN ANTIBODIES FROM TRANSCRYPOTOMOSOMIC BOVINES FOR PASSIVE IMMUNOTHERAPY

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No specific treatments of proven effectiveness for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infections are currently available. The International Severe Acute Respiratory & Emerging Infection Consortium (ISARIC) identified passive immunotherapy with neutralizing antibodies as a treatment approach that warrants priority study. A platform technology using transchromosomic bovines (Tc-bovines) that produce fully human, antigen specific, polyclonal IgG antibody (Tc-pAb) of all subclasses following immunization has been developed. The quantity of Tc-pAb that can be derived from each animal after plasmapheresis ranges from 150 to 300 grams per month. Purified Tc-pAb for intravenous or intramuscular administration has extremely low quantities of bovine proteins and no evidence of adventitious agents. The Tc-bovine platform can also rapidly produce a diverse repertoire of fully human monoclonal antibodies. Two experimental anti-MERS CoV Tc-pAb immunoglobulins were produced in Tc-bovines hyperimmunized with inactivated whole virion Jordan strain virus (clade A) or a recombinant spike protein derived from an Al-Hassa strain (clade B). Both Tc-pAb immunoglobulins, termed SAB-300 and SAB-301, demonstrated 50% plaque reduction neutralizing antibody titers > 10e4/ml and cross neutralized other MERS-CoV strains. SAB-300/SAB-301 were evaluated in recombinant mice expressing the DPP-4 receptor (5 mg/kg and 25 mg/kg IP as a single dose 12 hours before intranasal challenge) and SAB-300 in marmosets (80 mg/kg in 4 divided doses IV starting 24 hours after intratracheal challenge). Control infected mice had a lung viral titer of ~6.0 log10 PFU/mg through day 5 post inoculation but treated mice approached, or were below, the limit of detection (2.0 log10 PFU/mg) by 24-72 hours and displayed no toxicity. Treated marmosets displayed no toxicity and the clinical/pathologic data will be presented. Because of these encouraging pre-clinical findings, an IND application is in development.

1923

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.
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), Puromala (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.

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 Fottial 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.

SEROEPREVALENCE 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 reasortant virus composed of the S and L segments from Bunyamwera and the M segment from Batai 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 Sangailu, 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 aggressively investigated to determine the public health impact of these infections especially during seasons of high mosquito abundance.

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 reasortant 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 reasortant virus showed that there was more than a 4-fold difference in titer between these viruses, indicating that the new reasortant was serologically distinct from the prototype Caraparu strain. Serological analyses also confirmed that the novel reasortant 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 ZOONOTIC 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 trypan 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 TRYPSANOSOMA 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 pharmacological treatments are plagued by 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 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.
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 clinical manifestations of leishmaniasis, caused by more than 20 species of *Leishmania*. All NTDs have been categorized as “tool ready,” yet also “tool deficient” because many of these tools (i.e., drugs and diagnostics) and implementation strategies are inadequate to achieve the desired goals. New effective, safe, and affordable drugs, preferably oral, are needed. The general neglect that these diseases have encountered by the pharmaceutical industry has meant that basic research findings have not found their way into a drug discovery pipeline. In this paper we present an integral approach to the early drug discovery for the three major kinetoplastid NTDs, i.e. visceral leishmaniasis, Chagas disease and sleeping sickness. The GSK 1.8 million compounds diverse collection has been screened phenotypically against their causative parasites, respectively *L. donovani*, *T. cruzi* and *T. brucei*, using the state-of-the-art methodologies available in high throughput screening. As a result of this effort, three anti-kinetoplastid boxes of approximately 200 compounds each have been assembled, which represent all the chemical and biological diversity identified and are intended to serve as an open source of starting points for further lead discovery programs.

A FULLY INTEGRATED PARTNERSHIP PERFORMING DRUG DISCOVERY TOWARDS VISCERAL LEISHMANIASIS: PART 1

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GSK Kinetoplastid DPU and the Drug Discovery Unit, University of Dundee have formed a partnership to conduct drug discovery within kinetoplastid diseases (Visceral Leishmaniasis, Chagas disease and human African trypanosomiasis). The collaboration, with support from the Wellcome Trust has made significant progress, in building new methods and infrastructure to carry out drug discovery for these parasites. Our advances has resulted in the identification of a lead optimisation series for Visceral Leishmaniasis through phenotypic optimisation. Estimates suggest that Visceral Leishmaniasis worldwide causes 51,000 deaths per year. The current drugs are not fit for purpose, suffering from many issues including poor efficacy and unacceptable levels of toxicity. Part 1, by Paul Wyatt from the Drug Discovery Unit, will describe the transition of a *T. brucei* GSK3 kinase inhibitor series into a series that fulfils lead optimisation criteria for Visceral Leishmaniasis. This novel series is one of the few reported globally to show oral efficacy in an acute *in vivo* mouse model against Visceral Leishmaniasis. Part 2, by Tim Miles from GSK, will concentrate on the lead optimisation and progression of this series. As a number of issues were highlighted through critical path screening that have been overcome (i.e., solubility and exposure). Hence a discussion of medicinal chemistry strategies to solve these current set lead compounds within this series are being evaluated for pre-candidate selection.

CLINICAL EVALUATION OF CL DETECT™ RAPID TEST FOR CUTANEOUS LEISHMANIASIS: 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 with 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.

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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.
residual 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 input 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 to quantitatively examine the activity of expressed VSGs in a population of trypanosomes, isolated either from culture or from blood. This next-generation sequencing approach requires very little input 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.

### 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 (monAAM) are phenotypically and functionally distinct from AAM that arise from tissue macrophages (tissAAM). monAAM are F4/80+ and express the mitochondrial protein Ucp1, but low levels of MR1 and PDL2, while tissAAM were F4/80+ and expressed high levels of MR1 and PDL2. Here, we find that monAAM recruited to sites of inflammation remain plastic after activation and differentiation. In short-term transfer experiments, monAAM donor cells transferred into naïve recipient cells retain expression of MR1, but lose expression of PDL2. In long-term transfer experiments, monAAM can convert to F4/80+ macrophages with a phenotype similar to tissAAM. Hence, monAAM 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 monAAM 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 wound 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 CD11cCre x AMPKα1flox/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, INOS), greater numbers of T,17 cells, and defects in the generation of alternatively activated macrophages. Taken together, our data are consistent with an
important role for host metabolism in shaping inflammatory responses during helminth infection. Thus, AMPK activity within myeloid antigen presenting cells regulates host protection against GI parasites.

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**1943**

**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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Toxoplasma gondii is a widespread protozoan parasite 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 Toxoplasma-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 PVM. 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.

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**1944**

**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 liberated heme into reactive hemozoin. Several successful antimalarial drugs such as the aminoquinolines inhibit this process, presumably leading to the toxic accumulation of labile heme, which 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.

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**1945**

**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 Rho5 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 (PFRH5). Previous studies have shown that the PFRipr/PFRH5 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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**1946**

**HSP101/PTEX MEDIATES EXPORT OF DIVERSE MALARIA EFFECTOR PROTEINS INTO THE HOST ERYTHROCYTE**

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To mediate its survival and virulence the malaria parasite Plasmodium falciparum exports hundreds of proteins into the host erythrocyte. To enter the host cell, exported proteins must cross the parasitophorous vacuole membrane (PVM) within which the parasite resides, but the mechanism remains unclear. A putative Plasmodium translocon of exported proteins (PTEX) has been suggested to be involved for at least one class of exported proteins; however, direct functional evidence has remained elusive. Here we show that export across the PVM requires heat shock protein 101 (HSP101), a ClpB-like AAA+ ATPase component of PTEX. Using a chaperone auto-inhibition strategy, we achieved rapid, reversible ablation of HSP101 function, resulting in a nearly complete block in export with surmountable block across the PVM within which the parasite resides, but the mechanism remains unclear. A putative Plasmodium translocon of exported proteins (PTEX) has been suggested to be involved for at least one class of exported proteins; however, direct functional evidence has remained elusive. Here we show that export across the PVM requires heat shock protein 101 (HSP101), a ClpB-like AAA+ ATPase component of PTEX. Using a chaperone auto-inhibition strategy, we achieved rapid, reversible ablation of HSP101 function, resulting in a nearly complete block in export with surmountable block across the PVM. 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.

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.
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